SOME ASPECTS OF PROTEIN NUTRITION AND ITS RELATION TO WOOL GROWTH AND BODY WEIGHT CHANGES IN THE SHEEP

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A C K N O W L E D G M E N T S

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CHAPTER 1

INTRODUCTION

INTRODUCTION

Early studies involving dietary protein level effects on wool growth (Fraser and Roberts, 1933; Slen and Whiting, 1952; Ferguson, 1959) noted that over a wide range, dietary protein level remains relatively independent of wool growth rate. These observations led to the conclusion that once minimum protein levels were met, dietary protein was no longer a major factor limiting wool growth (Ferguson, 1959).

For some time dietary protein has been known to be involved in a complex series of biochemical reactions within the rumen (reviewed by Barnett and Reid, 1961). Most notable of the reactions related to this study are those involved with deamination and fermentation of protein by the rumen microorganisms.

After considering results of protein level experiments, knowledge of rumen microbial action on protein, and work with sheep indicating that nitrogen retention was increased by abomasal or duodenal protein infusions (Cuthbertson and Chalmers, 1950), Reis and Schinckel (1961) decided to by-pass rumen action by administering casein supplements through an abomasal cannula. The effect of "by-passed" protein supplements on wool growth rate was then assessed. Following supplementation, wool growth rate increased by 41 to 77%. These results suggested that protein nutrition is a major factor limiting wool growth.

The early protein-level results indicating that wool growth rate is relatively independent of dietary crude protein level, have subsequently been explained by the work of Hogan and Weston (1967). Using radioactive and organic marker techniques, the amount of non-ammonia nitrogen (representing

mainly protein and amino acids) reaching and absorbed from the intestines was found to be similar for isocaloric diets varying significantly in crude protein content. Thus, rumen action can regulate the amount of protein made available to the host with subsequent effects on wool growth rate.

The work of Marston (1935) suggested that the sulphur-containing amino acid content of protein may be important for wool growth. Reis and Schinckel (1963) used abomasal infusion as a "by-pass" technique for testing Marston's suggestions. L-cysteine and DL-methionine were infused, producing significant (35-130%) increases in wool growth rate which thus confirmed the importance of sulphur-containing amino acids for wool growth.

Work directly relating to the present experiments was conducted by Reis (1967) who gave abomasal supplements of MHA (methionine hydroxy analog). Substantial increases in wool growth rate resulted from this form of supplementation. Also directly related is the work of Ferguson et al. (1967) who treated casein with formalin to protect the protein from rumen degradation. Treated casein supplements were then fed to sheep and wool growth responses measured. Results indicated that the supplements were effective in producing significant wool growth rate increases.

It was with the preceding information that the present experiments were undertaken.

Experiment I was designed to test the effectiveness of two formalin treatment procedures for protecting casein and MHA from rumen degradation and to measure wool growth and body weight responses resulting from supplementation with these treatment materials.

Experiment II was designed to measure wool growth and body weight responses to different levels of protein nutrition. Romney sheep were used

and the result compared with those obtained from similar experiments with other breeds. In addition, results of the first experiment raised a question as to the possibility of an energy-protein level interaction for wool growth. Thus, a test for the existence of such an interaction was incorporated into the experimental design.

CHAPTER 2

REVIEW OF LITERATURE

I. PROTEIN METABOLISM IN THE RUMEN

This section reviews the causes, events and products of protein metabolism in the rumen. Some of the important carbohydrate and nitrogen interrelationships within the rumen are shown in fig. 2.1. Figure 2.2 presents some of the important aspects of microbial protein digestion, absorption and general utilization within the host animal.

A. Bacterial and Protozoal Proteolytic Activity

Protein, upon entering the rumen is known to undergo proteolysis. Sym (1939) was possibly the first to recognize that microorganisms were responsible for proteolytic activity within the rumen. Sym observed that proteinases found in rumen fractions, filtrates and centrifugates were capable of hydrolyzing casein. He concluded that the enzymes were of bacterial origin. Pearson and Smith (1943), using *in vitro* techniques, provided further evidence by demonstrating the proteolysis of casein and gelatin.

The distinction between microbial proteolysis and bacterial fermentation has not always been clear. Because proteolysis and fermentation occur consecutively and rapidly it is difficult to isolate proteolytic products in rumen fluid. Abou Akkada and Howard (1962) utilized *in vitro* suspension of *Entodinium* protozoa to demonstrate the formation of proteolytic products. When casein was suspended with the *Entodinium* the occurrence of amino acids and peptides was noted. Ammonia formation (amounting to 10% of the breakdown products) was also noted and is thought to have arisen from the hydrolysis of amide nitrogen in the casein. Abou Akkada and Blackburn

FIG 2.1 SOME IMPORTANT CARBOHYDRATE and NITROGEN INTERRELATIONSHIPS within the RUMEN

ADAPTED FROM HUNGATE 1966

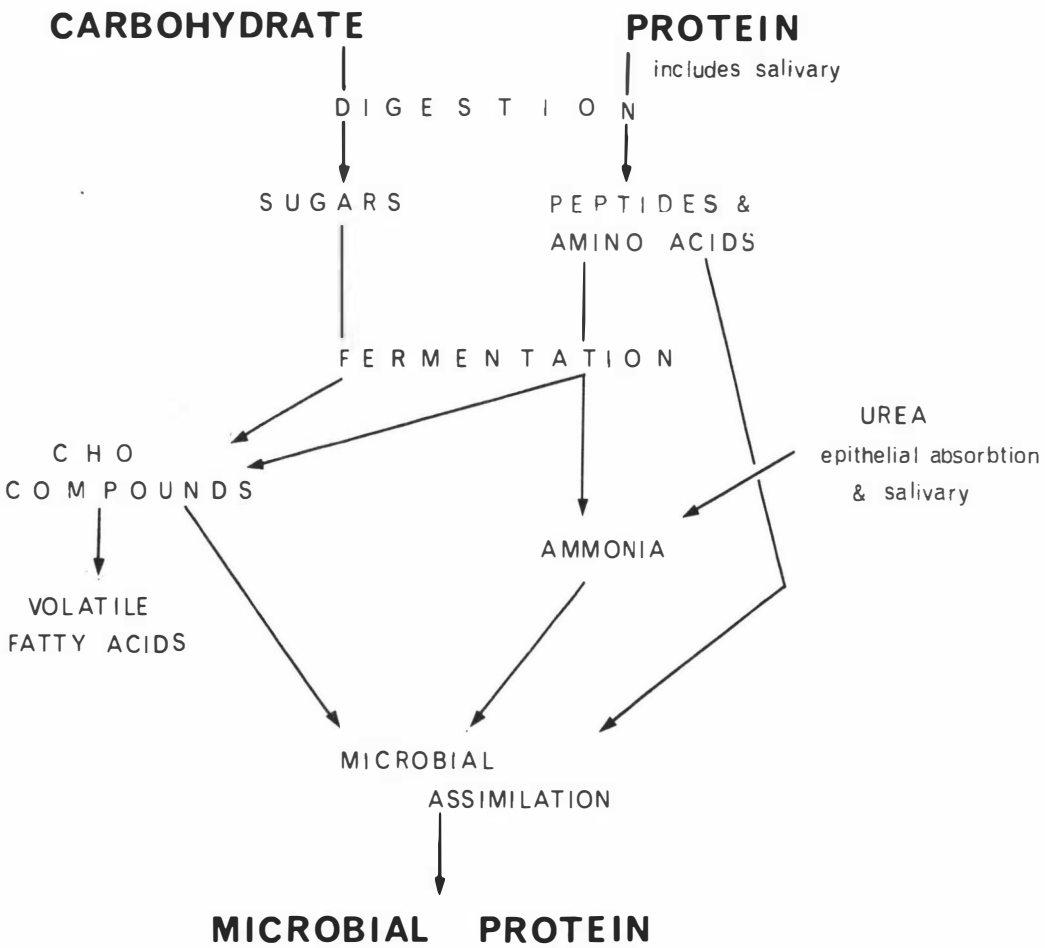
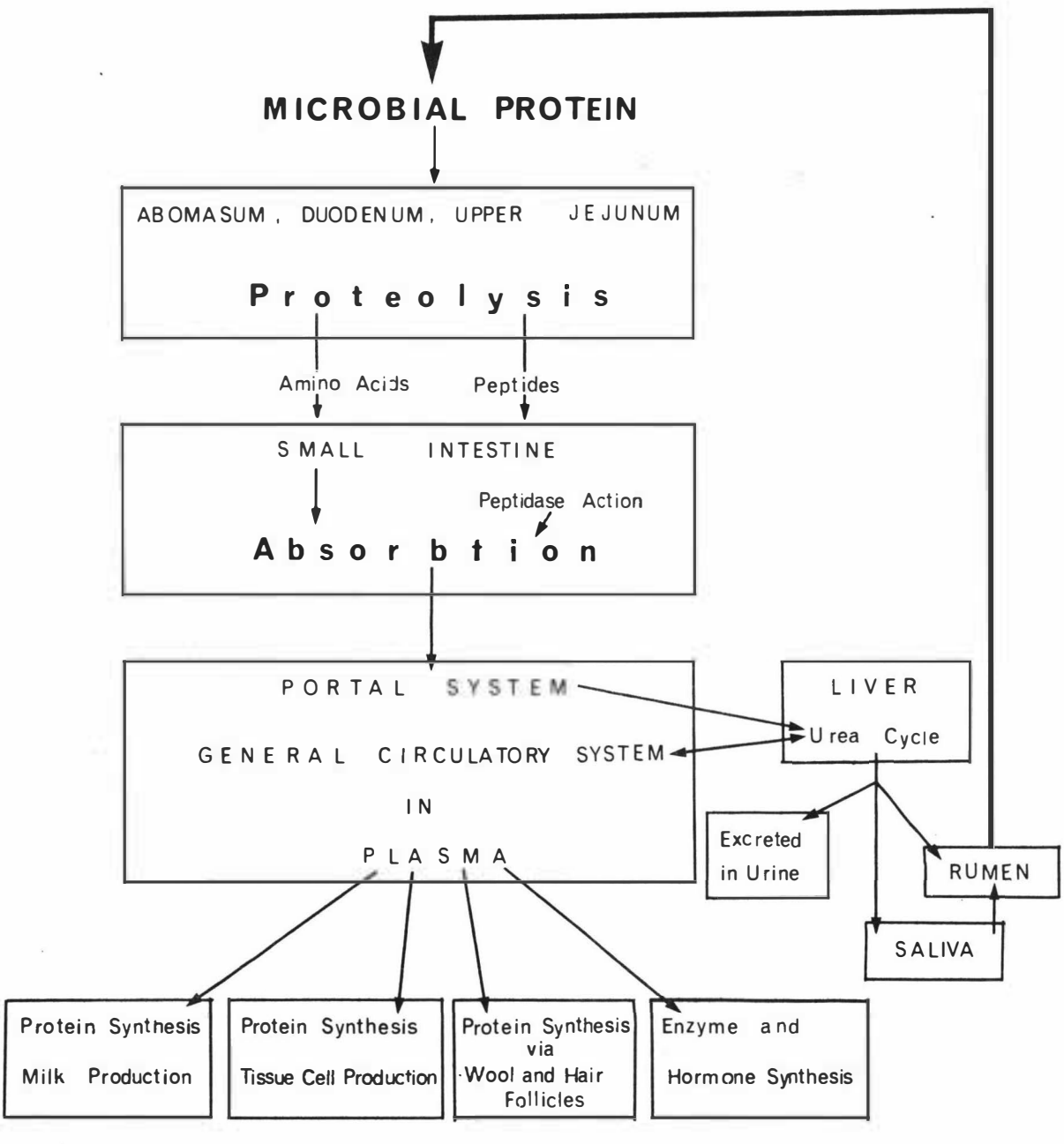


FIG 2.2 FLOW CHART SHOWING SOME of the IMPORTANT ASPECTS of MICROBIAL PROTEIN DIGESTION, ABSORBTION and GENERAL UTILIZATION



(1963) used pure cultures of rumen bacteria to demonstrate the formation of amino acids and polypeptides after digestion of casein. Most of the strains used in the study also exhibited exopeptidase activity in addition to proteolytic activity. This exopeptidase activity resulted in the liberation of free amino acids. Amidase activity was also noted in the strains under study and the authors suggested it would explain the results of Hale (1956) who found that propionamide could replace up to 50% of the protein in ruminant diets. Other relevant observations from this experiment were: few of the isolates demonstrated deaminase activity, and no significant urease activity was shown among any of the isolates. This latter observation poses an interesting question in that possession of urease has not yet been associated with any of the more common rumen microorganisms (Hungate, 1966) even though urea is rapidly hydrolysed in the rumen.

Warner (1956) used toluene to depress deamination in order to study and confirm proteolytic activity. It was shown that proteolytic activity remained constant independent of diet, an observation that is also supported by Blackburn and Hobson (1960a). This is in contrast to deamination which varies with the diet (el-Shazley, 1952).

Quantitative aspects of bacterial and protozoal involvement in proteolysis are of interest. Blackburn and Hobson (1960b) stated that a "fair" proportion of the proteolytic activity in the rumen can be attributed to protozoa. They also suggest that protozoa may be more active than bacteria in the hydrolysis of casein. Obviously, relative amounts of activity will vary with a number of factors. However, it is safe to conclude that both bacteria and protozoa can contribute significantly to proteolytic activity observed in the rumen.

B. Protein Solubility as Related to Rumen Degradation

el-Shazley (1952) found that the ability of rumen microbes to decompose amino acids to ammonia and other products was significantly affected by the content of soluble protein in the diet. Chalmers et al. (1954) results indicate that within the rumen highly soluble nitrogen sources (such as casein) are rapidly converted to ammonia with subsequent nitrogen loss via rumen wall absorption. It was inferred that protein sources of low solubility may have higher feed values for ruminants than proteins that are highly soluble. Chalmers and Synge (1954) observed that altering the solubility of casein changed the extent of attack by rumen microorganisms, and consequently ammonia production. It was noted that herring meal (relatively low in soluble protein) gave a higher nitrogen retention and lower ammonia levels than casein. el-Shazley (1958) suggests that protein solubility is the most important factor in determining the extent of ammonia production in the rumen. He notes it could be concluded that proteins with the highest solubility are the least retained in the body. However, nitrogen retention was found to be better with casein than with meat meal or beans, although it is more soluble than both. He indicates that this may be due to the non-nitrogenous part of the casein molecule stimulating microbial growth and propagation, the protein being subsequently utilized by the host animal.

Further work with microbial counts showed no correlation between nitrogen retention and the total counts of microorganisms in the rumen liquor. It was suggested that the lack of correlation between microbial counts and nutritive value of proteins could be accounted for by assuming a high biological value for the insoluble proteins (cottonseed cake and linseed oil meal in this case) which pass to the abomasum largely unaltered and, therefore, do not stimulate microbial growth and propagation to the same

extent as soluble proteins.

The rate of ammonia accumulation has been used as indirect evidence that protein solubility affects the rate of proteolysis (Preston et al., 1963). Blackburn and Hobson (1960c) have provided direct evidence by demonstrating that a soluble form of casein was more rapidly degraded than a relatively insoluble form.

Little et al. (1963) measured the solubility of various protein sources in rumen fluid, dilute sodium hydroxide and distilled water. Solubility was then compared with in vitro microbial production of free ammonia (see table). Solubility in rumen fluid was generally more closely related to ammonia formation than values obtained from other solvents. However, no definite relation between degree of nitrogen solubility and rate of ammonia production was evident.

It appears that a number of factors in addition to solubility are involved in producing observed differences between proteins in the rate and extent of rumen ammonia formation. Surface of the protein available to microbial attack, physical consistency of the protein, and the chemical nature of the protein as affecting its susceptibility to enzymic attack have been listed as possible additional factors, Chalmers and Synge (1954).

C. Bacterial Fermentation of Amino Acids

In contrast to proteolysis, amino acid fermentation involves not only enzymatic breakdown but may include the formation of high-energy phosphate molecules, (Hungate, 1966). Presumably, most amino acid fermentation within the rumen is attributed to bacteria. Rumen protozoa are known to ingest and assimilate bacteria and other proteinaceous materials

TABLE 2.1 NITROGEN SOLUBILITY AND IN VITRO MICROBIAL PRODUCTION OF FREE AMMONIA FROM DIFFERENT PROTEIN SOURCES*

	% TOTAL N SOLUBLE			FREE AMMONIA ON INCUBATION HR.[a]			
	Rumen Fluid	H_2O	0.2 N NaOH	1	2	3	4
SOYBEAN OIL MEAL	19	16	81	4.2	7.3	9.2	10.2
HEATED SOYBEAN OIL MEAL[b]	10	11	30	2.5	2.5	2.5	3.0
LINSEED OIL MEAL	45	39	68	3.0	6.9	7.4	8.0
CORN GLUTEN MEAL	13	11	32	2.2	3.1	3.2	1.6
PURIFIED SOY PROTEIN	7	2	99	3.0	5.5	7.6	10.2
PURIFIED CASEIN	81	2	98	3.8	7.0	11.4	12.5
PURIFIED ZEIN	3	0	99	0.1	2.9	0.5	0.9

a Values expressed as mg. NH_3-N per 100 ml. increase above control values.

b Heated in forced air oven at 110°C, for 24 hours.

* From Little, Burroughs and Woods (1963)

as a source of nitrogen (Hungate, 1966), and thus, many species may circumvent the need for exogenous amino acid fermentation.

There is ample evidence for the fermentation of amino acids in rumen fluid (Van de Hende et al., 1963; Lewis and Emery, 1962; Lewis and McDonald, 1962). This work indicates that all amino acids are fermented in the rumen to some extent. The various pathways and intermediates involved in amino acid fermentation will not be discussed here but their existence should be noted.

The end products of amino acid fermentation have been listed by Blackburn (1965) as being ammonia, carbon dioxide, and volatile fatty acids.

D. Role of Ammonia in the Rumen

The formation of ammonia is probably the key product in amino acid fermentation, in that ammonia is thought to be the basic substance from which bacteria synthesize cellular protein.

McDonald (1948, 1952) was one of the first to draw attention to the importance of ammonia in the rumen. This work provided indirect evidence that ammonia is utilized by the rumen microbes. Weller et al. (1962) sampled the rumen contents of sheep slaughtered at various times after feeding. Their work supports the views of McDonald and provides more indirect evidence for the breakdown of feed protein to ammonia, and for its subsequent utilization by the rumen bacteria. N^{15} tracer studies by Boggs (cited by Hungate, 1966) provide some direct evidence for the utilization of ammonia by the rumen bacteria.

Another aspect of ammonia's role in the rumen concerns its apparent

absorption through the epithelium. McDonald (1948) provided evidence for the absorption of ammonia through the rumen wall to the portal blood stream. Coombe et al. (1960) studied the rate of ammonia absorption and found that it is affected by the rumen pH. Also, absorption rate was found to be the highest in the nonionized (NH_3) form. Ruminal ammonia concentration is also thought to affect the rate of absorption (Lewis et al., 1957) as it was observed that increased levels of rumen ammonia gave rise to high levels in the portal blood.

The importance of ammonia absorption lies in the theory that when large amounts of protein are included in the diet, considerable wastage of the degraded protein nitrogen occurs via ammonia absorption and conversion to urea with resultant urinal excretion.

E. Utilization of Endogenous Urea

One of the most interesting characteristics of the ruminant is its capability of utilizing endogenous urea.

There are two major routes by which urea enters the rumen. One is via the salivary glands which are known to secrete up to 0.6 g. of nitrogen into the rumen daily (McDonald, 1948; Somers, 1958). Urea is the major nitrogen constituent of saliva, as Somers (1961a) determined that 60-70% of salivary nitrogen is found in that form. The other source of endogenous urea is that obtained by diffusion through the rumen wall (Houpt, 1959; Moir and Harris, 1962).

Quantitative estimates of total endogenous urea entering the rumen have been made. Kay and Hobson (1963) estimated that endogenous urea may contribute slightly over 1/5 of the nitrogen that the diet provides.

Dobson (1961) estimates that 1.5 g. of endogenous urea nitrogen enters the rumen daily. It should be noted that these estimates need qualification as the quantity of urea nitrogen entering the rumen is known to vary with the blood urea concentration which in turn varies with the nitrogen content of the diet (Somers, 1961b). Houpt (1959) measured the quantity of urea entering the rumen from the blood when 5 m.moles urea nitrogen/kg. body weight were injected intravenously into mature ewes receiving a less than maintenance level of nitrogen plus a carbohydrate supplement. Under these conditions it was estimated that 52% ± 10% of the injected urea entered the rumen. When the carbohydrate supplement was removed 22% ± 5% of the injected urea entered the rumen. Reduced microbial protein synthesis was assumed to account for the lower urea utilization on the unsupplemented diet.

The percentage of crude proteins in the diet was shown to affect body-pool urea retention (Cocimano and Leng, 1966). Urea entry and excretion rates were measured, using C^{14} urea isotope injection and infusion techniques, in sheep fed rations containing 3.5, 9, 17 and 27% crude protein. Urea excretion rates were calculated by measuring the urea in urine collected from a catheter in the bladder. The body-pool urea excretion percentages ($\frac{\text{urea excreted}}{\text{urea entering}} \times 100$) for the rations were: 8, 56, 67 and 70 respectively. It was concluded that urea recycling is a major process in ruminants and on low-protein roughages (3-4% CP) the amount of nitrogen actually entering the rumen may be as much as twice that in the feed.

Once urea enters the rumen it must be broken down to a utilizable form. Huhtaner and Gall (1955) have demonstrated that urea is hydrolized in the rumen to ammonia and carbon dioxide. Urease activity was demonstrated and it was suggested that the urease is in the anaerobic microorganisms of the rumen fluid. Ash and Dobson (1963) have also noted urease activity in rumen fluid. As previously mentioned (Section IA) urease activity has not

been attributed to any of the commonly occurring rumen microbes.

The importance of endogenous urea utilization in relation to ruminant protein metabolism becomes evident on low-nitrogen diets (Houpt, 1959; Cocimano and Leng, 1966). Under these conditions endogenous urea provides relatively substantial amounts of nitrogen for protein synthesis.

F. Synthesis of Microbial Protein

One of the essential processes in ruminant protein metabolism is the synthesis of microbial protein. Pearson and Smith (1943) and Smith and Baker (1944) utilized *in vitro* techniques to demonstrate decreases in non-protein nitrogen followed by increases in microbial protein.

Loosli *et al.* (1949) provided more direct evidence for microbial protein synthesis by feeding lambs a purified diet containing urea as the only nitrogen source. Arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine were shown to be formed in rumen fluid, presumably within the microbes. Similar evidence was provided by Duncan *et al.* (1953) who also demonstrated utilization of urea by rumen microbes.

There is much evidence to show that ammonia is utilized by many strains of rumen bacteria for amino acid synthesis. Bryant and Robinson (1961) noted utilization of ammonia by *Ruminococcus flavefaciens*, *R. albus* and *Bacteroids succonogenus* in preference to or regardless of the amount of amino acid or peptide nitrogen present. Bryant and Robinson (1962) cultured eighty-nine strains of rumen bacteria, 25% of which required ammonia for growth with 82% being capable of utilizing ammonia as their main source of nitrogen. Fifty-six percent of the strains under study grew well with

either amino acids or ammonia while only 6% required amino acids as a nitrogen source.

Volatile fatty acids have been shown to be essential or stimulatory substances for the growth of many rumen-bacteria strains. This may be the result of the bacteria's inability to incorporate or synthesize the carbon chain of amino acids (Blackburn, 1965). Bryant and Robinson (1962) used bovine bacterial isolates from whole rumen contents to demonstrate that, of the following volatile fatty acids: isobutyric, n-valeric, isovaleric and 2-methylbutyric, one or more were either required for or enhanced the growth of a large percentage of the eighty-nine strains under study. As the result of this and other work (Allison and Bryant, 1963) it was suggested that in the rumen a significant quantity of microbial branched-chain amino acids may be synthesized from branched-chain fatty acids.

Little information is available on the relative importance of rumen protozoa in microbial protein synthesis. It may be that their role is a minor one in relation to the amount of synthesis conducted by the rumen bacteria.

Present knowledge indicates that rumen bacteria use significant amounts of major fermentation products (i.e. ammonia and volatile fatty acids) in the process of synthesizing protein.

G. Composition of Microbial Protein

The rumen microbes constitute the major protein-source for the ruminant (Hungate, 1965). Therefore, a knowledge of their composition is essential to an understanding of the host's nutritional intake.

Based on the data of Weller (1957), the rumen bacteria are thought

to contain approximately 67% crude protein while the rumen protozoa are thought to contain approximately 33% crude protein. Digestibility estimates of mixed samples of rumen bacteria and protozoa were made by Abdo et al. (1964). When the samples were fed to rats true digestibility figures of 74-80% were obtained.

High biological values have been obtained for rumen bacteria and protozoa (80, 81 respectively) when fed to rats (McNaught et al., 1954).

The amino acid content of rumen fluid as used to compare two sources of nitrogen is shown in table 2.2 (Richardson and Tsein, 1963). It can be seen that, when compared with urea, the feeding of soybean-oil meal resulted in a higher concentration of amino acids but did not appear to significantly affect amino-acid proportions. These results also indicate that the amino acid proportions in rumen microbial proteins do not deviate significantly from the proportions found in many common herbage proteins.

Weller (1957) compared the amino acid composition of rumen bacteria and protozoa from sheep fed a variety of diets. The percentage composition for bacteria between diets appeared to be similar. However, protozoa composition demonstrated greater variation. The percentage of nitrogen in both bacterial and protozoal samples appeared to vary with the diet.

Microbial protein composition is relatively constant with variations in the diet, while protein concentration (based on nitrogen values) seems to vary with the diet. It appears that the rumen microbes provide a highly utilizable source of protein with a composition similar to that of many forage proteins. Like many forages however, the sulphur-containing amino acids may be limiting (Abdo et al., 1964). The significance of this limitation in relation to growth will be discussed in chapter 5.

TABLE 2.2 AMINO ACID CONTENT OF RUMEN FLUID FROM TWIN STEERS FED SOYBEAN-OIL MEAL OR UREA*

VALUES EXPRESSED AS Mg. AMINO ACID/LITER

	SOYBEAN-OIL MEAL		UREA	
ASPARTIC ACID	346.03	382.19	305.76	242.01
THREONINE	173.09	199.75	154.06	84.32
SERINE	137.38	150.73	97.74	32.52
GLUTAMIC ACID	496.91	517.14	491.60	377.24
PROLINE	299.62	283.42	251.63	190.78
GLYCINE	168.93	168.02	148.88	112.84
ALANINE	190.35	213.52	189.78	149.32
METHIONINE	89.75	93.73	79.52	71.89
ISOLEUCINE	229.40	249.98	197.85	156.80
VALINE	197.46	213.52	189.78	149.32
LEUCINE	291.69	303.31	254.42	220.90
TYROSINE	151.38	156.91	99.54	79.55
PHENYLALANINE	253.13	277.98	150.92	126.69
HISTIDINE	67.50	77.58	62.74	53.83
LYSINE	300.13	305.91	273.54	227.42
ARGININE	139.04	152.56	137.34	101.34
TRYPTOPHAN	78.20	129.23	55.99	45.78
CYSTINE & CYSTEINE	52.22	54.34	37.59	29.11

* From Richardson and Tsien (1963)

II. THE RELATIONSHIP OF PROTEIN NUTRITION TO WOOL GROWTH AND BODY WEIGHT CHANGE

A. Protein Level Feeding Experiments

A number of experiments were designed to interpret wool growth and body weight changes, as affected by feeding various levels of protein and energy, before the development of a general understanding of rumen protein and energy metabolism and the quantitative and qualitative nature of nutrient materials absorbed from the alimentary tract. Some of the frequently noted experiments from this period will be discussed.

Fraser and Roberts (1933) fed two groups of 20 wethers on isocaloric diets which contained different levels of protein. The two levels were estimated to contain approximately 78 and 118 g. digestible protein per day. No significant differences in rate of body weight change or wool growth were observed between the groups. The authors noted the difference in cystine content between forages and wool and indicated that cystine is probably synthesized in the sheep.

Slen and Whiting (1952) fed ewes isocaloric diets containing three levels of crude protein (7, 10 and 13% of the ration). No clear estimate of digestible energy intake was given but it can be extrapolated from the data that approximately 510 g. of digestible carbohydrate were consumed daily. Wool production was measured and the weight of clean wool was found to be significantly greater for the 10 and 13% CP diets when compared with the 7% CP diet.

Ferguson (1959) fed isocaloric diets to groups of ewes while varying crude protein as a percentage of intake (see table 2.3). No

TABLE 2.3 INFLUENCE OF DIETARY PROTEIN PERCENTAGE ON GROWTH OF WOOL*

PERIOD	DURATION (WEEKS)	GROUP	RATION	FEED INTAKE (GRAM/DAY)	CRUDE PROTEIN (PER CENT)	WOOL GROWTH (GRAM/DAY)
1	8	I	F6	509	16.9	4.90
		II	F6	517	16.9	4.40
		III	F6	507	16.9	4.81
		IV	F6	508	16.9	4.58
2	12	I	F6	1553	18.6	12.46
		II	F11	1389	18.5	11.54
		III	F12	1382	24.0	12.54
		IV	F13	1389	29.3	12.27
3	12	I	F6	499	18.4	6.38
		II	F11	500	18.3	6.43
		III	F12	500	24.5	6.89
		IV	F13	500	29.5	7.33
4	8	I	F11	500	18.1	5.79
		II	F11	500	18.1	5.53
		III	F11	500	18.1	5.89
		IV	F11	500	18.1	6.03
5	12	V	F16	447	7.5	5.19
		VI	F15	491	11.2	4.65
		VII	F14	500	13.6	4.88
		VIII	F11	495	17.2	4.93

* From Ferguson, K.A. (1959)

significant differences in wool growth were observed between groups fed isocaloric diets of differing protein content. Significant differences in wool growth were noted between levels of feed intake. Ferguson concluded that when the diet contains more than 8% crude protein the response in wool growth to increasing intake is not due to greater supplies of amino acids and therefore must be due to an increased supply of energy.

Feeding experiments of the type just described are difficult to compare due to variations in design and methods. It should be noted that the energy level used by Slen and Whiting is high, relative to the period 1, 3, 4 and 5 levels of Ferguson. It is probable that Slen and Whiting's observations were made with diets containing ample quantities of energy for microbial protein synthesis (this statement needs to be qualified as applying within the levels of protein used during the experiment). Thus, the supply of nitrogen was the main factor regulating protein synthesis and resulted in different wool growth rates between the 7 and 10 and 7 and 13% CP levels. Conversely, Ferguson's low-energy diets probably did not supply sufficient energy to meet potential demands for microbial protein synthesis. Thus, when the level of nitrogen was increased, energy supply became limiting for microbial growth and resulted in an unchanged rate of wool growth.

The upper limit for protein content (undergoing rumen fermentation) to significantly affect wool growth is not known. However, from work such as Slen and Whiting's it is suggested that, when energy is not limiting, dietary levels of crude protein much greater than 10% will not significantly affect wool growth.

Hogan and Weston (1967) have provided evidence which helps to explain results obtained in the earlier feeding experiments (as exemplified by Fraser and Roberts, 1933; Slen and Whiting, 1952; and Ferguson, 1959).

Merino wethers were fitted with rumen and abomasal cannulae and fed diets corresponding to Ferguson's (1959) period 5, F16 and F11 rations. The diets provided approximately 8 and 20% CP on a dry matter basis and were designated LP and HP respectively. The movement of digesta through the stomach was measured using dietary lignin and a radioactive chromium complex of ethylene-diamine-tetra - acetic acid (^{51}Cr-EDTA) as markers. Samples of rumen and abomasal digesta were regularly collected for analysis. Results of this work (see table 2.4) indicate that, although dietary N intakes were significantly different, the quantities of N leaving the abomasum and absorbed in the intestine were similar for both diets. It should be noted that, for digesta passing through the stomach, there was a net gain of nitrogen on the LP diet while on the HP diet there was a net loss. Therefore, the similarity of wool growth rates for these diets (observed by Ferguson, 1959) can now be explained on the basis of similar quantities of amino acids being absorbed through the intestine.

TABLE 2.4 AMOUNT OF NON-AMMONIA NITROGEN ABSORBED FROM THE INTESTINE*

DIET	TOTAL N INTAKE (g./day)	NAN[1] LEAVING ABOMASUM (g./day)	NAN[1] ABSORBED FROM THE INTESTINE (g./day)
HP[2]	13.8	8.8	6.8
LP[3]	5.5	8.1	6.1

1 NAN = Non-Ammonia Nitrogen

2 HP = High Protein Diet - approximately 20% CP

3 LP = Low Protein Diet - approximately 8% CP

NAN contains 70-80% α Amino Acid N, Hogan (1965), Hogan and Weston (1967)

* From Hogan and Weston (1967)

B. Circumvention of Rumen Fermentation as a Technique for Studying Protein Effects on Wool Growth and Body Weight Change

Since the realization that rumen fermentation limits the amount of protein available to the host a variety of methods have been utilized or suggested as routes for bypassing rumen action.

1. Abomasal and Duodenal Protein Infusion

For nearly twenty years this technique has been used for studies involving nitrogen retention. However, it is only recently that use has been made of it for observing wool growth and body weight responses.

Cuthbertson and Chalmers (1950) were of the first to utilize duodenal infusion for protein supplementation. Pregnant ewes were given casein via rumen and duodenal fistulae. Nitrogen retention was measured and found to be greater in those animals receiving casein via the duodenum.

Blaxter and Martin (1962) have compared the utilization of the energy of casein when given to sheep by infusion into the rumen with its utilization when given by infusion into the abomasum. The results (see table 2.5) indicate that superior utilization is obtained from the abomasal route. Rumen fermentation is suggested as the reason for the low nutritive value of proteins for lipogenesis in ruminants.

Little and Mitchell (1967) compared oral vs abomasal administration of soybean protein, zein, casein and gelatin. Nitrogen retention was significantly improved ($P < 0.05$) when soybean protein and casein were infused abomasally. Gelatin infusion also increased nitrogen retention but not significantly. This is possibly due to gelatin's comparatively low essential amino acid content. Nitrogen retention decreased after zein's infusion. This is thought to be due to the reduced digestibility

TABLE 2.5 CASEIN UTILIZATION IN THE SHEEP; COMPARING TWO SITES OF INFUSION*

SITE OF INFUSION	% N DIGESTED	INCREASE IN METHANE PRODUCTION Kcal/100 Kcal CASEIN	NET AVAILABILITY OF METABOLIZABLE ENERGY %	TOTAL ENERGY RETAINED Kcal/100 Kcal CASEIN
RUMEN	93.6 ± 2.7[1]	7.63 ± 1.16	50.2 ± 3.2	31.1 ± 3.6
ABOMASUM	99.8 ± 2.5	0.60 ± 0.92	64.7 ± 3.2	56.7 ± 3.6

[1] Standard Error of Mean

* From Blaxter and Martin (1962)

of zein when given abomasally and to zein's poor amino acid balance. It is noted that, when given orally, nitrogen retention is much lower for casein and gelatin when compared with soybean protein and zein. This probably reflects the differing rates at which the proteins are fermented within the rumen.

Increases in feed intake (a response that is probably indirectly related to changes in wool and body growth rate) have been observed after abomasal and duodenal infusions of casein (Egan and Moir, 1965; Schelling and Hatfield, 1967). Reasons for the increases are not clear but it has been shown that the responses are not simply related to changes in the rate of digestion within the rumen (Egan and Moir, 1965).

Reis and Schinckel (1961) were possibly the first to utilize abomasal infusions for the study of protein effects on wool growth. When a 600 g. basal diet (180 g. lucerne chaff, 180 g. wheaten chaff, 240 g. cracked corn) was supplemented with daily infusions of 12 g. casein N, wool production was increased by 41 to 77%.

Schinckel (1962) using a Merino ewe, studied the responses to two levels of nutrition, a low plane (300 g. lucerne chaff + 300 g. wheaten chaff) and a high plane (225 g. lucerne chaff + 225 g. wheaten chaff + 300 g. cracked corn). The high plane diet was supplemented with daily abomasal infusions of 185 $\pm$ 5 g. casein. During the high plane supplementation period the wool growth rate was about 156% greater than during the low plane period. Mitotic activity within the follicle bulb (as measured using colchicine injections) was found to be 56% greater during the period of high level intakes.

Short et al. (1965) have used Schinckel's (1962) material for a detailed examination of nutritional effects on wool growth at the cellular

level. Schinckel's high plane of nutrition, when compared to the low plane, was found to produce increases in follicle bulb cell proliferation rate, the germinal cell population and the volume of matrix and resulting cortical cells.

Reis and Schinckel (1963) conducted a further experiment with abomasally infused casein. Two Merino's were fed for a period of six weeks on a diet consisting of: 600-800 g. lucerne and wheaten chaff mixed in equal parts and supplemented with daily infusions of 60 g. casein. Wool growth, when compared with a control period, was found to have increased 84 and 102%. Body weight also increased (relative to a control period) during the infusion period, the increases being approximately 2 kg. for each sheep.

Reis and Schinckel (1964) compared wool growth responses to abomasal infusions of casein and gelatin. Two groups of Merinos (3 sheep/group) were infused daily with 60 g. of either casein or gelatin for a 9 week period. The sheep were on an 800 g. basal diet consisting of equal parts lucerne and wheaten chaff. Wool growth increases, based on control period figures, were 14% and 20% for casein and gelatin respectively. Mean body weight increases of 4.5 kg. (casein) and 3.0 kg. (gelatin) were recorded for the infusion period.

2. Heat Treatment of Proteins

Chalmers et al. (1954) were possibly the first to recognize heat treatment as a method for protecting proteins from rumen degradation. In a feeding trial with sheep, heat-treated casein when compared with untreated casein was found to increase nitrogen retention and reduce the rate of rumen ammonia formation. Tagari et al. (1962) fed sheep heat-treated soybean meal supplements. Heat treatment was shown to substantially reduce rumen

ammonia formation and increase nitrogen retention. It was demonstrated that heating reduced the protein's solubility in artificial saliva. This reduced solubility is thought to explain the value of heat treatment. It is theorized that untreated protein, being more soluble, liberates ammonia at a rate faster than the microorganisms can use it, the excess nitrogen being lost via rumen-wall absorption. Chalmers et al. (1964) fed heat-treated groundnut meal to sheep and lactating goats. Results were parallel with earlier work in that heat treatment reduced solubility and ammonia formation and increased nitrogen retention. Danke et al. (1966) fed heat-treated cottonseed meal to sheep. The effect of different heating times and treatments on nitrogen solubility and retention was studied. With both forms of treatment (autoclaving vs atmospheric steaming) solubility was reduced in a linear fashion with time. Autoclaving (with 1.05 kg./cm^2 pressure) was found to be superior, reducing the solubility 65% in 60 minutes while the same period of atmospheric steaming reduced the solubility by only 20%. Nitrogen retention differences between the treatments were not significant but retention tended to be higher with animals fed the autoclaved protein.

The use of heat-treatment as a means of partially protecting proteins from rumen degradation appears to give satisfactory results although, more information is needed concerning possible reductions in nutritive value due to treatment.

3. Chemical Modification of the Protein's Surface

A variety of methods for chemically altering a protein's surface have been envisioned. The object is to produce a reaction product that is resistant to microbial attack in the rumen but, is not resistant to the acid conditions of the abomasum thereby allowing digestion to begin at that site.

The reaction of a protein's surface with an aldehyde produces an acid reversible polymer complex which is resistant to rumen degradation. Chalmers et al. (1954) were possibly the first to utilize this technique. They mention the use of a formaldehyde-treated casein supplement in a feeding trial with sheep. Unfortunately the results were not clear and further experimentation was apparently not undertaken.

Ferguson et al. (1967) have successfully utilized the formalin-treatment technique in supplementation experiments with Merino sheep. Three groups of wethers (4 sheep/group) were fed an 800 g. basal ration consisting of equal parts lucerne and wheaten chaff for a 12 week control and an 18 week experimental period. One group was maintained on the basal ration during experimental period while a second was given a daily supplement of 80 g. formalin-treated casein; a third group was given 80 g. treated casein during the first 9 weeks of the experimental period and then switched to 80 g. untreated casein for the remainder of the period. When compared with control group values, the administration of treated casein was found to have increased wool growth rate by approximately 70% (4.5 g./day) after 9 weeks of treatment. The group which was switched to untreated casein in the latter half of the experimental period, displayed a marked reduction in wool growth rate which stabilised at a level approximately 15% (1 g./day) above the control group.

The reaction of protein with an aldehyde other than formalin has been reported (Ferguson et al., 1967). Glutaraldehyde was shown to be effective in in vitro tests.

4. Esophageal Groove Closure

Until recently this method had not been tried as a rumen-bypass

technique for protein supplementation although, Hungate (1966) has suggested its use. Mönning and Quin (1933, 1935) studied esophageal groove closure as a means for drug administration. A 5 to 10% solution of copper sulphate administered to sheep in 10 cc. doses was found satisfactory for stimulating closure. Using this treatment, the groove was found to remain closed 15 seconds or longer with closure occurring a few seconds after stimulation. Watson and Jarrett (1941) observed closure periods frequently greater than one minute and sometimes greater than six minutes when 10 cc. of a 10% solution of copper sulphate was administered to sheep.

Wickham (pers. comm.) has tried the use of sodium bicarbonate as a stimulant for closure in an attempt to place MHA (methionine hydroxy analog) directly into the abomasum of sheep. Individual variability in the response has posed a problem for the use of this chemical.

5. Other Methods

(i) The chemical coating of proteins or amino acids as mentioned by McDonald (1968), may prove to be a very useful technique for preventing rumen degradation. This is especially true for individual amino acids, which are unsuitable for forming polymer complexes. This technique would not involve a reaction but would involve placing a chemical polymer around the protein or amino acids. The coating would be resistant to rumen microbial attack but would break down in the acid conditions of the abomasum.

(ii) Circumvention of rumen fermentation via injection is a method which shows promise for specific amino acids. Marston (1935) gave subcutaneous injections of L-cysteine hydrochloride and significantly improved the rate of wool growth. However, Marston's experiment was not

specifically designed to test the use of injections as a technique for bypassing the rumen.

The injection of specific sulphur-containing amino acids may become a valuable technique for increasing wool growth if Downes' (1961a) theory is correct. This theory suggests the existence of a metabolic "pool" of cystine which can be drawn upon by the wool follicles. Thus, periodic injections of cystine might produce a continuous response in terms of an increased wool growth rate. It should be noted (Nickham, pers. comm.) that cysteine injections may produce undesirable physiological reactions which are associated with the form in which cysteine is injected.

(iii) The reduction of rumen retention time is a method which might be employed to increase the proportion of feed protein reaching the abomasum. Depending on protein fermentation rate, a marked reduction of particle retention time could allow significant amounts of undegraded protein to pass through to the abomasum and thereby be made available to meet wool and body growth requirements.

Increased water consumption, achieved by adding large amounts of sodium chloride to the diet, is thought to facilitate an increased rate of particle passage through the rumen (Hemsley, 1967).

C. Amino Acids and Wool Growth

1. The influence of Sulphur-Containing Amino Acids

It is only recently that the sulphur-containing amino acids (S-amino acids) have been positively identified as being stimulatory factors for wool growth. Early work by Marston (1935), based on the observation that the sulphur amino acid content of forages was much lower than that

found in wool, pointed to the importance of cystine for wool growth. Marston did demonstrate increases in wool growth rate after cysteine injections but it was not until the work of Reis and Schinckel (1963, 1964) that a relatively clear picture was obtained.

Reis and Schinckel (1963) used abomasal infusion to demonstrate the influence of S-amino acids. Merino and English Leicester x Merino sheep were fed diets as previously described; (section IIB) in addition, daily infusions of amino acids were given for a 6 week period. Wool growth was measured from tatooed mid-side and shoulder patches. When compared with pre-treatment period rates, 2 g. L-cysteine produced from 35-75% increases in wool growth rate, while 2.46 g. DL-methionine given to a single sheep produced a 130% increase.

Reis and Schinckel (1964) used basically the same sheep and diets of their 1963 experiment to give a further assessment of the effects of amino acids on wool growth. During a 9 week period, L-cysteine (1.5 or 3.0 g./day) and DL-methionine (3.7 g./day) were added to protein supplements (casein and gelatin) and infused into 6 sheep, each sheep receiving a different supplement. The results confirmed earlier observations that S-amino acids have a stimulatory effect on wool growth. Also, the wool growth response was greater from the 1.5 g. cysteine supplement than from the 3.0 g. cysteine supplement indicating to the authors the possibility of an amino acid imbalance at the higher level. The methionine supplement (which is equivalent to 3.0 g. cysteine in terms of sulphur) resulted in wool growth increases on a par with those obtained from the 1.5 g. cysteine supplement.

Reis (1967) has conducted further abomasal supplementation experiments with L-cysteine, DL-methionine, D-methionine and MHA (methionine

hydroxy analog). Results indicate all the forms of methionine and L-cysteine were capable of producing substantial increases in wool growth. However, it was demonstrated that relatively large amounts of DL-methionine (7.38 or 9.84 g./day) or L-cysteine (6.0 or 8.0 g./day) reduced the wool growth rate to a level below that produced by much smaller amounts (0.5 - 3.0 g./day) or to even lower levels which were below pretreatment period rates. It should be noted that for one sheep at the highest level of DL-methionine supplementation (9.84 g./day) treatment was stopped after 6 weeks due to a considerable drop in feed consumption and the development of mild scours.

Dryden (1968) conducted two experiments involving the intravenous infusion of cysteine.

In the first, three groups of Romney wethers (2 sheep/group) were infused daily for 31 days with 500 mls. of the following isotonic solutions: control-saline, low cysteine - 2.0 g. L-cysteine hydrochloride + saline, high cysteine - 4.0 g. L-cysteine hydrochloride + saline. Wool growth rates were estimated from mid-side samples. At the end of the infusion period wool growth rates (in relation to control group values) had increased by 53.5 and 40.7% for the 2.0 and 4.0 g. infusions respectively. A thirteen day post-treatment sample revealed wool growth rates to be 83.9 (low cysteine) and 80.5% (high cysteine) above control group values. By 35 days post-treatment, wool growth rates for both treatment groups had declined to levels similar to that displayed by the control group.

In the second experiment, twelve Romney wethers were randomly divided into four groups of three: low protein - cysteine (LC), low protein - saline (LS), high protein - cysteine (HC), high protein - saline (HS). The low protein diet provided approximately 70 g. digestible crude protein daily,

and the high protein diet provided approximately 109 g. Daily infusions of 2.0 g. cysteine in a 500 ml. isotonic saline solution were given to the LC and HC groups while the LS and HS (control) groups received 500 ml. of saline. The animals were infused for a five week period. Wool growth rates (based on group means adjusted to a pre-treatment control period) were 40.5% greater for the HC group when compared with the HS group while the LC group demonstrated a 130% greater rate than the LS group.

2. The Influence of Non-Sulphur-Containing Amino Acids

Reis and Schinckel (1963) observed that greater wool growth responses were obtained with 60 g. casein (supplying the equivalent of approximately 1.75 g. cysteine/day) than with 2.0 g. L-cysteine. This suggested to the authors that the greater response obtained with casein could be due to the presence of other "essential" amino acids (essential meaning those amino acids which cannot be synthesized by the sheep at a rate required for normal growth). The authors suggested that the major role of non-sulphur-containing, essential amino acids may be one of enabling more efficient utilization of the S-amino acids. This opinion is based on the fact that a higher proportion of S-amino acids (40-50%) were recovered from wool grown during the casein supplementation period as compared to the proportion (15-29%) recovered during the S-amino acid supplementation period.

This experiment also included the daily infusion of a sheep with 0.62 g. glycine plus 1.22 g. L-glutamic acid both of which are non-essential for the sheep (Downes, 1961b). The results are most interesting in that a small (16%) increase in wool growth rate during the supplementation period was followed by a marked increase (approximately 45%) which was

maintained for the duration of a 12 week post-treatment period. No explanation could be offered for the observed responses.

Reis and Schinckel (1964) compared the wool growth response between abomasal supplements of casein and gelatin (see section IIB). Casein is characterized by a high "essential" amino acid content while gelatin is characterized by a low "essential" amino acid content and a high proportion of the "non-essential" amino acid-glycine. The substantially greater increases in wool growth achieved with casein provide indirect evidence pointing to the importance of "essential" amino acids for wool growth.

The greater wool growth response obtained from a 60 g. casein supplement (containing 3.2% cysteine) demonstrates the importance of a protein's amino acid composition in addition to its S-amino acid content.

The present evidence concerning the importance of specific non-S-containing amino acids is sketchy. However, it does appear that the "essential" amino acids (which have not been strictly identified for the sheep but probably are the same as those essential to the rat with the exception of arginine; Downes, 1961b) are more important for wool growth than the "non-essential".

CHAPTER 3

SUPPLEMENTAL FEEDING OF TREATED CASEIN AND METHIONINE

I. INTRODUCTION

This chapter describes an experiment designed to: (1) test the effectiveness of two treatment procedures as means of protecting proteins and amino acids from rumen degradation, (2) measure any wool growth or body weight responses produced by the feeding of protected proteins or amino acids.

An analysis of plasma free amino acid levels was included in the experiment as it was hoped these levels would serve as an indicator of the protected materials absorption from the lower digestive tract.

II. MATERIALS AND METHODS

A. Experimental Periods

The experiment was divided into three periods: a pre-treatment "control" (duration 35 days), a treatment (duration 28 days) and a post-treatment (duration 10 days) period.

B. Animals

The experimental animals consisted of 12 mature Romney wethers 5 years of age or older. Nine of these animals had previously shown a wool growth response to cysteine infusion (Dryden, 1968).

At the beginning of the treatment period the animals were randomly divided into four groups of three as listed below:

GROUP	SHEEP NUMBERS	TREATMENT
C	6, 5, 10	Control
UC	11, 7, 12	Untreated Casein
TC	1, 4, 3	Treated Casein
M	9, 8, 2	Treated Methionine

Animals were housed in individual outdoor pens.

C. Rations

1. Composition (Dry Matter Basis)

During the pre-treatment period each sheep received a daily ration of 990 g. chopped grass-clover hay plus water ad lib. During the treatment

period each group received the following basal ration daily; 680 g. chopped grass-clover hay plus 270 g. barley meal. Water was provided ab lib. During the post-treatment period each group was continued on the treatment period basal ration.

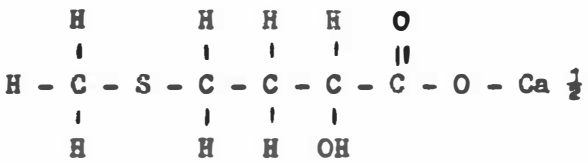
Crude protein (CP) was estimated from macro-Kjeldahl nitrogen analyses (CP equalling N x 6.25). The pre-treatment period ration was found to contain 14.6% CP. Of the treatment period rations, the control group's contained 12.2% CP while the casein supplemented groups contained 20.0% and the methionine groups 13.3%. The post-treatment ration contained 12.2% CP.

2. Supplementary Treatments

During the treatment period the basal rations were supplemented daily with the following:

GROUP	SUPPLEMENT
C	No Supplement
UC	100 g. Untreated Casein
TC	100 g. Treated Casein
M	6 g. Treated Methionine*

* A synthetic form of methionine - methionine hydroxy analog (MHA). It is of the general formula:



Note that no NH_2 group is attached to the molecule. The MHA was supplied by Monsanto Chemical Co., St. Louis, Mo., U.S.A.

3. Feeding Program

Treatment materials were mixed with barley meal and given daily at a 9 a.m. feeding. Chopped hay was fed separate from the meal at a 4 p.m. feeding.

D. Wool Growth

Wool growth was measured on each sheep from left mid-side patches approximately 100 cm^2. The wool was removed with the fine blade of small animal clippers (Oster, size 40) and the patch area measured with calipers. Samples were scoured with successive 4 minute elutions in petroleum ether, 95% ethyl-alcohol and hot water. They were then conditioned in a humidity room (RH 65%) for at least 48 hours and weighed. Wool growth rate was expressed as mg. clean dry wool per square centimetre per day. Patches were sampled on the last day of each experimental period.

E. Determination of Plasma Free Amino Acid Levels

1. Collection

A blood sample was taken from each sheep at the end of the pre-treatment period, on the sixth day of the treatment period and the fifth day of the post-treatment period. 40 ml. samples were collected from the jugular vein into heparinised vials.

2. Plasma Separation, Deproteinisation and Analysis

The blood samples were taken directly to the laboratory after collection and the plasma separated by centrifugation. The procedure used to isolate the free amino acids of plasma was that of Stein and Moore (1954).

Briefly, proteins were removed from 10 ml. plasma by precipitation with picric acid. The remaining supernatant liquid (containing the free amino acids) was passed through a Dowex AG-2X10 (200-400 mesh) resin column to remove excess picric acid, the total effluent was concentrated to a volume of approximately 1 ml. and stored under refrigeration (2-3°C). Samples were then transferred to the Wool Research Organisation's Lincoln laboratories where pH adjustments were made and analyses conducted according to procedures outlined in the Beckman Amino-Acid Analyzer Manual. All analyses were made using a Beckman 120-C amino-acid analyzer with values being expressed as micro-moles amino acid per ml. of plasma.

F. Preparation of Treatment Materials

1. Untreated Casein

No special preparatory procedure was necessary for this treatment material. Commercial grade ring-dried lactic casein was used.

2. Treated Casein

Four, five-pound batches of commercial grade ring-dried lactic casein were each treated with 6.85 l. water containing 2.5% commercial formalin. The material was then placed in a Z-blade mixer for 40 minutes. After removal of excess moisture the treated casein was dried in a fluid bed dryer and ground to pass through a 30 mesh screen.

3. Casein-Coated Methionine

Five-hundred g. of commercial grade spray-dried lactic casein were suspended in 2 l. water at 45°C. 14 g. of finely powdered calcium hydroxide was then added and the suspension mixed for approximately two hours. 100 g. MHA was then added, the mixture was stirred for a further one hour, then

poured into trays, frozen and placed in a freeze dryer where the bulk of the water was removed. The remaining water was removed in a fluid-bed dryer.

The dry material was treated with a solution containing 2.5% commercial formalin in 1.8 l. water and stirred for one hour at room temperature. After standing for a further one hour, an attempt was made to filter the mixture. This was unsuccessful, so the mixture was freeze-dried as previously, heated for 30 minutes in a fluid-bed dryer and ground to pass a 30 mesh screen.

G. In Vitro Ammonia Production From Treated Casein and Methionine

As a test of the treatment technique's effectiveness, 0.5 g. samples of untreated casein, treated casein and treated methionine were incubated at 39°C with 50 ml. of whole rumen fluid (obtained from a cow grazing grass-clover pasture). Ammonia production was measured using the boric-acid, hydrochloric-acid procedure of Conway and O'Malley (1942). Ammonia values were expressed as mg. NH_3 per 100 ml. rumen fluid.

H. Statistical Methods

1. Comparison of Regression Lines

For all variables measured in the experiment, the covariance technique was used to compare the slopes of the relevant regression lines and, if these were similar, the adjusted means.

Covariance model: $$y_{ij} = \mu_i + \beta(x_{ij} - \bar{x}_{..}) + \varepsilon_{ij}$$

where:

y_{ij} = value of the variable for the i th treatment and the j th sheep in the treatment or post-treatment period

μ_i = mean of the ith treatment group

β = the regression of Y on X

X_{ij} = value of the variable for the ith treatment and the jth sheep in the pre-treatment (control) period.

ε_{ij} = the error term, independent and normally distributed.

The slopes of the lines were compared by the F test as described by Snedecor and Cochran (1967).

The covariance technique increases the precision of estimates of differences between means by removing inherent variation that existed prior to the treatment period.

2. Analysis of Adjusted Means

If the analysis of regression lines indicated significant homogeneity amongst the slopes, analysis was continued to compare the adjusted means using the covariance model previously given. Adjusted means were compared by the F test as described by Snedecor and Cochran (1967).

All analyses were made by the use of an IBM 1620 computer using available programs.

III. RESULTS

A. Wool Growth

Wool growth rates are given in table 3.1a with group regression values and adjusted means in table 3.1b. Adjusted mean differences are shown in fig. 3.1.

Adjusted mean analysis indicated non-significant differences between groups in the treatment period but significant differences were shown ($P<0.05$) in the post-treatment period.

When compared with the control period general mean the treated methionine group demonstrated a 1.9% increase in wool growth rate in the treatment period and a 5.8% decrease in the post-treatment period, both values indicating no response to the administration of treated methionine. This observation is supported by the similarity of control group values which demonstrated a 0.9% increase in the treatment period and a 13.5% decrease in the post-treatment period. The untreated casein group displayed a 29.8% increase in the treatment period and a 52.9% increase in the post-treatment period. These responses are particularly surprising as rumen degradation was expected to nullify the nutritional value of untreated casein supplements. The treated casein group displayed a 31.7% increase in the treatment period and a 51.0% increase in the post-treatment period indicating a definite response to supplementation.

B. Body Weight

Body weight data are presented in table 3.2 and adjusted mean differences displayed in figure 3.2.

TABLE 3.1a EXPERIMENT I WOOL GROWTH RATES

SHEEP NUMBER	TREATMENT	WOOL GROWTH PRE-TREATMENT PERIOD	UNADJUSTED GROUP MEAN	WOOL GROWTH TREATMENT PERIOD	UNADJUSTED GROUP MEAN	WOOL GROWTH POST-TREATMENT PERIOD	UNADJUSTED GROUP MEAN
2	METHIONINE	1.14[1]		1.16		1.21	
8	"	0.80	0.96	0.96	0.99	0.75	0.91
9	"	0.95		0.86		0.78	
10	CONTROL	1.13		1.14		0.99	
6	"	0.89	1.02	1.15	1.03	1.03	0.88
5	"	1.04		0.82		0.62	
11	UNTREATED CASEIN	1.52		2.09		2.30	
7	" "	1.36	1.25	1.48	1.53	1.72	1.77
12	" "	0.86		1.02		1.29	
4	TREATED CASEIN	0.67		1.25		1.67	
3	" "	1.21	0.92	1.36	1.27	1.63	1.47
1	" "	0.89		1.21		1.12	

[1] = mg./cm^2/day

TABLE 3.1b SUMMARY OF COVARIANCE ANALYSIS OF WOOL GROWTH RATES

TREATMENT GROUP REGRESSION VALUES

TREATMENT PERIOD ON CONTROL

VARIABLE	GROUP: METHIONINE	STANDARD ERROR	CONTROL	STANDARD ERROR	UNTREATED CASEIN	STANDARD ERROR	TREATED CASEIN	STANDARD ERROR	F
WOOL GROWTH	0.632 ±	0.636	-0.262 ±	1.526	1.454 ±	0.564	0.223 ±	0.179	1.422

POST-TREATMENT PERIOD ON CONTROL

WOOL GROWTH	1.393 ±	0.585	-0.429 ±	1.814	1.370 ±	0.539	0.047 ±	1.128	0.802

TREATMENT GROUP ADJUSTED MEANS

TREATMENT PERIOD ON CONTROL

WOOL GROWTH	1.058[1] ±	0.130	1.053 ±	0.130	1.351 ±	0.130	1.372 ±	0.130	1.797

POST-TREATMENT PERIOD ON CONTROL

WOOL GROWTH	0.977 ±	0.169	0.896 ±	0.169	1.594 ±	0.169	1.571 ±	0.169	4.726*

1 = mg./cm^2/day

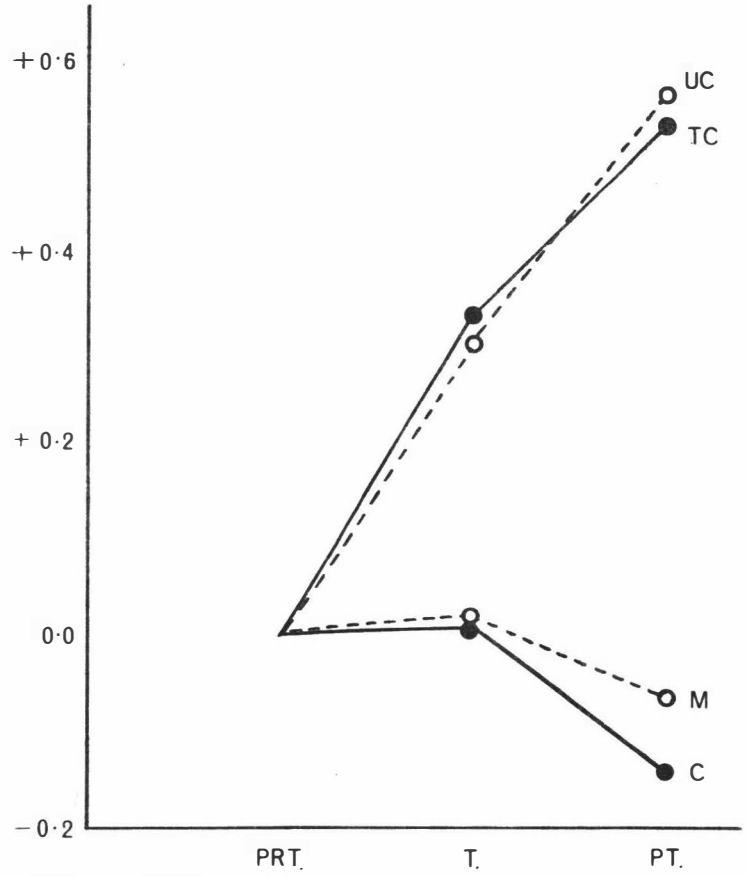
* = $P < 0.05$

FIGURE 3·1 **EXPERIMENT 1**

CASEIN & METHIONINE SUPPLEMENTATION

WOOL GROWTH RATE

DEVIATIONS OF ADJUSTED MEANS FROM PRE-TREATMENT PERIOD GENERAL MEAN EXPRESSED AS MG/CM²/DAY



UC = UNTREATED CASEIN
TC = TREATED CASEIN
M = METHIONINE
C = CONTROL

PRT = PRE-TREATMENT PERIOD
T = TREATMENT PERIOD
PT = POST-TREATMENT PERIOD

	GENERAL MEAN	ADJUSTED MEANS	
		TREATMENT PERIOD	POST-TREATMENT PERIOD
UC	1·04	1·35	1·59
TC	1·04	1·37	1·57
M	1·04	1·06	0·98
C	1·04	1·05	0·90

TABLE 3.2a EXPERIMENT I BODY WEIGHT DATA

SHEEP NUMBER	TREATMENT GROUP	BODY WEIGHT LAST DAY OF CONTROL PERIOD	UNADJUSTED GROUP MEAN	BODY WEIGHT LAST DAY OF TREATMENT PERIOD	UNADJUSTED GROUP MEAN	BODY WEIGHT POST-TREATMENT PERIOD	UNADJUSTED GROUP MEAN
10	CONTROL	45.25[1]		48.25		48.50	
6	"	47.00	48.92	47.00	50.58	46.50	50.33
5	"	54.50		56.50		56.00	
2	METHIONINE	53.50		54.25		53.00	
8	"	51.00	52.92	52.50	53.50	53.25	53.08
9	"	54.25		53.75		53.00	
11	UNTREATED CASEIN	38.25		39.75		40.50	
7	" "	53.75	45.67	55.50	47.25	55.00	47.67
12	" "	45.00		46.50		47.50	
4	TREATED CASEIN	52.25		54.25		54.00	
3	" "	51.25	51.50	53.75	54.08	54.00	54.17
1	" "	51.00		54.25		54.50	

[1] = Kilograms

TABLE 3.2b SUMMARY OF COVARIANCE ANALYSIS OF BODY WEIGHTS

REGRESSION VALUES

TREATMENT PERIOD ON CONTROL

VARIABLE	GROUP: CONTROL	STANDARD ERROR	METHIONINE	STANDARD ERROR	UNTREATED CASEIN	STANDARD ERROR	TREATED CASEIN	STANDARD ERROR	F
BODY WEIGHT	0.947	± 0.378	-0.083	± 0.020	0.932	± 0.051	-0.286	± 0.330	1.285

POST-TREATMENT PERIOD ON CONTROL

BODY WEIGHT	1.004	± 0.311	0.464	± 0.256	1.017	± 0.008	0.143	± 0.413	0.596

ADJUSTED MEANS

TREATMENT PERIOD ON CONTROL

BODY WEIGHT	51.08[1]	± 0.83	50.25	± 0.90	51.33	± 0.94	52.60	± 0.85	

POST-TREATMENT PERIOD ON CONTROL

BODY WEIGHT	51.41	± 0.60	50.36	± 0.65	51.30	± 0.68	52.35	± 0.61	1.825

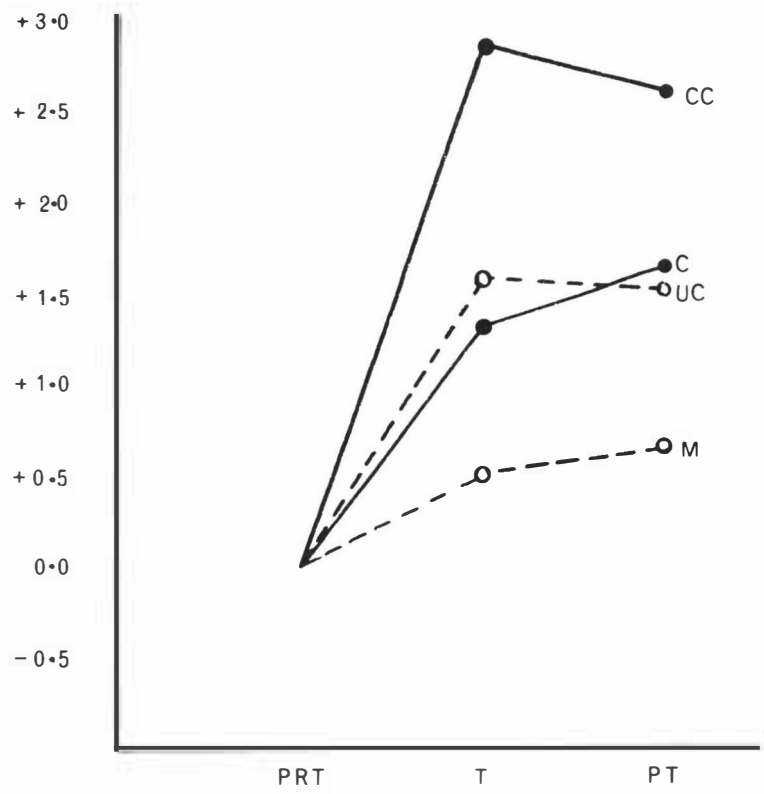
[1] = Kilograms

FIGURE 3·2

CASEIN & METHIONINE SUPPLEMENTATION EFFECT ON BODY WEIGHT

BODY WEIGHT

DEVIATIONS OF ADJUSTED MEANS FROM PRE-TREATMENT PERIOD GENERAL MEAN, EXPRESSED AS KILOGRAMS.



C = CONTROL
M = METHIONINE
UC = UNTREATED CASEIN
CC = TREATED CASEIN

PRT = PRE-TREATMENT PERIOD
T = TREATMENT PERIOD
PT = POST TREATMENT PERIOD

GROUP	GENERAL MEAN	ADJUSTED MEANS	
		TREATMENT PERIOD	POST-TREATMENT PERIOD
CONTROL	49·75	51·08	51·41
METHIONINE	49·75	50·25	50·36
UNTREATED CASEIN	49·75	51·33	51·30
TREATED CASEIN	49·75	52·60	52·35

Analysis of adjusted means for the treatment and post-treatment periods revealed no significant differences between the groups. However, there was an indication of a small positive response by the treated casein group and possibly a small negative response by the methionine supplemented group.

C. Plasma Amino Acid Levels

Treatment period on control regression values are given in table 3.3 for each variable in each treatment group. Significant differences between regressions are observable for taurine ($P < 0.05$), glutamic acid ($P < 0.05$) and cystine ($P < 0.01$). Post-treatment regression values are given in table 3.4. A significant difference between regressions is observable for 3-methyl histidine ($P < 0.05$).

Treatment period means adjusted to the overall control period mean are given in table 3.5 for each variable in each treatment group with the exception of those variables that displayed a non-significant within group regression or a significant difference between the slopes of regression lines. Significant differences between treatment group adjusted means are observable for citrulline ($P < 0.05$), proline ($P < 0.01$), valine ($P < 0.05$), leucine ($P < 0.05$), an unidentified amino acid referred to as "unknown" ($P < 0.001$) and histidine ($P < 0.05$). With the exception of histidine, all amino acids displaying significant differences between adjusted means show substantially higher values for the treated casein group. Post-treatment means adjusted to the overall control period mean are given in table 3.6. A significant difference between the adjusted means is observable for the unknown amino acid ($P < 0.001$). This difference is due to the substantially higher concentration displayed by the treated casein group. It should be

TABLE 3.3 PLASMA FREE AMINO ACID LEVELS

SUMMARY OF REGRESSION VALUES - TREATMENT PERIOD ON CONTROL

VARIABLE	GROUP: METHIONINE	STANDARD ERROR	CONTROL	STANDARD ERROR	UNTREATED CASEIN	STANDARD ERROR	TREATED CASEIN	STANDARD ERROR	F
TAURINE	0.790 ±	0.280	1.185 ±	0.071	0.993 ±	0.014	0.038 ±	0.297	6.246 ✓
UREA	0.225 ±	0.777	0.311 ±	1.569	1.011 ±	0.096	-0.116 ±	1.095	0.283
METH. SULPHOXIDE	0.013 ±	0.028	0.402 ±	0.062	0.595 ±	0.037	0.515 ±	0.279	2.114
ASPARTIC ACID	-0.405 ±	1.710	0.881 ±	0.151	1.457 ±	0.303	0.823 ±	0.152	1.575
METH. SULPHONE	0.314 ±	0.510	0.733 ±	0.397	0.780 ±	0.056	1.133 ±	0.122	2.062
GLUTAMIC ACID	0.683 ±	0.018	4.900 ±	1.386	1.071 ±	0.333	0.416 ±	0.241	6.557 ✓
CITRULLINE	0.462 ±	0.462	0.464 ±	0.876	0.915 ±	0.238	2.149 ±	0.624	2.424
PROLINE	1.747 ±	0.351	0.183 ±	0.688	0.796 ±	0.505	2.123 ±	2.548	0.310
GLYCINE	1.583 ±	0.433	-0.392 ±	1.156	1.928 ±	1.123	0.615 ±	0.095	1.225
ALANINE	-0.224 ±	0.353	0.572 ±	0.034	0.927 ±	0.838	0.771 ±	0.403	0.664
CYSTINE	1.375 ±	0.000	1.417 ±	0.144	1.000 ±	0.035	0.250 ±	0.433	21.126**
α-AMINO ADIPIC ACID	0.778 ±	0.001	0.311 ±	0.562	1.372 ±	0.094	0.875 ±	0.505	1.234
VALINE	1.375 ±	0.024	0.570 ±	0.232	1.221 ±	0.050	1.463 ±	0.973	0.108
METHIONINE	1.276 ±	0.205	0.962 ±	0.232	0.865 ±	0.033	1.062 ±	0.005	0.738
ISOLEUCINE	0.849 ±	0.515	0.404 ±	0.384	0.917 ±	0.435	2.547 ±	0.313	2.002
LEUCINE	1.012 ±	0.127	0.403 ±	0.041	1.357 ±	0.106	2.504 ±	0.905	2.770
TYROSINE	0.240 ±	0.057	0.964 ±	0.902	0.549 ±	0.009	0.472 ±	0.226	0.676
PHENYLALANINE	1.797 ±	0.498	0.663 ±	0.821	0.865 ±	0.274	1.047 ±	0.084	0.516
ORNITHINE	1.257 ±	0.023	0.840 ±	0.396	0.486 ±	0.049	1.280 ±	0.172	3.306
LYSINE	0.972 ±	0.133	1.047 ±	0.471	2.612 ±	1.409	1.115 ±	0.324	0.254
UNKNOWN	1.180 ±	0.440	1.736 ±	0.348	1.441 ±	0.183	3.093 ±	1.258	0.929
HISTIDINE	0.731 ±	0.312	1.211 ±	0.066	1.361 ±	0.223	0.857 ±	0.025	3.697
3-METHYL HISTIDINE	1.050 ±	0.109	0.772 ±	0.493	1.000 ±	0.144	0.261 ±	0.413	2.149
ARGININE	1.509 ±	0.780	0.446 ±	0.944	0.760 ±	0.965	0.899 ±	1.117	0.140

✓ = $P < 0.10$
** = $P < 0.01$

TABLE 3.4 PLASMA FREE AMINO ACID LEVELS

SUMMARY OF REGRESSION VALUES - POST-TREATMENT PERIOD ON CONTROL

VARIABLE	GROUP: METHIONINE	STANDARD ERROR	CONTROL	STANDARD ERROR	UNTREATED CASEIN	STANDARD ERROR	TREATED CASEIN	STANDARD ERROR	F
TAURINE	0.508 ±	0.297	0.778 ±	0.047	0.901 ±	0.136	1.245 ±	0.064	2.675
UREA	0.983 ±	0.822	-0.159 ±	0.279	1.466 ±	0.251	-0.993 ±	0.649	4.354
METH. SULPHOXIDE	0.005 ±	0.040	1.041 ±	0.079	0.570 ±	0.285	0.850 ±	0.625	1.815
ASPARTIC ACID	-0.152 ±	0.658	1.262 ±	0.756	1.142 ±	0.802	0.943 ±	0.268	0.195
METH. SULPHONE	0.514 ±	0.020	0.010 ±	0.440	0.594 ±	0.108	0.916 ±	0.532	0.432
GLUTAMIC ACID	0.774 ±	0.079	1.442 ±	0.926	0.865 ±	0.503	1.048 ±	0.612	0.186
CITRULLINE	1.201 ±	1.153	1.705 ±	0.021	0.747 ±	0.035	1.129 ±	0.344	1.086
PROLINE	1.435 ±	0.002	0.629 ±	0.121	0.766 ±	0.448	1.498 ±	0.102	2.477
GLYCINE	1.382 ±	0.006	0.470 ±	0.270	2.361 ±	0.658	0.665 ±	0.203	4.938
ALANINE	0.970 ±	0.846	0.238 ±	0.264	0.934 ±	0.209	0.184 ±	0.319	0.928
CYSTINE	0.856 ±	0.184	1.833 ±	0.001	0.840 ±	0.000	0.850 ±	0.779	0.168
α-AMINO ADIPIC ACID	1.222 ±	0.770	0.372 ±	0.176	0.837 ±	0.067	1.125 ±	0.072	1.385
VALINE	1.542 ±	0.143	0.447 ±	0.187	1.101 ±	0.101	0.868 ±	0.388	0.962
METHIONINE	1.645 ±	0.479	0.589 ±	0.121	1.019 ±	0.211	1.021 ±	0.002	4.084
ISOLEUCINE	0.959 ±	0.464	3.065 ±	1.750	0.723 ±	0.188	0.866 ±	0.086	0.997
LEUCINE	1.069 ±	0.183	-0.475 ±	0.280	0.658 ±	0.057	0.946 ±	0.414	3.866
TYROSINE	0.514 ±	0.090	0.969 ±	0.690	0.676 ±	0.737	0.893 ±	0.429	0.238
PHENYLALANINE	1.165 ±	0.654	0.400 ±	0.438	0.905 ±	0.045	0.814 ±	0.047	0.857
ORNITHINE	0.662 ±	0.243	1.056 ±	0.545	0.961 ±	0.209	0.804 ±	0.316	0.241
LYSINE	0.781 ±	0.437	0.509 ±	0.104	0.824 ±	0.381	0.813 ±	0.108	0.179
UNKNOWN	1.018 ±	1.197	2.580 ±	0.343	0.915 ±	0.207	5.296 ±	1.776	2.281
HISTIDINE	0.055 ±	0.463	1.038 ±	0.416	0.957 ±	0.091	1.449 ±	0.255	2.080
3-METHYL HISTIDINE	1.214 ±	0.114	1.052 ±	0.019	0.760 ±	0.126	1.994 ±	0.027	7.885*
ARGININE	0.985 ±	0.545	2.743 ±	3.690	0.437 ±	0.977	0.071 ±	1.083	0.314

* = $P < 0.05$

TABLE 3.5 PLASMA FREE AMINO ACID LEVELS

SUMMARY OF ADJUSTED MEANS - TREATMENT PERIOD ON CONTROL

VARIABLE	GROUP: METHIONINE	STANDARD ERROR	CONTROL	STANDARD ERROR	UNTREATED CASEIN	STANDARD ERROR	TREATED CASEIN	STANDARD ERROR	F
TAURINE[2]	-	-	-	-	-	-	-	-	-
UREA[2]	-	-	-	-	-	-	-	-	-
METH. SULPHOXIDE[2]	-	-	-	-	-	-	-	-	-
ASPARTIC ACID	0.080[1]	± 0.005	0.071	± 0.005	0.078	± 0.005	0.071	± 0.005	0.884
METH. SULPHONE	0.201	± 0.010	0.196	± 0.010	0.191	± 0.010	0.191	± 0.010	0.734
GLUTAMIC ACID[2]	-	-	-	-	-	-	-	-	-
CITRULLINE	0.421	± 0.046	0.479	± 0.046	0.582	± 0.046	0.710	± 0.046	6.820*
PROLINE	0.404	± 0.054	0.384	± 0.054	0.538	± 0.054	0.734	± 0.054	8.704**
GLYCINE	3.961	± 0.518	3.946	± 0.518	3.224	± 0.518	3.001	± 0.518	0.863
ALANINE	0.779	± 0.037	0.852	± 0.037	0.832	± 0.037	0.690	± 0.037	3.520✓
CYSTINE[2]	-	-	-	-	-	-	-	-	-
∝-AMINO ADIPIC ACID	0.030	± 0.002	0.033	± 0.002	0.035	± 0.002	0.029	± 0.002	1.381
VALINE	0.903	± 0.073	0.807	± 0.073	0.969	± 0.075	1.224	± 0.073	5.510*
METHIONINE	0.044	± 0.001	0.041	± 0.001	0.045	± 0.001	0.046	± 0.001	4.152✓
ISOLEUCINE	0.423	± 0.025	0.369	± 0.025	0.378	± 0.025	0.404	± 0.025	1.010
LEUCINE	0.486	± 0.032	0.463	± 0.032	0.557	± 0.032	0.660	± 0.032	7.087*
TYROSINE	0.259	± 0.006	0.248	± 0.006	0.237	± 0.006	0.246	± 0.006	1.319
PHENYLALANINE	0.164	± 0.006	0.160	± 0.006	0.152	± 0.006	0.164	± 0.006	0.858
ORNITHINE	0.359	± 0.013	0.341	± 0.013	0.342	± 0.013	0.323	± 0.013	1.028
LYSINE	0.448	± 0.025	0.443	± 0.025	0.424	± 0.025	0.386	± 0.025	0.926
UNKNOWN	0.327	± 0.061	0.243	± 0.061	0.336	± 0.061	0.887	± 0.061	20.356***
HISTIDINE	0.163	± 0.009	0.218	± 0.009	0.222	± 0.009	0.223	± 0.009	4.763*
3-METHYL HISTIDINE	0.160	± 0.010	0.142	± 0.010	0.134	± 0.010	0.138	± 0.010	1.080
ARGININE	0.248	± 0.021	0.224	± 0.021	0.225	± 0.021	0.275	± 0.021	1.141

✓ = P< 0.10
* = P< 0.05
** = P< 0.01
*** = P< 0.001
1 = Values expressed as micro-moles per ml.
2 = See table 3.7

TABLE 3.6 PLASMA FREE AMINO ACID LEVELS

SUMMARY OF ADJUSTED MEANS - POST-TREATMENT PERIOD ON CONTROL

VARIABLE	GROUP: METHIONINE	STANDARD ERROR	CONTROL	STANDARD ERROR	UNTREATED CASEIN	STANDARD ERROR	TREATED CASEIN	STANDARD ERROR	F
TAURINE	0.116[1]	± 0.006	0.104	± 0.006	0.103	± 0.006	0.106	± 0.006	1.007
UREA[2]	-	-	-	-	-	-	-	-	-
METH. SULPHOXIDE[2]	-	-	-	-	-	-	-	-	-
ASPARTIC ACID	0.072	± 0.006	0.078	± 0.006	0.080	± 0.006	0.073	± 0.006	0.360
METH. SULPHONE[2]	-	-	-	-	-	-	-	-	-
GLUTAMIC ACID	0.350	± 0.002	0.373	± 0.022	0.350	± 0.022	0.367	± 0.022	0.285
CITRULLINE	0.437	± 0.024	0.494	± 0.024	0.477	± 0.024	0.473	± 0.024	0.983
PROLINE	0.428	± 0.018	0.413	± 0.018	0.449	± 0.018	0.401	± 0.010	1.321
GLYCINE	3.746	± 0.498	4.138	± 0.498	3.518	± 0.498	3.340	± 0.498	0.409
ALANINE	0.805	± 0.037	0.859	± 0.037	0.828	± 0.037	0.875	± 0.037	0.460
CYSTINE[3]	0.045	± 0.003	0.044	± 0.003	0.043	± 0.003	0.046	± 0.003	0.186
α-AMINO ADIPIC ACID	0.032	± 0.002	0.033	± 0.002	0.034	± 0.002	0.034	± 0.002	0.112
VALINE	0.794	± 0.043	0.725	± 0.043	0.857	± 0.043	0.734	± 0.043	1.876
METHIONINE	0.042	± 0.002	0.041	± 0.002	0.043	± 0.002	0.046	± 0.002	0.617
ISOLEUCINE	0.384	± 0.019	0.335	± 0.019	0.376	± 0.019	0.314	± 0.019	3.062
LEUCINE	0.453	± 0.020	0.416	± 0.020	0.465	± 0.020	0.455	± 0.020	0.877
TYROSINE	0.269	± 0.008	0.257	± 0.008	0.229	± 0.008	0.256	± 0.008	3.828$\not$
PHENYLALANINE	0.157	± 0.004	0.153	± 0.004	0.151	± 0.004	0.165	± 0.004	2.670
ORNITHINE	0.331	± 0.015	0.330	± 0.015	0.324	± 0.015	0.325	± 0.015	0.046
LYSINE	0.409	± 0.015	0.420	± 0.015	0.412	± 0.015	0.448	± 0.015	0.908
UNKNOWN	0.289	± 0.113	0.203	± 0.113	0.373	± 0.113	1.308	± 0.113	18.171***
HISTIDINE	0.171	± 0.017	0.221	± 0.017	0.218	± 0.017	0.225	± 0.017	1.069
3-METHYL HISTIDINE[2]	-	-	-	-	-	-	-	-	-
ARGININE[2]	-	-	-	-	-	-	-	-	-

1 = Values expressed as micro-moles per ml.
$\not$ = $P < 0.10$
*** = $P < 0.001$
2 = See table 3.7
3 = Values 10 x actual

noted that with the exception of the unknown, all amino acids which responded to treated casein supplements in the treatment period returned to control period levels 5 days after supplementation was stopped.

Table 3.7 gives the unadjusted group means for those variables which displayed a non-significant regression within groups or a significant difference between the slopes of regression lines thus nullifying the usefulness of adjusted mean analysis. A non-significant regression within groups indicates that individual values were too variable for any meaningful interpretation of the data. A significant difference between the slopes of regression lines may be interpreted as being due to treatment, a variable response of the sheep in a group to a treatment, random error or a combination of these factors. Unadjusted amino acid concentration suggest that for taurine the administration of treated casein has appreciably reduced the plasma concentration. Evidence of taurine suppression as a result of treatment can be seen in the markedly lower treatment period on control regression values for treated casein. For the other variable displaying significant between-slope differences, random error and variable within-group responses probably are the major factors producing the differences.

Table 3.8 compares the mean within-group regressions with the residual sums of squares for 5 variables chosen at random and 5 variables which displayed significant variation between adjusted means. The relatively low residual sums of squares observable for both groups of variables indicates that the technique used to measure amino acid concentrations possessed a high degree of accuracy.

TABLE 3.7 SUMMARY OF UNADJUSTED MEANS[1]

(TREATMENT PERIOD)

	GROUP METHIONINE	CONTROL	UNTREATED CASEIN	TREATED CASEIN	
UREA	2.428	2.950	2.610	3.282	Non-Significant Regression within groups
METH. SULPHOXIDE	0.057	0.052	0.060	0.054	
TAURINE	0.113	0.112	0.071	0.083	Significant difference between slopes of regression lines
GLUTAMIC ACID	0.416	0.380	0.281	0.378	
CYSTINE[2]	0.107	0.026	0.037	0.025	

(POST-TREATMENT PERIOD)

UREA	2.543	3.253	2.644	2.373	Non-Significant Regression within groups
METH. SULPHOXIDE	0.053	0.057	0.059	0.058	
METH. SULPHONE	0.190	0.143	0.171	0.235	
ARGININE	0.316	0.191	0.214	0.285	
3-METHYL HISTIDINE	0.198	0.119	0.116	0.159	Significant difference between slopes of regression lines

1 = Values expressed as micro-moles per ml. plasma
2 = Values 10 times actual

TABLE 3.8 COMPARISON OF MEAN WITHIN GROUP REGRESSIONS WITH THE RESIDUAL SUMS OF SQUARES - TREATMENT PERIOD ON CONTROL

FIRST 5 VARIABLES CHOSEN AT RANDOM, SECOND 5 ARE THOSE WHICH DISPLAYED SIGNIFICANT VARIATION BETWEEN ADJUSTED MEANS

VARIABLE	REGRESSION VALUE	RESIDUAL S.S.
ISOLEUCINE	1.081	0.013
METH. SULPHONE	0.937	0.002
TYROSINE	0.422	0.001
GLUTAMIC ACID	0.715	0.021
ARGININE	1.100	0.009
CITRULLINE	1.264	0.044
PROLINE	1.282	0.062
VALINE	1.265	0.111
LEUCINE	1.408	0.022
UNKNOWN	2.437	0.077

D. In Vitro Incubations

The results of *in vitro* incubations of treated and untreated casein are given below:

NH_3 PRODUCTION (Mg. NH_3/100 ml. rumen fluid)*

INCUBATION TIME	3 HOURS	6 HOURS	24 HOURS
BLANK	34.4	35.4	51.4
UNTREATED CASEIN	42.0	48.2	84.9
TREATED CASEIN	31.9	37.3	52.6

* Values represent the mean of duplicate samples

RESULTS EXPRESSED AS DIFFERENCES FROM BLANK

INCUBATION TIME	3 HOURS	6 HOURS	24 HOURS
UNTREATED CASEIN	+ 10.6	+ 12.8	+ 33.5
TREATED CASEIN	+ 0.5	+ 1.9	+ 1.2

It can be seen that after 24 hours incubation, treated casein ammonia - values were comparable with those of the blank, indicating the treatment was effective in preventing protein degradation *in vitro*. As a comparison, untreated casein was shown to produce values substantially above the blank.

Unfortunately the incubation technique could not be applied to treated methionine. The MHA molecule contains no amino group and thus its degradation is not likely to produce increases in ammonia levels.

IV. DISCUSSION

Results indicate that supplemental feeding of 1 g. of MHA per day in the casein-coated form produced no observable effect on wool growth and possibly a negligible negative effect on body weight.

Reis (1967) has demonstrated a wool growth and slight positive body weight response to a 3.0 g. per day MHA supplement given by abomasal infusion.

Two possible reasons for the lack of response in this experiment appear. Firstly, the treatment techniques may not have been effective, the MHA being degraded by the rumen microorganisms. This possibility appears likely as there were no significant increases in plasma amino acid concentrations following MHA supplementation. Secondly, if the treated MHA did escape rumen action the dosage level may have been too small to produce an observable response. However, Wickham (pers. comm.) obtained a significant ($P<0.01$) wool growth response to subcutaneous injections of 1.5 g. per day of DL-methionine.

Supplemental feeding of 100 g. per day treated casein produced a significant wool growth response and possibly a small body weight response. Since the result of *in vitro* ammonia and plasma amino acid level tests indicate that treated casein was escaping rumen degradation, it may be suggested that observed responses were the result of treated casein being made directly available to the sheep.

The observed response with untreated casein conflicts with the lack of nitrogen retention response to casein supplements shown by McDonald (1952) and the absence of a wool growth response to protein in Ferguson's (1959) experiment. It is possible that the untreated casein while being

degraded in the rumen, provided a nitrogen source which when combined with the readily available energy provided by the barley meal supplement, produced an increase in the rumen microbial population sufficient to account for most of the observed increase in wool growth rate. Since microbial counts were not included in the experiment no direct evidence is available to support the above suggestion.

Another possibility is that sheep on low-energy diets can utilize high levels of dietary proteins more efficiently than sheep on high-energy diets. Since the sheep in this experiment were on a maintenance level of energy, those receiving untreated casein may have utilized some of the additional protein as an energy source for microbial synthesis.

Post-treatment wool growth responses observed in the treated and untreated casein groups are of interest as a post-treatment response after cysteine infusion has been noted by Dryden (1968). The existence of a metabolic "pool" of cysteine and other amino acids as suggested by Downes (1961a) and Downes *et al.* (1962), could possibly explain the post-treatment responses provided such a pool was filled during the treatment period and gradually depleted over the post-treatment period.

Downes (1961a) and Downes *et al.* (1962) suggested that part of the metabolic "pool" consisted of cysteine bound to plasma protein. Since analysis of plasma free cystine would not indicate variation in the level of bound cystine in the plasma, the cystine levels recorded in table 3.7 would not provide evidence for or against the existence of a metabolic "pool".

The effect of small group size on analyses of wool growth and body weight data should be noted since between-group differences had to be large to reach a level of statistical significance. Thus, while wool growth differences between groups in the post-treatment period were significant and

therefore probably attributable to treatment ($P<0.05$) it is likely that treatment period differences, while not being statistically significant, were also mainly attributable to treatment. The interpretation of body weight results is also affected by small group size. For example, the treated casein group appeared to give a positive response to treatment but group size is far too small to permit confirmation of this observation as being real and due to treatment.

The results of plasma amino acid analysis indicate that the level of several individual amino acids is raised after dietary supplementation with formalin-treated casein. The highly elevated levels found for the unknown amino acid may be particularly useful information. It is suggested that once the unknown is identified, it could be used as an indicator of whether or not formalin-treated casein is escaping rumen degradation.

CHAPTER 4

PROTEIN AND ENERGY LEVEL

EFFECTS ON WOOL GROWTH

I. INTRODUCTION

This chapter deals with two aspects of wool growth responses to the dietary level of protein.

Firstly, previous experiments measuring wool growth responses to various levels of protein nutrition have used breeds other than the Romney (Slen and Whiting, 1952; Ferguson, 1959). Thus, it was decided to investigate whether or not the results obtained from other breeds apply for the Romney.

Secondly, in the previous experiment wool growth rate appeared to be increased by untreated casein supplements, a result that is in marked contrast with the protein level responses obtained by Slen and Whiting (1952) and Ferguson (1959). One hypothesis arising from this observation is that an interaction exists between protein and energy levels. Thus, a test for the existence of an interaction was incorporated into the experiment. The method used was to measure wool growth responses from 4 levels of protein fed at 2 widely differing levels of energy.

II. MATERIALS AND METHODS

A. Animals

The experimental animals were Romney wether hoggets (27 - 33 kgs. liveweight) taken from a randomly bred flock.

Twenty-four of the animals were randomly divided into groups as listed below:

GROUP	SHEEP NUMBERS	TREATMENT
E1 P1	486, 381, 831	ENERGY LEVEL 1, PROTEIN LEVEL 1
E1 P2	300, 729, 560	" " 1 " " 2
E1 P3	895, 878, 827	" " 1 " " 3
E1 P4	858, 874, 789	" " 1 " " 4
E2 P1	319, 394, 385	" " 2 " " 1
E2 P2	807, 703, 879	" " 2 " " 2
E2 P3	901, 329, 792	" " 2 " " 3
E2 P4	327, 582, 506	" " 2 " " 4

An additional six animals (sheep numbers: 402, 468, 358, 465, 820 and 552) were used for nitrogen balance and digestibility determinations.

B. Housing

The 24 treatment-grouped animals were placed in individual pens within controlled-environment rooms where the temperature was maintained at 15.6°C and the light cycle regulated to correspond with outside day-lengths. The six animals used for nitrogen balance and digestibility studies were placed in metabolism cages that allowed for separate collection of feces and urine.

C. Rations

The experiment was divided into two periods, a 4-week pre-treatment and an 8-week treatment. The amounts and compositions of the rations fed during these periods are given in table 4.1a.

Crude protein content of the rations (N x 6.25) was determined by an automated Kjeldahl procedure. Triplicate samples of ration constituents were finely ground and 0.140 g. sub-samples digested in micro-Kjeldahl flasks using 5.0 ml. of the following digestion mixture: 100 g. K_2SO_4, 1.00 g. selenium powder and 1,000 ml. conc. H_2SO_4. Digestion time was approximately 4 hours. After digestion, samples were cooled and made to volume (70 ml.) with distilled water. With digestion complete the ammonia thus formed was measured calorimetrically utilizing a Technicon Auto Analyzer system, details of which are described by Warner and Jones (1967). Crude protein content of the experimental rations is given in table 4.1b.

Energy intake was expressed as kilocalories digestible energy (DE). Energy values were determined with an adiabatic bomb calorimeter. DE = Energy fed - Energy excreted in the feces. Energy values for individual rations and daily amounts fed are given in table 4.1b. The rations within energy levels were designed to be approximately isocaloric (i.e. ration E1 P1 contains approximately the same amount of digestible energy as rations E1 P2, E1 P3 or E1 P4).

Animals were fed once daily at approximately 9 a.m. All feed given and refused was weighed to the nearest 0.1 lb with intakes being recorded daily.

TABLE 4.1a RATIONS (DRY MATTER BASIS)

PERCENTAGE COMPOSITION

	RATION				
INGREDIENT	PRE-TREATMENT	E1 P1 and E2 P1	E1 P2 and E2 P2	E1 P3 and E2 P3	E1 P4 and E2 P4
MOLASSES	-	4.6%	3.8%	4.7%	5.0%
LUCERNE MEAL	38.8%	40.1	52.2	84.3	42.8
BARLEY STRAW (chopped)	-	34.1	8.0	-	7.4
BARLEY GRAIN (ground)	34.5	21.2	36.0	11.0	38.6
PEA HULLS	26.7	-	-	-	-
CASEIN	-	-	-	-	6.2

RATION	QUANTITY FEED DAILY
PRE-TREATMENT	1,015 g.
E1 P1	816
E1 P2	726
E1 P3	771
E1 P4	680
E2 P1	1,224
E2 P2	1,088
E2 P3	1,179
E2 P4	1,043

TABLE 4.1b RATIONS (DRY MATTER BASIS)

RATION	CRUDE PROTEIN CONTENT
PRE-TREATMENT	12.8%
E1 P1 and E2 P1	10.4
E1 P2 and E2 P2	13.1
E1 P3 and E2 P3	15.5
E1 P4 and E2 P4	17.2

RATION	DIGESTIBLE ENERGY VALUE	AMOUNT FED DAILY
PRE-TREATMENT	2.859 Kcal DE/g.	2,902 Kcal DE
E1 P1	2.788	2,275
E1 P2	2.948	2,140
E1 P3	2.992	2,306
E1 P4	3.290	2,237
E2 P1	2.656	3,249
E2 P2	2.908	3,166
E2 P3	2.804	3,306
E2 P4	2.935	3,061

D. Wool Sampling

Wool samples were obtained and processed according to procedures previously described (chapter 3, section IID). Samples were clipped at 4 week intervals.

E. Nitrogen Balances

The nitrogen balance technique was used to ascertain the extent to which nitrogen was retained at different levels of feeding. Using a single animal for each diet, three levels of nitrogen intake (P1, P3, P4) were compared at each energy level (E1 and E2).

For two five-day periods (A and B) urine and fecal samples were collected and weighed daily. Urine samples were bulked and stored at $4^{\circ}C$ while fecal samples were bulked and frozen.

To prevent ammonia loss through evaporation, urine samples were collected in plastic buckets containing 40 ml. conc. H_2SO_4. Fecal samples were collected in canvas bags attached to the animals by a harness.

Nitrogen content of feed and excreta (urine + feces) was measured using the automated Kjeldahl procedure described in section IIC. The procedure for urine samples deviated slightly from that used for feeds and feces (0.5 ml. of urine was digested and the digestion time was approximately twenty minutes).

F. Statistical Methods

Data from this experiment were analyzed by a two-way covariance analysis using the model:

$$y_{ijk} = \mu + a_i + c_j + (ac)_{ij} + \beta x_{ijk} + \varepsilon_{ijk}$$

y_{ijk} is the kth observation in the ijth group

μ is the general mean

i = energy levels 1, 2

j = protein levels 1 - 4

$(ac)_{ij}$ is the interaction term

β is the regression of Y on X

ε_{ijk} is the error term, independent and normally distributed.

This analysis not only adjusts for inherent variation that existed prior to treatment but allows for measurement of interaction between protein and energy levels.

Adjusted mean differences between protein and energy levels, and the interaction terms were tested for significance by the F test as described by Snedecor and Cochran (1967).

The analysis was made by an available program for an IBM 1620 computer.

III. RESULTS

A. Wool Growth

Results of covariance analysis of wool growth rate are presented in table 4.2 and figures 4.1 and 4.2. After the first 4 weeks of treatment (period A) there was no significant difference between the two energy or four protein levels, nor was there any evidence of an interaction between energy and protein level. After another 4 weeks of treatment (period B) a highly significant difference emerged between energy levels ($P < 0.001$). However, differences between protein levels, and the interaction term remained non-significant.

B. Body Weight

Results of covariance analysis of body weight are presented in table 4.3 and figures 4.3 and 4.4. A highly significant difference ($P < 0.001$) was found between energy levels at the end of period A. A significant difference ($P < 0.05$) was also found between protein levels. The interaction term between energy and protein levels was non-significant. At the end of period B the difference between energy levels was still highly significant ($P < 0.001$) but the difference between protein levels was no longer significant. There was a slight indication ($P < 0.10$) of an energy - protein level interaction in this period. The interaction appears to involve mainly protein levels 1 and 2.

C. Feed Intake

Table 4.4 gives the digestible energy intakes for each individual and group during the control and treatment periods. Note the variability

TABLE 4.2 WOOL GROWTH: COVARIANCE ANALYSIS RESULTS

ADJUSTED MEANS: TREATMENT PERIOD A ON CONTROL

ENERGY LEVEL	PROTEIN LEVEL 1	STANDARD ERROR	PROTEIN LEVEL 2	STANDARD ERROR	PROTEIN LEVEL 3	STANDARD ERROR	PROTEIN LEVEL 4	STANDARD ERROR
1	1.324[1]	± 0.133	1.369	± 0.129	1.607	± 0.149	1.430	± 0.124
2	1.373	± 0.129	1.644	± 0.126	1.727	± 0.126	1.482	± 0.124

TEST FOR ADJUSTED MEAN DIFFERENCES AND INTERACTION

	DEGREES OF FREEDOM	RESIDUAL MEAN SQUARE	F
BETWEEN ENERGY LEVELS	1	0.093	2.007
BETWEEN PROTEIN LEVELS	3	0.088	1.898
BETWEEN ENERGY X PROTEIN LEVELS	3	0.016	0.356
WITHIN ENERGY X PROTEIN LEVELS	15	0.046	

ADJUSTED MEANS: TREATMENT PERIOD B ON CONTROL

ENERGY LEVEL	PROTEIN LEVEL 1	STANDARD ERROR	PROTEIN LEVEL 2	STANDARD ERROR	PROTEIN LEVEL 3	STANDARD ERROR	PROTEIN LEVEL 4	STANDARD ERROR
1	0.902	± 0.160	1.017	± 0.154	1.197	± 0.178	1.011	± 0.149
2	1.305	± 0.149	1.566	± 0.152	1.398	± 0.151	1.503	± 0.149

TEST FOR ADJUSTED MEAN DIFFERENCES AND INTERACTION

	DEGREES OF FREEDOM	RESIDUAL MEAN SQUARE	F
BETWEEN ENERGY LEVELS	1	1.016	15.319***
BETWEEN PROTEIN LEVELS	3	0.044	0.656
BETWEEN ENERGY X PROTEIN LEVELS	3	0.025	0.381
WITHIN ENERGY X PROTEIN LEVELS	15	0.066	

[1] = Mg./cm^2/day

*** = $P < 0.001$

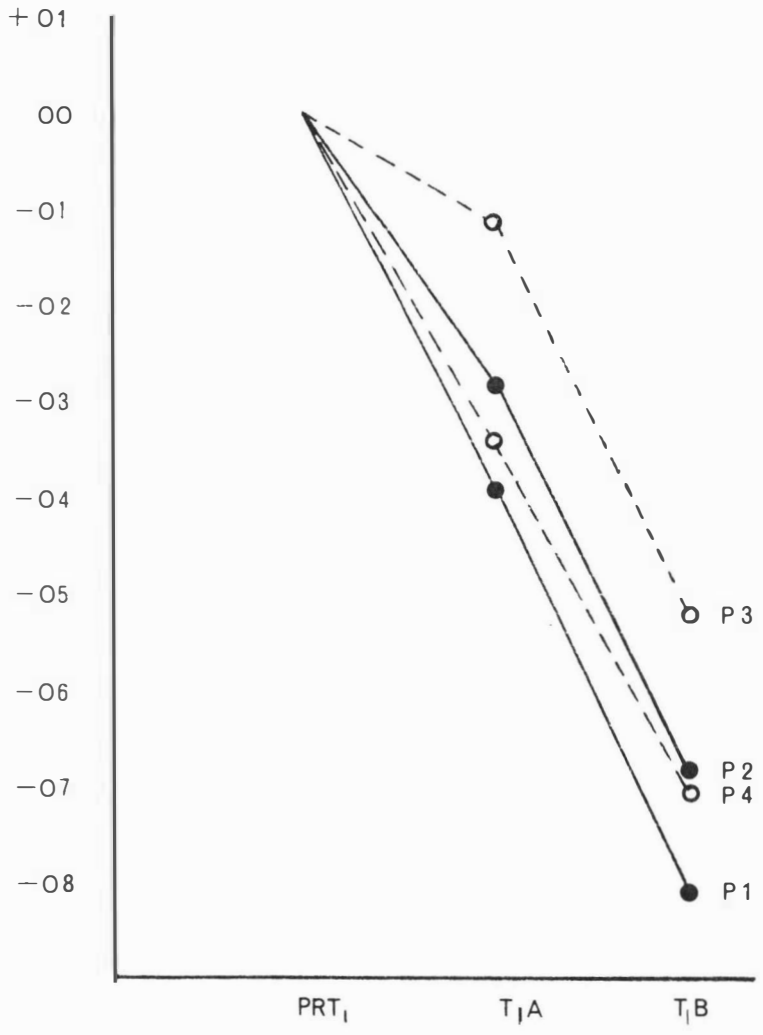
FIGURE 4·1

PROTEIN LEVEL EFFECT

ENERGY LEVEL E_1

WOOL GROWTH RATE

DEVIATIONS OF ADJUSTED MEANS FROM PRE-TREATMENT PERIOD GENERAL MEAN, EXPRESSED AS MG/CM^2/DAY.



PRT_1 = PRE-TREATMENT PERIOD
T_1A = TREATMENT PERIOD A
T_1B = TREATMENT PERIOD B

	GENERAL MEAN	ADJUSTED MEANS	
		TREATMENT A	TREATMENT B
P 1	1·711	1·324	0·902
P 2	1·711	1·369	1·017
P 3	1·711	1·607	1·197
P 4	1·711	1·430	1·011

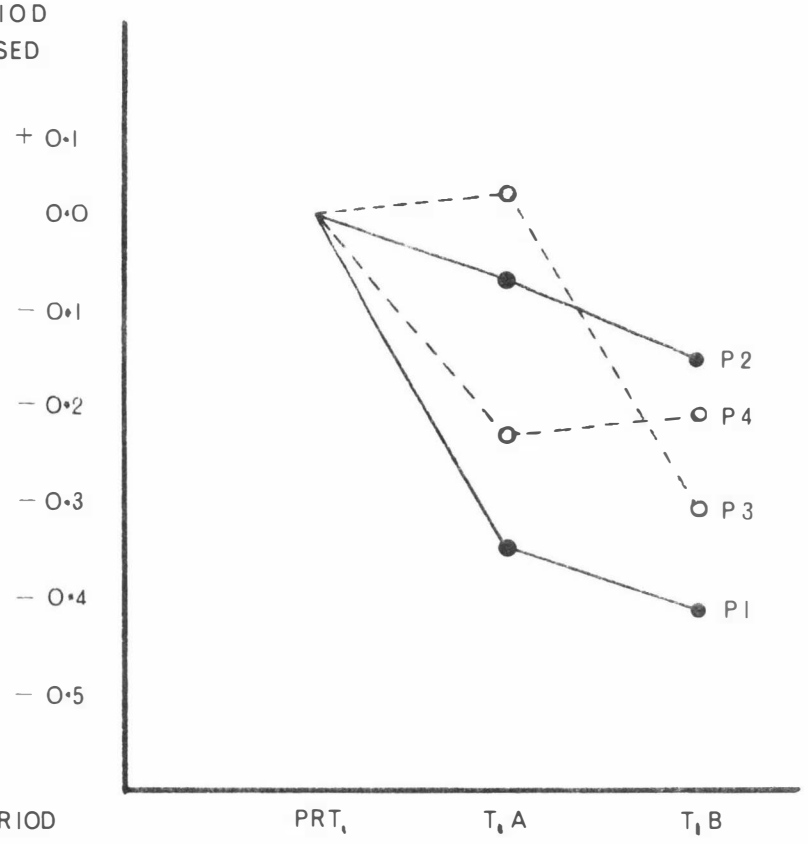
FIGURE 4·2

PROTEIN LEVEL EFFECT

ENERGY LEVEL E_2

WOOL GROWTH RATE

DEVIATIONS OF ADJUSTED MEANS FROM PRE-TREATMENT PERIOD GENERAL MEAN, EXPRESSED AS MG / CM^2 / DAY.



PRT_1 = PRE-TREATMENT PERIOD
T_1A = TREATMENT PERIOD A
T_1B = TREATMENT PERIOD B

	GENERAL MEAN	ADJUSTED MEANS TREATMENT A	TREATMENT B
P 1	1·711	1·373	1·305
P 2	1·711	1·644	1·566
P 3	1·711	1·727	1·398
P 4	1·711	1·428	1·503

TABLE 4.3 BODY WEIGHT: COVARIANCE ANALYSIS RESULTS

ADJUSTED MEANS: TREATMENT PERIOD A ON CONTROL

ENERGY LEVEL	PROTEIN LEVEL 1	STANDARD ERROR	PROTEIN LEVEL 2	STANDARD ERROR	PROTEIN LEVEL 3	STANDARD ERROR	PROTEIN LEVEL 4	STANDARD ERROR
1	30.87[1]	± 0.40	29.42	± 0.43	29.69	± 0.39	29.18	± 0.39
2	32.02	± 0.42	31.99	± 0.40	31.61	± 0.40	30.89	± 0.41

TEST FOR ADJUSTED MEAN DIFFERENCES AND INTERACTION

	DEGREES OF FREEDOM	RESIDUAL MEAN SQUARE	F
BETWEEN ENERGY LEVELS	1	19.457	41.963***
BETWEEN PROTEIN LEVELS	3	1.668	3.597*
BETWEEN ENERGY X PROTEIN LEVELS	3	0.491	1.060
WITHIN ENERGY X PROTEIN LEVELS	15	0.464	

ADJUSTED MEANS: TREATMENT PERIOD B ON CONTROL

ENERGY LEVEL	PROTEIN LEVEL 1	STANDARD ERROR	PROTEIN LEVEL 2	STANDARD ERROR	PROTEIN LEVEL 3	STANDARD ERROR	PROTEIN LEVEL 4	STANDARD ERROR
1	31.01	± 0.49	29.47	± 0.53	30.42	± 0.48	29.77	± 0.48
2	33.79	± 0.51	34.43	± 0.48	32.97	± 0.48	33.14	± 0.50

TEST FOR ADJUSTED MEAN DIFFERENCES AND INTERACTION

	DEGREES OF FREEDOM	RESIDUAL MEAN SQUARE	F
BETWEEN ENERGY LEVELS	1	67.309	98.503***
BETWEEN PROTEIN LEVELS	3	0.847	1.239
BETWEEN ENERGY X PROTEIN LEVELS	3	1.706	2.497 ∤
WITHIN ENERGY X PROTEIN LEVELS	15	0.683	

1 = Kilograms

*** = P< 0.001

∤ = P< 0.10

* = P< 0.05

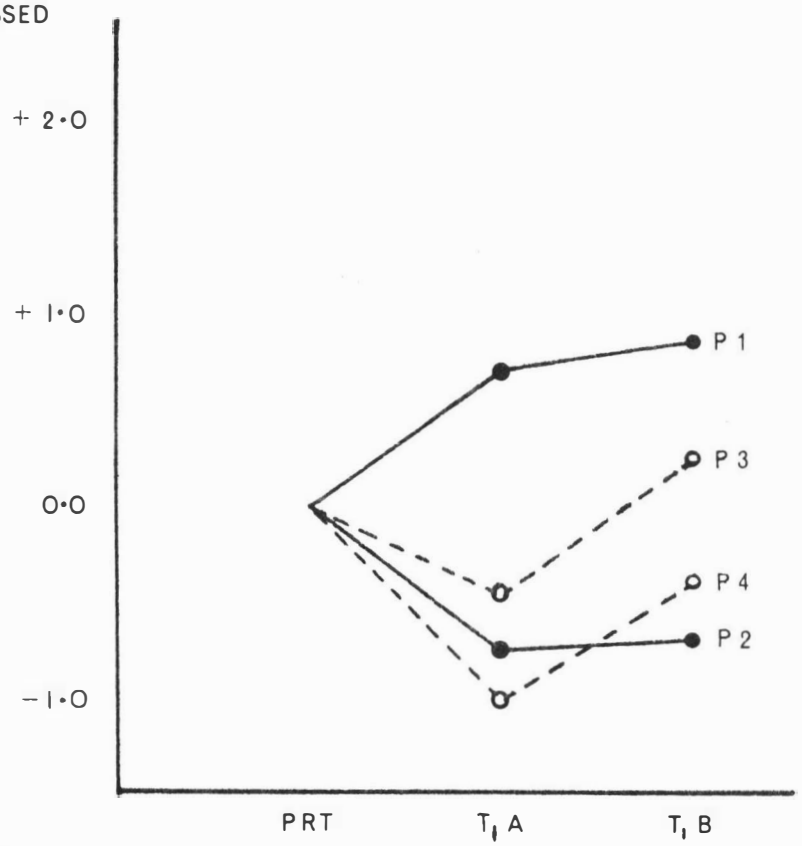
FIGURE 4·3

PROTEIN LEVEL EFFECT

ENERGY LEVEL E_1

BODY WEIGHT

DEVIATIONS OF ADJUSTED MEANS FROM PRE-TREATMENT PERIOD GENERAL MEAN, EXPRESSED AS KILOGRAMS.



PRT = PRE-TREATMENT PERIOD
T_1 A = TREATMENT PERIOD A
T_1 B = TREATMENT PERIOD B

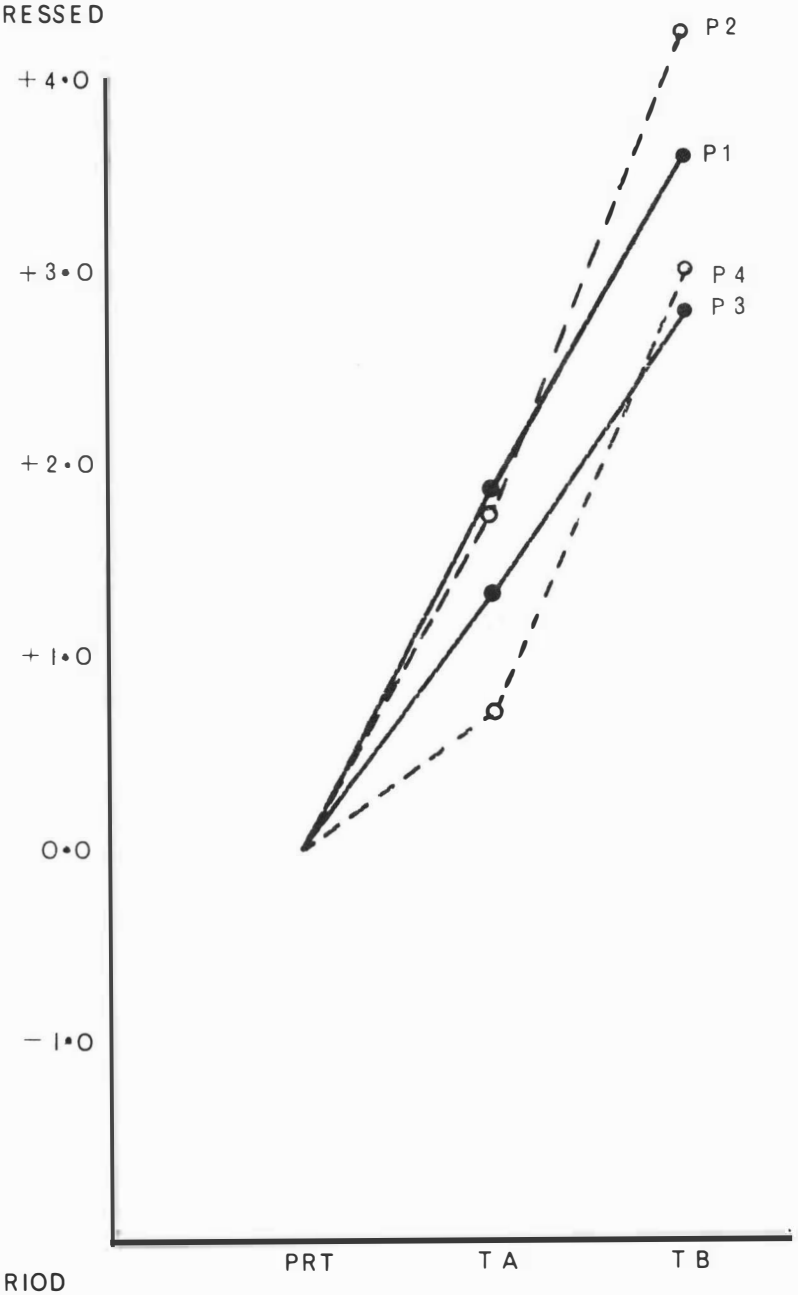
	GENERAL MEAN	ADJUSTED MEANS TREATMENT A	TREATMENT B
P1	30·17	30·87	31·01
P2	30·17	29·42	29·47
P3	30·17	29·69	30·42
P4	30·17	29·18	29·77

FIGURE 4·4

PROTEIN LEVEL EFFECT
ENERGY LEVEL E 2

BODY WEIGHT

DEVIATIONS OF ADJUSTED MEANS FROM PRE-TREATMENT PERIOD GENERAL MEAN, EXPRESSED AS KILOGRAMS.



PRT = PRE-TREATMENT PERIOD
TA = TREATMENT PERIOD A
TB = TREATMENT PERIOD B

	GENERAL MEAN	ADJUSTED MEANS TREATMENT A	TREATMENT B
P1	30·17	32·02	33·79
P2	30·17	31·99	34·43
P3	30·17	31·61	32·97
P4	30·17	30·89	33·14

TABLE 4.4 FEED INTAKE - KILOCALORIES DIGESTIBLE ENERGY (DRY MATTER BASIS)

EAR TAG NO.	TREATMENT GROUP	PRE-TREATMENT PERIOD SUMS*	GROUP MEAN	TREATMENT PERIOD A SUMS	GROUP MEAN	TREATMENT PERIOD B SUMS	GROUP MEAN
486	E1 P1	74,330		58,834		57,719	
381	E1 P1	76,617	75,093	61,065	58,743	57,719	57,347
831	E1 P1	74,330		56,325		56,604	
300	E1 P2	74,044		57,489		43,043	
729	E1 P2	57,177	62,800	54,541	53,853	53,362	48,740
560	E1 P2	57,177		49,529		49,824	
985	E1 P3	74,044		61,639		59,245	
878	E1 P3	58,034	66,513	49,969	56,553	55,355	57,353
827	E1 P3	67,469		58,048		57,449	
858	E1 P4	75,187		60,870		56,263	
874	E1 P4	71,471	73,853	59,224	60,210	56,592	56,043
789	E1 P4	74,902		60,540		55,276	
319	E2 P1	75,474		68,249		75,154	
394	E2 P1	67,469	72,520	60,282	67,453	71,170	74,003
385	E2 P1	74,616		73,826		75,685	
807	E2 P2	67,755		72,996		72,123	
703	E2 P2	77,475	69,380	80,848	75,810	78,812	74,837
879	E2 P2	62,895		73,578		73,578	
901	E2 P3	71,757		71,215		74,580	
329	E2 P3	74,902	71,947	83,552	74,953	72,056	72,150
792	E2 P3	69,184		70,094		69,813	
327	E2 P4	61,751		62,508		69,258	
582	E2 P4	76,046	65,850	71,605	67,400	76,008	71,707
506	E2 P4	59,750		68,084		69,845	

* SUM ADJUSTED TO A 28 DAY BASIS

between individuals in the control period (each animal was receiving approximately 81,000 Kcal digestible energy). Also note that when compared with period A, the within group variation is markedly reduced in period B. This observation may be more easily seen in the standard error for group mean intakes as derived from covariance analysis (see below).

PERIOD A

ENERGY LEVEL	GROUP: P1	P2	P3	P4
E1	2,157.7[1]	2,241.9	2,076.8	2,105.8
E2	2,064.3	2,029.4	2,051.2	2,098.1

PERIOD B

E1	1,827.6	1,898.9	1,759.0	1,783.6
E2	1,748.5	1,718.9	1,737.3	1,777.1

[1] = Standard error of mean; kilocalories digestible energy

Within energy level E2 the variation in intake between groups was markedly reduced in period B (when compared with period A). However, within energy level E1 intake variation between groups slightly increased in period B.

D. Nitrogen Balances

Results of nitrogen balance trials are given in table 4.5. Several of the sheep on trial refused their diets, the extent of the refusal being so severe that only results for diets E2 P1 and E2 P4 were comparable.

Even though the animal on the high energy - high protein (E2 P4) diet had a substantially greater nitrogen intake than the animal on the

high energy - low protein (E2 P1) diet, the difference between diets in the amount of nitrogen retained was small. When nitrogen output is separated into fecal and urine portions it can be seen that the proportion of nitrogen excreted via the urine is greater (approximately 50%) for the high protein diet.

TABLE 4.5 NITROGEN BALANCE DATA

PERIOD A

	INTAKE/ DAY	FECAL OUTPUT/DAY	URINARY OUTPUT/DAY	TOTAL OUTPUT/DAY	BALANCE
E2 P1	15.0[1]	6.7	9.1	15.8	- 0.8
E2 P4	24.3	7.6	14.2	21.8	+ 2.5

PERIOD B

E2 P1	14.2	5.2	10.1	15.3	- 1.1
E2 P4	24.3	7.4	16.3	23.7	+ 0.6

MEANS OF PERIODS A AND B

E2 P1	14.6	6.0	9.6	15.6	- 1.0
E2 P4	24.3	7.5	15.3	22.8	+ 1.5

[1] = grams of nitrogen

IV. DISCUSSION

Wool growth results indicate that for the levels fed, there was no significant interaction between protein and energy. Results also indicate that between-group wool-growth responses to various levels of protein intake were at no time significantly different.

The slightly higher wool growth rate observed for the P3 groups when compared with the P4 groups may be due to more efficient microbial utilization of "additional" protein when fed as lucerne versus when fed as casein ("additional" meaning that amount of protein by which a diet exceeds the supply necessary for efficient microbial growth and reproduction). More efficient microbial utilization might be attributable to a slower rate of degradation. If the protein fraction of lucerne meal was more resistant than casein to microbial degradation, the higher wool growth rate of the P3 group may also be due to relatively larger amounts of dietary protein reaching the abomasum.

The highly significant between-energy level differences in wool growth and body weight were expected as the E2 diets provided significantly greater amounts of carbohydrate and protein.

Period B body weight results indicated the presence of a slight interaction between energy levels for protein levels 1 and 2. However, feed intake data suggest that between-group variation in voluntary intake was the major factor producing the interaction.

The significant difference in body weight observed between protein levels in period A also appears to be due to between-group feed intake variation and not due to treatment.

Nitrogen balance results demonstrate that the protein of the P4 diet was not efficiently utilized and that P4 urinary nitrogen losses were proportionally higher than losses for the P1 diet. It might therefore be assumed that a substantial amount of the "additional" protein provided by the P4 diet, after undergoing rumen microbial degradation to ammonia, was not converted into microbial protein but was lost through rumen-wall absorption.

The results of this experiment indicate that wool growth in the Romney responds to various energy and protein levels in a manner similar to that observed for other breeds. The most notable feature of these responses being that dietary crude protein levels much greater than 12% (P3 and P4) were not efficiently utilized for wool growth when compared with lower protein level diets (P1 and P2).

CHAPTER 5

GENERAL DISCUSSION AND SUMMARY

I. GENERAL DISCUSSION

Until recently the relative roles of dietary energy and protein in controlling wool growth were not clearly understood. Some of the early work; Fraser and Roberts (1933), Slen and Whiting (1952), Ferguson (1959) indicates that when isocaloric diets are fed, wool growth remains independent of dietary protein level over a relatively wide range. On the basis of these observations Ferguson (1959) suggested that wool growth responses to increasing intake (when the diet contains more than about 8% CP) were wholly due to an increased energy supply. This interpretation fails to consider the importance of increased microbial protein synthesis resulting from increased intake.

The work of Reis and Schinckel (1961, 1963, 1964) has shown that the quantity of amino acids (particularly the S-amino acids) absorbed into the circulatory system is probably the major factor limiting wool growth. It has also been shown (Hungate, 1965, 1966) that the protein of rumen microorganisms is probably the principal supplier of these amino acids. Thus, the quantity of microorganisms made available to the host is suggested as being the major determinant of wool growth rate.

The main role of dietary energy and protein in controlling wool growth rate is suggested as being that of providing substrate for microbial growth, the cytoplasmic protein of the microbes being subsequently utilized by the host as an amino acid source. However, some dietary protein may escape rumen action and be used directly by the host (McDonald, 1954), the solubility of "availability" of the protein being a deciding factor as to the amount that escapes rumen action (Barnett and Reid, 1961).

From the results of the second experiment in the present work plus the observations of Ferguson (1959) and Slen and Whiting (1952) one might

contend that for most feedstuffs, dietary crude protein levels much greater than 12% (dry matter basis) are not efficiently converted into microbial protein, much of the excess protein being lost through the rumen wall as ammonia (McDonald, 1952). With microbial production not significantly increased and large protein losses occurring, high levels of dietary crude protein could not be expected to increase wool growth rate. This contention is supported by the observation of Hogan and Weston (1967) that the amount of non-ammonia nitrogen absorbed from the intestines is similar for isocaloric diets varying significantly in protein content. However, the results of the first experiment in the present work appear to be contradictory in that significant wool growth increases were observed in sheep that received a diet which was increased from 14.6 to 20.0% CP. No entirely satisfactory explanation for this discrepancy is apparent. In explaining the results of the first experiment it was suggested that the barley meal fraction of the ration provided a readily available source of energy which enabled the rumen microbes to efficiently utilize the 20% CP diet. In the second experiment using rations which also contained barley meal supplements, an increase in dietary crude protein from 12.8 to 17.2% (rations E1 P4 and E2 P4) produced no significant increase in wool growth rate. It is possible that differing protein demands by the two classes of sheep used in these experiments (aged versus hogget wethers) might account for the differences between experiments in wool growth response, however, no direct evidence to support this suggestion is available.

When dietary crude protein levels are considerably less than 12% an endogenous supply of nitrogen from the saliva (Somers, 1958) and rediffused blood urea (Houpt, 1959) is used extensively by the microbes (Cocimano and Leng, 1966). This mechanism ensures microbial growth when dietary nitrogen supplies are low and thus indirectly serves as a buffer against changes in wool growth rate.

The relation between dietary crude protein level and wool growth rate appears to be complexly inter-related with a number of associate factors such as: the quantity and availability of the energy supply, the quality and availability of the protein source, the supply of recycled nitrogen and possibly the class of animal being fed. Thus, generalization in regards to this relationship should be used with caution.

In addition to wool growth rate the amount of protein absorbed by the sheep also affects the rate and degree of body weight change. Small, positive body weight responses following abomasal protein or amino acid supplementation have been observed in mature sheep by Reis and Schinckel (1963, 1964) and Reis (1967), and following formalin-treated casein supplementation in the present work. Protein or amino acid effects on body weight are likely to be more marked in young growing animals whose protein demands are relatively high.

The results of experiments where proteins or amino acids were made directly available to the sheep suggest a number of areas where "direct" protein or amino acid supplements may prove useful (direct, meaning being made available to the host animal without subjection to rumen microorganisms).

Direct S-amino acid supplements would probably be effective in preventing tenderness or break in wool, as evidenced by the work of Reis and Schinckel (1963, 1964) and Dryden (1968). The problems to be overcome are: (1) determining and developing the most suitable method(s) of administration, (2) determining the dosage level necessary to prevent tenderness or break, (3) determining the required frequency of dosage at that desired level, and (4) determining the degree and extent of any secondary effects associated with amino acid supplementation such as increased fetal size.

Direct protein or amino acid supplementation may also be useful as

a stimulant to ovulation rate. Ovulation rate in the sheep is known to be influenced by gonadotropins (Averill, 1958). Gonadotropins are mainly composed of proteins or polypeptides (Zarrow, 1962). Thus, an increase in protein nutrition prior to ovulation might influence gonadotropin levels and subsequently ovulation rate. If an increase in ovulation rate was demonstrated as being associated with an increase in protein nutrition the possibility of direct protein supplementation as a flushing technique would become feasible.

Direct protein supplementation given via the feed to young cattle or sheep might significantly improve rate of gain and proportion of muscle to fat. In young animals growth primarily involves an increase in the structural tissues and the organs (Maynard and Loosli, 1962). The demands for such growth being met mainly by minerals such as calcium and by protein (ibid). Because the young growing animal has a high demand for protein a direct protein supplement might be expected to fill part of a demand not usually met by normal dietary channels.

II. SUMMARY

Given the experimental conditions reported, the present work has shown that:

(1) (On the basis of *in vitro* ammonia and plasma free amino acid level tests) formalin treatment can be an effective means of protecting protein from microbial degradation in the rumen.

(2) Formalin-treated casein supplements are capable of producing significant wool growth responses and possibly body weight responses.

(3) As treated, 1 g. daily MHA supplements were not effective in producing wool growth or body weight responses.

(4) Untreated casein supplements are capable of producing significant wool growth responses.

(5) The technique used to measure plasma free amino acid concentrations was highly accurate and could justifiably be used in experiments where group numbers are small.

(6) The concentration of an unidentified plasma free amino acid responds to formalin-treated casein supplements in a highly significant and positive manner.

(7) A significant protein-energy level interaction does not appear to exist for wool growth.

(8) The New Zealand Romney appears to respond to different levels of protein nutrition in a manner similar to that observed for other breeds.

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