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PROTEIN SUPPLEMENTATION AND WOOL GROWTH

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SUMMARY

A brief review is given of utilization of protein by ruminants and in particular of known means of providing for absorption of increased quantities or kinds of amino acids. A summary is given of three experiments concerned with feeding a supplement of formalin-treated casein to Romney and Corriedale ewes. Nitrogen retention was greatest when casein was formalin-treated but wool growth results were variable and no clear pattern of behaviour was evident.

GENERAL INTRODUCTION

CONSIDERABLE EFFORT has been directed towards a better understanding of the digestion and utilization of proteins by sheep. Apart from the academic challenge provided by investigation of a highly complex situation, many aspects of the overall problem have intense practical significance.

The most obvious interest lies in provision of the eighteen amino acids of wool protein (Alexander and Hudson, 1954). The composition of wool is reasonably constant and the inference must be that if one or more of these amino acids are in short supply then wool growth may be limited. Amino acids are, of course, required for ordinary body processes and there is undoubted complexity in this requirement. In general, the provision of animal feeding stuffs with moderate to high protein content requires considerable effort and expense and it is imperative that this protein be used with economy.

The physiology of digestion and absorption in the ruminant has been discussed by McDonald (1969). The processes, while undoubtedly complex, have several unique features that can be simply stated. The rumen, which in itself does not produce any digestive enzymes, contains a vast population of bacteria and protozoa capable of fermenting carbohydrates and breaking down proteins to constituent amino acids and then deaminating these.

The end product of carbohydrate fermentation is volatile fatty acids and McDonald draws attention to the fact

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that, while those resulting from fermentation of cellulose and related substances are a net gain to the host, the fermentation of starches and sugars by organisms in the rumen causes some loss to the animal because these same substances can be digested and absorbed more efficiently by the small intestine. The fate of protein materials is complex and, depending on the state of nutrition of the animal, the activities of micro-organisms in the rumen may provide either a gain or loss of protein to the animal.

The main source of nitrogenous materials in the usual animal feeding stuffs is protein, but high quantities of amino acids and amides may be consumed when animals are grazing young, rapidly growing plants. In the rumen, proteins are subject to the proteolytic action of bacteria and the resulting amino acids are rapidly deaminated, the microbial deaminase activity being strongly influenced by the nitrogen content of the diet. Soluble proteins such as casein are more readily attacked than those less soluble, for example, maize protein. The chief products of deamination are ammonia, volatile fatty acids, and carbon dioxide. Thus, nitrogenous material in the diet is subject to powerful breakdown in the rumen and only a portion of the more insoluble protein will pass undegraded into the animal's own digestive tract and be subject to proteolytic activity to provide amino acids for direct absorption.

Ammonia produced in the rumen by proteolytic and deaminase activity may be disposed of in two ways. Some is immediately used by micro-organisms in providing for their own bodily protein which eventually passes to the duodenum and intestine and is subject to the ordinary processes of digestion. Utilization of nitrogen by this route is highest when the diet contains high amounts of readily fermentable carbohydrate which provides for high populations and activity of rumen micro-organisms and when proteins in the diet are moderately insoluble. If ammonia production is rapid, as it is when protein content of the diet is high and the proteins are highly soluble, excess ammonia is rapidly absorbed into the portal blood stream and conveyed to the liver where it is rapidly converted to urea. Some of this urea may be recirculated to the rumen by two routes, by passage through the rumen wall from the portal blood system and via the saliva. In any event, urea entering the rumen is rapidly degraded owing to high ureolytic activity for which bacteria and not protozoa are responsible. Thus there exists a diffusion of ammonia and urea in opposite directions, and because of swift conversion, into areas of negligible concentrations.

When protein content of the diet is high and in excess of requirements for maintenance, growth and productive processes, excess of nitrogen is converted chiefly to urea, blood urea concentration is high, there is a high ratio of urea nitrogen to total nitrogen in urine, renal excretion is rapid, and there is considerable loss of nitrogen. These events take place in a cycle removed from that of conventional digestion and absorption.

If protein content of the diet is low, it is possible for the digesta entering the intestine to have a higher nitrogen content than the food. In such circumstances, the excess of energy producing carbohydrates ensures that ammonia produced in the rumen from nitrogenous food materials, and from urea entering the rumen by diffusion and in saliva, is rapidly incorporated into microbial protein. At the same time, there is a marked fall in blood urea concentration, reduced urinary excretion of urea, and a considerable fall in the ratio of urea to total nitrogen in urine. In these circumstances, the percentage nitrogen in the digesta entering the absorptive regions of the stomach will be higher than that in the diet. One important side issue in this situation is that the improved nitrogen status due to blood and salivary urea enhances the utilization of roughages and may even lead to increased intake.

It is obvious that reserves of urea must eventually be utilized and that any situation in which there is a net gain to the animal must be transitory. However, despite this, the nitrogen cycle of ruminants is a remarkably adaptable system which provides for considerable economy if dietary nitrogen intakes are low. At the same time, it is clear that on high protein diets wastage can be high. It will be highest when diets contain the more highly soluble proteins and carbohydrates of low availability either because of quantity or slow digestibility.

The experimental work which led to firm establishment of the above principles was significantly affected by the observation by McDonald (1948, 1952) that considerable ammonia production occurred in the rumen, that the addition of starch to the diet caused a decline in ammonia concentration; further, that ammonia production was greater during ruminal infusion of casein than it was when zein, which is relatively resistant to proteolysis, was fed. These principles were substantiated by the work of Chalmers *et al.* (1954) who further noted that casein, if administered by duodenal fistula, was better utilized, and that heat treatment of the casein caused it to be less soluble and led to lower ammonia production.

Further information on behaviour of different proteins was provided by Annison *et al.* (1954) and Chalmers and Synge (1954). The latter authors made what might now be considered a quite significant observation. They noted that treatment of herring meal with formalin had no deleterious effect on palatability or utilization and their work indicated that growth rate of sheep fed a supplement of formalin-treated herring meal was greater than that of sheep fed supplements of ordinary herring meal or casein. It appears that the use of formalin was motivated chiefly by a necessity to stop deterioration of herring before processing.

An important observation by Chalmers and Synge (1954) was that in their opinion it seemed "impossible to assign to individual protein feeds biological values that have any practical significance over a wide range of diets". They drew attention to the fact that any component of the diet could be operative which controlled either the development of a deaminating flora or availability of substrates capable of being deaminated.

A further difficulty was emphasized by Ferguson (1959) who measured wool growth after feeding approximately isocaloric rations but in which the percentage protein varied from 7.5% to 29%. There were no significant differences in wool growth due to percentage protein intake during *ad lib.* feeding or feeding at approximately a maintenance level, but greater wool growth during *ad lib.* feeding prompted Ferguson to propose that energy intake was the most significant factor when diets containing more than 8% crude protein were fed. Subsequently, Hogan and Weston (1967) showed, using a high protein and a low protein diet similar to two of those used by Ferguson (19.8% and 7.8% crude protein), that the amount of nitrogen leaving the abomasum was approximately the same for both diets. There was considerable loss of nitrogen as ammonia on the high protein diet and a net gain of nitrogen on the low plane diet.

Recently, Colebrook *et al.* (1968) have shown an approximately linear relationship between the percentage protein in the diet and wool growth rate. In this work the various protein concentrates were fed as a 50:50 ration with lucerne hay and, in the case of whale oil meal, in varying ratios to lucerne hay.

If wool growth rate is to be used as a measure of utilization of proteins, it is not enough to attempt to equate this with either the amount of protein fed in the ration (Ferguson, 1959; Colebrook *et al.*, 1968) or perhaps the amount of nitrogen passing the duodenum or the balance between nitrogen and digestible organic matter (Hogan and Weston, 1967), but attention must be given to possible differences between proteins in their capacity to provide, via the synthetic activity of rumen micro-organisms, the assemblage and proper proportions of amino acids essential for wool growth.

Downes (1961) has proposed that the amino acids regarded as "essential" for the growing rat are substantially the same as those required by sheep. Of the ten, arginine, "essential" for the rat, is evidently not necessary for sheep. In the study by Downes, cystine and tryptophane were not measured. The sheep receives its "essential" amino acids from two sources, amino acids liberated by digestion after the material has escaped deamination in the rumen, and from the digestion of micro-organisms.

Apart from the amino acids regarded as "essential" for bodily function, there is the possibility that others may be necessary for optimum wool growth. In wool there is a high content of sulphur-containing amino acids and it has been proposed that cystine, the chief sulphur amino acid, was most likely to be a limiting factor if indeed wool growth was restricted by an imbalance of amino acids. There is now ample evidence that wool growth rate is enhanced and sulphur content of wool increased if sheep on moderate dietary intakes are given supplements of the sulphur amino acids in such a way that they are not subject to deamination in the rumen. For example, Marston (1935) reported increased wool growth after subcutaneous injection of cystine hydrochloride, and in a series of experiments Reis and Schinckel (1963, 1964) and Reis (1965) clearly established that administration of cystine or methionine via the abomasum or duodenum will bring about substantial increases in wool growth and sulphur content of wool far in excess of those following administration of similar amounts of amino acid supplements in the form of gelatin glycine and *l*-glutamic acid.

Apart from work done with the sulphur amino acids and in some cases allied with it, a good deal of work has been done in the general field of supplementary protein feeding. This work has been reported by Reis and Schinckel (1961, 1963, 1964), Ferguson *et al.* (1967), Weston (1967), Little and Mitchell (1967), Reis (1969), and Reis and Tunks (1969). Enhancement of wool growth has been the main target of these studies and in general the basic ration has been a modest one, generally a mixture of lucerne and wheaten hay given at a maintenance or near maintenance level. For the most part, protein supplementation has been achieved by infusing solutions containing casein into the abomasum or duodeum. Alternatively, casein has been treated with formalin and fed in the diet. Treatment with formalin as described by Ferguson *et al.* (1967) causes the casein to be virtually insoluble in the rumen (pH approximately 6.0), and thus unavailable to micro-organisms, and to be soluble in the abomasum (pH below 3.0) and thus available for digestion and absorbtion.

Supplementation of moderate basic diets with casein, either formalin-treated or by duodenal or abomasal infusion, has led to higher nitrogen retention, greatly increased wool growth, and high efficiency of conversion of dietary protein to wool. In general, approximately 40 to 50% of the increased wool growth is due to greater fibre diameter, and sulphur content of wool grown during supplementation is increased. It has been noted that individual response to protein supplementation is variable but that the response of individual sheep is repeatable. Body weight shows a small and consistent rise during supplementation.

Reis (1969) has observed that similar maximum wool growth can be reached in various ways. As would be expected, the magnitude of the effect of protein supplementation depends on the level of wool growth reached on the basal diet, but it is notable that the maximum can be reached by appropriate supplementation of a low basal diet (400 g/day of 50:50 chopped lucerne and wheaten hay + 200 g/day abomasal casein + 3 g/day *DL*-methionine). The second point of note is that two sets of wool growth measurements were available for some of the sheep used, those while the sheep were pasture-fed and those after the sheep had been used in work on supplementary protein feeding. For the three sheep so observed, in no case did wool growth rate on supplementary feeding exceed 90% of maximum wool growth on pasture. It is pointed out, too, that response to supplementary feeding might be less in breeds of sheep with a marked seasonal rhythm of wool growth (Doney, 1966) and in inherently low producers of any breed.

The reservations expressed by Reis must inevitably influence choice of ways by which we might influence sheep or wool production by exploiting our knowledge of supplementary protein feeding. There are many possibilities, especially in a system of intensive sheep farming based on pasture feeding with its attendant periods of plenty and shortage, and using as our chief breed one in which wool growth is rather sensitive to nutritional changes. The higher nitrogen retention which accompanies appropriate supplementary feeding suggests that there may be advantages in forms of production other than wool growth and these deserve investigation.

EXPERIMENTAL

Three trials concerned with feeding of both ordinary and protected casein were conducted. The type of sheep used, the basic ration and the experimental design varied.

However, a number of procedures were common. For example, all sheep were weighed weekly, kept in individual pens, fed once daily and had constant access to water. Wool production was measured by periodic clipping of a midside patch of approximately 100 sq. cm. Wool was cleaned by extraction with petroleum ether followed by rinsing in warm water and unless otherwise stated the weight is given as dry conditoned weight (16% regain). Fibre diameter was determined by measurement of 250 fibres per sample by projection microscope and fibre length by measurement of approximately 100 fibres per sample by a technique whereby the fibre was mounted on tape and the projected image measured at a magnification of 10 ×.

EXPERIMENT 1

GENERAL DESIGN

The purpose of the experiment was to investigate the effectiveness of feeding formalin-treated casein to 5½-year-old Romney ewes at two levels superimposed on two levels of basic feeding (Table 1).

The work extended over five periods each of 28 days (May 1 to September 17, 1968). Prior to this, sheep, which had been chosen for similarity of body condition, had been penned for 2 months and settled on to a maintenance ration. Of the five periods for which wool growth was measured, Period 1 was considered to be pre-experimental (third month of maintenance feeding).

Despite the intention to use non-pregnant sheep, 8 pregnant ewes were subsequently found to be distributed among the groups; the lambs were removed within 48 hours of birth. Three sheep died during the experiment,

TABLE 1: BASIC RATIONS AND SUPPLEMENTS FED IN EXPERIMENT 1 AND CRUDE PROTEIN INTAKES OF SHEEP ($n = 5$)

0.5 Maintenance Ration 159 g chaffed meadow hay + 272 g whole oats/day			*1.0 Maintenance Ration* 318 g chaffed meadow hay + 545 g whole oats/day		
Group	*Daily Supplement Casein*	*Daily Intake Crude Protein* (g)	*Group*	*Daily Supplement Casein*	*Daily Intake Crude Protein* (g)
1	30 g untreated	75	5	30 g untreated	124
2	60 g untreated	101	6	60 g untreated	150
3	30 g treated	74	7	30 g treated	123
4	60 g treated	99	8	60 g treated	148

but all others remained healthy and there was no refusal of food.

RESULTS

(1) *Liveweight*

Because of complications due to pregnancy, it is difficult to judge liveweight changes accurately. However, it was clear that sheep on a basic maintenance ration remained at substantially the same weight throughout, while the weight of those on 0.5 maintenance declined by approximately 6 kilograms.

(2) *Wool Growth*

The data were examined using covariance analysis and allowance was made for missing values, the effects of pregnancy, and initial differences in wool production between groups as determined in Period 1. Figures 1, 2 and 3 describe treatment effects in terms of the difference produced by each separate treatment. It is clear that treatment of casein was responsible for a major effect on rate of wool production and that this was achieved largely by increasing fibre diameter of the sheep fed formalin-treated casein. The effect of treated casein on fibre diameter increased as the experiment progressed, but its effect on length remained relatively stable after initiation of a highly significant difference at the beginning of treatment. Doubling the level of casein fed was responsible for a small but consistent difference in wool growth which was of variable significance.

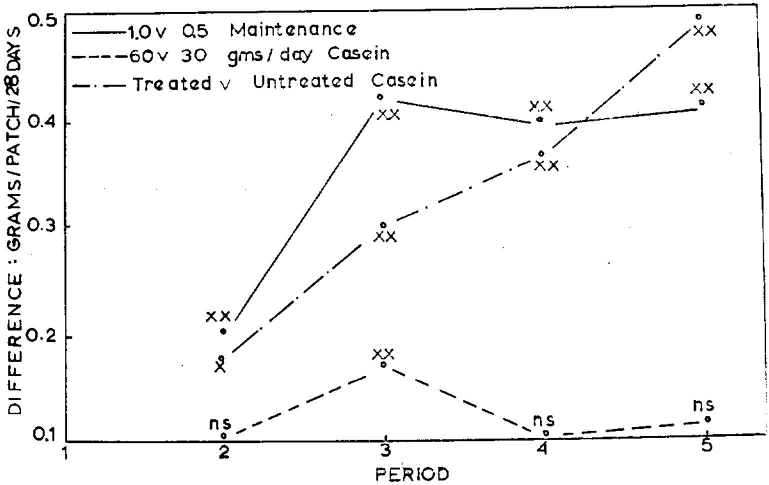

FIG. 1: *Wool weight responses — Experiment 1.*
n.s. = Difference not significant; × *P < 0.05;* ×× *P < 0.01.*

Level of basic ration is shown to be of considerable importance and this is to be expected. In this experiment, the effects due to level of basic ration could conceivably be confounded with liveweight in that at the start of the experiment the sheep allocated to a basic ration of 0.5 maintenance were on average 5.8 kg lighter than those allo-

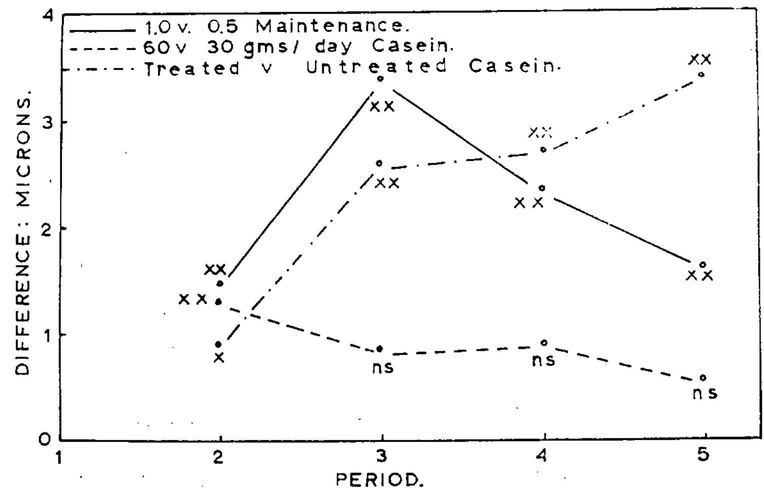

FIG. 2: *Fibre diameter responses — Experiment 1.*
n.s. = not significant; × *= P < 0.05;* ×× *= P < 0.01.*

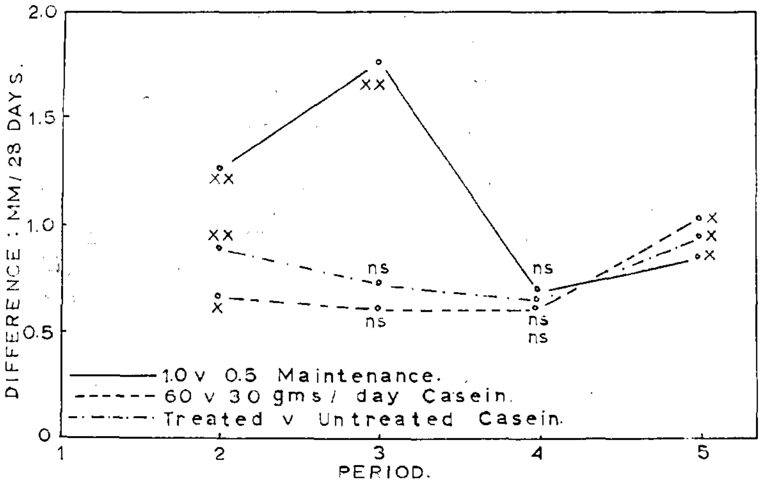

FIG. 3: *Fibre length responses — Experiment 1.*
n.s. = not significant; $\times = P < 0.05$; $\times\times = P < 0.01$.

cated to a basic maintenance ration. There is ample evidence of a positive correlation between total fleece weight and body-weight (Henderson, 1953) but there is negligible association between body-weight and wool growth per unit area (Morley *et al.*, 1955). In the present experiment wool growth measurement is based on a specific area and, taking note of the fact that errors associated with the size of the sample patch tend to cancel, measurement is technically based on unit area production. A covariance analysis which included initial liveweight showed no significant association between liveweight and patch wool production after allowance had been made for treatment and pregnancy effects, and it is concluded that confounding, if present at all, is not important. It will be noted that differences in level of basic ration produced an immediate and substantial difference in wool production, which increased over two periods and then remained at a stable high level.

Of 48 possible first and second order interactions, five were significant and three, all second order, were highly significant. At this point it is difficult to attach clear importance to these interactions.

EXPERIMENT 2

Young, non-pregnant Romney ewes having a unit area wool production approximately twice that of the aged ewes used in the first experiment, were fed formalin-treated casein at what were considered to be strategic times. The basic ration, different from that fed in Experiment 1, was a pelleted proprietary mixture containing 14 to 16% crude protein and casein was incorporated at the rate of 50 g per pound of mixture. The second difference from Experiment 1 was that, because of a change of method during formalin treatment of casein, only 30% was "protected" by the formalin treatment.

The trial covered a 28-day pre-experimental period (May 1 to 28, 1969) and four 28-day experimental periods (May 29 to September 18). The plan of the experiment is given in Table 2.

TABLE 2: DESIGN OF EXPERIMENT 2 AND CRUDE PROTEIN INTAKES OF SHEEP
($n = 5$)

Group	Treatment: Period	Treatment: Daily Feed	Daily Intake Crude Protein (g)
1	1, 2, 3, 4	817 g nuts	123
2	1, 2, 3, 4	817 g nuts containing 90 g untreated casein	199
3	1, 2, 3, 4	817 g nuts containing 90 g treated casein	199
4	1, 2	409 g nuts + 409 g nuts containing 45 g treated casein	155
	3, 4	817 g nuts	123
5	1	817 g nuts containing 90 g treated casein	199
	2, 3, 4	817 g nuts	123
6	1	817 g nuts	123
	2	817 g nuts containing 90 g treated casein	199
	3, 4	817 g nuts	123

RESULTS

(1) *Nitrogen Retention*

At the conclusion of the trial, six of the trial sheep were used in a nitrogen retention study using the diets containing untreated casein (Group 2) and 30% protected casein (Group 3). Results of this study are given in Table 3.

It will be noted that nitrogen retention was greater when "partially protected" casein was fed.

TABLE 3: NITROGEN RETENTION BY ROMNEY EWES FROM EXPERIMENT 2

Daily Feed	*N* (%)	*Digestibility* (*DM* %)	*N Intake* (*g/day*)	*N Retention* (*g/day*)	%
800 g nuts containing 88 g unprotected casein	3.81	72.3	27.06	4.28	15.8
800 g nuts containing 88 g of 30% protected casein	4.03	71.7	28.36	5.06	17.8

(2) *Liveweight*

Liveweight change was similar for all groups and after allowing for cumulative increase in fleece weight the average gain per sheep during the experiment was approximately 2 kg. The inference is that all groups of sheep were constantly in positive energy balance.

(3) *Wool Growth*

Patch wool production of Groups 1, 2 and 3 are shown in Fig. 4. Although there is a suggestion that supplementary protein feeding did cause differences in wool production, these were not significant.

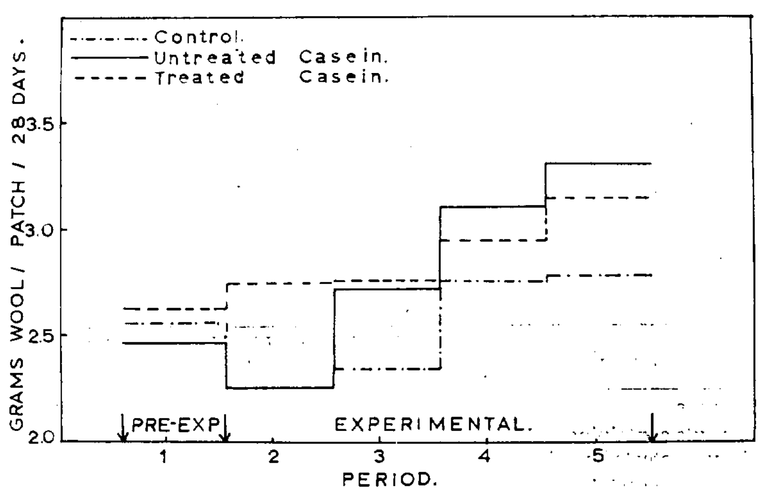

FIG. 4: *Wool growth responses — Experiment 2.*

EXPERIMENT 3

This experiment was designed to investigate the effect of feeding formalin-treated casein during pregnancy and lactation in 1½-year-old Corriedale ewes (three groups, $n = 6$). One group was non-pregnant, two were pregnant, and each pregnant ewe subsequently reared a single lamb. Selection of groups was based on mean lambing date and estimated efficiency of wool production, the aim being to have sheep lamb over a short period and to have groups with approximately equal potential for wool production.

At the time treatment began, ewes of two groups had been pregnant for approximately 28 days so that the experiment covered the last four months of pregnancy, two months of lactation, and one month post-lactation.

The basic ration was the same proprietary pellet mixture used in Exp. 2. Casein was incorporated in the mixture during pelleting and was fed at the rate of 45 g/day throughout.

RESULTS

(1) *Nitrogen Retention*

The results of nitrogen retention studies on each of the diets used are given in Table 4. It is clear that the percentage of nitrogen retained is greater when a supplement of casein is fed and greatest when the casein has been formalin-treated. It is interesting to note that the percentage of nitrogen retained was much higher with Corriedale ewes than it was with Romney ewes. The breeds were of comparable age and history and the untreated casein diet differed only in the amount of casein.

(2) *Liveweight*

Pregnancy and lactation induced a difference in liveweight of approximately 10 kg between the two groups

TABLE 4: NITROGEN RETENTION BY CORRIEDALE EWES

Daily Feed	*N* (%)	*Digestibility* (*DM* %)	*N Intake* (*g/day*)	*N Retention* (*g/day*)	%
800 g nuts	2.70	69.6	19.06	4.02	21.10
800 g nuts containing 45 g unprotected casein	3.31	70.1	23.62	6.20	26.24
800 g nuts containing 45 g protected casein	3.25	70.1	23.01	6.30	27.37

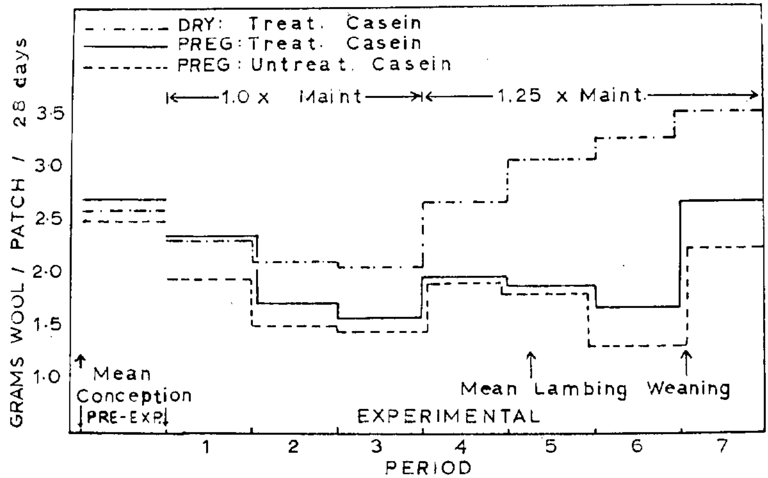

FIG. 5: *Mean wool growth for groups of sheep in Experiment 3.*

supplemented with treated casein. However, there was no apparent change in the relative weights of both pregnant groups, although both gained slightly in effective body-weight during pregnancy and lost weight rapidly during lactation.

(3) *Wool Growth*

Wool production of the groups is shown in Fig. 5. As would be expected, pregnancy and lactation were responsible for a difference in wool production to the extent that wool production rate of ewes in the second month of lactation was less than half that of dry ewes receiving the same ration. It will be noted that a difference was established quite early in pregnancy.

Despite higher nitrogen retention when formalin-treated casein was fed, there was no apparent response during the course of the experiment until the last month of lactation and this continued after weaning. The significance of this difference has not yet been tested but it is in effect similar to that reported by Barry (1969).

Mean fibre diameters of the three groups are shown in Fig. 6 and the differences produced as a result of treatment are consistent with those for wool growth rate shown in Fig. 5. It is clear that formalin-treated casein was not responsible for any particular effect.

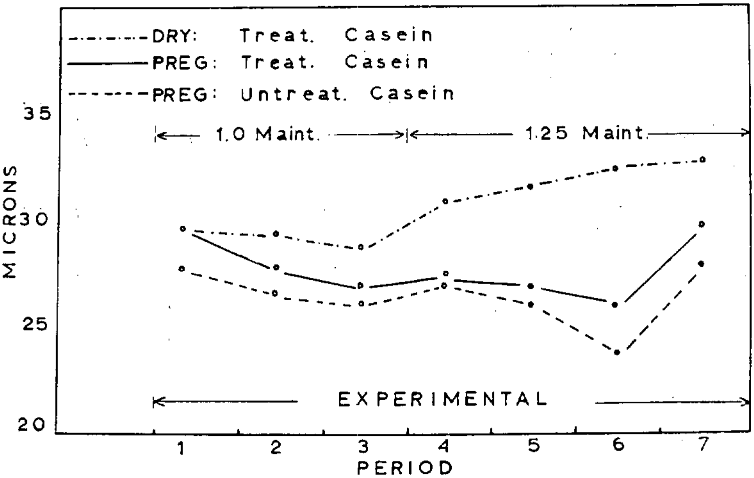

FIG. 6: *Mean fibre diameter for groups of sheep in Experiment 3.*

CONCLUSION

The present series of experiments indicate that response to supplementary protein feeding is variable. It would appear that the response to added protein in the ration, or to formalin-treatment of the protein, depends on the prior establishment of certain conditions in the animal and, at this stage, these are by no means clear. From these experiments it might be suggested that type and amount of base ration are important and deserve further intensive study. The nitrogen retention work indicates that breed of sheep could be an important source of variability in response and over all experiments some considerable variability in individual response has been noted. Individual response is likely to be different because of inherent differences in efficiency of wool production, and perhaps the degree to which overall efficiency has declined because of aging.

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