RELATIVE VALUE FOR WOOL GROWTH AND NITROGEN RETENTION OF SEVERAL PROTEINS ADMINISTERED AS ABOMASAL SUPPLEMENTS TO SHEEP

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Summary

Supplements (supplying *c.* 100 g protein per day) of whole egg protein, egg albumen, maize gluten, and gelatin were given to sheep via the abomasum and the effects on wool growth rate, body weight gain, and nitrogen retention were compared with those of casein.

Casein supplements increased wool growth rate substantially (140–200%). Wool growth rates obtained with supplements of whole egg protein and egg albumen were approximately equal to those obtained with casein; maize gluten supplements resulted in less than half the response in wool growth rate obtained with casein, while gelatin supplements had little effect on the rate of wool growth.

All protein supplements were at least 90% digestible, except for egg albumen, which was 60–80% digestible. All protein supplements, except gelatin, were effective in stimulating body weight gain and enhancing nitrogen retention; egg albumen appeared to be inferior to whole egg protein and maize gluten.

I. Introduction

Investigations by Colebrook *et al.* (1968), with a large number of protein concentrates added to the diet of sheep, showed that wool growth rate was influenced by the amount and type of protein. These differences between protein sources may be due to differences in the extent of degradation in the rumen or to differences in the assemblage of amino acids absorbed from the intestines. Wool growth rates with these protein concentrates were low compared to those obtained with casein supplements given per abomasum (Reis and Schinckel 1964; Reis 1969). It has also been shown that, in contrast to oral supplementation, the abomasal or duodenal administration of casein results in enhanced nitrogen retention (Chalmers, Cuthbertson, and Synge 1954; Reis and Schinckel 1961; Little and Mitchell 1967; Schelling and Hatfield 1968). This is due to the rapid deamination of orally administered casein by rumen microorganisms and subsequent loss of nitrogen in the urine (McDonald 1952; Chalmers and Synge 1954).

There is very little information available regarding the utilization of proteins other than casein, given via the abomasum. Little and Mitchell (1967) obtained high nitrogen retentions with casein and soya bean proteins per abomasum, but obtained low values with zein and gelatin, an indication of the importance of amino acid composition. Reis and Schinckel (1964) found only a small response in wool growth

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rate with supplements of gelatin given per abomasum; the addition of cysteine or methionine increased the value of the gelatin supplement, but wool growth was still markedly inferior to casein.

On the basis of results with abomasal supplements of casein and sulphur-containing amino acids (*S*-amino acids), it was suggested by Reis and Schinckel (1964) that any good quality protein, available for digestion and absorption in the intestines, should stimulate wool growth. Preliminary results (Colebrook and Reis, unpublished data) with some protein concentrates infused into the abomasum have indicated differences between proteins in their value for wool growth; moreover those investigated appeared to be inferior to casein. The aim of the present study was to compare the nutritional value of different proteins given as abomasal supplements, and in particular to test the hypothesis that good quality proteins would give "maximum" wool growth rates equivalent to those obtained with casein (Reis 1969). Supplements of casein, egg albumen, whole egg protein, maize gluten, and gelatin (each providing approximately 16 g nitrogen/day) were given and effects on wool growth rate, body weight gain, and nitrogen retention were measured.

II. Materials and Methods

(*a*) *Sheep and Diets*

The experimental sheep were mature Corriedale wethers, fitted with an abomasal cannula near the pylorus. The sheep were kept in metabolism cages in a room maintained at a temperature of 23 ± 1°C. A supplement of 1,000,000 i.u. of vitamin D was given to each sheep once every 3 months. The daily ration was 600 g of a mixture of equal parts wheaten and lucerne chaff; water was available *ad libitum*. The sheep were fed once daily, between 9 and 10 a.m., and were weighed once weekly prior to feeding. The body weights were corrected for cumulative fleece growth.

(*b*) *Experimental Treatments and Protein Supplements*

During a pretreatment period of 12 weeks, the sheep received the roughage diet only; wool growth rate and body weight were stable during this period. There were three consecutive treatment periods, each of 7 weeks, during which protein supplements (supplying 15·2–15·9 g nitrogen/day) were given per abomasum. During period 1 all sheep received supplements of casein; during periods 2 and 3 the supplements were varied as indicated in the following tabulation:

Sheep	Period 1	Period 2	Period 3
4476	Casein, 99 g	Egg albumen, 110 g	Maize gluten, 142 g
4489	Casein, 99 g	Gelatin, 92 g	Egg albumen, 110 g
4493	Casein, 99 g	Maize gluten, 142 g	Whole egg protein, 114 g
4494	Casein, 99 g	Whole egg protein, 114 g	Gelatin, 92 g

Throughout the experiment all sheep received 600 g/day of the roughage diet. During the post-treatment period of 8 weeks the sheep again received only the roughage diet.

The protein supplements were casein (Casinal, Glaxo–Allenburys), gelatin (Davis Edible Gelatine, grade B), maize gluten (Amigold corn gluten, Corn Products Co., New York), hen egg albumen (spray dried grade A, New South Wales Egg Marketing Board), and whole egg protein (prepared from spray dried whole egg powder, grade A, New South Wales Egg Marketing Board).

Whole egg protein was prepared by extracting the egg powder with chloroform, to reduce the fat content to approximately 5%, followed by drying at 45°C.

All proteins were infused into the abomasum as aqueous solutions or suspensions, volume approximately 2 litres, 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 administration of the proteins. Gelatin was dissolved in hot water and the pH was adjusted to 5·5 with hydrochloric acid; the solution was kept at 40°C during infusion to prevent gel formation. The infusion of hydrochloric acid into the abomasum, in amounts equivalent to those given with the gelatin, has no effect on wool growth rate (Reis and Schinckel 1964). Gluten, egg albumen, and whole egg protein were suspended in water (albumen was largely dissolved), and were stirred continuously during infusion to prevent sedimentation and to allow a uniform rate of infusion. It was necessary to add 40 mg of activated methylpolysiloxane (Mylicon, Parke Davis and Co.) to albumen and whole egg protein to minimize foam formation. Methylpolysiloxane has been shown to be physiologically inert (Paul and Pover 1960).

(c) *Collection of Excreta and Nitrogen Analysis*

Collections of excreta were carried out during the last 10 days of each experimental period as described by Reis and Tunks (1969).

Representative samples of the roughage diet and the protein supplements were taken for analysis. For calculation of tissue nitrogen retention, wool was regarded as containing 16% nitrogen.

Nitrogen was determined by a Kjeldahl method, and dry matter content by drying at 104°C for 24 hr.

(d) *Wool Growth*

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, with the exception of the first clipping in each treatment period 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).

III. Results

(a) *Wool Growth*

The responses to the various protein supplements are shown in Figure 1. Casein supplements substantially increased wool growth rate in period 1; the effect of casein may have been slightly underestimated as wool growth was not stable after 7 weeks supplementation. Stable rates of wool growth were achieved in response to all protein supplements given during periods 2 and 3, suggesting that the results were not influenced by the sequence of supplementation. Substantial increases in wool growth rate were obtained with whole egg protein and egg albumen, moderate increases with maize gluten, and the effect of gelatin was negligible. The wool growth rate of all sheep returned to about the pretreatment level within 6 weeks of the end of protein supplementation.

The increases in wool growth rate obtained with each supplement are given in Table 1, together with the effects of the protein supplements given in periods 2 and 3 relative to the effect of casein given in period 1. Whole egg protein and egg albumen were approximately equivalent to casein, maize gluten gave less than half the response obtained with casein, and gelatin had only a slight effect on wool growth. However, when wool growth rates were compared on the basis of digestible nitrogen supplied by the supplements, the two egg proteins appeared to be superior to casein.

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

The digestibility of the protein supplements was estimated from the increase in faecal nitrogen output during protein supplementation. Casein supplements were almost completely digested; whole egg protein, maize gluten, and gelatin were greater than 90% digestible, while the digestibility of egg albumen was lower and variable (Table 2). During egg albumen supplementation both sheep produced abnormal, soft faeces.

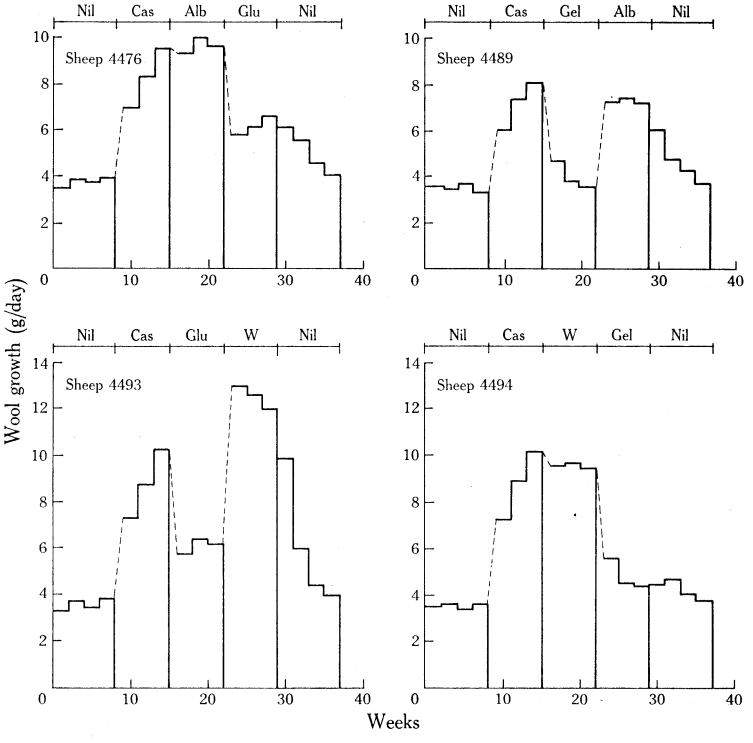

Fig. 1.—Effect of protein supplements on the growth rate of clean, dry wool. Protein supplements (Alb, egg albumen; Cas, casein; Gel, gelatin; Glu, maize gluten; W, whole egg protein) were infused into the abomasum as indicated; they provided approximately 100 g protein per day (15·2–15·9 g nitrogen). All sheep received 600 g/day of the roughage diet throughout the experiment.

During the pretreatment period all sheep were in nitrogen equilibrium and fleece-free body weights were stable. Casein supplements during period 1 resulted in substantial increases in body weight with all sheep (Fig. 2), and in positive tissue nitrogen retentions of 1·4–2·1 g/day (Table 2). In contrast to the wool growth results the changes in body weight and nitrogen retention, in response to the proteins given in periods 2 and 3, are difficult to assess due to the sequential supplementation. Both nitrogen retention and the rate of body weight gain would be expected to decrease

with time (Reis and Schinckel 1961; Reis 1969). In this regard it should be noted that the nitrogen retention obtained with each protein was lower in period 3 than in period 2 (Table 2). It is apparent that gelatin was of little value, either for body weight gain (Fig. 2) or for nitrogen retention (Table 2). Bearing in mind the probable influence of the sequence of supplementation, it would appear that maize gluten and whole egg protein are approximately equivalent to casein for body weight gain and nitrogen retention, whereas egg albumen appears to be somewhat inferior (Fig. 2; Table 2).

TABLE 1

EFFECT OF VARIOUS PROTEIN SUPPLEMENTS ON WOOL GROWTH

The increases in wool growth due to supplement are calculated from the final wool growth rate on each protein supplement compared with the mean pretreatment wool growth rate. Amounts of protein supplements infused into the abomasum are given in tabulation on p. 1508

Supplement	Sheep	Increase in Wool Growth (g/day)	Increase Relative to Casein (%)*		(%)†	
Casein	4476	5·7	—		—	
	4489	4·6	—		—	
	4493	6·7	—		—	
	4494	6·7	—		—	
Whole egg protein	4493	8·4	125	107	135	116
	4494	6·0	89		98	
Egg albumen	4476	5·8	102	92	126	128
	4489	3·8	83		130	
Maize gluten	4476	2·8	49	44	50	44
	4493	2·6	39		39	
Gelatin	4489	0·1	2	8	2	8
	4494	0·9	13		14	

* Calculated from column 3.

† Calculated from wool growth data expressed as grams per gram digestible nitrogen in the supplement.

Except for sheep 4493, body weights declined only slowly after protein supplementation was stopped (Fig. 2), and body weights 6 weeks after supplementation ceased were well above pretreatment weights in all sheep. This is in marked contrast to wool growth which had returned to the pretreatment rate within 6 weeks (Fig. 1).

IV. DISCUSSION

This study has demonstrated that there are substantial differences between proteins, when given via the abomasum, in their value for the synthesis of wool proteins. Further, the relative values of the proteins for wool growth on the one hand and for body weight gain and nitrogen retention on the other were not identical.

A stable wool growth rate was not obtained with casein in period 1 following a long period of low nutrition, but any further increases in wool growth rate during continued casein supplementation should be slight (Reis 1969). It is unlikely that wool growth rates obtained with the various protein supplements given in periods 2 and 3 were influenced by the sequence of supplementation. Thus, new stable rates

TABLE 2

NITROGEN RETENTION AND DIGESTIBILITY OF VARIOUS PROTEIN SUPPLEMENTS

Amounts of protein supplements infused into the abomasum are given in tabulation on p. 1508. The basal diet provided 12·6–12·9 g nitrogen/day, and the protein supplements 15·2–15·9 g nitrogen/day. Nitrogen in wool was calculated assuming 16% nitrogen in clean dry wool. Tissue nitrogen retention = nitrogen intake−(faecal nitrogen+urinary nitrogen+wool nitrogen). Digestibility of protein supplements was calculated from the increase in faecal nitrogen output during protein supplementation

Supplement	Sheep	Period	Digestibility of Protein Supplement (%)	Nitrogen Retention (g/day)	Tissue Nitrogen Retention (g/day)
Nil	4476	Pre-treatment	—	0·6	0·0
	4489		—	0·2	−0·2
	4493		—	0·8	0·2
	4494		—	0·2	−0·4
Casein	4476	1	99	3·6	2·1
	4489	1	96	2·7	1·4
	4493	1	99	3·6	1·9
	4494	1	100	3·0	1·4
Whole egg protein	4494	2	91	2·6	1·1
	4493	3	90	2·3	0·4
Egg albumen	4476	2	80	2·1	0·6
	4489	3	61	1·4	0·2
Maize gluten	4493	2	96	2·7	1·8
	4476	3	93	2·6	1·6
Gelatin	4489	2	92	1·3	0·7
	4494	3	92	0·4	−0·3

of wool growth were achieved with all supplements in periods 2 and 3. Also, the responses obtained with gelatin were similar to those obtained by Reis and Schinckel (1964) when gelatin was given as the initial supplement. Further, wool growth rates returned to the basal rates within 6 weeks after the prolonged period of protein supplementation ceased (Fig. 1), although body weights, and presumably protein stores, were still well above the pretreatment values. This result is in accord with previous experiments with casein supplements, although body weight data have not been reported in detail (Reis and Schinckel 1964; Reis 1969). Thus, it would appear that wool growth rate is dependent mainly on the current nutrition. In contrast to the effects on wool growth rate, it was difficult to evaluate the effects of the protein

supplements given during periods 2 and 3 on nitrogen retention and body weight gain, as these parameters may be influenced by the cumulative effects of the previous supplements. In general, tissue nitrogen retention and body weight gains obtained with each protein supplement were higher in period 2 than in period 3 (Table 2; Fig. 2).

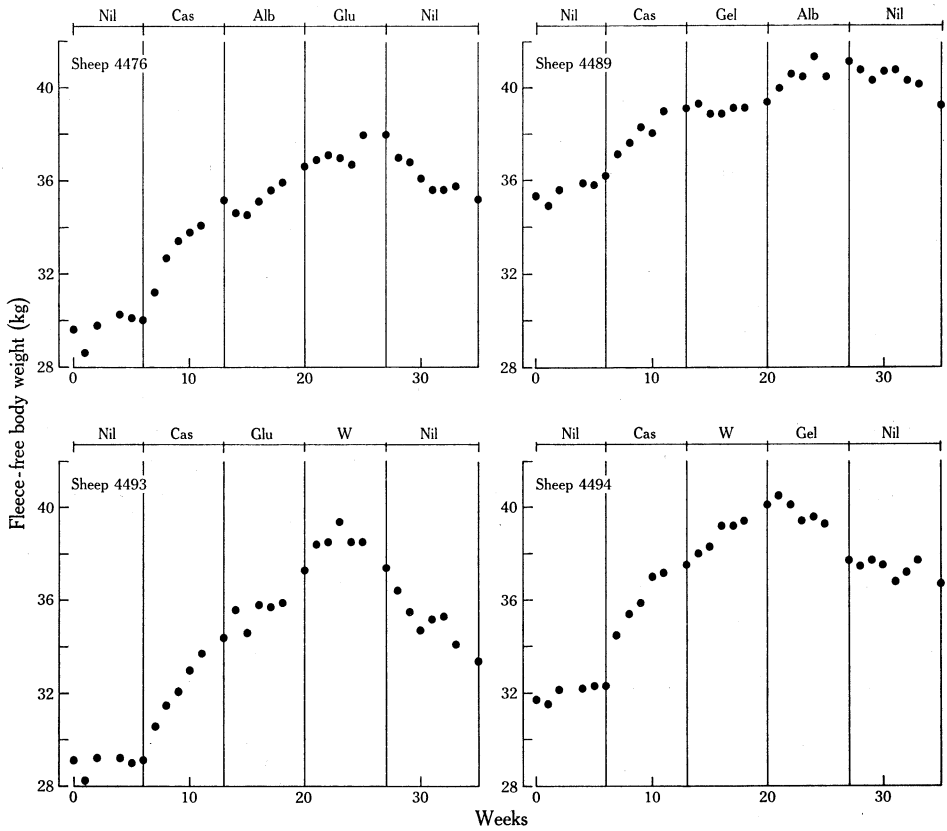

Fig. 2.—Effect of protein supplements on the rate of fleece-free body weight gain. Protein supplements (Alb, egg albumen; Cas, casein; Gel, gelatin; Glu, maize gluten; W, whole egg protein) were infused into the abomasum as indicated; they provided approximately 100 g protein per day (15·2–15·9 g nitrogen) All sheep received 600 g/day of the roughage diet throughout the experiment.

With the exception of egg albumen, all protein supplements were at least 90% digestible. However, only apparent digestibility was measured and proof is needed that these values represent digestion in and absorption from the small intestine; the possibility of protein escaping digestion in the small intestine, but being degraded by microbial enzymes in the large intestine, must be considered. This consideration is most likely to apply to maize gluten; the other proteins studied (casein, gelatin, and the egg proteins) are known to be readily digested in the small intestine of the rat (Forbes, Vaughan, and Yohe 1958; Rogers *et al.* 1960). The rather poor apparent digestibility of egg albumen may have influenced the responses obtained. This

reduced digestibility may be related to a trypsin inhibitor present in raw egg white (Balls and Swenson 1934). The abnormal, soft faeces observed during albumen supplementation also suggest a digestive upset; moreover soft faeces were one of the symptoms of egg albumen toxicity in rats noted by Peters (1967).

It is probable that the differences in wool growth rate and nitrogen retention observed in these experiments are due to differences in the amino acid composition of the proteins and hence to differences in the amounts and proportions of individual amino acids absorbed. The high apparent digestibilities of all proteins, except albumen, are presumptive evidence for substantial digestion in, and absorption of amino acids from, the small intestine. Plasma amino acid levels (Colebrook and Reis, unpublished data) also support this view, as there were large changes in plasma levels of individual amino acids which reflected the composition of the protein given. Also, a low true digestibility of gluten does not seem likely in view of good responses obtained in body weight and nitrogen retention.

Whole egg protein, egg albumen, and casein appear to stimulate wool growth to a similar extent; more extensive data would be needed to assess any differences that may exist between them. When allowance is made for the poorer digestibility of albumen, both egg proteins may have a slightly higher value than casein for wool growth. In view of the increase in wool growth obtained when *S*-amino acids are added to casein supplements or given as the sole supplement per abomasum (Reis and Schinckel 1964; Reis 1967), a bigger response to whole egg protein and egg albumen might have been expected due to their high content of *S*-amino acids. The approximate *S*-amino acid contents (cystine+methionine, g/100 g protein) are 4·0, 6·5, and 8·0 for casein, whole egg protein, and egg albumen respectively (Block and Mitchell 1946; Block and Bolling 1951; Spector 1956). However, the amounts of the proteins given in this experiment (about 100 g) approximate the quantity of casein estimated to be necessary for "maximum" wool growth rate (Reis 1969), and may have been too high to allow differences between the egg proteins and casein to be expressed.

As maize gluten contains similar amounts of *S*-amino acids to casein, the relatively small increase in wool growth rate in response to this protein is presumably related to an imbalance of amino acids, namely low levels of lysine and tryptophan and an excess of leucine (Block and Mitchell 1946; Block and Bolling 1951; Spector 1956). A lack of lysine or tryptophan may well reduce wool growth, although it has been observed that lysine as a sole supplement into the abomasum does not influence wool growth rate (Reis, unpublished data). The high content of leucine may increase the requirement for isoleucine and valine and adversely affect wool growth; such effects have been observed on the growth of rats and chickens (Lewis and D'Mello 1968). Many workers have observed that maize gluten has a low value for growth of rats (e.g. Tagle and Donoso 1965; Christensen, Lloyd, and Crampton 1967). Although maize gluten gave satisfactory nitrogen retention and body weight gains in this experiment, the high total nitrogen intake may have masked any effects of the amino acid imbalance.

The low value of gelatin for wool growth was in accord with earlier results (Reis and Schinckel 1964) and is presumably related to a deficiency of most essential amino acids, particularly tryptophan and *S*-amino acids. The complete lack of tryptophan in gelatin may have a direct adverse effect on the rate of protein synthesis

(Wunner, Bell, and Munro 1966). The high proportion of non-essential amino acids in gelatin, especially glycine, proline, and hydroxyproline, may also adversely affect synthesis of wool and tissue proteins. Such an effect has been observed on growth in rats (Swendseid, Hickson, and Friedrich 1962; Young and Zamora 1968). The poor nitrogen retention obtained with gelatin is in agreement with the results of Little and Mitchell (1967), with supplements of gelatin given per abomasum. Diets containing a large proportion of the protein as gelatin, or diets which are deficient in tryptophan, also are of little value for nitrogen retention and growth in monogastric animals (Krehl, Sarma, and Elvehjem 1946; Bressani 1962).

The results indicate that there are some differences in the amino acid requirements for the synthesis of wool proteins and of tissue proteins, although proteins such as casein and whole egg protein were satisfactory for all productive purposes while gelatin was uniformly of little value. Wool growth may be especially sensitive to the balance of amino acids absorbed; the low value of maize gluten for wool growth could be explained in this manner. The sensitivity of wool growth to the level of methionine absorbed has been demonstrated previously (Reis 1967).

V. Acknowledgments

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