STUDIES IN RUMINANT NUTRITION WITH PARTICULAR REFERENCE TO NON-PROTEIN-NITROGEN UTILIZATION.

Summary of a Thesis presented for the degree of
Doctor of Philosophy of the University of Glasgow

by James Joseph Parkins, B.Sc.

Section 1 of this thesis gives a review of the use of urea as a non-protein-nitrogen source in ruminant nutrition together with a description of the major biochemical reactions in the ruminant associated with an increas in the dietary intake of urea. The potential toxicity of urea is discussed.

In Section 2 the analytical methods available for the determination of ammonia in biological tissues (blood, rumen fluid and milk) are reviewed and the analytical techniques used have been described and evaluated. In particular, a modified technique involving the use of an ion-exchange resin was found to be the best procedure for the analysis of large numbers of blood samples.

Section 3 describes the formulation and manufacture of a molassed sugar beet pulp nut containing added urea, phosphate, trace elements and vitamins and containing about 17% crude protein and about 0.5% P considered suitable for a wide range of purposes in ruminant feeding. A series of experiments indicated that the rate of release of urea in the rumen from such a product was slower and caused a reduced degree of ammonia production than was the case for a barley nut containing an equivalent amount of urea.

Section 4 describes the use of this supplemented sugar beet product as a major component of the milk production concentrate fed to dairy cows. In four of five trials where the material was used in 25 - 50% substitution of a

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barley/vegetable protein mixture entirely comparable yields of milk of similar composition were obtained. The product was shown to be completely non-toxic and was well accepted by cattle.

In Section 5 a series of experiments describe the results of trials where the supplemented sugar beet pulp formed 50% of an all-concentrate diet fed to growing cattle. When compared with the more normal 85% cereals + 15% protein supplement type of diet virtually identical daily liveweight gains were recorded but there was a reduction of some 5% in food conversion efficie

In Section 6 three experiments with housed ewes are described. Inclusi of urea in the sugar beet nut was shown to give improved and fully satisfacto liveweight gains in ewes during pregnancy, fully adequate birthweights in lan and satisfactory liveweight gains. Balance trials were conducted to assess the extent of utilization of dietary urea in late pregnancy. The performance of the ewes and their lambs was shown to be related to the changes in plasma urea and free fatty acid concentrations recorded during the period of the tri

Section 7 describes a number of investigations concerning experimentall induced urea toxicity in sheep. Starvation was found to have an important effect on increasing the potential toxicity hazard. Investigations were conducted to assess the effects of certain combinations of amino acids when administered intravenously for both the protection and alleviation of urea toxicity in sheep. When the liver function of sheep was progressively impair by oral administration of either carbon tetrachloride or copper sulphate over a long period, the sheep were demonstrated to be increasingly susceptible to urea toxicity.

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by

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CONTENTS

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SECTION I.

A review of the literature on the use of urea in animal feedingstuffs together with a consideration of the major biochemical reactions concerned with the metabolism and toxicity of urea in the ruminant animal.

a). Introduction.

b). Principal areas of use of urea.

c). Economic value of urea in practical rations.

d). Historic background.

e). Digestion and absorption of nitrogenous compounds in the ruminant.

f). The 'Adaptation response' to urea feeding.

g). The nature and source of blood ammonia and its toxic effects.

Introduction

Several exhaustive reviews and bibliographical collections have been published recently on the feeding of urea to ruminants (e.g. Goss, I942-3; McNaught & Smith I947-8; Reid, I953; Reid & Bentley, I955; Stangel, I964; Loosli & McDonald, I967; Conrad & Hibbs, I968; Waldo, I967; C.A.B., I968; Briggs, I967; Helmer & Bartley, I97I). Each of these contains references to several hundred publications and the literature is continually expanding.

It is the intention of this thesis to review only those parts of the literature which bear more directly on the work to be undertaken and its subsequent interpretation. More detailed literature reviews involving specific aspects of use of urea feeding are included in the introductions to the appropriate experimental sections.

In the introduction a brief consideration of some economic aspects of urea feeding has been undertaken to demonstrate some of the cost advantages existing at present. This is the clear incentive for the continuing rapid development in the use of urea.

Ruminant animals can utilize non-protein nitrogen compounds by the means of the activities of appropriate rumen microorganisms. Ammonia, the product of non-protein nitrogen digestion in the rumen, can be incorporated into microbial protein. This microbial protein is subsequently available to the ruminant as a dietary source of protein. These events were partly appreciated as long ago as I880 in Germany by Weiske and his co-workers. (Henderickx I967)

Urea, which can be produced relatively cheaply in quantity, is the only non-protein nitrogen compound which has been used to date on a large scale in ruminant feeding. Hodges, Thigpen, Mahan, Baumann, Davis, Ross, Clough & Poats, (I965) recorded that I3.6% of all protein in high protein feeds for ruminants in the U.S.A. was derived from urea.

In I964 consumption of urea in animal feeds in the United

Kingdom was in the order of 1,800 tons. (Stangel, 1967). Published statistics on the use of urea in animal feeds are difficult to obtain but it would appear that the consumption in the U.K. is now (1971) at least 15-16,000 tons. per year.

Recent published estimates of feed grade urea usage (British Sulphur Corporation Ltd., 1969) give the following rates of consumption (1000 tons/annum); U.S.A., 190; U.S.S.R., 70; Great Britain, Canada, 16; South Africa, Poland 10; East Germany, 4; Australia, France, West Germany, Italy, Japan, 1-2. Total consumption is accordingly in excess of 300,000 tons per annum. This is probably a gross under-estimate as the United States Department of Agriculture in 1969 put the annual rate of use at 300,000 tons for the U.S.A. alone.

Dramatic changes in the acceptance of urea for feeding to all classes of ruminant livestock have occurred in recent years. Cooper (1967) pointed out that before the livestock producer could confidently accept urea and similar compounds as major protein sources, further experimental testing would be necessary under various farming conditions to realize the full potential of the available knowledge. The continual increase in the use of urea and other non-protein nitrogen sources in animal feeds indicates the increasing importance of these materials in modern animal husbandry methods and the acceptance by farmers of experimental findings.

Much of the recent emphasis on the use of non-protein nitrogen (NPN) compounds is concerned with increasing the efficiency of utilization of these materials in the ruminant. One of the main interests in this context has been the attempts to slow down the rate of dissolution of urea in the rumen so that more efficient utilization of the ammonia produced might occur. Experimental materials produced in this category include urea prills coated with wax and other 'protective' substances

designed merely to reduce the solubility rate of urea in the rumen. (e.g. Szabo, 1966 a.b.). In the U.S.A. gelatinised starch has been used as a protective matrix to reduce the solubility rate of the contained urea. This Product 'Starea' is now commercially available and was first described by Deyoe, Bartley, Pfost, Boren, Berry, Anstaett, Helmer, Stiles, Snug and Meyer (1968).

One of the main lines of work presented in this thesis is specifically concerned with the experimental work associated with a product of similar properties.

Urea (NH_2. CO. NH_2) theoretically contains 46% N. Typical analyses of commercially obtained urea show the range of nitrogen content to be between 42.0-45.0% (i.e. a crude protein of 262-281%). There is very little moisture content (0.6% maximum) and the ash content is between 5.0-8.0%. A conditioner may often be included in the material at a rate between 2-7%. The density is about 48lbs/cu.ft. and the maximum content of biuret is in the order of 1%.

Urea is manufactured by the reaction of carbon dioxide and ammonia under high temperature and pressure. It is available in solid form as prills, and as crystals, with an off-white to tan colour. It has a cool saline taste but no odour. It is very soluble in water (40 & 87g / 100g at 0 & 100° C) slightly soluble in ethyl alcohol and virtually insoluble in other common solvents. Urea is stable both in solution and in crystalline form at room temperature. Crystalline urea decomposes at its melting point (132°C) to form biuret and other related compounds. A saturated solution of urea does not decompose appreciably below 70°C when hydrolysis produces detectable quantities of carbon dioxide and ammonia. Urea is unlikely to react under normal manufacturing and storage conditions with the cereal products and oil-seed residues used in compound animal feedingstuffs. Soyabean meal is not used in urea - containing feeds since it contains the enzyme urease,

which will decompose urea. Heat-treated soya-bean meals, however, are inactive.

The use of urea in animal feedingstuffs is subject to the Fertiliser and Feedingstuffs Regulations, 1968. In the case of compound cakes and meals the regulations require, among other things, statements of (a) The amount of protein and (b) The protein equivalent of urea, if any, which is included in the total.

For this purpose the amount of protein means the amount of nitrogen including urea nitrogen (but not ammoniacal or nitrate nitrogen) multiplied by 6.25. The amount of protein equivalent of urea means the amount of urea nitrogen multiplied by 6.25. In the case of mixtures of molasses and urea the statutory statement must include the protein equivalent of urea.

In the rumen urea is rapidly hydrolysed by the enzyme urease to yield carbon dioxide and ammonia. The ammonia may be incorporated into microbial protein. Pearson & Smith (1943) showed the rate of urea hydrolysis to be independant of the ability of the microflora to utilize the ammonia produced. If unfavourable conditions for the utilization of this ammonia exist in the rumen, the ammonia concentration in the rumen liquor increases markedly and a potentially toxic situation may subsequently arise.

Pearson & Smith (1943) using an in-vitro technique, also demonstrated that the synthesis of protein from ammonia predominated only when starch or simple sugars were also present as a source of energy for the bacteria. Smith & Baker (1944) found the protein-containing material synthesized in-vitro to consist mainly of bacteria. The bacterial protein contained 36% crude protein and its nutritive value to the host ruminant is

high. (McNaught & Smith, 1947).

There have been numerous subsequent in-vitro and in-vivo experiments using rumen contents, together with nitrogen balance studies and extensive feeding trials. A review of this work is given by Fairbairn (1965).

Principal areas of use of urea

Urea is of particular advantage when it can be fed frequently in relatively small doses, (Lowe, 1967). This practice will minimise any possible toxic situations and enables the total daily intake to be increased above the level which could be effectively introduced in one or two feeds per day. Such ad-lib feeding systems are fairly common for fattening cattle especially in America. The protein concentrate products presently available in the U.K. and which are intended for use with several times their own weight of cereals generally contain between 5-10 % urea.

There have been many trials conducted with dairy cows designed to investigate the effect of the partial substitution of the crude protein in the production ration by urea on milk yield and composition. The results are conflicting (Helmer & Bartley, 1971). It has been recognised, however, that some inefficiency in the utilization of urea may occur in the higher yielding cows which are fed relatively large amounts of urea in only two feeds a day. (McNaught & Smith, 1947; Reid, 1953; Fairbairn, 1965). In investigating the extreme limit to which NPN might replace vegetable protein, Virtanen (1966) demonstrated that Ayrshire dairy cows maintained normal milk yields over three lactations when fed on diets based on cellulose, potato starch and sucrose, when almost all of the dietary nitrogen was supplied from urea. Under present day practical conditions the amount of urea commonly included in dairy rations is limited to about 1-2 % of a mainly cereal based production ration, so that the protein equivalent contributed by urea does not exceed one third

of the total protein of the concentrates (i.e. 3-6 % in 15-18 % crude protein).

The ability of calves to utilize urea has been reviewed by Reid (1953). Generally, calves are allowed to become fully ruminant before urea-containing rations are fed although Loosli and McCay (1943) showed that very young calves (2 weeks old) were able to utilize urea when fed a protein-deficient diet.

Fundamental studies on the utilization of urea nitrogen by growing lambs were made by Johnson, Hamilton, Mitchell & Robinson (1942). They domonstrated protein synthesis to be a function of bacterial metabolism and that protozoa apparently did not play an essential part in the process. Urea added to a low-protein diet to produce a ration with 12 % crude protein was digested as well as soya-bean meal and casein. However, further addition of urea gave no increase in nitrogen retention. Feeding costs are of particular inportance in systems of sheep intensification. The economics of the incorporation of urea into foods for sheep are of considerable interest.

Urea is also of potential value when suckler cows, dairy replacements and store beef cattle are fed on straw and poor quality hays. It has been shown by Campling, Freer & Balch (1962) that the voluntary intake of poor quality roughage can be greatly increased by urea. An apparent increase in the digestibility of the crude fibre and nitrogen-free-extract of the straw was achieved when 75-150 g. urea/day was supplied in solution to non-lactating cows by continuous intraruminal infusion. In the establishment of an efficient, practical feeding method the urea should be fed frequently and in small quantities.

Economic Value of Urea in Practical Diets.

Little work has been performed to date on the economic aspects of including urea in diets for ruminants as a major protein source. This may partly be due to the continually changing costs of all protein sources, and partly because the experimental feeding trial results concerning the production of milk and beef are often conflicting.

Urea is cheap, it is produced from inorganic materials whose sources are independant of land and imports and is water soluble. The relatively low cost of urea may prove to be of no value if the urea is inefficiently utilised by the animal or if it is mixed with expensive ingredients or if the final product is too costly. The costed examples of urea-containing diets given subsequently are considered to be fair at prices prevailing at the present time (1972). The normally accepted rate of use at the present time is about 28 lbs / ton (i.e. $1\frac{1}{4}$ %) giving an addition of about 3.5 % crude protein. It is generally accepted that satisfactory results may be obtained from feeding a ration of cereal including $1\frac{1}{4}$ % urea together with the necessary minerals and vitamins to growing beef animals, dairy replacements and to store beasts. The consideration to include $1\frac{1}{4}$ % urea to replace one-half of the normal protein sources used to supplement production rations for dairy cows (16-18 % crude protein) is not nearly so well accepted at this time. There is considerable difference of opinion regarding the value of urea for dairy cows. It is considered that urea can satisfactorily be included in all types of sheep diets, other than for lambs below 6-8 weeks old.

Approximate Comparative Prices.

	% N.	% C.Protein.	Cost/Ton.	Cost of I% C.Protein.
Groundnut	8	50	£60	£I.20
Fish Meal	I0	62	£I00	£I.60
Urea	46	288	£50	£0.I7

A simplified example of the possible savings to be obtained by including urea in concentrate rations containing I6-I7 % crude protein is given below. Approximate amounts/ton are used for simplicity of calculation. Barley is assumed to cost £30/ton.

An all-vegetable protein diet composed of 85% barley and I5% groundnut having an approximate crude protein content of I6.0% is calculated to cost £34.50/ton. In direct comparison, a diet containing urea as the sole source of additional nitrogen (ie.97.5% barley + 2.5% urea) having an approximate crude protein content of I7.0% is calculated to cost £30.40/ton.

In the assesment of any cost advantage which urea may have in direct comparison to a vegetable protein source it is thought necessary to make an allowance for the value of the energy component of the vegetable protein as it has been argued (Owen, I967) that urea has a negative starch equivalent since energy is required for the metabolic processes involved in incorporating it into microbial protein.

Present commercial forms of presentation of urea containing products include urea/vitamin/mineral protein concentrate cakes, urea/molasses liquid feeds and solid blocks containing urea, (some containing additional minerals and vitamins.) An assesment of the costs per lb. of crude protein in these products in comparison with groundnut and fishmeal, with an allowance made for the cost difference in energy value is given below.

	Urea/ Molasses	Groundnut	Fish Meal
Cost	£0.25 per gall.	£60/ton	£100/ton
% C.Protein	30	50	61
Amount to supply I-lb. C.Protein	2 pints	2 lbs.	I½ lbs.
Cost	£0.06	£0.05	£0.07
Energy provided lbs. S.E.	I.0	I.2	I.0
Value @ £43/ton S.E. (Barley = £30/ton)	£0.02	£0.02	£0.02
Cost of I lb. C.Protein less energy	£0.04	£0.03	£0.05

Solid blocks containing urea (6% urea) of about 23% crude protein presently have a commercial price of about £50/ton. The cost of I% crude protein is thus about £2.20. This can be compared with groundnut (of similar energy value) costing about £I.20 for I% crude protein.

On the present evidence the advantage of using urea lies in its cheapness. The proprietary products of the solid block and liquid type which are currently available are somewhat dearer sources of crude protein in comparison with conventional protein sources. Their particular advantage would appear to be one of convenience. Solid blocks blocks may be left outside until consumed and the reservoirs merely require refilling periodically in the case of liquid feeds.

The second use of urea is in the concentrate ration fed mainly to beef animals with some brands now available for feeding dairy cattle. Armstrong & Trinder (I966) calculated the theoretical savings possible by feeding urea-containing rations to dairy cows and beef cattle.

The experimental data collected showed a mean reduction in milk yield of I2.9 lbs. when a ration containing 2¼% urea was fed to cows yielding 3 gallons a day. A theoretical costing over a I83 day lactation period, allowing for both reduction in feeding costs and the reduction

in return due to the lowered milk yield showed no definite advantage in using the urea ration. It must be noted however that this costing was performed using the prices for foodstuffs and milk of that time. A recalculation of the data using current prices shows an economic advantage of using urea even though some theoretical loss in yield is accounted for. Dairy cows are usually fed concentrates twice a day and the apparent lack of utilization of urea hitherto recorded in dairy cows may well be accounted for by the inefficiency of the rumen microorganisms to fully utilize all of the liberated ammonia. This would particularly apply to high yielding cows receiving relatively larger amounts of urea.

Armstrong & Trinder (1966) comment "on theoretical grounds urea can best be used in frequent feeding regimes. The so-called "barley-beef" system would seem to be ideal for the point of view of maximum utilization of urea, since th animals are allowed unlimited access to the concentrate feed". They demonstrated a considerable saving of food costs when urea is used even allowing for some reduction in liveweight gain.

Historic Background

In 1773 Rouelle identified urea in urine and by 1823 Prevost & Dumas had shown that the kidneys removed urea from the blood and had suggested that it was formed in the liver. Liebig in his book "Aninal Chemistry" (1842) indicated the chemical basis of protein and showed urea to be one of the end products of protein digestion. Later (1847) he recognised the existance of the large number of intermediates between the nitrogenous constituents of food and the end-product of urea.

The first balance studies involving nitrogen were reported by Boussingault in 1839. Voit (1831-1908) then developed the technique into a quantitative method for studying nitrogen metabolism. Zuntz (1891) indicated that the rumen microflora played a significant role in cellulose digestion and that the protein-sparing action of asparagine and other amides was observed only with ruminant animals. During the following 35 years much work was performed, mainly by researchers in Germany, on the ability of rumen organisms to utilize non-protein nitrogen.

In a series of experiments (1907-1924) Morgen and his co-workers demonstrated that 30-40 % of the protein in sheep rations could be replaced by urea. Then Voltz (1920) reported the maintenance and growth of lambs on a low protein, semi-purified diet composed of starch, alkali-washed straw, inorganic salts and urea. In 1911 Armsby produced a review concerning work on the utilization of non-protein nitrogen by ruminants and non-ruminants. He deduced the role of rumen microorganisms in converting NPN to protein, their subsequent digestion by the host animal and conversion to the protein required for milk production and growth. He also noted that in the presence of adequate protein, NPN usually did not increase production of the "nitrogenous constituents" of the animal.

During the following 20 years nearly all research on this topic was conducted by European workers, primarily German. The development of the Haber-Bosch synthetic ammonia process with the production of large

amounts of carbon dioxide as a by-product stimulated the successful development of a process to synthesize urea from ammonia and carbon dioxide. In an excellent monograph, Curtis (1932) surveyed the industrial technology of ammonia and urea synthesis.

Continued work by Honcamp, Koudela & Muller in the 1920's showed that urea could be used in practical rations for dairy cows. Up to the 1930's there was a rather surprising school of thought that believed the protein-sparing effect of urea was due to the "neutralization of organic acids formed in digestion".

During their studies Hart and his co-workers (1938-39) used an in-vitro system to study non-protein metabolism in the rumen. The disappearance of inorganic nitrogen was stimulated in the prescence of readily available energy sources. Both urea and ammonium bicarbonate were shown to be converted into bacterial protein. Wegner, Booth, Bohstedt and Hart (1940-1) by sampling rumen contents from a fistulated heifer showed that both the total protein and level of natural protein in the ration influenced the utilization of urea. High total protein with a high percentage of natural protein gave rise to poor urea utilization. When the protein level of the rumen contents exceeded 12%, urea utilization was markedly decreased.

Schmid (1939). was able to maintain the liveweight of sheep over a three year period using ammonium salts as the only supplementary nitrogen source. Some changes in the relative propartions of the different rumen microorganisms were observed. His opinion was that digestion and absorption of the protein-like compounds formed from these soluble nitrogen sources occured in the intestine.

Research on the feeding of urea and other nitrogen compounds was now intensified on a worldwide scale and by 1940 a considerable body of knowledge had been accumulated. It was concluded that up to one third of the total nitrogen in a ration could be replaced by ammonium salts or urea.

Studies with rats, dogs, chickens, pigs and other monogastrics showed these nitrogen sources to be of little or no benefit for this class of animal. During the years 1940-1945 feed manufacturers and farmers began to accept the use of urea inclusion in practical rations for ruminant livestock. This trend was most noticeable in the U.S.A.

After this time the emphasis in research on the use of urea was more concerned with increasing the efficiency of urea utilization in the ruminant. The amount of urea which can be used in ruminant rations was found to depend upon; the potential toxicity level, the amount and type of carbohydrates present and the amount and type of true protein present. By 1955 the need to supplement urea-containing rations with energy, minerals and vitamins was recognised and was being recommended to the commercial feeder.

Digestion and Absorption of Nitrogenous Compounds in the Ruminant

McDonald (1948, 1954) showed that large quantities of ammonia were produced in the rumen under normal feeding conditions. The concentration was associated with the amount and type of protein fed and also upon the kind of carbohydrate materials present. Ammonia was shown to be absorbed directly from the rumen into the ruminal veins and also, urea was seen to be returned to the rumen in the saliva (0.5 g. per day in the case of sheep). Most of the accepted views on the pathways of nitrogen metabolism in the ruminant is expressed in schematic form (adapted from Annison,& Lewis, 1959) in Fig I .

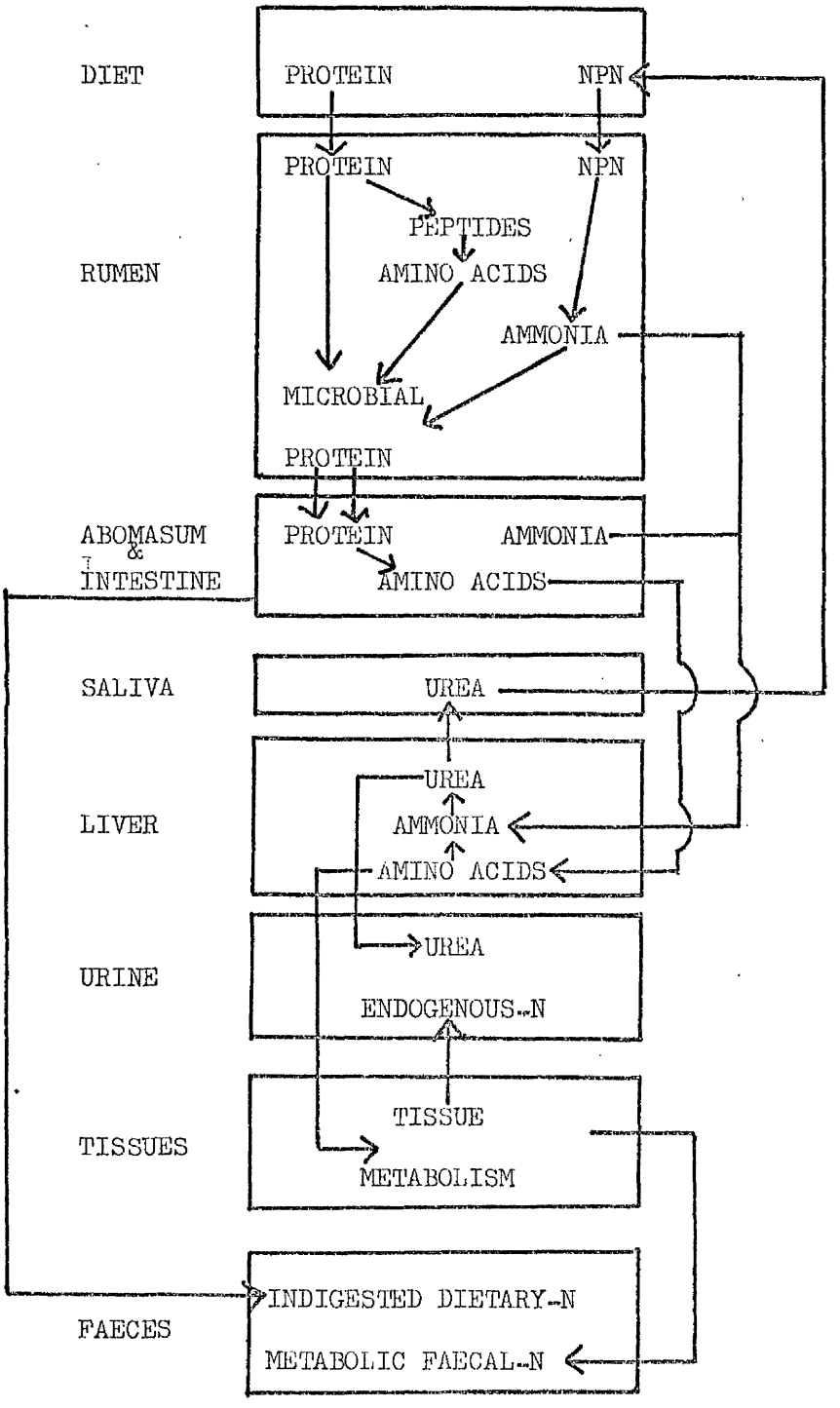
a) Ruminal sources of Ammonia

Ammonia is produced in the rumen by the metabolism of proteins, peptides, amino acids, urea, nitrates and some other non-protein (NPN) compounds. Microbial deaminases in the rumen rapidly metabolise free amino acids to produce ammonia (Warner, 1956). However Annison (1956) showed the actual concentration of amino acids in the rumen to be low. Microbial amidase action on glutamine, asparagine, and protein amidic groups also produces ammonia. (Abou Akkada & Blackburn, 1963; Warner, 1964). Proteins were observed to be hydrolysed in the rumen to varying degrees dependant upon their individual solubilities. For example, insoluble zein was only 40 % degraded (McDonald, 1954) whereas a similar value for soluble casein was 90 % (McDonald & Hall, 1957). Pearson & Smith, (1943) showed urea to be hydrolysed very rapidly in the rumen by bacterial urease to produce ammonia and carbon dioxide. Apart from the dietary route urea is known to enter the rumen endogenously by salivary recirculation (McDonald, 1948) and by diffusion across

Figure I.

Principal Sources and fates of Nitrogen in Ruminant metabolism.

(Adapted from Annison & Lewis, (1959).



the rumen wall itself. (Houpt, 1959, 1968). The in-vitro utilization of many NPN compounds by rumen bacteria has been studied by Belasco (1954). The actual concentration of ammonia in rumen liquor which may vary between 0-130 mgs/100 ml.,(Johns, 1955), represents a complex dynamic situation. A balance exists between the bacterial ammonia uptake, diffusion and metabolism of ammonia across the rumen wall, passage into the omasum and the dietary nitrogen and recycled urea intakes producing ammonia at different rates according to the various specific enzyme lytic activities.

b) Metabolism of Ammonia by ruminal flora

Ammonia is an essential nutrient for the growth of several bacterial species in the rumen, (Bryant, 1963; Hungate, 1966). It stimulates both cellulose digestion (Belasco, 1954; Chalupa, 1963; Little, 1963) and starch digestion, (Acord, Mitchell & Little, 1968; Acord, Mitchell, Little & Karr, 1966). Several researchers have observed that carbon from a wide spectrum of organic compounds could be used in the synthesis of amino acids in the rumen (Tillman & Sidhu, 1969). In some cases a specific stereochemical carbon chain requirement was noted for the synthesis of certain amino acids. The energy for these syntheses is derived from dietary carbohydrate and other organic compounds. The exact biochemical mechanisms of amino acid synthesis in the rumen are still uncertain. Possibly the ammonia is "fixed" by amination reactions, since NAD and NAD phosphate linked glutamic acid dehydrogenases are present in the rumen. (Burchall, Niederman & Wolin, 1964; Palmquist & Baldwin, 1966, Hoshino, Saramaru and Morimoto, 1966). Other suggestions include transmination reactions as α- keto acids were found in the rumen after glucose plus grass feeding, (van der Horst, 1961).

Ammonia is apparently metabolised in the rumen mucosa itself (McLaren 1961-2; Hoshino et al, 1966), where glutamine is the postulated "storage form" in the mucosa. Actual ammonia absorption across the

rumen wall (McDonald, 1948) is governed by both rumen pH (Hogan, 1961), and the concentration gradient, (Hogan, 1961; Lewis, Hill & Annison, 1957). Ammonia is a weak base (pKa 8.8-9.2) and an alkaline pH causes the NH_4^+ ion which is relatively tissue-impermeable, to be converted to uncharged, free NH_3, which is tissue-permeable. (Hogan, 1961; Bloomfield, Kearley, Creach & Muhrer, 1963). The absorbed ammonia is carried via the portal circulation to the liver where conversion to urea occurs. Lewis et al, (1957) reported an upper limit of 0.8 m M/litre of ammonia in portal blood when the ammonia concentration in the rumen was observed to be 60 m M/litre. Above this concentration in portal blood the peripheral blood ammonia concentration increased as the liver was unable to complete conversion to urea effectively,

c) Urea recycling in the ruminant

Several researchers have demonstrated that endogenous urea enters the rumen by a salivary route and by diffusion across the rumen wall (e.g. Houpt, 1959; Packet & Groves, 1965; Juhasz, 1965; Cocimano & Leng, 1967; Houpt & Houpt, 1968). The main route would now appear to be by diffusion across the ruminal epithelium (Houpt, 1959; Juhasz, 1965). However, blood urea is also diffused into the abomasum and the intestinal tract, (Le Bars, 1967; Cocimano & Leng, 1967). Somers, (1961 a.b.c.), reported a positive relationship between nitrogen intake and urea excretion in saliva. It was also noted that 60-70 % of the total nitrogen in saliva is derived from urea.

Nitrogen conservation in the ruminant is governed by the activities of the kidneys and salivary glands. The concentration of urea in saliva increased (Somers, 1961 d) when sheep were fed on a nitrogen-deficient diet, and the rate of urine urea clearance falls. (Schmidt-Nielsen & Osaki, 1958).

The recycled urea is utilized as dietary urea (Moir & Morris, 1962). Thus dietary urea is in no way an alien metabolite to the rumen. Packet & Groves, (1965) showed that dietary carbohydrates improved the utilization of recycled urea. It is of interest to note the efficiency with which the ruminant can conserve nitrogen. Even that portion of dietary or recycled

urea which diffuses into the abomasum and the intestines can by hydrolysed by intestinal urease, (Sidhu, Jones & Tillman, 1968) and the resultant ammonia absorbed by the tissues of the intestinal tract, (McDonald, 1948).

d) Increasing the efficiency of utilization of dietary urea

Ever since Bloomfield et al (1960) reported that the rate of urea hydrolysis was about four times greater than the corresponding uptake of ammonia by rumen microorganisms, there have been several attempts to improve urea utilization by decreasing rumen urease activity. Tillman & Sidhu (1969) in an excellent review article, give an account of experimental inhibition of ureolysis and proteolysis by the use of suitable chemical inhibitors. Other methods of increasing the efficiency by which the ammonia is utilized has included the feeding of urea phosphate. (Perez, Warner & Loosli, 1967). Urea phosphate lowered the pH of ruminal fluid and thereby decreased the rate of ammonia absorption. Biuret may also be utilized by ruminants (Waite & Wilson, 1968). This material being relatively insoluble in water (2 g/100 ml at 25°C) affords a slow release of ammonia after hydrolysis by the bacterial enzyme, biuretase. (Schroder & Gilchrist, 1969; Bauriedal, Craig, Ramsey & Camehl, 1969). Isobutylidene diurea (IBDU), a condensation product of urea and isobutyraldehyde, is a sparingly water soluble material containing 32 % N. The manner of the slow release of urea from IBDU when fed to ruminants was described by Parkins, Ritchie & Hemingway, (1971 a). In a trial with growing lambs a ration containing IBDU was shown to produce significantly better growth rates than a comparable ration containing urea. (Parkins, Ritchie & Hemingway, (1971 b). Urea phosphate, biuret and IBDU are all much less toxic than urea. Deyoe et al (1968) developed a new " complexed" urea produce called "Starea" by cooking and extruding the gelatanized product in pellet form. Growth trials showed the product to be superior to urea as a protein supplement.

A direct approach for decreasing gastrointestinal urease activity

is by producing circulating antibodies to urea. (Dang, Visek, 1960; 1964; Glimp & Tillman, 1965; Sidhu, Jones & Tillman, 1968). A critical review of current progress in this area of research has been given by Tillman & Sidhu (1969).

The 'Adaptation Response' to urea feeding.

Urea feeding is best introduced at low levels and then gradually increased to allow the rumen microorganism population to adapt itself to the increased ammonia and to enable the liver to adjust to the increase in blood ammonia concentration. The phenomenon of 'adaptation' to urea is well known. The utilization of absorbed nitrogen in sheep has been reported to be increased by the prolongation of the preliminary period in which the sheep received the NPN containing diet. (McLaren, Anderson, Welch, Campbell,& Smith, 1959, 1960). The effects of the amounts of carbohydrate and length of time of urea feeding on the nitrogen retention by lambs fed semi-purified diets containing 75 % of the total nitrogen from urea were studied by McLaren, Anderson, Tsai & Barth, (1965). Regression analysis indicated that the retention of absorbed nitrogen was improved by three percentage units with each 10-day period of feeding and by two percentage units for each 100 Kcal of available carbohydrate in the diet.

Clifford & Tillman (1968) showed that the improved nitrogen retention with time by sheep fed extra isolated soya protein or urea was closely associated with a reduced urinary nitrogen output. The maximum retention was noted after 30 days of feeding. Caffrey, Hatfield, Norton & Garrigus (1967) reported that the time for ruminal microorganisms to assimilate ammonia at a maximum rate was 19 days after an abrupt change from a diet containing soya bean to a diet containing urea.

In an extensive review article Oltjen (1969) surveyed results from experiments investigating the adaptation response to urea feeding. He observed that some experimental results showing an adaptation response could be (a) a reflection of metabolic adjustment to some other components

of purified diets rather just the urea alone or (b) "animal adaptation from an adequate protein diet to a lower protein diet".

It is of interest to note that from a review of experiments an adaptation response is noted for biuret. (Campbell, McLaren, Smith, Welch, Shelton & Anderson, 1956; Welch, McLaren, Smith, Campbell, Shelton & Anderson, 1956; Smith, Anderson, McLaren, Campbell, & Welch, 1957; Ewan, Hatfield & Garrigus, 1958). This may be because the biuretase enzymes have only minimal activity in animals which have never been fed biuret and increase in activity after biuret is included in the diet. (Schroder & Gilchrist, 1969).

Also of interest is that apparent digestibility of IBDU in adult sheep, fed a barley diet containing 3 % of added crude protein from IBDU, increased from 64.6 % two weeks after feeding commenced to 100 % after six weeks of continuous feeding (Parkins, Ritchie & Hemingway, 1971 a).

The nature and source of blood ammonia and its toxic effects.

Historic introduction

The forced feeding of meat to dogs in which a shunt had been established between the portal system and the peripheral venous system and thus bypassing the liver resulted in "meat intoxication". (Nencki & Pawlow, 1895-96; 1896-97; Hahn Massen, Nencki & Pawlow, 1893). The symptoms observed were dizziness, ataxic gait, anorexia and blindness followed by death. These results did not occur when the dogs were fed on a cereal diet. The explanation proposed was that the symptoms could be due to ammonia intoxication.

Van Caulaert, Deviller and Halff (1932) demonstrated the toxic effect following administration of ammonium salts to human patients with cirrhosis of the liver. Symptoms of stupor appeared when blood ammonia concentrations rose to abnormal levels. Continued work in this line of study by Gabuzda, Phillips & Davidson (1952) and Phillips, Schwartz, Gabuzda & Davidson (1952) showed that any method producing large increases in blood ammonia concentration also caused symptoms of "impending hepatic coma".

Nature and source of blood ammonia

Under certain pathological conditions the volatile base appearing in the blood is ammonia (Bessman 1959). There is a small amount of ammonia normally present in blood which may be liberated by mild alkalinization. At a pH of 7.4 ammonium is almost completely ionized according to the equation: $NH_4^+ \rightleftharpoons H^+ + NH_3$. The free ammonia penetrates cell membranes rapidly, whereas tissues are relatively impermeable to the ammonium ion. (Jacobs, 1927; Jacobs & Parpart, 1938).

Source of ammonia

a) Non-ruminant studies

There are three main sources of ammonia; it may arise in the gut, the kidney and the muscle. Folin & Denis (1912) found that blood leaving the intestinal tract in the portal system contained far greater amounts of ammonia than the blood of the peripheral venous or arterial systems. Enzyme action on protein in the gut liberates the γ- amide of glutamic acid (glutamine) which is hydrolysed by bacterial glutaminases to release ammonia (Damodaran & Narayanan, 1938).

Urea is normally present in all the gastrointestinal secretions in amounts equal to its distribution in other body fluids, since urea easily penetrates all membranes (Bessman, 1959). The enzyme urease hydrolyses urea to ammonia and carbon dioxide. Large amounts of ammonia are formed in the intestines in a condition known as uraemia. The ammonia is thought to cause ulceration and necrosis of the colonic and intestinal mucosa, (Bessman, 1959).

Renal venous blood is another source of ammonia in the organism. Here the concentration of ammonia was shown to be greater than that in the arterial blood. This is thought to be a result of the diffusion of ammonia from the kidney tubules, (Loeb, Atchley & Benedict, 1924). Urinary ammonia was thought to be derived from the oxidative deamination of glutamine and other amino compounds. Van Slyke, Phillips, Hamilton, Archibald, Futcher & Hiller, 1943). It has been known for some time that the contractile activity of muscle gives rise to ammonia. (e.g. Parnas, 1928; Embdem & Wassermeyer, 1928). The exact mechanism for this is not known. In an excellent review Bessman (1959) details some pathological alterations connected with ammonia metabolism, these include;

hepatic failure, cardiac failure, shock, asparagine intoxication, ureterocolic anastomosis and erythroblastosis foetalis. In all these conditions substantial increases in blood ammonia concentrations are observed.

b) Ruminant studies

Urea toxicity The occurence of urea toxicity in the ruminant animal is directly associated with an excess production of ammonia in the rumen and the absorption of this ammonia into the blood. Hence, dietary urea toxicity is virtually synonymous with ammonia toxicity.

In their review article Armstrong & Trinder (1966) state that if the concentration of ammonia nitrogen in the blood reaches a critical level, (above 1650 µg/100 ml) acute toxicity occurs leading to rapid death. They described the symptoms of acute toxicity as muscular twitching, excessive salivation, bloat, ataxia and laboured breathing followed by tetany, complete collapse and death within one to three hours.

Lewis et al (1957) demonstrated symptoms of poisoning when the blood ammonia nitrogen level reached 840 - 1300 µg/100 ml while a standard reference, Garners' Veterinary Toxicology, states that ataxia occurs at a blood ammonia concentration of over 2000 µg/100 ml. The first signs of toxicity described by Lewis et al (1957) were twitching and a general hypersensitivity. Lewis (1960) further indicated signs of poisoning when the blood ammonia concentration was less than 500 µg NH_3 - N/100 ml. and noted an initial metabolic alkalosis demonstrated by stertorous breathing due to a direct stimulation of respiratory exchange by the ammonium ion. A direct toxic effect was considered to occur at 800 µg ammonia nitrogen/ 100 ml blood. That sheep became somnolent in the initial stages

was noted by Lewis et al (1957) and that generalized tetany and death occurred at levels beyond 1500 µg NH_3 - N/100 ml after toxic symptoms had first been observed at 1020 µg NH_3 - N/100 ml.

Repp, Hale, Cheng, Burroughs (1955) recorded an absence of clinical symptoms until the blood ammonia-nitrogen concentration rose to 1000 µg/100 ml and in cases where the concentration was greater than 1158 µg/100 ml acute toxicity and death occurred. The maximum recorded blood ammonia concentration was 3585 µg NH_3 - N/100 ml, just prior to death. Repp et al (1955) found that lambs appeared to be able to withstand blood NH_3 - N values of about 1000 µg/100 ml without showing clinical signs of toxicity. They stated that mean normal blood NH_3 - N concentrations was 148 µg/100 ml (range 80 - 250 µg/100 ml) and concluded that the critical range of blood ammonia has rather narrow limits. The first symptoms following above normal concentrations were that the animals became restless and then shortly afterwards exhibited ataxia. These symptoms were usually of a 10 - 15 minute duration after which the animal collapsed. During this stage laboured breathing was encountered accompanied by frothing at the mouth. Bloating sometimes occurred suggesting a cessation of rumen mobility. About 25 minutes after collapse the animal usually went into tetany. Laboured breathing continued and bloating were obvious. The skin appeared to have a blue colour, which suggested anoxia, and death usually occurred $1\frac{1}{2}$ - 2 hours after dosing. Extensive epicardial and endocardial haemorrhages were nearly always found and the blood vessels were cyanotic. The kidneys were usually swollen and congested and in the blood methaemoglobin formation was suggested by a brownish discolouration. A certain degree of haemorrhaging was noticed in the abomasum and digestive tract.

These clinical symptoms of toxicity and necropsy findings of Repp et al (1955) are similar to those reported by Clark, Oyaert, & Quin (1951).

Pierson & Aanes (1959) described affected animals as suffering from twitching, ataxia, frothing, salivation and bloat, slow and deep respiration followed by death within 15-30 minutes of the onset of symptoms. Post mortem findings included subendocardial haemorrhages and mild abomastitis.

An observation from the work of Clark et al (1951), later confirmed by Szwabowiez (1962), was that the description of the whole syndrone was similar to that noted for strychnine poisoning. Clark et al (1951) also noted that stimulation of a poisoned animal led to aggravation of the symptoms.

Symptoms described by these various workers also included severe toxicity of the skeletal musculature and regurgitation before death. Post mortem examination indicated circulatory collapse, severe venous stasis and epocadial haemorrhages, liver and kidney degeneration with fatty degeneration. A distinct smell of ammonia was noted from the rumen contents.

McInnes (1964) recorded staggers, ataxia, collapse, slow deep and balanced respiration and frothing at the mouth with regurgitation in poisoned sheep. Death followed 30-60 minutes after administration.

Although poisoning has been observed and recorded by several research workers there is still some confusion according to Austin, (Briggs, 1967) as to the exact chronological order of the symptoms, possibly due to inaccurate field observations and controversy over the respiration defects.

A brief summary of the evidence suggested an initial metabolic alkalosis and an apparent complete recovery to a normal level by the stage at which the most obvious signs of toxicity are

observed. This may be accounted for as a respiratory alkalosis which occurs not as a response to blood pH but to a direct stimulation of respiratory exchange by the ammonium ion. Changes in the acid-base balance of body fluids cannot account entirely for all the signs of toxicity subsequent to the pharmacological effect. The symptoms would seem to be in some way directly related to the blood ammonia concentration.

Mode of Action of the Toxic Agent

Austin (1967) reported that it is generally accepted that ammonia is the toxin involved in urea poisoning. Lewis (1960) had previously reached this conclusion, considering that ammonia toxicity was due to the circulating ammonium ion.

The suggestion made by Head & Rook (1955), and subsequently supported by Voisin (1963) that urea poisoning could be attributed to hypomagnesaemia has now been largely discounted. Wilson (1963) concluded from his experiments that supplemental urea did not decrease serum magnesium in sheep.

Dukes (1955) described urea as causing electrolyte and water imbalance, while Warren (1962) suggested a co-relation between ammonia intoxification and potassium deficiency. Lewis (1961) had thought that an electrolyte imbalance accompanied by a potassium leak from the cells could explain the toxicity of urea.

Increased muscular and nervous activity ending in convulsions was shown by McIlwain (1959) to result from an increase in cerebral ammonia, and the association of these signs with the adverse effects that a potassium leak could be expected to have on the nerve and muscle cells is particularly striking.

It has been shown that the potassium content of the red blood cells of sheep vary about two or more distinct means (Evans 1954).

This characteristic is of a genetic nature with the potassium concentration of whole blood being within the range 30-40 m-equiv./ litre (a recessive characteristic) or with the lower range of 8-16 m-equiv./litre. A survey of the frequency of the two red blood cell types was undertaken by Evans & Mounils (1957). Of Scottish Blackface sheep studied, 52% were within the higher concentration range of 30-40 m-equiv. potassium/litre blood. If ammonia toxicity was associated with a cellular potassium leak as suggested by Lewis (1961), the wide variations which was found by Austin (1967), in the tolerance of individual animals to urea may possibly be due to differing blood potassium concentrations.

SECTION II.

ANALYTICAL METHODS.

a). The Determination of Ammonia in biological fluids - a review.

b). Methods of separating ammonia from biological fluids.

c). Methods for the estimations of the diffused or exchanged ammonia.

d). Experimental.
- I). The determination of blood ammonia concentrations by microdiffusion and ion-exchange techniques.
- 2). Possible interference effects of Ca and Mg in the determination of blood ammonia by ion-exchange.
- 3). Suitability of Analytical Method.
- 4). Storage of blood samples.
- 5). Colorimetric end-point determination.
- 6). Determination of the concentration of ammonia in milk.
- 7). Determination of the concentration of ammonia in rumen liquor.

e). The determination of urea in blood and milk.

f). Other Methods Employed.

The Determination of Ammonia in Biological Fluids

During the course of experiments described in this thesis analyses of ammonia concentrations in blood, rumen fluid and milk samples were performed. A survey of the various techniques available was undertaken. A short review of these methods with particular reference to those for blood follows.

The determination of the ammonia concentration in blood has long been regarded as being particularly difficult. Early reviews are given by Parnas & Heller (1924), Schneller (1928), and by Stanoyevitch (1938). One of the original interests in the determination of blood ammonia was concerned with defining the 'normal' ammonia concentration range in healthy human subjects. Reported values for this concentration ranged between zero and up to 300 μg NH_3-N/100 ml. An important factor emerged when Conway (1935-1939) described an increase in blood ammonia concentration with time after collection (or 'shedding') of the blood sample. Two stages in the ammonia development in shed blood are described. (Conway, 1957). 'Alpha' ammonia release occurred up to five minutes after shedding and its concentration had a mean value of 46 μg NH_3-N/100 ml. Further ammonia release, varying with temperature, was termed 'beta' ammonia and was accounted for by the breakdown of adenyl pyrophosphates, although part of this ammonia was later thought to be derived from glutamine hydrolysis. (Archibald, 1944). Formation of additional ammonia, after blood is shed, and upon the addition of alkali for the diffusion of ammonia during its determination has been termed "artifactual" ammonia.

All methods for the determination of ammonia in biological fluids are based on two general processes. The first is the isolation of the ammonia and the second is the application of some technique for its quantitation.

Methods of Separating ammonia from biological fluids

(a) Distillation. Steam distillation of the mildly alkalinized fluid removing the liberated ammonia to an aliquot of standard acid was introduced by Parnas & Heller (1924). Some modifications of this technique are still used for the determination of ammonia in certain biological fluids, for example deproteinized rumen fluid.

(b) Aeration. This technique involves the alkalinization of the fluid and aeration of the liberated ammonia into an acid solution (Folin, 1919, Folin & Denis, 1912). Using this technique Folin studied the content of ammonia in the various vessels of the body and found the highest concentrations in the portal system.

(c) Microdiffusion. Essentially this is a distillation conducted at room temperature where the volatile base ammonia diffuses from an alkaline to an acid solution.

Conway & Byrne (1933) developed the technique using the apparatus known as the 'Conway dish'. This consists of a small circular dish divided into two compartments, an inner compartment and a concentrically arranged outer compartment, the whole dish being hermetically covered by a glass plate. Acid is placed in the inner compartment and the biological fluid under investigation placed into the outer compartment and alkalinized. After a suitable period the ammonia which had diffused into the inner solution is estimated by one of several methods. This particular method is still extensively used today.

Seligson & Seligson (1951) developed the second microdiffusion technique in common use. The diffusion vessel consists of a small vial (30 ml capacity, through the stopper of which projects a glass rod with an enlargement of the end. The biological fluid is placed in the vial and is alkalinized, and the rod is wetted with dilute sulphuric acid. The vessel is then placed in a horizontal position and rotated on a wheel so that the fluid is distributed uniformly and continuously over the sides of the bottle. The liberated ammonia diffuses to the acid on the glass rod. The ammonium

sulphate so formed can then be washed off the rod into a suitable reagent for the estimation of ammonia.

Modifications of the original Seligson technique, are reported by Seligson & Hirahara (1951). In the original technique the total ammonia evolved from a mixture of blood and saturated sodium carbonate was observed to increase with increasing time of diffusion. For this reason the modification involved adding the blood directly to a dry mixture of K_2CO_3 $1\frac{1}{2}H_2O$ and $KHCO_3$ so that the final pH of the mixture did not exceed 11. A further refinement was added by Reinhold & Chung (1961), who realizing that the main source of artifactual ammonia production was due to the alkaline decomposition of protein material, used a sodium borate buffer to displace the blood ammonia at a pH of 10.8 that avoided this spontaneous production of ammonia.

Nathan & Rodkey (1957) further modified the Seligson & Seligson technique by first precipitating the blood proteins with ice cold TCA immediately after the blood sample was withdrawn. After centrifugation, the ammonia in the supernatant liquid was diffused using potassium carbonate. The application of this technique to cattle blood has been investigated by Hendriks (1964).

Bessman (1959) has discussed some of the limitations of the various procedures described above. The question whether adequate mixing of the materials can be effected in the outer compartment of the Conway dish has been considered by Seligson & Hirahara (1951). Also, unless a direct titrimetric method is used for estimating the ammonia content of the solution in the central well, the solution must be quantitatively removed by pipetting where scrupulous care must be taken to avoid any exposure to atmospheric ammonia. The volume in the central well is critical and the preparation and cleaning of the Conway dish of the utmost importance.

Bessman (1959) considered the Seligson technique to offer

several advantages. Firstly, the diffused ammonia is concentrated into a very small volume which, being a thin film covering the surface at the end of the glass rod may be quickly transferred into a prepared colorimetric tube. The volume of acid coating the diffusion rod is not critical since it will represent less than one percent of the final volume of the solution used in the colorimetric analysis.

Preston (1969) compared three procedures for the determination of blood ammonia concentrations in sheep. The procedures were those outlined by Seligson & Hirahara (1957), Nathan & Rodkey (1957) and Reinhold & Chung (1961). Blood samples were analysed simultaneously from sheep which had previously been drenched with a urea solution. Blood ammonia concentrations were considerably elevated and an excellent correlation ($r. = .99$) was obtained between all procedures. Preston commented that all three procedures gave proportional values which were valid for relative comparisons but considered that from the standpoint of routine laboratory determination, the procedure of Reinhold & Chung to be the easiest to employ if blood samples could be analysed immediately.

(d) Ion exchange techniques. Methods for the estimation of whole blood and plasma ammonia by ion-exchange have been proposed for several years. (e.g. Fenton, 1960, 1962; Fenton & Williams 1968; Dienst, 1961; Hutchinson & Labby, 1962). The general principle is that sodium and/or potassium ions are exchanged for ammonia. After elution the ammonia is determined by one of several colorimetric methods. The method of Hutchinson & Labby, (1962) involves the addition of freshly drawn blood to a prepared graduated glass stoppered centrifuge tube containing a quantity of resin in the Na^+/K^+ form. The tube is then immediately shaken. Subsequently the blood is washed off by several shakings with ammonia-free distilled water. The ammonia is determined directly by colorimetry after the addition of a volume of a diluted Nessler's reagent which is shaken with the resin. The method of Fenton & Williams (1968)

utilizes plastic columns charged with resin to which plasma is added and allowed to flow through, after which the protein is washed off with ammonia-free distilled water. Colorimetric analysis is performed using a phenol-hypochlorite method. In both methods analysis may be held at the point when the blood proteins have been washed off the resin for later colorimetric analysis.

(e) Other methods. Generally other reported methods for the analysis of blood ammonia concentration fall into the category of direct colorimetric procedures where plasma is mixed with a colour reagent directly. (e.g. McCullough, 1967; Muramatsu, 1967).

Rubin & Knott (1967) have reported an enzymatic fluorometric method for the determination of blood ammonia.

Methods for the estimation of the diffused or exchanged Ammonia.

(1) Titration

Conway (1947) backtitrated the ammonia in a standard volume of acid with a barium hydroxide solution. However, for this purpose only small quantities of the standard alkali were required and the use of a microburette was necessary. Later a borate titration was used. Even so several other volatile vases e.g. n-butylamine and isoamylamine, present in blood would be included as "ammonia" by this technique (Richter, 1937). Bessman (1959) considered that the result from any acidimetric technique should be reported as 'volatile base' rather than ammonia.

(2) Nessler's Reagent

The Nessler reagent develops a brown colour in the presence of ammonia but not with aliphatic amines. The reagent has been employed with considerable success in the Seligson and ion-exchange techniques for the measurement of blood ammonia concentrations.

(3) Other methods

In the presence of ammonia, a phenol and hypochlorite solution

forms a dye colour. (Bertholet reaction). Van Slyke and Hiller (I933) reported its use in the determination of blood ammonia. The reagent can be made more sensitive by the addition of nitroprusside as a catalyst. (Lubochinsky & Zalta, I954). Nathan & Rodkey (I957) used the ninhydrin reaction with both the Conway and Seligson methods. A method devised by Stone (I956) uses the stoichiometric decolourization of phenosafranine by hypobromite. Seligson & Hirahara (I95I) used this technique by adding a standard amount of hypobromite and determining the residual reagent.

Bessman (I959) described the Nessler reagent as being the most siutable although the least sensitive colorimetric reagent available for the determination of ammonia. It requires no heating or incubation and uses only one easily prepared and stable solution. In addition it is subject to the least variable error of all the colour methods. The time required for the performance of an analysis using Nessler's reagent is considerably less than with any other reagent.

Experimental

1). The determination of blood ammonia concentrations by Microdiffusion and ion-exchange techniques.

After careful consideration of the literature a study was conducted in order to decide which would be the most suitable method for the determination of blood ammonia concentrations in ruminant animals. Consideration had to be made upon the number of samples which could be handled in batches and upon the time and labour involved in the analyses, together with an assessment of the reliability and reproducibility of the method.

From a survey of the literature two methods emerged as being the most promising in these respects, namely the modified microdiffusion technique of Seligson described by Reinhold & Chung (1961) and the ion-exchange method described by Hutchison & Labby (1962). In the present study these methods will be referred to as microdiffusion and ion-exchange procedures respectively.

Materials and Methods

1) Microdiffusion

The rotation apparatus, which was built at low cost, consisted essentially of a 12" diameter wooden wheel, fitted with spring clips on the circumference, maintained in the vertical plane. A variable speed laboratory stirrer motor (400 - 1000 r.p.m.) was linked by a system of 'V'-belts and pulleys to the driving shaft. The apparatus could rotate up to 24 diffusion vessels, each of 30 ml capacity, at one time, at rates varying between 20 - 60 r.p.m.

Reagents

a) Glass distilled ammonia-free water.

b) Borate buffer. Prepared by adding 48 gms. of $Na_2B_4O_7$ and 9.8 gms. NaOH pellets to 400 ml. of water and boiling for 15 minutes with stirring. After cooling at room temperature the final solution was diluted to 500 ml. The

separation of any sodium borate crystals was disregarded. The pH should be 10.8 ± 0.2 and if the pH exceeded 11.0 the solution was diluted with water to the desired alkalinity.

c) Sulphuric acid: 1 Normal.

d) Nessler's reagent. (B.D.H. Ltd.)

Diluted 1/5 immediately before use with distilled ion-free water.

e) Stock standard Ammonium Sulphate Solution. (0.1 mg N/ml)

Prepared by dissolving 0.472 g $(NH_4)_2SO_4$ per litre of distilled water.

f) Working standard

Prepared when required by the dilution of 1 ml. of stock standard to 100 ml. with distilled water, so that 1 ml. contained 1 ug. NH_3-N.

Analytical Procedure

All analyses were performed in duplicate. 1.5 ml of borate buffer was pipetted into a sufficient number of bottles to accommodate all specimens, standards and blanks. This was delivered by a multi-delivery pipette (A.R. Horwell Ltd.) which was sufficiently accurate for this purpose. Glass beads were added to facilitate mixing. 1 ml of freshly drawn blood was added and the vessel immediately capped with the stopper and receiving rod which had been dipped into 1 N Sulphuric Acid. Care was exercised to avoid touching the rod to the neck or wall of the bottle. The samples, which were accompanied in all determination by standards, were rotated for 30 minutes at about 40 revolutions per minute. The glass rods were carefully removed, immediately transferred directly into test tubes containing 6 ml of the diluted Nessler's solution and inverted to wash off all the ammoniated acid. After standing for 5 minutes the absorbancy of the solution was measured at 415 mu in an E.E.L. Spectrophotometer. A standard graph was prepared from the rotated standards.

2) Ion exchange

Reagents

a) Cation exchange resin. (Dow Chemical Co.). Dowex 50 W by 12, 200 - 400 mesh, hydrogen ion form, sulphuric acid type, with a total capacity of 2.8 m.Eq. per moist gram. The resin was rendered ammonium free and converted to the sodium potassium form before use by the following procedure. 50 g resin was added to a 2 litre 'ammonia free' flask and washed 3 times with 500 ml portions of ion-free water in order to remove excess sodium hydroxide. The resin was washed 3 times with 300 ml portions of 0.2 M sodium potassium phosphate buffer at pH 7.40. The resin was washed 3 times with ion-free water to remove excess buffer. The material was transferred to an ammonia-free 500 ml glass stoppered reagent bottle with 400 ml of ion-free water. This stock resin suspension was tightly stoppered and stored in the dark.

b) Nessler's Solution (B.D.H. Ltd.)

c) Standard potassium phosphate buffer (0.2 M, pH, 7.40). 27.22 g KH_2PO_4 per litre adjusted to pH of 7.40 with sodium hydroxide.

Ammonia-free glassware

All glassware was washed in a hot detergent solution, rinsed in hot tap water, rinsed again 3 times in distilled water, soaked in 0.1N NaOH and rinsed 10 times with ion-free water before drying.

Method

4 ml of resin suspension was added to a graduated glass stoppered centrifuge tube. 2 ml of freshly drawn blood was added from a glass pipette. The tube was stoppered and shaken vigorously for 3 minutes. The tube was then allowed to stand to allow the resin-ammonium complex to settle by gravity. The supernatent was decanted carefully and ion-free water added to the 6 ml graduation mark. The tubes were shaken for 30 seconds, and allowed to settle before decanting. This was

repeated for a total of 4 washes. 6ml of I/5 diluted Nessler's reagent was added and the tube shaken vigorously for 3 minutes. The Nessler's reagent was then decanted and the absorption measured at 415 mu in a spectrophotometer.

Preliminary laboratory studies were conducted with the micro-diffusion technique in order to investigate the optimum rotation speeds and diffusion times. The optimum rotation time for the complete diffusion of ammonia standards was 35 minutes when the rotation rate was 40 r.p.m. Results obtained when aqueous standards containing between 0 and 600 µg NH_3 - N / 100 ml were diffused under these conditions agreed very closely with values obtained from the direct Nesslerization of aqueous standards. The standard graph obtained in this concentration range was linear. Recovery of known ammounts of ammonia from freshly drawn whole blood (total of 16 samples with additions of 100 - 1000 µg NH_3 - N / 100 ml) was 96.8 % (range 80.1 - 102.3 %). Diffusion times of over 35 minutes resulted in the apparent production and recovery of ' artifactual ' ammonia (ie. recovery was then in the range 116 - 142 %). Standard graphs obtained using aqueous standards under the given conditions of rotation rate and time were highly reproducible (Error $\pm$ 4.6 %) . During the remainder of this section analysis of ammonia by the described micro-diffusion technique was performed under these conditions.

Analysis of ammonia by the resin technique was performed according to the method as described previously (p. 39). The repeated production of standard calibration graphs agreed extremely well ($\pm$ 3.6 %) and a linear relationship existed between the optical density of the nesslerized aqueous standards and concentrations of ammonia equivalent to 0 to 1000 µg / 100 ml of whole blood.

I). A comparison of values obtained for blood ammonia concentration using resin and microdiffusion techniques.

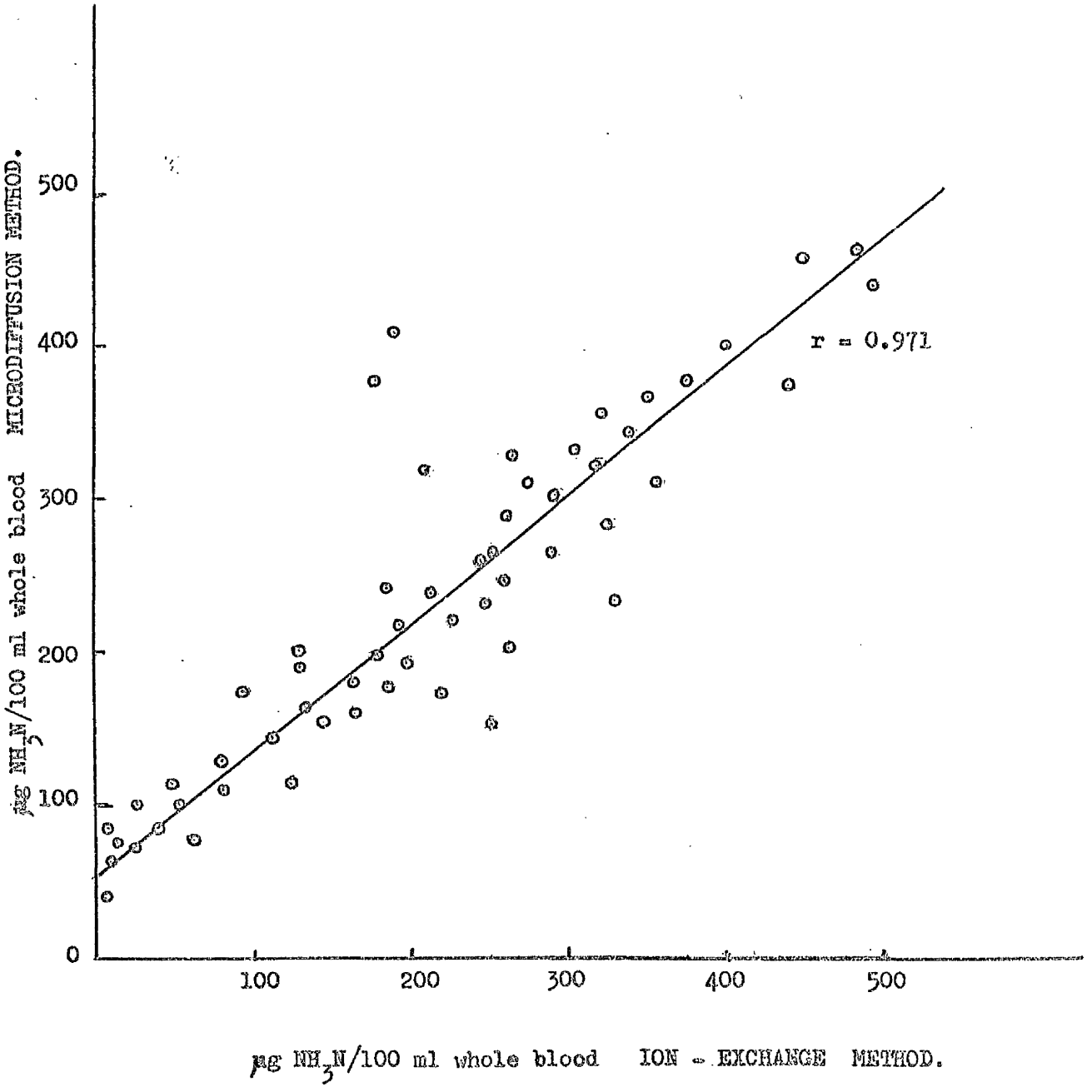
The two procedures were compared simultaneously using jugular blood from both normal sheep and sheep following oral administration of urea (0.3 - 0.6 Kg liveweight) to yield various concentrations of ammonia in the peripheral circulation. A total of I3 Blackface lambs were used for this purpose. This study was performed over a period of several weeks (I0/I0/68 - 2/I/69). A total of 54 samples were collected and analysed for ammonia concentration by both methods. All analyses were performed in triplicate which were in good agreement. Typical values for ten samples covering the range 0 - 450 µg NH_3N / I00ml were as follows.

	NH_3.N / I00 ml.			Triplicate samples.
Sample No.	a	b	c	
I	I20	I32	I22	
2	50	42	52	
3	2I2	2I0	222	Microdiffusion.
4	308	298	306	
5	444	427	430	
6	68	66	60	
7	382	37I	390	
8	I54	I59	I68	Ion-exchange.
9	320	309	322	
I0	20	I5	30	

The results are presented graphically in Fig. 2 . The correlation between procedures was very good. Thus both procedures give proportional concentrations which are quite acceptable for relative comparisons.

Values obtained for normal blood ammonia concentrations (ie. below I00 µg NH_3-N / I00 ml whole blood) however differed somewhat. Results from the diffusion technique were invariably higher than those obtained using the resin method at this lower concentration. However normal values

Figure 2. The comparison between a microdiffusion and an ion-exchange method for the determination of blood ammonia in ruminants.



for sheep blood determined by both procedures generally agree with those reported by Reif (I960) who used the Conway procedure with appropriate corrections and with normal values for humans (Reinhold & Chung, I96I). The report by Mc. Baron & McInnes (I968) recording normal ammonia concentration in sheep to be over 200 µg / I00 ml and sheep apparently recovering after exhibiting in ammonia intoxicosis concentrations of I,600 µg % must be viewed with considerable caution.

Experiment. 2.

Possible interference effects of Calcium and Magnesium ions in the determination of blood ammonia by cation exchange resin.

This experiment examined the optical density noted at 415 mμ following Nesslerization after varying quantities of magnesium and calcium had been added to aqueous ammonia standards. The results obtained from Experiment I. indicated that at concentrations of ammonia below about 100 μg % the resin technique yielded values somewhat below those obtained by microdiffusion. The possibility exists that plasma calcium and magnesium may be interfering.

Aqueous ammonia standards were prepared that contained :-

a) a range of calcium concentration from 2.0 - 10.0 mg %

b) a range of magnesium concentration from 0.5 - 2.0 mg %

c) No additions.

In addition , a ' plasma standard ' was prepared where suitable additions of ammonia had been added to freshly separated plasma. The magnesium and calcium concentration in the plasma was determined by an atomic absorption technique.

Each determination was performed in duplicate.

Results The results are shown in Table I.

For the aqueous samples magnesium but not calcium, addition depressed the optical density of the Nesslerized sample. Plasma (containing 2.07 mg Mg and 10.25 mg Ca %) with added ammonia exhibited optical densities upon Nesslerization very close to that of the aqueous ammonia standards.

The optical densities noted are detailed below.

Table. I. Concentration of NH_3- N /100 ml in Standard.

	0	100	200	300	400
Aqueous Samples					
No addition	10	15	19	21	26
+ 10 mg Ca	10	14	-	-	26
8 mg Ca	9	14	-	-	24
6 mg Ca	11	14	-	-	26
2 mg Ca	10	15	-	-	25
+ 20 mg Mg	6	6	-	-	10
1.5 mg Mg	6	8	-	-	12
1.0 mg Mg	7	7	-	-	21
0.5 mg Mg	8	10	-	-	18
10 mg Ca + 2 mg Mg	10	11	11	13	12
PLASMA (2.07 mg Mg & 10.25 mg Ca %).	9	14	18	21	25

A test-tube examination of the loss of colour formation when magnesium is included in the aqueous ammonia standard revealed that loss of stable colour occured only when the resin was added to an otherwise colour-stable solution of ammonia and added magnesium with Nesslers solution. A precipitate quickly formed which rapidly settled.

It must be concluded however that the lower ammonia concentrations observed in normal sheep from analysis by the resin method cannot be attributed to magnesium ion interference since the plasma standard (containing 2.07 mg Mg %) exhibited similar optical densities to those obtained from the Nesslerization of the aqueous ammonia standards.

It is likely that even with the careful conditions imposed upon the microdiffusion method that some ' artifactual ' ammonia formation may still occurr.

3). Suitability of Analytical Method.

Careful consideration was made regarding the suitability of each of the methods for the analysis of blood ammonia. The resin method offered several important advantages for the purpose of analyses intended during the course of this thesis. The resin procedure was by far the easiest to employ especially when repeated blood samples were collected for immediate analysis from several animals at intervals of 30 minutes or less. The tubes once prepared and charged with resin were easily transported and handled in situations where large numbers of samples were to be analysed. The blood samples were easily handled immediately upon collection. Labelling of samples was simplified , (one tube per sample). Samples could be handled with great efficiency and simplicity if required in the field. In contrast, although analytically yielding valid results, the micro-diffusion technique is the form employed in this study, had several disadvantages. Whereas the analysis could be held at the point when the blood proteins had been washed off the resin and resumed at a more convenient time, the nature of the microdiffusion technique did not allow the procedure to be held at any point. The microdiffusion equipment is somewhat cumbersome and requires an electric power source. Time is therebye lost in transporting the blood samples to the laboratory increasing the likelihood of ' artifactual ' ammonia production. (Conway I957).

It was accordingly decided to adopt the resin procedure for all further blood ammonia determinations. Acland & Strong (I968) concluded from their experiment comparing a Seligson microdiffusion technique and an ion-exchange method for the determination of blood ammonia " Recoveries

by the ion-exchange method are adequate and consistent. Recoveries by the microdiffusion method are inconsistent . and unsatisfactory".

One other major disadvantage with the microdiffusion technique is in the actual performance and handling of the method. For example, if the glass rod should touch the side of the diffusion vessel upon removal, the analysis is useless, as any accidental splashing of the acid-wetted rod voids the analysis. The whole procedure requires much care and precise handling whereas in comparison the resin technique is simpler and is not liable to such handling errors.

4). Storage of blood samples.

Investigations were performed to examine the possibility of storing blood samples for the analysis of blood ammonia concentration at a later date using the ion-exchange procedure. The results of these investigations are summarised as follows. Only whole blood ammonia concentrations proved to be of value for comparative purposes, since the ammonia levels in whole blood and plasma did not appear to bear a constant relation to each other. Immediate deproteinization of whole blood by TCA and subsequent freezing proved of no value since the low pH of the clear supernatent denatured the resin material. Immediate freezing of heparinised whole blood by solid CO_2 on the spot was the most promising procedure examined. Good correlation ($r = +0.9I$) was obtained between the analysis of the freshly drawn blood and the thawed frozen treated blood provided the analysis was performed no later than 72 hours after collection and freezing. No problems were encountered upon thawing.

5). Colorimetric end-point determination.

Two other procedures for the colorimetric determination of ammonia by the resin method were examined. These were the Bertholet reaction as described by Emmet (I968) and a Ninhydrin procedure described by

Jacobs (I959). Both proved to be excessively lengthy in procedure, (requiring periods of incubation for the colour development) and both gave values for ammonia concentration in excess of that obtained using Nesslers solution.

For the purposes of blood ammonia analysis described in this thesis the ion exchange procedure of Hutchison &Labby (I96I) together with the Nessler solution colour formation was hereafter adopted.

6). Determination of the concentration of ammonia in milk.

Two procedures , the microdiffusion and resin methods were employed in a study undertaken to examine the ammonia content in milk. The results of the examination can be summarized as follows.

Determination of the ammonia concentration of whole milk using the resin technique proved worthless since on the addition of the milk to the resin the protein precipitated and quickly coated the resin surface. Recovery of added ammonia to whole milk by this method yielded recoveries in the order of only I5 - 34 % . (I0 samples) In contrast, the microdiffusion technique appeared to be perfectly satisfactory. Added ammonia to whole milk resulted in excellent recoveries. (98 - I04 % , 32 samples with a concentration range from 0 - I000 μg NH_3/ I00ml). Storage of milk samples by either TCA precipitation or by the freezing of whole milk proved to be totally unsatisfactory. Milk samples were analysed only when fresh.

7). The determination of the concentration of ammonia in rumen liqour.

Three methods were examined for the determination of ammonia content in rumen samples . These were the microdiffusion and resin techniques as previously described and a modification of the technique outlined by Waite & Wilson (I968).

In all determinations freshly drawn rumen liqour was strained

through cheesecloth (4 layers) before any further manipulation occured. The ammonia content of rumen liquor is generally in the range 0-I20 mgs. NH_3-N /I00 ml. (Johns I955) For this reason dilutions of up to I/I000 were necessary before analysis by either microdiffusion or ion-exchange resin was performed. The modified method of Waite & Wilson (I968) used in this study was as follows:-

I ml of strained liquor was added to 9 ml of a solution of acidified sodium tungstate. (0.75% sodium tungstate in 0.075 N Sulphuric acid). Following centrifugation at 3,000 r.p.m. for I0 minutes, 5 ml of the clear deproteinized supernatent was taken and placed in a Hoskins distillation apparatus to which was added 2 ml 20% sodium hydroxide. The liberated ammonia was steam distilled into I0 ml 2% Boric acid with Methylene red / Methylene blue (mixed indicator). This solution was titrated against a standard hydrochloric acid. (approx. 0.0I5 N)

Some 48 rumen samples of varying ammonia content were analysed simultaneously by each of the three different procedures. The results obtained can be summarised as follows.

The ion-exchange procedure involved diluting raw strained rumen fluid with deionized water in the range I/I00-I/I000. Results were generally unsatisfactory in that triplicate analysis did not agree well, (Error $\pm$ I8%) and that recovery of added ammonia to the raw rumen fluid was subject to considerable error (62-I44%).

Microdiffusion analysis of diluted rumen fluid (I/I00-I/I000 with deionized water) gave somewhat better results in that triplicate analysis agreed fairly well (Error $\pm$ 5.0%) and recovery of added ammonia to the raw rumen fluid was quite good, (92-I06%). An unfortunate feature of both microdiffusion and ion-exchange resin methods for the determination of rumen ammonia concentration was that it was not possible to treat the raw rumen fluid with an acidic deproteinizing agent (eg. Sodium Tungstate or TCA) and necessarily analyses had to be performed as soon as possible after collection.

In contrast, the modified method of Waite and Wilson (I968) gave excellent recoveries of added ammonia (98 - IOI %, Error $\pm$ 2.I %) and afforded both a deproteinization of the material and no large dilutions of the raw fluid. It is interesting to note however that correlation between both microdiffusion and steam distillation methods were quite good. ($r = + 0.78$).

The modified method of Waite and Wilson (I968) was subsequently adopted for rumen ammonia determinations performed during this thesis.

Further investigation showed that rumen samples once deproteinized and centrifuged could be stored by freezing indefinately for subsequent analysis. Deproteinization with ice-cold TCA proved to be ineffective. No increase in ammonia was noted if urea was added to raw rumen fluid in quantities calculated to yield increases of c.IOO mg NH_3-N / IOO ml if the urea was to be hydrolysed.

Estimation of Blood Urea

Principle

The sample of blood is incubated with the enzyme urease which converts the urea to ammonia. The proteins are precipitated and the colour produced with Nessler's Reagent compared colorimetrically with the colour produced under the same conditions with a standard urea solution.

Procedure

Test: Add 0.2 ml. of blood (serum or plasma) to a I5 ml. tapered centrifuge tube containing 3 ml. of isotonic sodium sulphate solution.

Standard: Add 0.2 ml. of standard urea solution to a I5 ml. tapered centrifuge tube containing 3 ml. of isotonic sodium sulphate.

Blank: Add 0.2 ml. of distilled water to a centrifuge tube containing 3 ml. of isotonic sodium sulphate solution.

Place "test""standard" and "blank" tubes in a water bath until contents reach 37°C.

Add to each tube 0.2 ml. of urease suspension prepared by grinding one urease tablet in 5 ml. of 30% methanol.

Stopper tubes with rubber bungs; mix and incubate for 20 minutes at 37°C.

To each tube add 0.3 ml. zinc sulphate solution and 0.3 ml. 0.5 N sodium hydroxide to precipitate proteins, mixing by inversions after each addition.

Centrifuge and treat 2 ml. of supernatent with 5 ml. ammonia-free distilled water and I ml. Nesslers Reagent and read against water after standing for 30 seconds in the colorimeter at 450 mu.

(N.B. Measure out tests, standards and blanks and only add Nessler's Reagent prior to reading.)

It was necessary to employ a standard curve for calculation of

urea concentration since the colour formed does not obey Beer's law at all concentrations encountered.

REAGENTS:

1) Nessler's Reagent - (B.D.H. Ltd.)

2) Standard urea solution - Dissolve 100 mg. urea in 100 ml. distilled water. Dilute as required.

3) Isotonic sodium sulphate solution - 30 g. crystalline Na_2SO_4 $10.H_2O$ or 13.2 g. anhydrous Na_2SO_4 are dissolved in water and made to 1 litre.

4) Zinc sulphate - 10 g. crystalline $ZnSO_4$ 7 H_2O are dissolved in water and made to 100 ml.

5) 0.5 N - sodium hydroxide

N.B. For values greater than 200 mg. urea/100 ml. blood, dilute the protein free supernatent with distilled water and measure optical density obtained with 2 ml. diluted supernatent, 5 ml. distilled water and 1 ml. Nessler's Reagent. Multiply result obtained by the dilution factor used for the supernatent.

Estimation of Milk urea (+ ammonia).

The procedure as described for the estimation of blood urea was adopted. No problems were encountered. Recovery of added urea to whole fresh milk (giving a range of final concentrations between 30-120 mgs.%) was excellent. (Error $\pm$ 3.0%)

Other Methods.

For the analysis of all other constituents of foodstuffs, faeces, urine and blood described here standard analytical techniques were adopted. Only a brief outline of these various methods is given below.

I. Foodstuffs and faeces.

a). Dry matter. By drying in a hot air oven at 90°C for 48 hrs.

b). Crude protein. Semi-micro Kjeldahl procedure followed by the steam distillation of an aliquot of the alkalinized solution into boric acid and the resultant ammonia determined by titration with standardised N/70 HCl.

c). Ether extract. Repeated extraction with petroleum ether (40-60°C B.Pt.) in Soxhlet apparatus followed by the evaporation of ether and weighing the residue.

d). Crude fibre. By successive treatment with dilute acid and alkali.

e). Ash. By ignition at dull red heat.

f). Urea in foodstuffs. Removal of protein material by treatment with zinc acetate and potassium ferrocyanide solution followed by decolourisation of the solution with activated charcoal. A colorimetric end-point is effected by reaction with p.dimethyl-aminobenzaldehyde.

g). Phosphorus. According to method described by Cavell, A.J. J. Sci. Food Agric. 6,479.

h). Calcium. According to method described by Piper, C.S. "Soil and Plant Analysis" p.279.

2. Blood constituents.

a). Plasma Calcium and Magnesium. By Atomic Absorption Spectrophotometry.

b). Phosphorus. Deproteinization with TCA followed by reaction with acidified ammonium molybdate and aminonaphthosulphonic acid.

c). Potassium. By Flame Photometry.

SECTION III.

The Production of Sugar Beet Cubes with added urea, phosphate and trace elements.

a). Introduction.

b). Proposed composition of the product.

c). Experimental.

Experiment I. The solubility of urea contained in SBP urea cubes in water.

Experiment 2. The in-vitro solubility of the total nitrogen content of SBP urea in rumen liquor.

Experiment 3. The production of ammonia in the rumen from dietary urea presented as different physical forms of sugar beet pulp.

Experiment 4. Blood ammonia concentrations in Blackface lambs fed urea-containing diets.

Experiment 5. Blood ammonia concentrations in Dairy Cows fed SBP urea cubes.

Discussion.

The Production of Sugar Beet Cubes with added urea, phosphate and trace elements

Introduction

Some 500,000 tons of molassed sugar beet pulp are available in Britain each year for livestock feeding. In recent years an increasingly large proportion of this output has been cubed (0.5 ins diameter, 0.5 - 1.0 ins length) and magnesium supplemented cubes are available. Molassed sugar beet pulp, as fed, has the following approximate composition; 10 % crude protein, 12.5 % crude fibre and 58 % soluble carbohydrate. The crude protein is about 60 % digestible but some 90 % of both the crude fibre and soluble carbohydrate are digested by ruminants. Evans (1960) has indicated that the starch and protein equivalents of molassed sugar beet pulp are 58.3 and 4.6 respectively. More recent work (to be described in subsequent sections) suggests that molassed sugar beet pulp nuts as currently produced has rather higher values than these. (e.g. Present-day material contains less fibre (12.5 %) than the 15 % quoted by Evans (1960) probably because of a currently higher inclusion of molasses). The approximate mineral composition is; 0.8 % Ca, 0.08 % P, 0.4 % Na, 0.4 % Cl, 0.6 p.p.m. Co, 15 p.p.m. Cu, and 50 p.p.m. Mn. Molassed sugar beet pulp is generally used as an energy source at about 4 lbs (1.8 kg) per day for adult and growing cattle. Compared with feeding cereals for an equivalent energy intake it supplies appreciably more calcium but markedly less phosphorus. When used as a supplement to poor hay and/or straw the inadequacy in phosphorus content is of particular concern.

The inclusion of some 40 % molasses and the production of a rather hard cube which takes some time to soften and disintegrate in contact with liquid media (e.g. rumen fluid) would seem to make this an ideal material for the inclusion of urea. The high content of

readily soluble carbohydrate and the physical nature of the product might combine to make the urea inclusion both a safe and at the same time a more efficient nitrogen source to the ruminant and with a measure of slow-release.

This present and succeeding sections describe the formulation, production, properties and nutritional value of a urea and phosphate-supplemented molassed sugar beet cube including both vitamins and trace elements suitable for a wide range of feeding purposes.

Proposed Composition of the Product

Individual sugar beet factories have large (several 100 tons/day) outputs of cubed material and it was considered important to formulate a single product suitable for use in diverse practical circumstances. Work was begun in this sphere following suggestions by Hemingway of Glasgow University in 1967. Fortification of the molassed sugar beet pulp to give 16 - 17 % crude protein by inclusion of about 2.8 % urea was adopted throughout; initially some 2 % of dicalcium phosphate plus trace minerals was added to give a product containing about 0.4 % P but for the 1971 trials the dicalcium phosphate/trace element supplement addition was increased to about 3.0 % (giving 0.53 % P). This has been further increased to 4 % (giving 0.67 % P) in subsequent (1972) commercial production.

The procedure adopted by the British Sugar Corporation Ltd. for the production of molassed sugar beet nuts with about 17 % crude protein, up to 0.67 % P and with added trace elements and vitamins uses normally available raw materials and existing large scale production processes. After extraction of the beet juice in large continuous diffusers, the residual shredded beet pulp is pressed in twin screw presses which yield the pressure pulp at about 22 % dry substance. The conventional procedure provides for continuous additions of molasses to the pressed pulp and the mixture is then dried in direct fired rotary

driers to produce molassed dried pulp in a loose form. In some factories this material is then cubed to produce molassed dried pulp nuts.

In the plant for production of fortified molassed beet pulp, urea is added continuously to the molasses stream in a proportioning mixer. The urea dissolves readily in the molasses but this addition takes place in the same plant as is used at other times during the processing season for adding insoluble magnesite in production of molassed sugar beet pulp with added magnesite. The automatic control of the proportioning between molasses and urea is adjusted so that the final nutted product contains about 2.8 % urea.

The urea-containing molasses is mixed with the pressed pulp and dried as in the conventional process and then the dicalcium phosphate, trace minerals and vitamins are added as a powder to the dried molassed pulp in a proportioning mixer and this mixed product is then fed to the nutting machines. The proportioning between the dried molassed pulp and the phosphate/mineral/vitamin mixture is adjusted so that the final nutted product contains up to 0.67 % P together with the desired content of trace minerals and of Vitamins A and D.

This product (Triple Nuts, British Sugar Corporation Ltd.) could be used (a) as a proportion of an existing milk production concentrate by admixture and fed at 4 lbs (1.8 kg) per gallon, (b) with an equal weight of cereals (giving about 13.5 % crude protein) for growing cattle on a "barley beef" system or with some hay for more traditional cattle rearing, (c) with the higher phosphorus inclusion as a straw balancer in a range of cattle diets and (d) for pregnant or lactating ewes. A product with about 1.4 % Ca and about 0.45 % P fed at 4 lbs (1.8 kg) per gallon would provide about 25 g Ca and about 8 g P. (The A.R.C. (1965) recommended requirements are 12.5 g Ca and 7.7 g P). A mixture of 50 % of such a product with 50 % cereals fed ad lib would contain about 0.75 %

Ca and about 0.35 % P and would provide more of each element than the A.R.C. (1965) recommendations for 300 lb (140 kg) cattle gaining weight at 2.2 lbs (1 kg) per day. Pregnant or lactating sheep receiving 2 lbs (900 g) per day would thereby receive their full calcium requirement and an appreciable part of the A.R.C. (1965) recommended intakes from this supplementary food alone.

Additions of trace elements (iron, manganese, cobalt, iodine, latterly zinc and magnesium but not copper) and Vitamins A and D have also been included so that in each case the added concentrations are greater than those recommended by the A.R.C. and so that they could contribute towards the correction of any of these inadequacies present in other dietary components. For this latter reason sodium chloride was added in 1971 and the phosphorus level was further increased in the commercial product.

The experimental programme described in this and succeeding sections took place during the years 1968-69, 1969-70 and 1970-71. During each of these three winter sugar beet harvesting seasons, the British Sugar Corporation Ltd. manufactured experimental products by a variety of processes but using essentially the normal production procedures for adding molasses, drying, cubing etc. The additions of urea, dicalcium phosphate, trace elements and vitamins were broadly comparable during each of these three years. Several tons and latterly hundreds of tons of the materials described in Table were manufactured. In addition, in each year a comparable range of products were prepared which differed only in that no urea was included. These contained some 9.4 - 10.1 % crude protein compared with 16.6 - 17.1 % crude protein where urea was included. Throughout the remainder of this and succeeding sections the following abbreviations have been used as appropriate and infer the inclusion of dicalcium phosphate, trace elements and vitamins as in Table 2. e.g. molassed sugar beet pulp with urea (S.B.P. urea) and

Table 2. Composition of experimental supplemented molassed sugar beet pulp products as fed. (87% dry matter).

			1969	1970	1971*
Crude Protein %	With no urea		9.8	9.4	10.1
	Added as urea		7.2	7.2	7.0
	Total		17.0	16.6	17.1
Total Calcium	Ca %		1.25	1.32	1.40
Phosphorus.	P %		0.38	0.44	0.45
Manganese.	Mn.	p.p.m.	80	80	125
Copper	Cu.	p.p.m.	14	15	15
Cobalt	Co.	p.p.m.	13	13	8
Iodine	I.	p.p.m.	3	3	3
Added/ton. Vitamin A.		I.Us.	4	20	10
Vitamin D.		I.Us.	1	5	2.5

* In addition, 0.25 % sodium chloride, 0.12 % Mg and 40 p.p.m. Zn were included.

molassed sugar beet pulp without urea (SB.P. no urea) as appropriate for the particular year of manufacture.

Experimental

A number of experiments have been conducted to assess the rate at which the 2.8 % of urea incorporated in the nuts from the 1969 production material with 17.0 % crude protein (Table 2) appeared in solution. Further experiments have investigated the effects of feeding various quantities on the blood and rumen ammonia concentrations of both sheep and cattle.

Experiment 1. The Solubility of urea contained in SBP urea cubes in water.

This experiment was undertaken to measure the pattern of diffusion of urea into water at room temperature from different sizes of the urea - containing molassed sugar beet nuts. Material of constant diameter (0.5 inch) was subdivided into three fractions; (a) whole nuts (1 inch in length), (b) halved nuts (0.5 inch in length) and (c) quartered nuts (0.25 inch in length). A further sample (d) was crumbled by pulling the cubed material apart. Other material (e) was ground through 1 mm sieve using a laboratory hammer mill.

The same quantity (100 g) of each of the five materials were suspended separately in 800 ml water in 2 litre beakers at room temperature (21^{o}C). The contents were stirred gently with a glass rod every fifteen minutes. Portions of supernatant liquid (3 ml) were withdrawn for determination of urea at frequent intervals over seven hours and a final sample was obtained after overnight standing at 24 hours.

Results

Figure 3 details the amounts of urea present in the samples of supernatant liquid withdrawn at intervals over the 24 hour period of immersion in water at room temperature. The final concentration reached in each case was in the range 360 - 370 mg urea/100ml

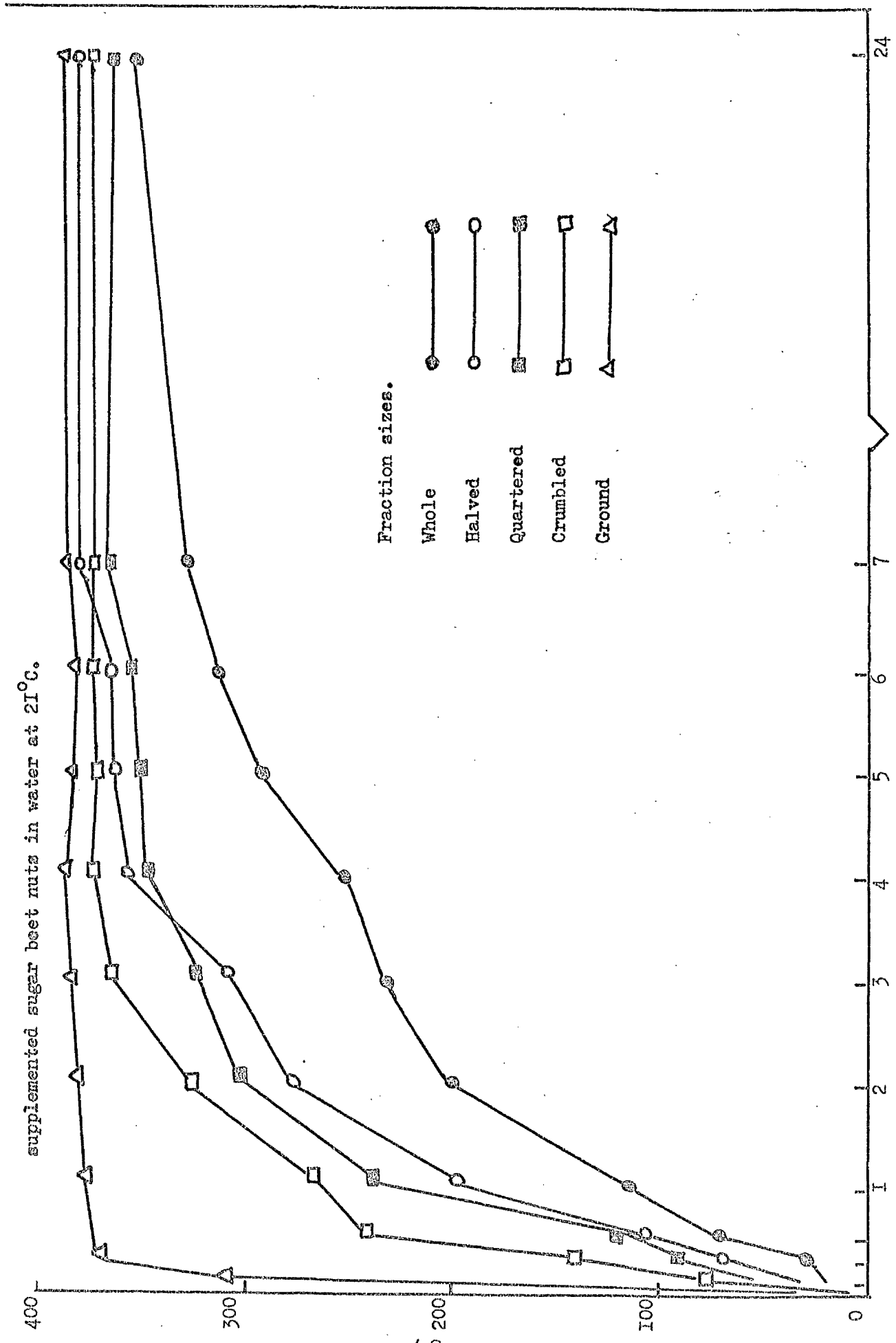


Figure 3. Concentrations of urea (mg/100 ml) in the supernatent liquid from various sized fractions of urea-supplemented sugar beet nuts in water at 21°C.

supernatant liquid. The concentration of urea in solution increased most rapidly in the finely divided materials. For the ground material the urea concentration was 312 mg/100 ml after only 5 minutes. A value of 240 mg/100 ml was found for the crumbled material after 30 minutes, and the urea was substantially in solution after one hour. In contrast, the full amount of urea appeared in the supernatant liquid after about 4 hours with the quartered nuts and halved nuts but was not fully attained after as long as 7 hours with the 1 inch length whole nuts.

The contrasting rates of appearance of the urea in solution can be partially explained on a basis of initial differences surface area and partially because the larger fractions took an appreciable time to fully absorb water. Moisture did not fully penetrate the whole or halved nuts for about 2 hours. Disintegration of these sizes of material did not take place until after a further 2-3 hours.

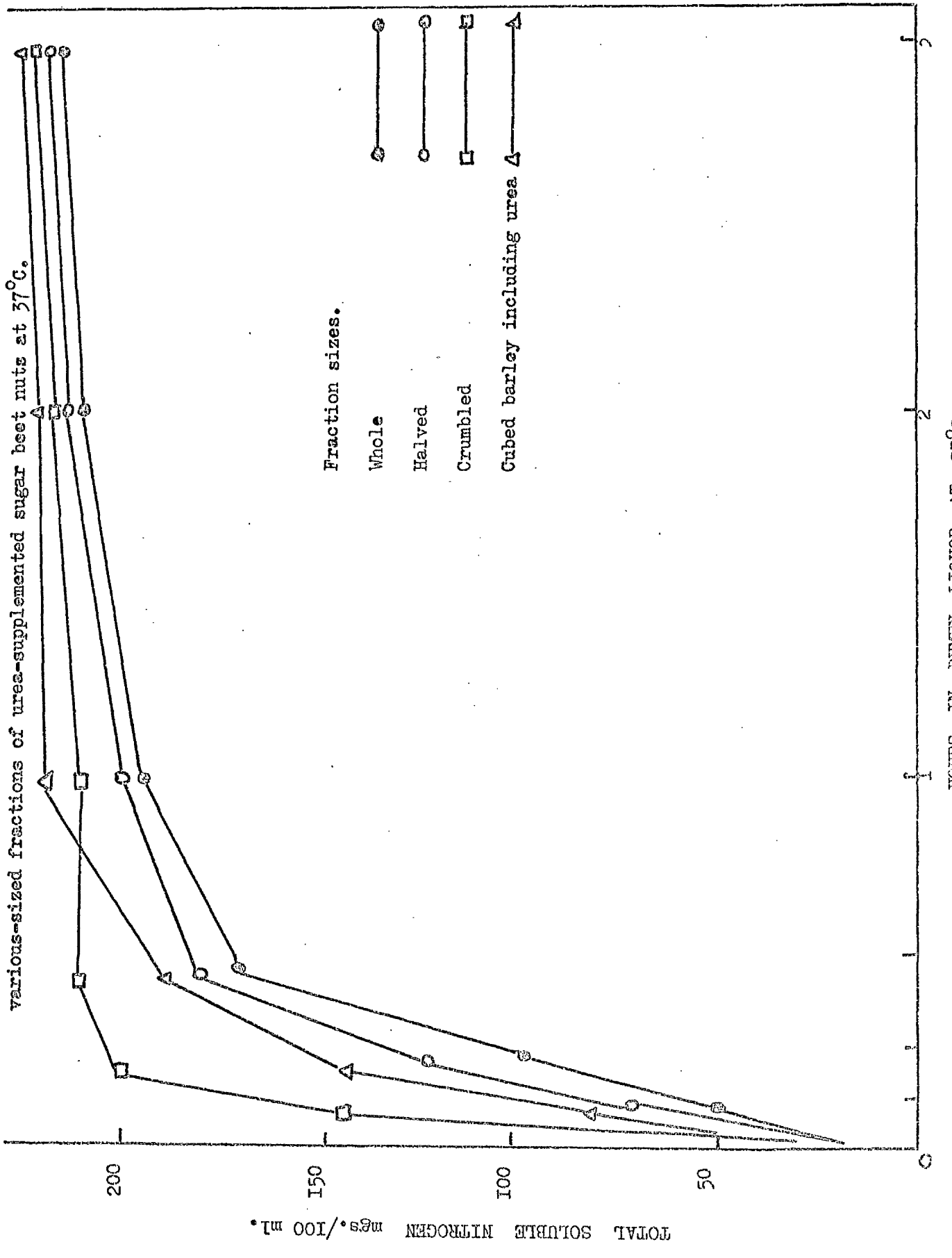
Experiment 2. The in-vitro solubility of the total nitrogen content of SBP urea in rumen liquor.

Separate 50 g portions of (a) whole (1 inch), (b) halved (0.5 inch) and (c) crumbled nuts containing 2.8 % urea as in Experiment 1 together with (d) 50 g of cubed barley of similar diameter and 1 inch in length and containing 2.8 % urea were placed in 400 ml portions of freshly drawn rumen liquor from a hay-fed cow fitted with a rumen fistula. The samples were kept under an atmosphere of nitrogen at 37°C and were agitated gently every 15 minutes. Samples of rumen liquor were withdrawn at intervals over three hours for the determination (after straining through muslin) of total nitrogen.

Results

When the urea-containing molassed sugar beet nuts were placed in rumen liquor at 37°C the final concentration of soluble nitrogen attained was about 220/mg/100 ml (Figure 4). A concentration of about 200 mg N/100 ml was reached after 15 minutes in the rumen liquor

Figure 4. Concentrations of total soluble nitrogen (mg/100 ml) in rumen liquor following addition of various-sized fractions of urea-supplemented sugar beet nuts at 37°c.



containing the crumbled material but only after about 1 hour for the halved or whole nuts. The appearance in solution of urea from the barley cube was intermediate between these two extremes and a concentration of 200 mg/100 ml was reached after about 30 minutes. The barley cube disintegrated within a few minutes in the rumen fluid but entire sugar beet nuts were not fully saturated with rumen liquor for almost 1 hour.

Experiment 3. The production of ammonia in the rumen from dietary urea presented as different physical forms of sugar beet pulp.

Four materials each containing 2.8 % urea were prepared; (a) whole molassed sugar beet nuts with urea (1 inch), (b) crumbled molassed sugar beet nuts with urea, (c) crumbled molassed sugar beet nuts made originally without urea but including an admixture of 2.8 % crystalline urea and (d) barley cubes (1 inch) containing 2.8 % urea as described in Experiment 2.

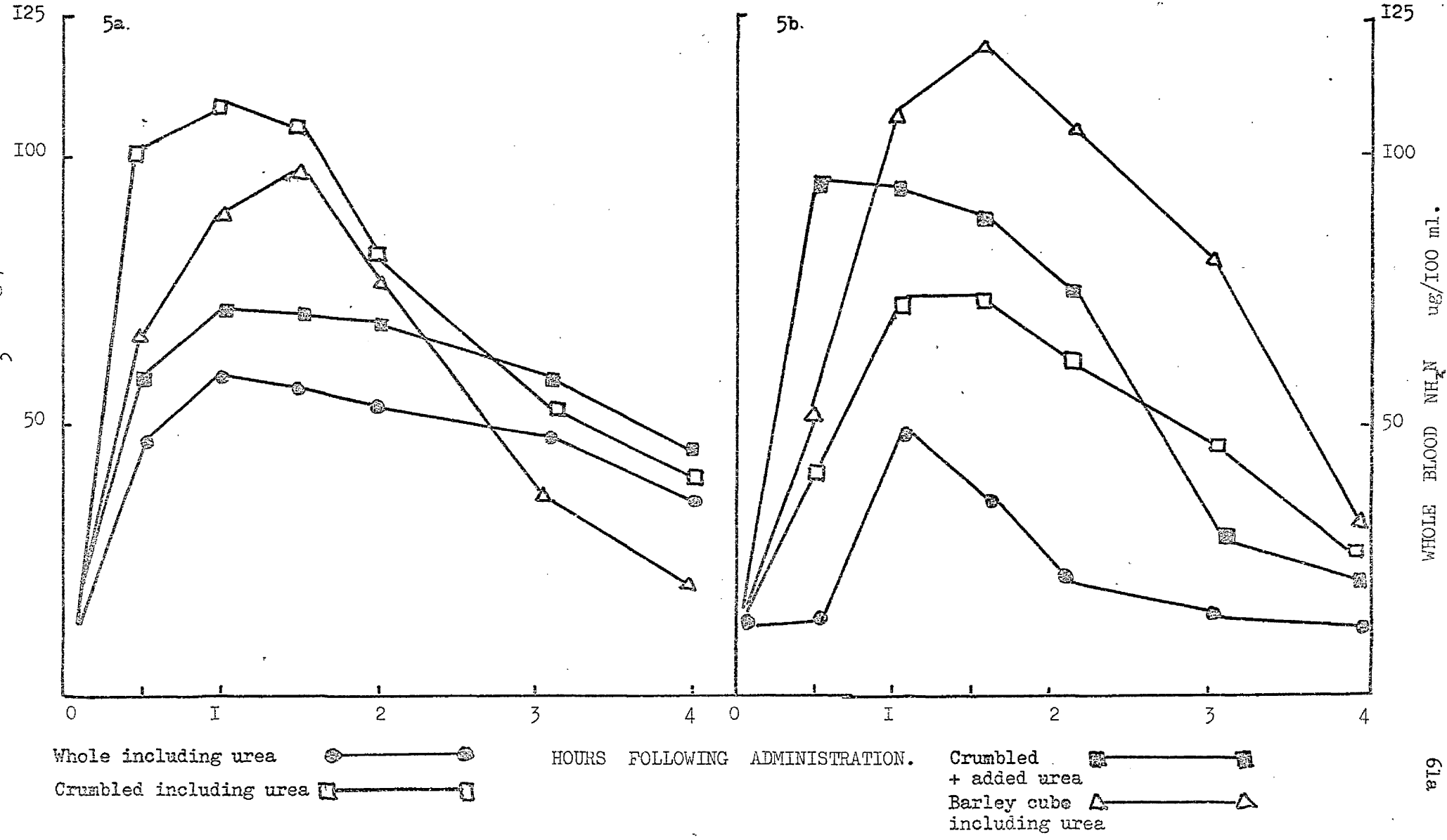
Eight lbs (3624 g) of each material containing 101 g urea were placed separately in turn directly into the rumen of a fistulated cow. Each material was introduced to the cow on two separate occasions and the total of eight investigations were conducted at weekly intervals. The cow was fed a daily ration of 20 lbs of hay in two equal feeds but none was given on the morning of each investigation. Samples of rumen liquor and jugular blood were obtained at regular intervals over four hours after introduction of the experimental materials and analysed for ammonia nitrogen.

Results

Figures 5 a and 5b indicate the changes in ammonia concentrations in blood and rumen liquid, each point being the mean of two similar observations. Rumen ammonia concentrations after 1 hour were in the order of 100 - 110 mg. NH_3.N/100 ml for both the barley cube and the

Figures 5a & 5b.

Concentrations of ammonia nitrogen in rumen liquor (mg/I00 ml) and blood (ug/I00 ml) following administration of various sized fractions of urea-supplemented sugar beet nuts per fistula.



crumbled sugar beet containing an admixture of 2.8 % crystalline urea. In contrast the concentration for the whole (1 inch) sugar beet nuts in which urea had been incorporated at the time of manufacture never increased beyond 60 mg NH_3.N/100 ml.

Blood ammonia concentrations followed a similar pattern. The highest values (100 - 120 µg NH_3.N/100 ml) were recorded after 30 - 60 minutes for both the crumbled sugar beet and the barley cube containing admixed crystalline urea. The concentration was much less (50 µg/100 ml) where the urea was included in the whole (1 inch) nuts and was intermediate where the manufactured material was crumbled before additions to the rumen.

Experiment 4. Blood ammonia concentrations in Blackface lambs fed urea-containing diets.

Two groups, each of five Blackface lambs (mean liveweight 25 kg) were fed 1.0lbs (454 g)/day over two weeks of either (a) a molassed sugar beet nut with no urea inclusion or (b) cubed barley. After a two-day fast the sheep were fed 1.0 lb/head of either (a) the standard 2.8 % urea-supplemented nut or (b) cubed barley of similar dimensions containing 2.8 % urea. The two foods were eaten readily by all the sheep within 15 minutes and the amount of urea consumed was 12.7 g. This is equivalent to 0.5 g urea/kg liveweight which is in the order of the dose which is potentially toxic if given on one occasion in solution to a hungry sheep (Austin, 1967). Blood samples were taken periodically during the three hours after feeding.

Results

The maximum concentration of ammonia in the blood of the two groups of lambs was attained about 45 minutes after feeding the two concentrates. The mean concentration for the lambs after feeding the urea-supplemented barley for the first time was 84.0 $\pm$ 26.2 µg NH_3.N/100 ml and that for the urea-supplemented sugar beet nut was 15.0 $\pm$ 6.9 µg/100 ml This difference was highly significant ($P = 0.001$).

Experiment 5. Blood ammonia concentrations in Dairy Cows fed SBP urea cubes.

The standard 2.8 % urea-supplemented sugar beet nut was fed to a total of 14 milkimg cows which had not previously consumed urea in any form. Feeding took place at the morning milking at 6 a.m. and the cows had not received roughage since the previous day at 6 p.m. The amounts fed were 4, 8 or 12 lbs supplying respectively 51, 102 and 153 g urea. The basal diet of the cows was either hay (3 cows) or silage (11 cows).

Blood samples were taken at regular intervals during the four hours after feeding and analysed for blood ammonia concentration.

Results

Examination of the results of the blood analyses resulting from the feeding of urea-supplemented sugar beet nuts for the first time to dairy cows indicated that the peak ammonia concentrations occurred between 60 and 90 minutes after feeding. The mean maximum concentrations recorded are detailed in Table 3 .

Table 3 . Mean maximum concentrations of ammonia (µg NH/100 ml) in the plasma of cattle 75 minutes after feeding urea-containing molassed sugar beet nuts.

Basal diet	Hay	Silage	Silage	Silage
No. of cows	3	6	2	3
S.B.P. incl. 2.8 % urea (lbs)	4	4	8	12
Urea (g.)	51	51	102	153
Plasma NH_3.N. (µg 100 ml)	195	58	240	334

No single individual exceeded 360 µg NH_3.N/100 ml. It is generally considered (e.g. Repp, Hale, Cheng and Burroughs, 1955) that toxicity signs do not develop below about 600 µg. NH_3.N/100 ml. The feeding of 12 lbs of urea-supplemented sugar beet pulp nuts (153 g urea) at one of the two daily milkings would, if repeated at the subsequent

milking, provide a total nutrient intake approximating to that for the production of 6 gallons of milk per day and is unlikely to be exceeded in practice. Accordingly, there appears to be an adequate safety margin for normal levels of feeding even when the material is introduced suddenly and without sensible practical precautions.

Subsequent experiments have shown that the continued feeding to dairy cows of 8 lbs per day of urea-supplemented sugar beet nuts of the formulation described in Table 2 resulted in an apparent measure of adaptation to urea feeding. In contrast to the concentration of 200 - 300 µg NH_3.N/100 ml blood recorded after the first feeding, the amounts were found to be reduced markedly after a few days and after 3 - 4 weeks were in the general range of 30 - 50 µg/100 ml measured one hour after feeding.

Discussion

If a less readily soluble form of urea were available for ruminant feeding it is generally accepted that there would be a decrease in potential toxicity and a possible increase in the efficiency of dietary utilisation. Various approaches which have been made to formulate non-protein nitrogen containing materials which have a reduced tendency to elevate rumen and blod ammonia concentrations have included, inter alia investigations involving; (a) the relative insolubility of materials such as biuret (e.g. Waite and Wilson, 1968) and isobutylidene diurea (e.g. Parkins, Ritchie and Hemingway, 1971), (b) the acidity of urea phosphate (e.g. Perez, Warner and Loosli, 1967), (c) the association between urea and gelatinised starch-rich materials (e.g. Helmer, Bartley and Deyoe, 1955) and (d) the coating of urea prills with insoluble materials (e.g. Johnson, Bentley and Hershberger, 1962). Mostof these materials are inherently expensive to produce relative to urea and have a lower nitrogen content.

In the present section preliminary experiments with molassed

sugar beet pulp nuts fortified with urea, minerals and vitamins indicate that the high content of molasses (40 %) and the hardness of the nuts (which results in a relatively slow diffusion of urea into water or rumen fluid) combine to produce a urea-containing product which has advantages comparable to more expensive and sophisticated non-protein nitrogen containing materials.

SECTION IV.

The Use of Sugar Beet Pulp Products in the feeding of Dairy Cows.

a). Introduction.

b). Experimental.

Analytical methods.

Dairy Experiment I. (1969).

Dairy Experiment 2. (1970).

Dairy Experiment 3. (1971).

Dairy Experiment 4. (1970).

Dairy Experiment 5. (1971).

Discussion.

c). The influence of diets containing sugar beet pulp ($\pm$ urea) on the composition of milk. (Dairy Experiments I - 3).

1). Ammonia content in milk. (Expt. I. 1969).

2). The interrelationship of dietary protein intake, blood and milk urea concentrations and the total amount of urea secreted in the milk.

THE USE OF SUGAR BEET PULP PRODUCTS IN THE FEEDING OF DAIRY COWS.

Introduction

A large amount of research has been conducted to assess the nutritive value of urea for feeding lactating dairy cows. Large variations in response have been recorded. Most of the experiments have involved the inclusion of 2-3 % urea (i.e. about 5.5-8.5 % crude protein) in the concentrate part of the ration which otherwise consisted of cereals with about 10% crude protein. Such mixtures have generally been fed at about 4 lbs per gallon for the whole of the milk production and have been compared with equal protein intakes based in the main on vegetable proteins, especially extracted soya bean meal. Frequently the cattle used have had low mean milk yields in the order of 2-3 gallons per day. The results of these trials have generally indicated that urea-containing rations are inferior to those based on more traditional sources. The extent of the reduction in yield has been in the order of 1.8 lbs of milk per day based on a mean yield of about 30 lbs per day for an inclusion of about 2.25 % urea in the concentrate ration i.e. a reduction of some 5-6 % in total yield.

Several exhaustive reviews have been published containing sections dealing with the feeding of urea to dairy cows (e.g. Briggs, 1967; Helmer & Bartley, 1971; Reid, 1953, 1966; Hungate, 1966; Armstrong & Trinder, 1966; Van Horn, 1967; Chalupa, 1968; Waldo, 1968). Table 4 gives a summary of the results from the principal experiments conducted under controlled conditions.

Several important features concerning the difficulties and limitations of conducting comparative trials with dairy cattle with particular reference to urea have been ennumerated by Helmer & Bartley (1971). In practice there may be difficulties experienced in obtaining a sufficiently large number of cows of similar age, yield and stage of lactation for experimental purposes. Other problems involve practical

TABLE 4.

Effect of Urea on Milk Production

(after Armstrong & Trinder, 1966)

Source	Reduction in Milk Yield lb./day	% Urea in Production Ration Approx.	Yield of Milk Gal./Day Approx.
Campbell, (1963)	6.40	3.0	4.0
Wetteraw et al., (1959)	3.55	1.5	3.0*
Balch and Campling, (1961)	3.45	3.0	3.0
Schmidt et al., (1940)	3.23	3.0*	2.5
Ulvesli, (1949)	2.87	2.5	3.0
Weston, (1948)	2.85	3.0	5.5
Felinski, (1962)	2.70	1.25	2.5
Willett et al., (1946 b)	2.40	2.5	2.75
Rys et al., (1960)	2.32	2.0	3.0
Burt, (1965)	2.24	2.0	3.0
Thompson et al., (1952)	2.20	2.75	3.0
Bartlett and Blaxter, (1947)	2.15	2.0	4.0
Archibald, (1943)	2.10	3.0	4.25
Rust et al., (1956)	2.10	1.5	2.5
Davis, R.F., (1953)	1.85	2.25	3.5
Willett et al., (1946 b)	1.80	1.25	2.75
Archibald, (1943)	1.70	3.0	4.0
Davis, R.F., (1953)	1.55	2.25	3.5
Ulvesli, (1949)	1.55	2.5	3.0*
Willet et al., (1946 a)	1.30	2.5	2.5
Chugunkov, (1958)	1.27	3.0	2.5
Bartlett and Blaxter, (1947)	1.21	2.0	3.0
Rupoletal, (1943)	1.20	3.0	3.0
Loosli, (1956)	1.10	2.0*	4.25
Davis, C.L. et al., (1956)	0.80	1.5	2.75
Ulvesli, (1942)	0.72	2.5	3.0*
Popov, (1946)	0.65	1.25	2.75
Thompson et al., (1952)	0.60	2.75	3.0
N.I.R.D. (1947)	0.58	2.0	2.5
Lassiter et al., (1957)	0.20	1.5	2.5
Thompson et al., (1952)	0.10	2.0	2.5
Chugunkov, (1958)	-0.17	1.75	2.5
Mean	1.84	2.75 (equivalent to 6.3% C.P)	3.0

* Assumed.

feeding difficulties, variation in quality of roughage, the comparatively short effective feeding periods and illnesses occurring during the experiment. Indeed much of the work involving dairy cows has indicated some methodological deviation from the ideal. For example, a small number of animals, too short a period of investigation, variation in ad-lib intake of roughage, use of crystalling or prilled urea as an admixture rather than as a constituent of cubed, molassed diets, etc.

Differences in experimental conditions have probably led to some of the variation in results obtained when urea has been fed to dairy cows. For example, many of the earlier experiments in the literature were conducted with low yielding cows (e.g. Schmidt, 1940; Ulvesli, 1942). Other early experiments were conducted where cows received nearly ad-libitum amounts of good quality roughage leaving little and in some instances no additional protein required for production. In many of these experiments concentrate intakes were low by present day standards and little variation in yield was observed between diets containing different nitrogen sources. The difference in palatability of urea-containing diets may have accounted for some of the dissimilarities of yield in some experiments. (e.g. Archibald, 1943; Huber & Sandy, 1965; Horn & Mudd, 1971).

The conditions under which an experiment is conducted can influence the result obtained. The ideal technique should incorporate the full lactation period in the experiment. This is however rarely practical. Experiments conducted to assess the relative merits of different nitrogen sources in the production rations fed to dairy cows should properly include a diet providing inadequate protein. This would demonstrate more effectively the production effect of the nitrogen source under investigation. A low protein diet included as an integral part of a dairy experiment is always an aid in interpreting experimental results obtained in other countries where recommended feeding standards may often differ. Reid, Moe & Tyrell (1966), emphasized the difficulties of

evaluating protein requirements of lactating dairy cows from short term experiments when the contribution of body protein resulting from live-weight loss to maintaining milk yield is an uncertain factor.

It is important to appreciate that, at best, urea can only be expected to give results which are as good as (but no better than) the other protein-containing food stuffs with which it is compared. Frequently urea is used experimentally in comparison with diets which are the best which can be devised and which have been in regular use. Armstrong & Trinder (1966), concluded that "No clear recommendations can be made for cows giving low yields of milk i.e. below $2\frac{1}{2}$ gallons of milk daily. As regards cows giving more than $2\frac{1}{2}$ gallons daily it appears that urea is almost always inferior to conventional forms of protein in sustaining milk production". (See Table 4.). Urea usually produces better results than otherwise protein-deficient diets but it can be a milk depressant if added to diets already well supplied with protein (Opletalova & Lizal, 1963; Bartlett & Blaxter, 1947). Some inefficiency in the utilization of urea for milk production may occur, particularly in the higher yielding cows which are fed relatively large amounts of urea in only two feeds a day. (McNaught & Smith, 1947; Fairbairn, 1965). In contrast to the possible inefficiencies of twice a day feeding of urea the 'barley beef' ad-lib system as well as regularly supplying small amounts of dietary urea also maintains an acidic rumen pH as a result of the high cereal intake. At an acidic pH, the liberated ammonia is in the form of the charged ammonium ion which is tissue impermeable. (Hogan, 1961). This effectively reduces loss of ammonia across the rumen mucosa. The pH of the rumen of dairy cows are more often between pH 6.0-7.0, and loss of liberated 'free' ammonia from dietary urea may occur to some extent.

The Use of Sugar Beet Pulp in Diets for Lactating Cows

The use of urea in rations for dairy cows was extensively investigated in Germany in the late 1930's. Frequently, the urea was

incorporated in sugar beet residues. Nehring (1937, 1939), Ehrenberg, Nitsche & Muller (1938), and Schmidt & Kleisch (1937) in their experiments replaced about 30% of the total nitrogen of the concentrate diet with urea nitrogen. This was most often given as "Amidschnitzel" (amide-slices) i.e. a mixture of beet pulp, molasses and urea. The decrease in yield recorded amounted to 13 % of the milk yield compared with that where a vegetable protein source was fed. Wetterau, Schlegel & Holzschuh (1961) studied the possibility of using sugar beet pulp as a grain replacement in a urea-containing ration. The experiment compared the yields obtained when the following diets were fed for production; a low protein concentrate of sugar beet pulp alone, sugar beet pulp plus urea (where the urea constituted 25% of the crude protein of the concentrate diet), and potato flakes plus urea. The results showed greater yields of fat-corrected milk when the sugar beet plus urea was fed. This trial has only limited value however in that a diet containing conventional protein as the only supplemental nitrogen source was not included in the treatments. Musiál & Rys (1962) fed Friesian cows a diet of ensiled sugar beet pulp together with either urea or ammonium sulphate and reported a decrease in fat-corrected milk yield compared to that obtained when the normal concentrate was fed. There were no differences in the intakes of digestible crude protein per kg. milk produced or in the liveweight changes of the cows. Chomyszyn, Brelinski & Slabon (1962), concluded that up to 65 % of 'high protein concentrates' may be replaced by ammoniated or urea-containing beet pulp without any loss in milk yield or milk fat. The cows were fed according to Polish standards. Kaemmerer & Bollman (1968) reported feeding a ration of 'Dormaschnitzel' (beet pulp and urea) containing up to 240 g. urea per day to dairy cows without any reduction in the yield or quality of the milk. Previous literature seems to have a distinct lack of knowledge concerning the effect of the inclusion of large amounts of beet pulp in diets for lactating cows.

More recently studies by Bhattacharya & Lubbada (1970), and Bhattacharya & Slieman (1970), have included attempts to investigate more fully the use of sugar beet pulp as a grain replacer in dairy rations. A lactation trial involving 8 cows in a changeover design was conducted. A control ration containing 57 % of barley was compared with a ration containing 55 % dried sugar beet pulp. No significant differences were observed with regard to changes in bodyweight or yields of fat-corrected milk. In a further experiment Bhattacharya & Lubbada (1971), studied the effect of feeding four different amounts of beet pulp as a replacement for maize (i.e. 0, 50, 75 and 100 % of the maize) in the diets of dairy cows on liveweight change, milk yield and milk composition.

The experiment involved four cows in a 4 x 4 Latin square design. Each of the four cows were quoted as being fed a constant ration of 11 lbs alfalfa hay and 42 lbs of a 15 % crude protein concentrate per day, irrespective of yield! Milk yield and milk fat were reported not to be influenced by the rations.

Ronning & Bath (1962), studied the relative milk production value of barley, dried sugar beet pulp, molassed dried beet pulp and concentrated Steffen-filtrate dried beet pulp. They reported no difference in milk yields when the various beet pulps replaced about 25 % of the energy of a basal ration comprised of 70 % alfalfa hay and 30 % barley, The experiments so far reported tend to indicate that dried beet pulp properly supplemented is equal to maize or barley as an energy source for milk production.

The present study has concentrated on two main aspects;

(a) The inclusion of molassed sugar beet pulp in dairy rations at high levels and

(b) the effect of the added urea present in the sugar beet cube as a partial protein replacement for milk production.

Experimental

Three main experiments 1,2 and 3 were conducted during the winter periods of each of the years 1969, 1970 and 1971. Each experiment followed the same general design and the following information summarises their common features, more precise experimental details are outlined in the descriptions of the individual experiments.

In each of the trials three milk production concentrates were compared. The actual materials fed differed from one year to another but essentially in each year the comparisons were between

a) A low-protein concentrate.

b) A urea-containing supplemented concentrate.

c) A concentrate containing supplementary protein of only vegetable origin - generally equal parts of ground nut and cotton seed cakes.

The experimental design adopted in each case was that given by Cochran, Autrey & Cannon, (1941) for a changeover trial. This involved the feeding of each of the three separate concentrate rations to each of the cows on the trial in three consecutive periods which varied from 4-5 weeks from one experiments to another. The total number of cows involved in each of the years was eighteen (Trials 1 and 2) and twelve (Trial 3). In each case the cows were grouped in sets of three, i.e. Six sets each of 3 cows (Trials 1 and 2) and four sets of 3 cows (Trial 3). Each individual set of three cows was composed of animals of similar calving date and age, previous lactation yields, and the amount and stage of the present lactation. Only cows which had already attained their lactation peak were included in the trials. Both Friesian and Ayrshire cows were used but the individual cows in each set of three were invariably of the same breed.

The cows were housed in a traditional byre and stood in pairs. The maintenance diets were fed twice per day after each milking.

Concentrates were also fed twice per day at each of the milkings which commenced at 06.30 hours and 16.30 hours.

The various concentrates were fed at a constant rate of 4 lbs (1.8 kg) per gallon. As each of the three feeding periods inherent in each trial lasted 4-5 weeks, the concentrate allocation for each cow was adjusted at the end of each week according to the actual mean yield recorded over the previous week, (Lucas, 1943).

The cows were milked by machine employing a "round the byre" pipe-line. Individual yields (and milk samples when required) were obtained by use of Milkoscope Recorders (Foss Electric). These are approved for use in the official milk recording scheme of the Scottish Milk Marketing Board, and were calibrated before use. (A recording error of $\pm$ 2.0% was noted). Morning and evening yields were separately assessed and, where necessary, individual or combined samples were stored in bottles for subsequent analysis.

Analytical Methods

Milk samples were analysed as required (generally twice weekly) for fat (Gerber, BSI Publ 696, Parts 1 and 2), total solids (by both drying and density determination) and total protein (Kjeldahl technique). In Trial 2 milk fat and protein was additionally determined by the laboratories of the Scottish Milk Marketing Board on automated apparatus. Urea-plus-ammonia N in the milk was determined after incubating a buffered, protein-free milk filtrate with a Jack-bean meal urease preparation (B.D.H. Ltd.). Ammonia was determined by a modified micro-diffusion technique. (Reinhold & Chung, 1961).

The daily yields of fat-corrected milk (FCM) were calculated according to the equation given by Gaines (1928).

DAIRY EXPERIMENT I. (1969).

The changeover design (Cochran et al., 1943) was employed which involved eighteen cows divided into six groups each of three cows. They were allotted at random to the treatment sequences in 2 orthogonal 3 x 3 Latin squares. Six Friesian and twelve Ayrshire cows were used. The average lactation number of the cows was 3, with only three cows having had more than 3 lactations. Each cow had been milking for 50-70 days before the experiment started. The three concentrate mixtures used in the experiment were:-

Diet	A	B	C
% Constituents			
S.B.P.	100	-	-
S.B.P. + urea	-	100	-
Barley	-	-	75
Protein Concentrate *	-	-	25
Mean Crude Protein %	9.8	17.0	17.1

* 36 % crude protein (B.O.C.M. Ltd.) supplementary protein being largely in the form of ground nut and cotton seed cakes.

Diets A and B were fed for the first two gallons of milk produced followed by the normal farm concentrate (C) for any additional production. Diet C was fed for all the milk produced. The concentrates were fed at a standard rate of 4 lbs (1.8 kg) per gallon of milk produced. At no time during the experiment did any individual cow fed on Diets A and B receive less than 8 lbs (3.6 kg) of one or other of the sugar beet pulp materials per day. Table 5. details the experimental design.

The basal diet consisted solely of good quality hay having the following analysis. (Dry matter basis):- Dry matter 86.0 %, crude protein 8.9 %, crude fibre 32.6 %. (Mean of 18 samples obtained at intervals over the period of the trial). The average energy value of the hay, expressed

TABLE 5.

DAIRY EXPERIMENT 1 1969

EXPERIMENTAL DESIGN

	COW	1	2	3	4	5	6	7	8	9
	Herd No.	50	6	21	31	47	55	33	13	46
Period.	I	A	B	C	A	B	C	A	B	C
	II	B	C	A	C	A	B	B	C	A
	III	C	A	B	B	C	A	C	A	B

	COW	10	11	12	13	14	15	16	17	18
	Herd No.	1	12	27	9	53	36	29	5	37
Period.	I	A	B	C	A	B	C	A	B	C
	II	C	A	B	B	C	A	C	A	B
	III	B	C	A	C	A	B	B	C	A

PERIOD I - 4th. December 1968 - 6th. January 1969.

PERIOD II - 6th. January 1969 - 3rd. February 1969.

PERIOD III - 3rd. February 1969 - 1st. March 1969.

on a fresh matter basis was calculated from the crude fibre percentage and the regression equations of Alderman, Collins, Jones, Morgan & Ibbotson (1967) and was 34 S.E. The average protein equivalent content was calculated as 3.4. This was estimated to supply 6.9 lbs S.E. and 0.69 lbs P.E. when fed at 20 lbs/head/day to the Friesian cows and 6.1 lbs S.E. and 0.61 lbs P.E. when fed at 18 lbs/head/day to the Ayrshire cows. The energy intake was just adequate for maintenance purposes according to the suggested requirements of both Woodman (Evans, 1960) and of the Agricultural Research Council (1965). The estimated protein intake was barely adequate for maintenance according to Woodman but more than adequate (about 125 % of that recommended) according to the A.R.C. The estimated energy content of the pulp concentrates A and B fed for the first two gallons of milk was 58 S.E., and 69 S.E. for the normal farm mix C. The crude protein content of diets B and C (about 17.0 %) is that commonly fed for milk production, but some 42.0 % of the total crude protein of diet B when fed for the first two gallons is derived from urea. Diet A containing only 9.8 % crude protein is apparently severely deficient in protein for milk production, and acted as a negative control.

Each cow received each of the three diets in sequence over three periods each of four weeks. Daily yields were recorded for the entire second fourteen days of each feeding period. Milk samples were collected for analysis twice weekly. Statistical evaluation of the data was kindly undertaken by computer by Henderson and Shukla of the A.R.C. Unit of Statistics, Edinburgh University.

Results

All three diets were palatable and there were no refusals at any time during the experimental period. It is recognised that some apparent energy deficit exists in the rations containing sugar beet pulp over the normal farm concentrate. The energy requirement for the production of two gallons of milk is 5.0 lbs S.E. according to Woodman (Evans, 1960). Calculation of the energy supplied from 8 lbs (3.6 kg) of sugar beet pulp gives 4.6 lbs S.E.,

whereas 8 lbs of the normal mix is estimated to supply 5.5 lbs S.E. This marginal deficiency of energy occurred however only when sugar beet pulp diets were fed for the production of the first two gallons of milk. (See Table 6.).

Table 6.

Diet	A	B	C
Galls Milk			
3	7.35	7.35	8.25
4	I0.I0	I0.I0	II.00
5	I2.85	I2.85	I3.75
6	I5.60	I5.60	I6.50

This experiment (and experiments 2 and 3) produced a very large number (several hundreds) of daily recordings and analyses. Fig. 6a. illustrates the type of record kept. The whole collection of data involving 250 pages have been filed in the Animal Husbandry Department, Glasgow University.

Fig. 6a.

DAIRY EXPERIMENT 1969 COCHNO FARM, GLASGOW UNIVERSITY.

DATE: _____.

Diet.	Cow and No.	Yield lbs. a.m.	p.m.	TOTAL	Analysis. Fat%	T.S.%	Urea.
A							
B							
C							

Table 7

Dairy Experiment 1, 1969

Main Results of Analysis

		Milk lbs/day	F.C.M. lbs/day	Total solids %	Fat %
	Weeks	3 & 4	3 & 4	3 & 4	3 & 4
	D.F.		Mean Sum of Squares		
Cows	17	285.2623	259.9877	13.9151	1.2758
Periods	12	93.0224	79.2215	4.2035	0.3584
Residual (Corrected)	2	1.2401	1.8892	0.0423	0.01954
Treatment (Corrected)	2	17.2512	19.6758	1.0936	0.1235
Error	18	3.0214	3.0154	0.1468	0.01816
Direct Effect Means					
A		30.89	30.55	12.37	3.93
B		31.09	31.66	12.64	4.12
C		33.01	33.05	12.57	4.01
S.E.		0.4580	0.4577	0.1793	0.0632
	S.E. diff	0.6476	0.6471	0.2535	0.0894
Residual Effect Means					
A		31.51	31.21	12.47	3.93
B		31.33	31.87	12.63	4.11
C		32.15	32.19	12.47	4.01
S.E.		0.6145	0.6140	0.2406	0.0846
	S.E. diff	0.8689	0.8682	0.3402	0.1196

The main statistical analysis giving the principal direct effect results are as follows:-

Diet	A Low Protein	B Urea	C Veg.protein	Significance P.
Milk yield lbs/day	30.89	31.09	33.01	C > A (0.01)
				C > B (0.05)
F.C.M. lbs/day	30.55	31.66	33.05	C > A (0.01)
				B > A (0.05)
Total solids %	12.36	12.60	12.60	N.S.
Fat %	3.92	4.12	4.06	B > A (0.05)

An apparent depression of about 2,0 lbs/day in the mean milk yield is observed for both Diets A and B, where 8 lbs of sugar beet pulp was substituted for some of the barley/protein ration. The mean milk yield on the vegetable protein diet (C) was significantly greater than both the SBP + urea diet ($P = 0.05$) and the negative control diet consisting of sugar beet pulp with a crude protein of 9.8 % ($P = 0.01$) fed for the first two gallons. Milk fat % was significantly greater for diet B containing 8 lbs of sugar beet pulp + urea than the low protein control diet A i.e. 4.12 compared with 3.92 (significant at $P = 0.05$). Accordingly, the calculated Fat Corrected Milk (FCM) yields partially reflect this difference in fat content.

The FCM yields on diet B (31.66 lbs) is greater ($P = 0.05$) than the FCM yield from the low protein diet A. The FCM yield on the vegetable protein diet C is significantly greater than both the urea diet (B) and the negative control diet A. i.e. 33.05 lbs compared with 31.66 and 30.55. The total solid content of the milks from diets B and C were both somewhat greater than that produced from the low protein concentrate diet A. i.e. 12.60, 12.60 and .2136.

DAIRY EXPERIMENT 2 (1970).

The experimental design was the same as that employed in Experiment 1. Table 8 details the layout of the experiment. The design of six groups - each of three cows, allowed each cow to receive three different rations in successive periods of five weeks. Twelve Ayrshire and six Friesian cows were selected for the experiment. The mean number of completed lactations was 3 with only five cows having more than three lactations. The mean daily yield at the start of the experiment was 4.5 gallons and had declined to 3.2 gallons after the 15 weeks of the experiment.

The experiment compared three concentrate rations, each of which contained 50 % sugar beet pulp. The concentrate mixtures were fed for all the milk produced at a rate of 4 lbs (1.8 kg) per gallon. The protein source used for comparison with the urea contained in the sugar beet cubes was a mixture of equal parts of ground nut and cotton seed cake. These were combined with some barley, minerals and vitamins into a cube containing 36 % of crude protein. The three concentrate mixtures used in the trial were:-

Diet	A	B	C
% Ingredients			
S.B.P.	50.00	-	50.00
S.B.P. + urea	-	50.00	-
Barley	43.75	37.50	25.00
Protein Concentrate	6.25	12.50	25.00
Mean Crude Protein % (by analysis)	11.70	16.60	16.55

The physical nature and energy content of the three mixtures were broadly comparable. Diet A acted a low protein (11.7 %) control. Diet C, containing 50 % S.B.P. was supplemented with protein from vegetable protein sources to give a ration with a crude protein content of 16.5 %. Diet B

DAIRY EXPERIMENT 2 1970

Table 8

EXPERIMENTAL DESIGN

Period.	COW	1	2	3	4	5	6	7	8	9
	Herd No.	36	21	50	39	43	14	17	45	52
	I	A	B	C	A	B	C	A	B	C
	II	B	C	A	C	A	B	B	C	A
	III	C	A	B	B	C	A	C	A	B

Period.	COW	10	11	12	13	14	15	16	17	18
	Herd No.	49	12	9	5	29	31	26	24	13
	I	A	B	C	A	B	C	A	B	C
	II	C	A	B	B	C	A	C	A	B
	III	B	C	A	C	A	B	B	C	A

PERIOD I - 25th. Janaury 1970 - 3rd. March 1970

PERIOD II - 3rd. March 1970 - 7th. April 1970

PERIOD III - 7th. April 1970 - 12th. May. 1970

contained a similar total amount of crude protein (16.6 %) but where 22.4 % of the total crude protein was derived from urea contained in the sugar beet pulp cubes. The basal diet consisted of 18-20 lbs of hay per day. The mean crude protein of the hay fed during the first experimental period was of superior quality to that fed subsequently. The mean crude protein was 10.6 % and was calculated to supply sufficient protein for maintenance and the production of one gallon of milk. The mean crude protein content of the hay fed during the last two periods was 7.78 % (14 samples) and the apparent digestibility (by sheep) was 61 %. Calculation of the protein supplied to the Friesian and Ayrshire cows when fed at 20 and 18 lbs per day respectively gave values of 0.7 and 0.6 lbs P.E. and thus be adequate protein for maintenance. The energy supplied was calculated from the equations of Alderman et al (1967) and values of 7.1 and 6.1 lbs S.E. were obtained for the Friesian and Ayrshire cows respectively; thus supplying sufficient energy for maintenance (Evans, 1960).

The milk yields of all the experimental cows were recorded on five days of each week over the entire fifteen week period. There were no difficulties at all with regard to the palatability of the various mixtures and all the cows consumed the whole amount of foods offered in a normal way. The experimental data were kindly examined by Henderson and Shukla of the A.R.C. Unit of Statistics in Edinburgh.

Results

The two sets of statistical tables (i.e. Tables 9 and 10) refer to a) an analysis based on the data collected from all three periods of five weeks, and b) an analysis based on the data from the last two periods alone. These separate analyses were performed in an attempt to assess and remove the contribution of the extra protein of the hay fed during the first period. This hay had a mean crude protein of 10.6 % which was unfortunately rather high for maintenance requirement purposes. In both cases the analyses were performed using firstly the entire five week period of each feeding regime,

TABLE 9.
DAIRY EXPERIMENT 2 (1970)

Statistical analysis using data collected from all three periods.

		Milk lbs/day	% Fat	Total % Solids	% Protein	FCM lbs/day
Weeks included in analysis		5	5	5	5	5
Sources	D.F.			Mean Sum Squares		
Cows	17	193.0684	.5640	1.0404	.0812	155.5218
Periods within pairs of squares	6	291.0234	.0195	.0253	.0271	307.8343
Direct (corrected)	2	5.9846	.0039	.0066	.0004	8.3021
Residual (corrected)	2	.8024	.0415	.0789	.0110	.1870
Error	26	1.8479	.0630	.0746	.0026	2.5376
Direct effect means						
A		33.801	4.049	12.754	3.172	34.018
B		33.985	4.022	12.783	3.180	33.645
C		34.998	4.051	12.795	3.169	35.106
S.E.		.3582	.0661	.0728	.0135	.4198
Residual effect means						
A		34.139	4.035	12.721	3.171	34.221
B		34.621	3.971	12.719	3.138	34.124
C		34.024	4.115	12.892	3.212	34.424
S.E.		.4806	.0887	.0966	.0181	.5632

TABLE IO.
DAIRY EXPERIMENT 2 (1970)

Statistical analysis using data collected from all three periods.

		Milk lbs/day	% Fat	Total % Solids	% Protein	FCM lbs/day
Weeks included in analysis		2	2	2	2	2
Sources	D.F.		Mean Sum Squares			
Cows	17	192.7769	.6331	1.2098	.0671	157.9319
Periods within pairs of squares	6	252.5836	.1993	.1698	.0197	292.5454
Direct (corrected)	2	19.7039	.0396	.0813	.0031	15.2355
Residual (corrected)	2	1.4471	.0295	.0477	.0158	0.0050
Error	26	2.3587	.0726	.1362	.0050	1.9361
Direct effect means						
A		31.938	4.018	12.779	3.175	32.036
B		32.614	3.944	12.740	3.152	31.935
C		33.584	4.046	12.845	3.148	33.765
S.E.		.4047	.0710	.0973	.0187	.3667
Residual effect means						
A		32.415	4.017	12.797	3.166	32.550
B		33.199	3.936	12.707	3.110	32.593
C		32.522	4.055	12.861	3.198	32.593
S.E.		.5730	.0953	.1305	.0251	.4920

and secondly the last two weeks of each period. This was performed in an attempt to determine if there were any adaptative responses to urea after its initial introduction.

None of these statistical approaches makes any major changes in the interpretation of the data. There is no real evidence in this experiment of an adaptation response by cows fed urea after a period of three weeks has elapsed.

The main findings in respect of the three treatments when the entire five weeks of all three periods are considered are detailed as follows:-

Diet	A Low Protein	B Urea	C Veg.protein	Significance
Milk yield lbs/day	33.80	33.98	35.00	C > A (.05)
Fat Corrected Yield	34.02	33.65	35.11	C > B (.05)
Total Solids %	12.75	12.78	12.80	N.S.
Fat %	4.05	4.02	4.05	A+C > B (.05)
Crude Protein %	3.17	3.18	3.17	N.S.
Milk urea mg/100 ml	22.25	34.71	34.78	B+C > A (.001)
Milk urea g/day	3.31	5.29	5.71	

With the treatments used and under the conditions of the experiment only the vegetable protein supplement had a significant effect on yield.

If the following values for protein equivalent in the different concentrate ingredients are

Barley	7.0	
S.B.P	6.0	
S.B.P. + urea	13.5	(9.75 if credited with only 50 % of the contained urea).
Veg. protein conc.	27,0	(36 % crude protein, largely ground nut and cotton seed cakes).

then 4 lbs (1.8 kg) of each mixture fed per gallon would supply

Mixture A.	Low protein	0.32 lbs P.E.
Mixture B.	S.B.P. urea	0.52 lbs P.E.

Mixture C. Veg. protein 0.46 lbs P.E.

If the urea contained in Diet B were credited with only 50 % of its presumed protein equivalent, the total supplied in 4 lbs would be 0.44 lbs P.E.

The starch equivalent of all three mixtures would be in the same order as each contained about 50 % barley and 50 % sugar beet. If this were say 65, 4 lbs would provide 2.6 lbs S.E./gallon.

The total amount of P.E. supplied would be in the following order:

		A Low protein	B Urea	C Veg.protein	Requirement
M.	20 Hay	0.95	0.95	0.95	0.6 - 0.7
M+1	4 lbs	1.27	1.47 (1.39)	1.41	1.15
M+2	8 lbs	1.59	1.99 (1.83)	1.87	1.65
M+3	12 lbs	1.91	2.51 (2.27)	2.33	2.15
M+4	16 lbs	2.24	3.03 (2.71)	2.79	2.65
M+5	20 lbs	2.56	3.55 (3.15)	3.25	3.15

(Values in brackets credit the urea with only 50 % of its presumed value)

It is unfortunate that the hay apparently provided rather more protein than was required for maintenance. In consequence, it could be argued that the low protein diet was only noticeably deficient at yields of over perhaps 3, but certainly over 4 gallons. From this it might follow that it is not surprising that the urea addition had no effect. But this cannot explain the significant yield increment from the use of vegetable protein at about the same level as the urea supplement.

Both vegetable and urea additions have a marked and highly significant effect on milk urea concentrations relative to the low protein diet. ($P = 0.001$).

DAIRY EXPERIMENT 3 1971

This experiment compared three production rations, two of which contained 50 % sugar beet pulp. The basal diet consisted of 20-25 lbs of good silage, 8 lbs of medium hay and 4 lbs barley. This formulation was calculated from laboratory analyses of the feedingstuffs to be sufficient for maintenance plus one gallon of milk. The calculation is detailed below.

The silage had a mean analysis of 27.8 % dry matter, with 26.2 % crude fibre and 15.1 % crude protein (on dry matter basis). This was calculated (Alderman et al, 1967) to provide 15.2 lbs S.E. and 2.7 lbs P.E. per 100 lbs of fresh matter. The hay had a mean analysis of 86.0 % dry matter, with 32.1 % crude fibre and 7.8 % crude protein (on dry matter basis), and was calculated (Alderman et al, 1967) to provide 35 lbs S.E. and 2.9 lbs P.E. per 100 lbs of fresh matter. The barley was assumed to supply 70 lbs S.E. and 7 lbs P.E. per 100 lbs.

The Friesian cows received 25 lbs silage, 8 lbs hay and 4 lbs barley supplying in total 9.4 lbs S.E. and 1.18 lbs P.E. The Ayrshire cows received 20 lbs silage, 8 lbs hay and 4 lbs barley, supplying a total of 8.6 lbs S.E. and 1.08 lbs P.E. These values are marginally adequate for maintenance and the production of one gallon of milk according to the standards of Woodman. (Evans, 1960).

All the concentrate mixtures were fed for all the milk produced over the first gallon, at a constant rate of 4 lbs (1.8 kg) per gallon.

The experiment involved 12 cows in a double changeover design similar to that described by Cochran et al, (1943). Each cow received three different rations in successive periods of four weeks. TableII outlines the design. Six Friesian and six Ayrshire cows were selected having a mean lactation number of 3 with only two cows having had more than four lactations. All the cows had been milking for 50-70 days before the experiment commenced. The mean daily milk yield at the start of the experiment was 4.5 gallons and this declined to an overall mean yield of 3 galls/day after 12 weeks of experimentation.

The three treatments fed after the first gallon were:-

Diet	A	B	C
% Constituent			
S.B.P.	50.0	-	-
S.B.P. + urea	-	50.0	-
Normal Concentrate *	-	50.0	100
Barley	50.0	-	-
Crude Protein % (by analysis)	10.20	16.5	16.4

* The normal concentrate consisted of a mixture of 50 % barley, 25 % oats and 25 % of a concentrate containing 34 % crude protein compared mainly of ground nut and cotton seed cakes together with suitable additions of minerals.

Diet A acted as a low protein (10.2 % crude protein) control containing 50 % S.B.P. and Diet C as a vegetable protein control diet contained 16.5 % crude protein, where 50 % of the concentrate was composed of sugar beet pulp and 21.1 % of the total protein was derived from urea. The milk yields were recorded on five days of each week over the whole 12 week experimental period.

Venous blood samples were collected at the end of each period at 10.00 hours.

Results

There were no difficulties with regard to the palatabilities of the various mixtures, except that one individual on two occasions refused the vegetable protein concentrate pellets contained in the normal concentrate mixture fed to Group C.

Tables I2 and I3 detail the statistical results obtained when the data from a) all four weeks, b) last two weeks of each of the three feeding periods are used. The principal results obtained from an analysis using data recorded throughout the experiment were as follows.

Table II.

DAIRY EXPERIMENT 3 1971

EXPERIMENTAL DESIGN

COW	1	2	3	4	5	6
I	A	B	C	A	B	C
II	B	C	A	C	A	B
III	C	A	B	B	C	A

COW	7	8	9	10	11	12
I	A	B	C	A	B	C
II	B	C	A	C	A	B
III	C	A	B	B	C	A

PERIOD I - 25/1/71 - 22/2/71

PERIOD II - 22/2/71 - 22/3/71

PERIOD III - 22/3/71 - 19/4/71

TABLE I2.

Dairy Experiment 3, 1971

Statistical Analysis using Data collected from all four weeks of each period

	d.f.	Milk lb/day	FCM lb/day	Fat %	Total Solids %	Urea mg %	Total Urea g/day
		Mean Sum of Squares					
Cows	11	245.021	233.898	0.1046	0.1387	52.03	4868.05
Periods	4	115.056	123.025	0.0173	0.0715	88.34	3406.07
Direct (T)	2	9.287*	6.885*	0.0088	0.0126	17044.5**	12792.29**
Residual (R)	2	0.153	1.247	0.0142	0.0151	134.6*	775.76
Error	16	2.025	1.445	0.0293	0.0574	27.12	549.19
C.V. in percentage		4.05	3.45	4.31	1.94	7.87	15.06
Direct T_A		33.96	33.90	3.99	12.37	23.85.	3.589
T_B		35.60 *+	35.42*+	3.98	12.31	34.59***	5.548***
T_C		35.73*+	35.31*+	3.93	12.30	34.79***	5.628***
S.E. of T difference		0.650	0.549	0.078	0.109	1.117	0.338
Residual R_A		35.29	35.24	3.99	12.38	28.80	4.579
R_B		34.97	34.33	3.91	12.28	30.90	4.853
R_C		35.04	35.06	4.00	12.31	33.52	5.333
S.E. of R differences		0.871	0.736	0.105	0.147	1.498	0.453

(*+, p = 0.02)

TABLE I3.

Dairy Experiment 3. 1971

Statistical Analysis using Data collected from last two weeks of each period.

	d.f.	Milk lb/day	FCM lb/day	Fat %	Total Solids %	Urea mg %	Total Urea g/day
		Mean Sum of Squares					
Cows	11	240.532	223.207	0.0907	0.1496	68.72	5350.21
Periods	4	86.386	96.114	0.0325	0.1152	163.41	3781.15
Direct (T)	2	8.911	7.262	0.0252	0.0311	1793.12**	13330.89**
Residual (R)	2	2.127	3.024	0.0014	0.0221	62.35	513.23
Error	16	2.751	2.605	0.0291	0.0484	48.81	876.05
C.V. in percentage		4.81	4.75	4.36	1.80	10.30	18.66
T_A		33.44	32.99	3.91	12.28	24.47	3.655
T_B		34.74+	34.50+	3.96	12.24	35.86***	5.672***
T_C		35.32*	34.50+	3.86	12.17	35.31***	5.720
S.E. of T difference		0.757	0.737	0.078	0.100	1.498	0.427
R_A		35.23	34.85	3.92	12.29	31.36	5.053
R_B		34.08	33.45	3.92	12.24	30.59	4.689
R_C		34.20	33.68	3.89	12.16	33.68	5.305
S.E. of R differences		1.016	0.988	0.104	0.135	2.010	0.573

* p = 0.05

+ Just misses at 0.05

Diet	A Low protein	B Urea	C Veg.protein	Significance
Direct effects				
Daily yield lbs	33.96	35.60	35.73	B+C > A (0.02)
FCM lbs	33.90	35.42	35.31	B+C > A (0.02)
Fat %	3.99	3.98	3.93	N.S.
Total Solids %	12.37	12.31	12.30	N.S.
Milk Urea g/day	3.59	5.55	5.63	B+C > A (0.001)
Urea mg/100 ml.	23.85	34.59	34.79	B+C > A (0.001)

These results indicate that both the urea-containing diet and the normal concentrate (Diets B and C respectively) to be significantly better than the low protein concentrate (Diet A). The milk yields produced on diets B and C are about 1.5 lbs greater than that produced from diet A. Differences in total solids or fat contents were not significant. A highly significant difference is seen in the daily output of urea in the milk between treatments. Diets C and B produced about 5.5 g of urea daily in the milk whereas the low protein control diet A produced only 3.6 g urea.

The principal results obtained from an analysis based on data obtained collected in the last two weeks of each feeding period is given below.

Diet	A	B	C	Significance
Direct effects				
Milk yield lbs	33.44	34.74	35.32	C A (0.05)
FCM lbs	32.99	34.50	34.50	C+B A (0.05)
Milk urea g/day	3.65	5.67	5.70	C+B A (0.001)
Milk urea mg/100 ml	24.47	35.86	35.31	C+B A (0.001)
				no other significance.

This analysis produced a similar set of results to that obtained from the previous analysis utilizing the whole 4-week data. The separate analysis performed on data obtained from the last two weeks of each feeding period was undertaken to examine the possibility of there being any evidence of a time-lag in

adaptation to urea after a preliminary two weeks of feeding the urea-containing diet. Comparisons between these sets of analyses apparently do not indicate any such period necessary for adaptation to the utilisation of urea when incorporated into a sugar beet cube.

DAIRY EXPERIMENT. 4. 1970

This particular trial was conducted with cooperation of Mr. A. Loudon, Dykescroft Farm, Moscow, Ayrshire and was undertaken to assess the value of the sugar beet pulp + urea product under more practical farm conditions.

The dairy herd consisted of about 90 Friesian cows. The cows had 24 hours access to self-feed silage in an open yard. Milking took place in two byres and concentrates (4 lbs/gallon) were fed at each of the two milkings. This particular trial was conducted with a total of thirty two cows (two groups of 16) which had calved mainly during September and October 1969, but there were a few in each group which had calved more recently. Milk yields were recorded at approximately 10-day intervals.

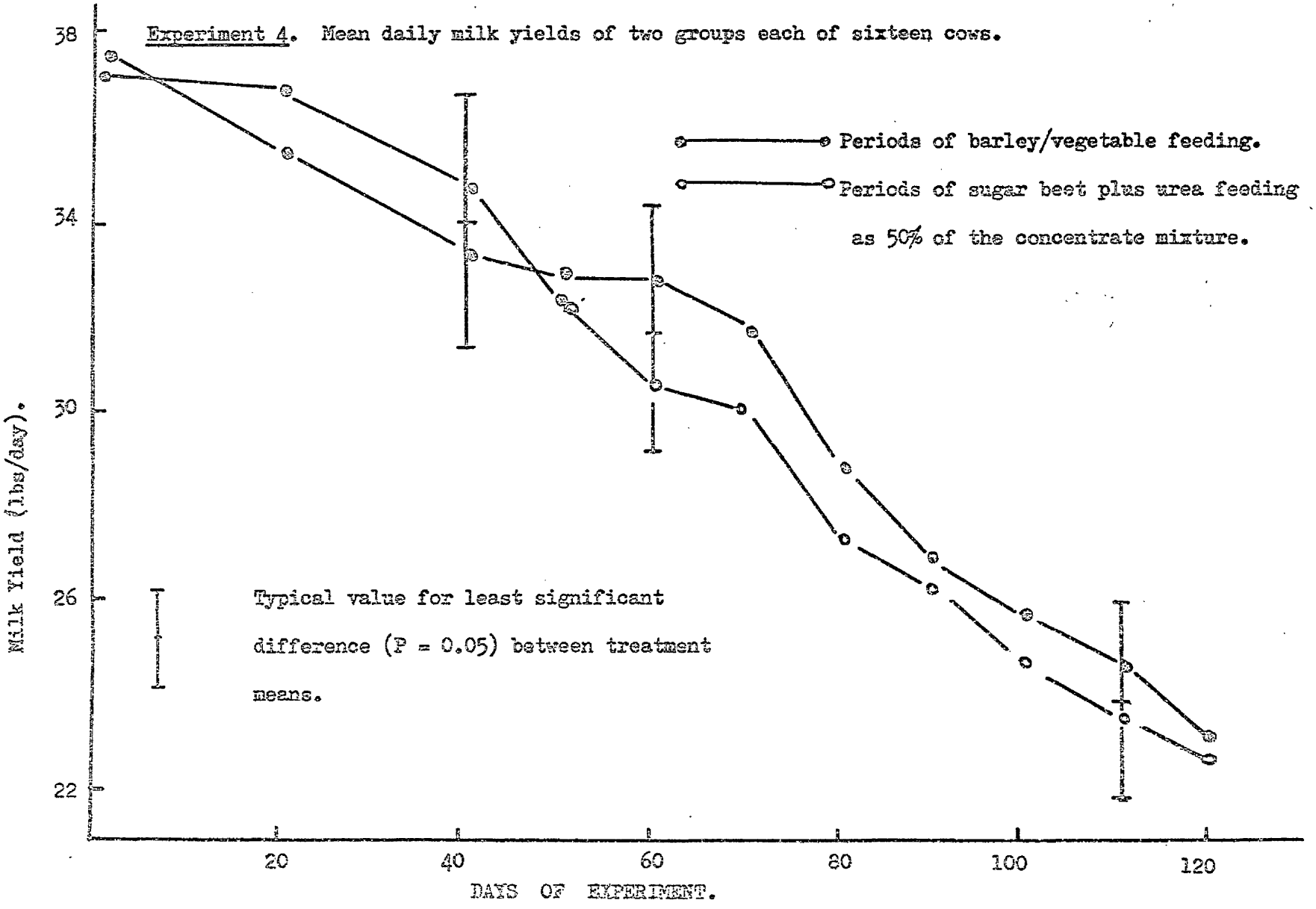
The trial commenced on 10 December 1969 when the overall mean yield was almost 37.5 lbs/day. From 10 Dec to 30 Jan 1970 both groups of cows received the normal farm concentrate fed at 4 lbs/gallon. This consisted of 4 parts barley plus 1 part of a proprietary protein/vitamin/mineral concentrate which contained 40 % crude protein. The mixture accordingly contained about 16 % crude protein.

From 30 Jan until 10 April one group continued to receive this mixture. The other group was fed the same mixture at the morning milking but received sugar beet + urea (16.6 % crude protein) at the evening milking. i.e. one-half of the total concentrate ration fed to this group was sugar beet + urea. Individual yields ranged up to 45 lbs/day and so some cows received as much as 9 lbs/day of the sugar beet + urea product. It was observed that about half of the cows (irrespective of yield) left small amounts (about $\frac{1}{2}$ lb) of sugar beet nuts + urea at the end of each milking. This was however consumed shortly after milking.

The mean recorded yields of the two groups are shown in Tables I4 and I5 and the results are plotted in Fig 6b . There was no significant difference in mean yields and any differences are small relative to those required for significance at P = 0.05 (Fig 6b). The fact that the addition of sugar beet + urea seemed to result in an apparantly reduced

Figure 6b.

Experiment 4. Mean daily milk yields of two groups each of sixteen cows.



yield is believed to be largely coincidental. It seems to arise mainly because that particular group showed a relatively large reduction in yield from 20 Jan - 30 Jan before feeding actually commenced. Equally, the group fed the normal concentrate throughout the whole period did not enhibit the normal decline in lactation yield between 20 Jan and 10 Feb.

It is concluded from this farm-scale trial that inclusion of sugar beet pulp + urea at 50 % of the normal concentrate intake did not influence the mean mid-lactation yield of cows when this was in the order of 30-35 lbs/day.

Table I4. Diary Cow Experiment No 4. Mean milk yields (lbs/cow/day) of 16 cows fed the normal concentrate ration.

Cow No	10 Dec 69	30 Dec 69	20 Jan 70	30 Jan 70	10 Feb 70	20 Feb 70	2 Mar 70	10 Mar 70	20 Mar 70	30 Mar 70	10 Apr 70
1	40	38	36	36	33	32	26	25	23	21	18
5	35	34	33	29	28	28	19	21	16	16	17
8	47	42	39	35	36	30	31	26	24	23	22
15	36	34	31	26	26	25	23	21	24	22	19
16	36	35	33	32	38	30	26	24	23	24	23
19	33	31	27	26	27	25	22	23	20	19	19
46	50	52	48	48	46	43	40	35	30	29	27
48	32	30	28	26	28	25	24	18	21	21	20
49	32	27	24	28	30	30	30	27	28	27	26
52	33	30	28	27	28	26	25	23	20	19	18
53	-	-	32	44	44	44	40	38	39	35	33
54	33	32	30	29	26	26	24	21	23	21	18
55	32	30	27	27	26	26	22	20	18	17	16
56	50	48	51	47	49	47	44	44	39	38	37
58	36	34	33	27	29	30	27	24	26	25	24
61	-	-	34	34	42	42	40	39	37	36	33
Total No	14	14	16	16	16	16	16	16	16	16	16
Mean	37.5	35.5	33.4	32.6	32.9	31.8	28.9	26.8	25.6	24.6	23.1
S. Dev ±	6.64	7.23	7.34	7.60	7.93	7.64	7.81	7.79	7.06	6.76	6.49

Table I5 . Diary Cow Experiment No 4. Mean milk yields (lbs/cow/day) of 16 cows fed the normal concentrate ration but including 50 % sugar beet pulp + urea.

Cow No	10 Dec 69	30 Dec 69	20 Jan 70	30 Jan 70	10 Feb 70	20 Feb 70	2 Mar 70	10 Mar 70	20 Mar 70	30 Mar 70	10 Apr 70
22	31	32	29	25	25	22	22	20	18	18	18
23	35	33	30	25	27	26	21	20	19	18	18
24	52	54	49	40	35	27	30	31	27	25	23
26	30	31	27	26	23	23	25	27	22	20	18
28	45	43	39	38	37	34	32	28	27	26	26
32	35	33	29	27	28	28	24	24	26	24	21
35	35	34	33	32	29	30	25	27	24	25	26
37	38	37	37	33	30	30	30	31	24	24	23
63	50	51	47	37	33	36	31	26	28	25	23
65	33	31	28	26	22	27	22	21	19	18	17
69	-	-	49	38	36	42	36	36	34	33	32
71	35	34	33	32	28	29	25	23	22	23	22
72	32	33	33	30	28	29	26	23	23	22	22
73	33	32	29	30	29	24	24	24	22	21	22
75	-	-	30	46	42	43	35	35	34	32	30
80	38	36	33	32	32	29	28	22	24	21	19
Total No	14	14	16	16	16	16	16	16	16	16	16
Mean	37.3	36.7	34.7	32.3	30.3	29.9	27.3	26.1	24.6	23.4	22.5
S. Dev ±	6.89	7.38	7.49	6.10	5.34	6.01	4.63	5.50	4.70	4.43	4.30

DAIRY EXPERIMENT. 5 (1971).

This trial was also conducted with the cooperation of Mr. Loudon, Dykescroft. The basal maintenance diet was self-feed silage. Two concentrate mixtures were compared in a changeover trial. These were (a) The normally used concentrate mixture of 3 parts barley + 1 part of a proprietary protein/vitamin/mineral concentrate (34 % crude protein) having an overall crude protein concentration of about 16 %. This was fed at 4 lbs/gall and (b) The same mixture but where 4 lbs of sugar beet pulp + urea (17.1 % crude protein) were fed for the first gallon of milk produced. There were no palatibility problems.

There were two groups of cows, A and B. Eighteen cows in Group A and fifteen cows in Group B were recorded at approximately 20-day intervals from 20 Nov 1970 to 20 March 1971. The diets fed to each group were changed over on 11 Jan 71 and 6 March 71. The mean yields are shown in Tables I6 and I7 and are shown graphically in Fig 7 .

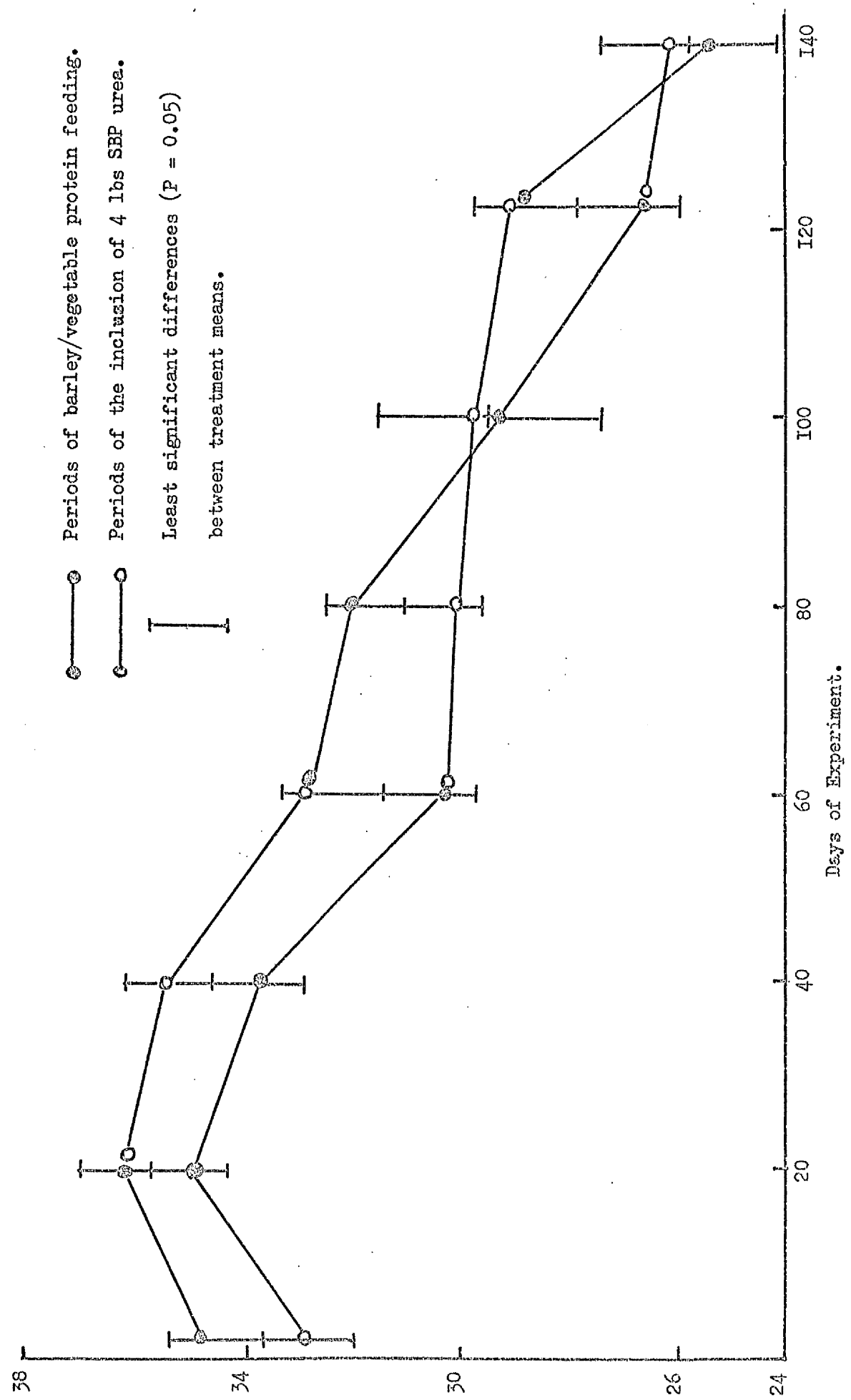
The overall mean yield of all the cows was rather more than 35 lbs/day at the start of the trial on 20 Nov 70 which was reasonable as most of the cows had calved in August/September. By 20 March when the cows started to go to grass for part of the day, the overall mean yield had fallen to about 25 lbs/day.

Fig. 7. indicates that at no time during the trial did the two groups have mean yields which differed significantly from each other. The least difference between the two means which is necessary for significance at P = 0.05 averaged about 3.5 lbs milk/day during the period of the trial. Although this difference was approached at the end of each of the two principal feeding periods (2.6 lbs actual of 3.47 lbs required on 3 Jan and 2.6 lbs actual of 3.57 lbs required on 2 March) no clear significant difference was recorded.

However, during all three periods of feeding, yields fell most rapidly when sugar beet pulp + urea was not included in the diet. viz.

Figure 7.

Experiment 5. Mean daily milk yields of two groups each of I8 cows.



	Reduction in Mean Yield (lbs/day)	
	+ S.B.P. urea	Normal Conc.
20 Nov - 3 Jan	3.3	4.7
3 Jan - 2 March	1.1	6.3
2 March - 20 March	0.5	4.0

There is no additional means of determining whether this was a real (i.e. significant) effect or not as the circumstances of the trial were such that yields were only recorded at infrequent intervals. It is, nevertheless an interesting observation. Although sugar beet pulp + urea was only fed at a maximum of 4 lbs/day, it nevertheless represented about 30 % of the concentrate intake at the commencement of the trial and some 40 % at the end of the investigation.

Table I6 . Dairy Cow Experiment No 5. Mean milk yields lb/cow/day of 18 cows in Group A.

Cow No	20 Nov 70	10 Dec 70	3 Jan 71	20 Jan 71	10 Feb 71	2 March 71	20 March 71
22	32	36	33	30	26	25	26
24	42	36	30	32	32	30	33
26	40	31	34	35	26	27	24
27	34	35	36	40	41	31	27
28	40	37	32	35	35	32	28
35	39	36	34	39	32	32	30
36	30	28	26	25	25	25	22
37	44	47	40	37	31	33	34
39	43	38	37	35	29	31	32
63	37	30	33	27	27	25	23
64	35	38	32	29	27	22	22
65	35	33	31	32	27	27	24
68	33	32	30	27	23	23	22
71	39	41	36	31	34	32	35
72	31	32	29	29	26	24	26
73	35	40	35	31	31	27	27
77	35	30	28	28	22	16	20
80	30	39	36	39	35	25	27
Mean	36.3	35.5	33.0	32.3	29.4	26.7	26.2
S. Dev ±	4.39	4.72	3.53	4.55	4.88	4.75	4.01

Group A feeding

20 Nov - 11 Jan 4 lbs sugar beet + urea
11 Jan - 6 March Normal concentrate
6 March - 20 March 4 lbs sugar beet + urea

Table I7 . Dairy Cow Experiment No 5. Mean milk yields (lb/cow/day) of 15 cows in Group B.

Cow No	20 Nov 70	10 Dec 70	3 Jan 71	20 Jan 71	10 Feb 71	2 March 71	20 March 71
1	36	37	35	32	27	30	29
2	32	30	25	26	25	25	24
3	31	31	26	28	25	24	22
4	35	30	27	27	25	25	22
7	33	33	30	28	31	26	22
9	36	30	26	28	30	29	33
11	32	27	26	21	20	24	17
15	30	28	27	27	28	30	21
45	36	39	39	36	37	35	32
49	36	34	28	31	32	29	28
51	36	30	29	30	28	25	19
54	37	34	25	33	32	30	25
55	39	39	35	33	29	31	30
56	35	43	42	36	43	44	35
58	42	41	36	35	35	32	31
Mean	35.1	33.7	30.4	30.1	29.8	29.3	25.3
S. Dev ±	3.13	4.98	5.54	4.20	5.63	5.23	5.22

Group B feeding

20 Nov - 11 Jan	Normal concentrate
11 Jan - 6 March	4 lbs sugar beet pulp + urea
6 March - 20 March	Normal concentrate

DISCUSSION

Although the five experiments described are each somewhat different in design, each essentially contains a comparison between diets based on (a) barley supplemented with vegetable protein and (b) molassed sugar beet plus urea, minerals and vitamins, included at between 25 and 50 % so that each contained 16 - 17 % crude protein. There were accordingly simultaneous differences in both the possible energy supply and the nature of the supplementary nitrogen in that (a) contained no urea whereas some 25 % of the crude protein depending on milk yield in (b) was in the form of urea. The normally suggested energy values for barley and molassed sugar beet pulp are S.E. 70 and 60 respectively (Evans, 1960). The inclusion of a combined total of about 5 % urea and dicalcium phosphate, trace elements and vitamins in the molassed sugar beet pulp might have the apparent effect of reducing the normally suggested starch equivalent from 60 to about 57. The inclusion of up to 50 % S.B.P. with urea might thus appear to reduce the energy content of the concentrate mixtures of type (b) by some 10 % i.e. from about S.E. 70 to about S.E. 63.

Experiments 1 and 2 indicate that barley and vegetable protein (largely ground nut and cotton seed cakes) were superior to S.B.P. with urea in increasing milk yields over those fed the low protein diet. In Experiment 3 however, both diets increased the yield of fat corrected milk to the same degree. In Experiment 1, the superiority of the diet containing barley and vegetable protein over that containing a substantial amount of S.B.P. with urea could have been partially a response to the presumed superiority of barley over molassed sugar beet pulp as an energy source. This is, however, not the case in Experiments 2 and 3 where all the diets (including the low protein control) contained some 50 % molassed sugar beet pulp ($\pm$ urea) differ markedly in available energy although it is appreciated that in Experiments 4 and 5 there could have been compensating differences in the intake of self-feed silage. It is therefore perhaps reasonable to conclude that for milk production purposes the energy value of molassed sugar beet

pulp is more similar to that of barley than is generally accepted. Additional investigations, presented elsewhere in this thesis, indicate that this may also be the case for intensive beef production.

Armstrong & Trinder, (1966) have reviewed the findings of more than thirty trials concerning the use of urea (generally at rather more than 2 % inclusion to raise the crude protein content of cereals from about 10 to about 16 %) in milk production rations for dairy cows. They concluded that urea was generally less effective as a protein source than vegetable protein supplements when such urea-containing mixtures were fed for the whole of milk production which averaged 3 gallons per day over the trials reviewed. On average the urea-containing diets produced about 2 lbs of milk less per day. It is probable that most of the concentrate mixtures used in the experimental work summarised by Armstrong & Trinder, (1966) were based on either ground or crushed cereals with a low (if any) molasses inclusion and contained an admixture of crystalline or prilled urea.

It has been suggested that the relative inefficiency of urea for milk production as opposed to intensive beef production arises because concentrates are normally offered only twice per day to dairy cows and that this leads to a relatively large and rapid increase in rumen urea and the resulting ammonia concentration. It has previously been suggested in this thesis that the molassed sugar beet plusurea cubed product produced a less rapid and smaller production of ammonia in the rumen. In three of the five experiments with dairy cows (Experiments 3, 4 and 5) there were no significant differences in the milk yields of cows fed 25 -50 % of this product in the concentrate diet compared with diets composed of cereals and vegetable protein. In Experiments 1 and 2 inclusion of S.B.P. with urea gave rather poorer results than the normal concentrate. This could have resulted from a possible difference in energy intake in Experiment 1 but not in the case of Experiment 2. The apparent reductions

in daily yields of fat corrected milk relative to the cereal/vegetable protein diets were 1.39 lbs (Expt. 1) and 1.83 lbs (Expt. 2). These reductions are in the same order as the mean found by Armstrong & Trinder (1966) for a large number of trials with urea-containing diets.

The influence of Diets containing Sugar beet pulp + urea on the composition of milk. (Dairy Experiments 1-3).

The literature holds a considerable number of reports concerning the effect of feeding urea-containing diets to dairy cows on the composition of the milk. The general opinion is that urea had no deteterious influence upon the chemical composition or aroma of milk (Rys, 1967).

Many experimental reports show that the protein content of the milk of cows fed urea-containing diets is not significantly different from milk obtained from the feeding of vegetable protein diets. Wetterau, (1959) replaced some 30 % of the crude protein of the concentrate mixture with urea and showed there to be no change in the crude protein, casein, albumins or globulins. Other workers have reported a tendency for the casein content of milk to increase in urea-fed cows. (e.g. Karysheva & Kudrasov, 1962, Semprini & Annibaldi, 1961). The results obtained from the study conducted inter alia on the protein contents of milk produced during Expt. 2. (1970) indicate there to be no obvious differences in milk protein contentregardless of whether urea was included in the diet to replace part of the normal vegetable protein or if a low crude protein diet were fed. i.e. protein concentrations g/100 ml of 3.17, 3.18 and 3.17.

Previous studies on the effect of urea-containing diets upon the total solids content of milk have generally shown there to be little difference between treatments when urea-containing diets have been compared with vegetable protein diets. Small decreases however have been reported by Karysheva & Kudrasov (1962), and also Wetterau, (1959). The results obtained from the Dairy Experiment 1, 2 and 3 show there to be no significant difference between dietary treatments in respect of the total solids content of the milk of the different dietary groups.

The influence of urea-containing diets upon the fat content in milk is not clear. Some reports have indicated an increase in milk fat

when urea is fed, although this most often is associated with a simultaneous decrease in the milk yield. There are however several reports in which there is no essential difference in fat content in milk produced from cows fed urea-containing diets. e.g. Kniga, (1961). The interpretation of the results obtained from Experiments 1, 2 and 3 is also uncertain. The results obtained from Experiment 1 shows a significant increase in milk fat % when SBP + urea was fed for the first two gallons of milk over that obtained from both the vegetable protein diet and the low protein control diet (SBP fed for the first two gallons only). i.e. 4.12 % compared with 4.00 % and 3.92 % respectively. This increase in fat content would at first sight appear to be associated with an apparent decrease in the mean yield of some 2.0lbs/day over that produced on the vegetable protein concentrate. However the low protein control diet also produced a decrease in the mean yield of over 2.0 lbs/day and no similar increase in milk fat content was recorded. The results from Experiment 2, where the three contrasting diets fed for all the milk produced each contained approx. 50 % sugar beet ($\pm$ urea) show a small but non-significant decrease in milk fat content when the diet containing SBP + urea was fed compared with both the vegetable protein diet and the low protein control diet. i.e. 4.02 % compared with 4.05 % and 4.05 %. In Experiment 3 there was no significant difference between treatments with respect to milk fat content.

There are several instances in the literature where an increase in urea content of milk occurs when urea-containing diets are fed. (eg. Wetterau, I959; Rys, I959, Kniga, I96I; Kniga, Babak, Klicenko & Hmelik, I96I; Karyseva & Kudasov, I962). Rys (I959) has suggested the possibility of utilizing the changes in the urea content of milk as an index of the changes in the ammonia content in the rumen. Different results have been obtained by Briggs & Hogg (I964) who did not show any increase in the urea content of milk produced by cows fed

a ration containing urea. Individual cows showed significant variations in urea excretion from day to day which were independent of milk yield. In most of the experiments no low protein diet was included in the experimental treatments.

The non-protein nitrogen fraction of milk accounts for about 5% of the total nitrogen. In this fraction are water-soluble, freely diffusible compounds including urea, uracil-4-carboxylic acid, hippuric acid, creatine, uric acid, ammonia and amino acids. Their concentrations in milk varies directly with that in the blood plasma. The quantitatively most important variation is in the urea concentration. (Rook, I97I).

During the course of Dairy Experiments I, 2 and 3 a study was undertaken inter alia to investigate the possible relationships existing between dietary nitrogen intake, blood urea concentration, milk urea (+ammonia) concentration and total daily urea output in milk in healthy lactating cows. The results of these studies are outlined below.

I). Ammonia content in milk. (Expt. I. I969).

Milk samples collected during Expt. I. (I969) were analysed at intervals of I4 days for ammonia-N concentration by the microdiffusion method described in the Analytical Section of this thesis. Milk samples were also analysed on each day of one 6-day period. The results of this study showed there to be a large daily variation in ammonia output. ($\pm$ 60% !). Concentrations of ammonia were observed between I0-600µg NH_3N/I00 ml, realising total daily outputs in the range I0-35 mgs. NH_3-N/day. The mean NH_3 N. output (mgs./day) for each of the treatment diets was as follows. (mean of 8 determinations).

Diet A. (Low Protein) 2I.3 mg.

B. (Urea) 22.7 mg.

C. (Vegetable) I5.0 mg.

These values were not statistically different.

Since the amount of nitrogen secreted in the milk as ammonia was relatively small in comparison with the urea fraction further studies on ammonia excretion in milk were not pursued.

2). The interrelationship of dietary protein intake, blood and milk urea concentrations and the total amount of urea secreted in the milk.

Expt. 2 (I970).

Analyses of the urea concentration of milk produced by cows fed the various diets in Expt, 2 (p.8I) showed a marked and highly significant difference (P=0.00I) between treatments. Cows fed the low protein diet (A) had a concentration of 22.25 mgs urea/I00 ml compared with 34.7I and 34.78 mgs/I00 ml for those cows fed the urea (B) and vegetable (C) diets. These results indicated that a direct relationship might exist between dietary protein intake (irrespective of nitrogen source) and the amount of urea secreted in the milk.

Expt. 3 (I97I).

During the course of this experiment a milk sample from each cow was analysed for urea concentration once a week. Blood samples were collected from each cow and analysed for urea content at I0.00 hours on one occasion during each of the three feeding periods. Careful note of the actual nitrogen intake of each cow was taken during the study. The statistical analysis of the results (Table I2) shows a marked and significant (P=0.00I) increase in milk urea concentration and total daily milk urea output in cows fed either the vegetable or urea-containing diets over that obtained from cows fed the low protein diet. (i.e. concentrations of 34.59 and 34.79 mgs % compared with 23.85mgs % total urea content of 5.55 and 5.63 gms compared with 3.59 gms) .

Correlations were tested between the calculated nitrogen intake (g), blood urea concentration (mgs/I00 ml), milk urea concentration (mgs/I00 ml) and the calculated total daily milk urea output (g), using the collected

data from the whole experiment (when each cow received each of the three diets in turn-Table II), with the following results :-

Test.	Correlation.(r)	Significance(P).
a). Nitrogen intake-blood urea.	+0.562	0.0I
b). Blood urea-milk urea conc.	0.7I3	0.00I
c). Blood urea-total milk urea.	0.452	0.0I
d). Nitrogen intake-milk urea conc.	0.528	0.0I
e). Nitrogen intake-total milk urea.	0.902	0.00I
f). Milk urea conc.-total milk urea.	0.658	0.00I

These preliminary findings show a highly significant correlation between nitrogen intake and daily milk urea output. This degree of significance is somewhat surprising in view of the fact that the correlation coefficient between nitrogen intake and blood urea concentration was less significant (r=+0.562).

Clearly the analysis of urea concentration in milk may provide an indication of the adequacy of the protein intake from the diet. Individual cows fed the low protein diet secreted significantly less urea in the milk than those cows fed either the normal vegetable diet or the urea-containing diet. These results confirm the findings of Rook & Line (I962) who described experiments where significant changes in the NPN content of milk of cows occured when the protein intake was varied. Cows fed 20% below the protein requirement of Woodman (I96I) exhibited a decrease in milk urea concentration of 4.I mgs % . Cows fed 65% above the protein standard exhibited an increase of I0.9 mg urea/I00 ml milk produced. Rook & line (I962) commented, "the non-protein nitrogen content of the milk provides an index of the plane of protein nutrition ".

Experiments on a herd basis performed subsequently to the work described in this thesis have shown clearly defined increases and decreases in milk urea content when the protein ration fed for milk production was either raised or lowered.

SECTION V.

EXPERIMENTS WITH BEEF CATTLE.

a). Introduction.

b). Urea in diets for fattening cattle.

c). Sugar beet pulp and urea in diets for fattening cattle.

d). Experimental

Fattening Cattle Experiment I. (1969).

Fattening Cattle Experiment 2. (1969-70).

Fattening Cattle Experiment 3. (1970-71).

Fattening Cattle Experiment 4. (1971).

Fattening Cattle Experiment 5. (1969-70).

Fattening Cattle Experiment 6. (1969-70).

Discussion.

EXPERIMENTS WITH BEEF CATTLE

Introduction

Growth responses from additional nitrogen in the form of urea are only to be expected when the basal diet contains insufficient crude protein to allow for the full utilization of the available energy of the diet. One problem confronting research on this topic is the apparent disagreement between various estimated protein requirements made by different recognised authorities such as the Agricultural Research Council (1965) and the National Research Council (1963). For example, the available protein requirement (g/day) for cattle weighing 180 kg (400 lbs) gaining 1 kg (2.2 lbs)/day is given as 390 (NRC 1963) and 270 (ARC 1965).

Preston and Willis (1970) have discussed some of the reasons for the differences in the present recommendations for desirable protein intake. Their main points are as follows. The A.R.C. (1965) recommendations are based on the use of a factorial method which is stated to estimate the minimum protein requirement for any specific liveweight and rate of growth. The N.R.C. (1963) standards are based primarily on the results obtained from feeding trials in which the amounts of protein fed were equated to the growth responses obtained. At a liveweight of 450 kg the factorial estimate is only some 50 % of that recommended by the N.R.C. (1963). i.e. 640 (N.R.C.) and 320 (A.R.C.) g/day of available protein.

Preston and Willis (1970) showed that the addition of protein to diets considered adequate according to the factorial system have led to significant responses in growth rate. These authors consider the variables determining protein requirements to be bodyweight, rate of live-weight gain, protein digestibility and voluntary energy intake expressed as Mcal ME/kg liveweight. They concluded "... as presently constituted the A.R.C. (1965) factorial system is totally misleading as regards the needs of growing and fattening cattle", and accordingly considered that the N.R.C. (1963) proposals were to be recommended.

Urea in diets for fattening cattle

In an extensive review on the feeding of urea to fattening cattle Reid (1953), concluded that urea could satisfactorily replace up to 25 % of the total dietary nitrogen intake. The evidence showed poorer rates of growth however when diets containing urea in such an amount were fed to younger animals below about 230 kg liveweight. There are instances in the literature where the addition of urea to diets already adequate in protein (according to A.R.C. 1965) had led to significant increases in liveweight gain and food conversion. For example, Morris (1966), Thrasher, Scott & Hansard (1967), Preston, Willis & Elias (1967), Blaylock, Neagle & Goihl (1965), Kercher & Paules(1968). Results of fattening experiments comparing different nitrogen sources have been difficult to evaluate because the basal rations have often provided nearly all the required nitrogen without any supplementation, and frequently a negative control was not included in the design. It is widely recognised and accepted that the protein requirement, in terms of crude protein percentage of the diet, decreases as the animals grow heavier. Finishing rations may not contain more than 8-9 % crude protein (i.e. that concentration from cereals alone) for animals growing from about 360 kg (800 lbs) to slaughter weight. (Putnam, Oltjen and Bond, 1967). Many experiments have been reported where the initial liveweight of the animals is over 500 lbs (227 kg) and often no significant difference has been observed between treatments regardless of the nitrogen source included in the diet. They may not have needed any additional protein.

Sugar beet pulp and urea in diets for fattening cattle

Much of the published work concerning the urea supplementation of diets containing sugar beet pulp has been performed in Eastern European countries. Limited research has been performed on all-concentrate or concentrates with limited roughage systems of fattening. Again, in many of the experiments the initial liveweight of the animals has been over 500 lbs (227 kg).

Significantly improved food conversions and liveweight gains have been reported for steers fed concentrate rations containing sugar beet pulp when urea was included e.g. Modjanov and Sul'ga, I965, Sadovnikova, I959, Tisserand & Zelter (I960), and Wetterau & Holzchuh (I960). The literature contains little information however on the use of sugar beet pulp as one of the major energy sources in all-concentrate diets. One study by Kercher, Smith & Paules (I966) used IIO Hereford steers and showed that replacing either barley or maize with beet pulp led to reductions in liveweight gain and deterioration in food conversion ratios.

TABLE

Beet Pulp in All-Concentrate Diets

(After Kercher et al, I966)

	% Cereal replaced by beet pulp *			
	0	33.3	66.7	I00
Days of trial				
Barley	I47	I57	I65	
Maize	I57	I70	I64	I76
Daily gain (kg)				
Barley	I.38	I.30	I.I8	
Maize	I.23	I.2I	I.24	I.I5
F.C.R.				
Barley	6.96	7.36	7.87	
Maize	6.97	7.49	7.72	8.28

* 7% of diet composed of a protein, mineral, Stilboestrol and chloratetracycline supplement.

Connolly, Calvill, Caffrey & Ruane (I967) compared maize, barley and beet pulp fed ad-lib to housed Friesian steer calves from two weeks of

age to slaughter at about 380 kg (830 lbs). There were only small differences in liveweight gains but food conversion ratios were some 20 % poorer with beet pulp. (Table I8).

TABLE I8.

Beet Pulp and Cereal feeding. (Mean, 5 animals/group).

(After Connolly et al, 1967).

	Maize		Barley		Beet Pulp	
	Ground	Flaked	Ground	Flaked	Unmolassed	Molassed
Initial wt. (kg)	42.0	44.0	44.5	44.0	42.5	42.5
Final wt. (kg)	383.0	373.0	376.0	375.0	379.0	370.0
Daily Gain	1.13	1.03	1.01	0.98	1.03	0.90
F.C.R.	4.01	4.19	5.19	4.94	6.09	6.04

* All diets supplemented with 0.45 kg (1 lb) hay/head/day.

Boucque, Buysse & Cottyn (1967) compared ensiled pulp (11.7 % DM), ensiled pressed pulp (16.9 % DM) and dried pulp (90.1 % DM) fed ad-lib together with 2.5 kg (5.5 lbs) of a 30 % crude protein concentrate. Daily gains of beef bulls growing from 200 to 510 kg liveweight were 1.17, 1.18, 1.29 kg respectively; corresponding food conversion ratios were 4.8, 5.1 and 5.2 kg DM/kg gain.

Many standard agricultural texts do not advocate the use of sugar beet pulp in proportions above 25 % of the concentrate ration for fattening animals. Experiments described in this thesis include diets where 50 % of the concentrate ration is composed of a sugar beet material. A molassed sugar beet product containing about 16-17 % crude protein (i.e. inclusion of about 2.8 % urea) and with about 0.4 % P (i.e. inclusion of about 1.8 % dicalcium phosphate) would appear to be a suitable material for use in diets for growing cattle. It may be mixed with an equal weight of cereals (giving about 13.5 % crude protein) for fattening cattle on a "barley beef" system or with some hay for more traditional cattle rearing. The following experimental section describes experiments performed using the

urea and phosphate supplemented molassed sugar beet cube including vitamins and trace elements in the fattening of beef animals. The SBP urea cubes containing about 17% crude protein, 1.4% Ca, 0.45% P plus added trace elements and up to 20 m. I. Us Vitamin A and 5 m. I. Us Vitamin D, (Table 2) would when mixed with an equal weight of barley, provide a diet containing about 0.75% Ca, about 0.35% P, trace elements and up to 10 m. I. Us Vitamin A and 2.5 m. I. Us Vitamin D and thus supply more than the suggested requirements (A.R.C. 1965) of 300 lbs (140 Kg) cattle gaining weight at 2.2 lbs (1.0 Kg) /day.

EXPERIMENTAL

FATTENING CATTLE EXPERIMENT 1 1969

Twenty two Friesian calves, 15 weeks of age, were allocated at random to four groups. Two groups consisted of six animals and two groups consisted of five animals each. The mean initial liveweight in each group was about 220 lbs (100 kg). Four different diets were fed for 16 weeks on an ad-lib basis, The experimental design was that of a direct comparison type. The experiment commenced on 22nd May, 1969 and was completed on 11th September, 1969.

The animals were housed in a semi-enclosed building in which four pens each provided 30 sq. ft. of bedded resting area per animal. Feeding and watering arrangements consisted of 18" of trough space/head situated outside the bedded area and two "gravity-feed" water bowls per pen. The animals were fed ad-lib and Table19 details the composition of the diets fed. The animals were weighed on the same day each week at 10.30 a.m. Food residues were weighed twice weekly.

The contrasting diets each contained about 13.5 % crude protein (by analysis on fresh matter) and were calculated to have a Starch Equivalent of about 65 (Evans, 1960). A control diet (Diet 1) consisting of barley and vegetable protein (ground nut and cotton seed cakes) was compared with three experimental diets of which two contained approx. 50 % sugar beet pulp. Supplementary nitrogen was supplied from urea. Diet 3 contained 50 % S.B.P. + urea whereas Diet 4 contained 50 % S.B.P. but where the required urea was mixed with the crushed barley. Diet 2 was composed of barley and urea alone. All the diets were supplemented with adequate (A.R.C. 1965) minerals and vitamins. (Table I9).

TABLE I9.

Diets fed in Fattening Cattle Experiment 1 1969

Diet	1	2	3	4
Diet Ingredient %				
Barley	86.7	95.9	49.0	48.3
S.B.P. + urea	-	-	49.0	-
S.B.P.	-	-	-	48.3
Ground nut	7.0	-	-	-
Cotton seed	3.5	-	-	-
Urea	-	1.3	-	1.4
Salt	0.4	0.4	0.4	0.4
Dicalcium Phosphate	0.8	0.8	0.8	0.8
Limestone	1.6	1.6	0.8	0.8
Vitamin * Trace Elements	+	+	+	+
Crude protein % (by analysis)	13.6	13.5	13.8	13.5
S.E. (by calculation)	69	68	64	64

* Cooper Nutrition Products Ltd. B TE 34 T.E.

All mixes contained 40 g/100 lbs adding in each case to 1 ton of the final mix.

Vitamin A	8 m.	I.u.s.	Iodine	5	p.p.m.
Vitamin D_3	1 m.	I.u.s.	Copper	8	p.p.m.
Iron	100	p.p.m.	Zinc	60	p.p.m.
Cobalt	1.0	p.p.m.	Magnesium	190	p.p.m.
Manganese	20	p.p.m.			

TABLE 20.

Fattening Cattle Experiment 1 1969

Mean liveweight (lbs) and overall Food Conversion Ratios.

Diet	1	2	3	4
	Barley/Veg.	Barley/urea	B/SBP+urea	Barley/urea/SBP
No. of cattle	5	6	5	6
Mean liveweight (lbs)				
Day 0	220	220	224	220
14	238	244	248	245
28	285	280	276	283
42	308	309	300	307
56	358	353	346	350
70	390	375	370	382
84	434	418	417	416
98	471	458	456	449
112	509	496	495	490
Total Gain (lbs)	289	276	271	270
Overall F.C.R. (Fresh Matter basis)	3.82	3.98	4.26	4.43
Mean liveweight Gain lbs/day	2.58	2.46	2.42	2.41

L.W. G/day S.E. difference between 2 means = 0.120 lbs.

L.S.D. = 0.26 lbs.

i.e. no significant difference between treatments.

Results

No difficulties were experienced in feeding any of the four diets. All the animals grew well over the 112 day feeding period. Table 20. outlines the main results of the experiment. Individual increases in liveweight and feed intakes are lodged in the Department of Animal Husbandry, Glasgow University Veterinary School.

The mean daily liveweight gain was greatest (2.58 lbs/day) in the vegetable protein supplemented control ration (Diet 1) and least (2.41) in Diet 4. These values are not significantly different. The diets composed mainly of barley (Diets 1 and 2) gave rise to slightly better daily liveweight gains than those diets composed of 50 % sugar beet pulp. (Diets 3 & 4) i.e. 2.58 and 2.46; 2.42 and 2.41. Food conversion ratios were similarly somewhat better in the diets composed mainly of barley. i.e. 3.82 and 3.98, 4.26 and 4.43. A slightly better food conversion is noted for animals fed on Diet 3 (4.26) where the urea is contained within the sugar beet cube than for animals fed on Diet 4 (4.43) where the equivalent amount of urea was mixed with the barley. Generally,all diets containing urea compared favourably with the vegetable protein control diet.

Fattening Cattle Experiment 2 1969-70

This experiment compared four similar diets to those described in Experiment 1. The animals used were of a heavier initial liveweight than those used in Experiment 1, and the crude protein content of the four contrasting diets was appropriately lower. The experimental design included the measurement of the individual feed intakes of the animals involved. Twenty Friesian steers were allotted at random to the four experimental diets, five animals per group. The steers were chained by the neck in cow stalls each equipped with a water bowl and ground-level feeding trough. The animals had a mean age of 31 weeks and had an initial liveweight of about 540 lbs (245 kg). The experiment commenced on 30th September, 1969 and was completed on 4th March, 1970 (135 days). Table 2I details the composition of the diets fed. All four diets contained about 12.0 % crude protein (Fresh Matter basis) and were calculated to have Starch Equivalents of 66-69 (Evans, 1960). All diets were supplied with adequate minerals and vitamins. The steers were fed twice daily in the stalls. The contrasting diets fed were, 1) Barley 91.5 % , vegetable protein (ground nut) 7.0 %. 2) Barley 97.5 %, urea 1.0 %. 3) Barley 46.3 %, S.B.P. 46.3 %, Ground nut 7.0 %. 4) Barley 49.8 %, S.B.P. 17.8 %, S.B.P. + urea 32.0 %. (All diets containing added minerals and vitamins). Diet 1 acts as a vegetable protein control, Diet 2 has added urea as the only supplemental nitrogen source, Diets 3 & 4 both contain about 50 % sugar beet pulp. The supplementary nitrogen (in Diet 4) is in the form of urea in sugar beet pulp nuts and/as ground nut in Diet 3.

Table 21.

Results of Fattening Cattle Experiment 2.

Diet	1	2	3	4
Dietary Ingredient %				
Barley	91.5	97.5	46.3	49.8
S.B.P.	-	-	46.3	17.8
S.B.P. + urea	-	-	-	32.0
Ground nut	7.0	-	7.0	-
Urea	-	1.0	-	-
Limestone	1.1	1.1	-	-
Salt	0.4	0.4	0.4	0.4
Vitamins* - As in Table I9.				
Crude protein % (by analysis)	12.2	12.0	11.9	12.2
S.E. (by calculation)	68	69	66	66
No. of Animals	5	5	5	5
Mean Initial wt. (lbs)	536	539	536	528
Mean Final wt. (lbs)	833	848	849	830
Mean Total Gain (lbs)	297	309	313	302
L.W.G. lbs/day	2.20	2.29	2.33	2.24
S.E. difference between 2 means, 0.14 lbs.				
F.C.R.	8.62	8.66	9.84*	9.44*
S.E. difference between 2 means, 0.42 lbs.				

Results

The principal results of the experiment are detailed in Table 2I. Individual liveweight and feed consumption records are housed in the Department of Animal Husbandry, Glasgow University Veterinary School. The animals grew reasonably well throughout the experiment. There were no significant differences between the daily liveweight gains of the animals on each of the treatments. Animals fed barley, sugar beetand ground nut (Diet 3) grew somewhat better than those fed on the contrcl Diet 1. i.e. 2.33 lbs/day and 2.20 lbs/day. Diets containing approximately 50 % sugar beet pulp (Diets 3 & 4) compared most favourably with those containing mostly barley. (Diets 1 & 2). i.e. 2.33, 2.24 and 2.20, 2.29 lbs/day. Food conversion ratios were significantly poorer ($P = 0.05$) in those groups fed in diets containing 50 % sugar beet pulp. (Diets 3 & 4), when compared with those fed on diets containing nearly all barley (Diets 1 & 2) i.e. 9.84, 9.44 compared with 8.62 and 8.66.

This particular system of experimental management where the animals are tied and fed individually is known to reduce performance (Rowett Research Institute Annual Report, 1964). It was, however, not possible to make other arrangements for the individual feeding of this size of animal. The most unfortunate feature of this experiment was that the arrangement of the troughs led to considerable wastage due to individual cattle placing their forelegs in the troughs. Wastage occurred equally in all four groups and the best attempt possible has been made to allow for this.

The experiment does demonstrate some agreement with the results obtained by Kercher et al (1966) and Connolly et al (1967) in that 50 % cereal replacement by sugar beet pulp does not alter the rate of liveweight gain, but that some reduced efficiency in the food conversion ratio is apparent.

FATTENING CATTLE EXPERIMENT 3. 1970-71

Thirty two Friesian steers aged about 16 weeks and with a mean liveweight of about 280 lbs were divided at random into four comparable groups. They were housed in equal sized pens allowing about 25 square feet of bedded area per animal. Each group fed together from an 8 ft. trough which provided fully adequate space as feeding was on an ad-lib basis throughout 24 hours.

The experimental diets were as follows: Diet A consisted of 85 % barley mixed with 15 % of a protein concentrate composed of equal parts of ground nut and cotton seed cakes and some barley having 34 % crude protein. The crude protein concentration of the mixture was 13.6 %.

In Diet B one half of the barley was replaced with sugar beet pulp nuts (no urea). The same quantity of the 34 % vegetable protein concentrate was added as for Diet A. The crude protein concentration of the overall mixture was 13.4 %.

Diet C was composed of 50 % barley and 50 % Sugar beet nuts with urea. Urea was accordingly the only form of additional protein and the total concentration of crude protein was 13.5 %.

Diet D was the negative control diet composed of 50 % barley and 50 % Sugar beet pulp nuts with no added urea. (10.4 % crude protein for the mixture). Accordingly, three of the diets (B, C and D) contained 42.5-50.0 % Sugar beet pulp in contrast to Diet A which was predominantly barley. Further details of the diets fed are presented in Table 22.

Table 22.

Diets fed during Fattening Cattle Experiment 3. 1970-71

Diet	A	B	C	D
Ingredient %				
Barley	85.0	42.5	50.0	50.0
Sugar beet + urea	-	-	50.0	-
Sugar beet	-	42.5	-	50.0
34 % CP. Protein Conc*	15.0	15.0	-	-
CP. %	13.6	13.4	13.5	10.4

* Composed of 32.6% Groundnut, 37.8% cottonseed, 18.4% barley, 2.6% dicalcium phosphate, 2.6% sodium chloride plus trace elements.

The first stage of the experiment lasted 70 days from 3 November 1970 - 12 January 1971. On 12 January certain of the treatments were interchanged and the trial continued for a further 56 days to 30 March 1971. The interchanges in diets were as follows:

1. Group D diet (50 % barley/50 % SBP) changed to the Group C diet of 50 % barley + 50 % SBP urea i.e. Increase in urea.
2. Group C diet changed to Group D diet (as above) i.e. Removal of urea.
3. Group A diet (barley + vegetable protein) changed to the Group B diet i.e. Replacement of half the barley with SBP.
4. Group B diet changed to Group A diet (as above) i.e. Replacement of SBP with barley.

All the rations were consumed well over the whole period of the experiment and there were no palatability problems. The overall growth rate averaged between the groups was about 2.5 lbs/head/day. The cattle were weighed once a week and the total amounts of food consumed by each group were recorded at regular intervals.

During the first period of the trial (70 days) Group A, fed the barley/vegetable protein diet, had the best liveweight gain (2.66 lbs/day) and the best food conversion ratio (3.40 lbs/lb L.W.G.). Growth (2.52 lbs/day) and food conversion (3.70) were almost as good for Group B where half the barley was replaced with Sugar beet. When Sugar beet + urea fed at 50 % with barley formed the only protein supplement (Group C), growth rate was maintained at 2.49 lbs/day but food conversion was poorer (4.36). The three supplemented groups all had liveweight gains which were significantly greater (Table) than that for the 10.4 % crude protein diet (2.14 lbs/day) which also had an inferior food conversion ratio (4.88).

Following the interchange of diets, the removal of urea from the Sugar beet (Group C to Group D) resulted in a fall in the mean liveweight gain from 2.49 to 2.31 lbs/day, and an increase in the food conversion ratio from

4.36 to 5.36. Addition of urea (Group D changed to Group C) increased the rate of gain from 2.14 in the first period to 2.67 in the second. A decrease in the food conversion from 4.88 to 4.58 occurred. The averaged values for liveweight gain and food conversion for Diets C and D are detailed in Table 23. Accordingly addition of urea resulted in a substantial increase in liveweight gain and an improvement in food conversion ratio.

In the second period when one half of the barley in Diet A was replaced with Sugar beet pulp, (Diet A changed to Diet B), the daily liveweight gain hardly changed from 2.66 to 2.63 lbs but the food conversion ratio changed from 3.40 to 4.38. When the reverse change was undertaken in Period 2, i.e. replacement of Sugar beet pulp in Diet B to form a predominantly barley based ration (Diet A), the daily liveweight gain changed from 2.52 to 2.16 lbs and the food conversion ratio increased from 3.70 to 4.94. The effects of substituting Sugar beet pulp for one half of the barley when vegetable protein was the only supplement is summarised in Table 23. There was apparently a slightly better mean liveweight gain for Diet B but the mean food conversion ratios were very similar.

It can be concluded that in diets when Sugar beet pulp forms approximately one half of the diet, daily liveweight gains are favourably comparable to a diet based on barley alone (about 2.5-2.6 lbs/day) and the food conversion is only inferior when all the supplementary protein is derived from urea. (4.57 compared with 4.08).

Table 23.

Fattening Cattle Experiment 3. 1970-71

Diet	A	B	C	D	S.E. diff
	Barley Veg.protein	Barley S.B.P. Veg.protein	Barley S.B.P. + urea	Barley S.B.P.	
% Crude Protein	13.6	13.4	13.5	10.4	
Period 1					
Days	70	70	70	70	
Group No.	1	3	4	2	
Daily L.W. Gain (lbs)	2.66**	2.52*	2.49*	2.14	0.13
F.C.R.	3.40	3.70	4.36	4.88	
Period 2					
Days	56	56	56	56	
Group No.	3	1	2	4	
Daily L.W. Gain (lbs)	2.16	2.63**	2.67**	2.31	0.14
F.C.R.	4.94	4.38	4.58	5.36	
Mean. Periods 1 and 2					
Daily L.W. Gain (lbs)	2.44*	2.57**	2.57**	2.21	0.08
F.C.R.	4.08	4.00	4.57	5.09	

Sig. Diff. from basal Diet B.

* P = 0.05 ** P = 0.01

In this experiment an attempt was made to relate mean blood urea concentrations to the mean liveweight gain and daily crude protein intake for the separate groups. Blood samples were obtained from each animal on the last day of both Periods 1 and 2 and the mean plasma urea concentrations are given in Table 24

Table 24.

Mean Plasma urea concentrations obtained at the end of each feeding period

		After Period 1	After Period 2	Mean
A.	Barley/Veg. Protein	16.25**	17.75	17.05 **
B.	Barley/S.B.P./Veg. Protein	11.68*	23.00*	17.34 **
C.	Barley/S.B.P. urea	17.78**	21.40*	19.59 **
D.	Barley/S.B.P. (Control)	5.89	12.87	9.38

The control diet D (with no protein supplementation and with the lowest growth rate) had a mean plasma urea concentration of 9.38 mg/100 ml which was significantly ($P \leq 0.01$) lower than that for the other three groups which were in the range 17.05-19.59 mg/100 ml. The value for the group fed sugar beet + urea of 19.59 mg/100 ml was not markedly different from those fed vegetable protein (17.05 and 17.34). The blood samples obtained at the end of Period 2 were also analysed for calcium, magnesium and phosphorus. The mean concentrations obtained were; Ca, 11.8 mg/100 ml (range 11.4-12.4); Mg, 2.38 mg/100 ml (range 2.20-2.66); P, 9.35 mg/100 ml (range 8.10-10.10). There were no differences between the various dietary groups and it is accordingly concluded that mineral supplementation was fully adequate.

FATTENING CATTLE EXPERIMENT 4. 1971

The three experimental treatments used in this trial were intended to be comparable to three of those used in Experiment 3. The actual diets employed are detailed in Table 25.

Table 25.

Diets fed in Fattening Cattle Experiment 4. 1971

Diet	A	B	C
Ingredient %			
Barley	34.8	50.0	50.0
S.B.P. + urea	-	50.0	-
S.B.P.	50.0	-	50.0
36 % CP. Protein Conc.*	15.2	-	-
Crude Protein % ($\frac{\text{Mean}}{\text{Analysis}}$)	13.7	13.6	10.2

* See table for Experiment 3. on p. I25.

Each contains essentially 50 % Sugar beet pulp. In Diet A the protein supplement is based, as in Experiment 3, on vegetable protein, i.e. ground nut and cotton seed; in Diet B the protein supplement is urea included in the Sugar beet cube; in Diet C no protein supplement has been added.

Eighteen steer calves (mean liveweight 220 lbs aged about 14 weeks) were available for this trial and consisted of approximately equal numbers of Hereford/Ayrshire cross, Hereford/Friesian cross and Friesians. Three comparable groups of calves were formed which were housed separately and allowed some 20 sq. ft. of bedded area per calf.

The previous trial (Experiment 3) had employed a measure of interchange of diets and it was decided to conduct this present trial in a Latin Square Design so that each group of calves received each of the three diets for 4-week periods over a total experimental time of 12 weeks from 29 December 1970 - 23 March 1971. It was appreciated that difficulties in interpretation of the overall results might be encountered due to alterations of

growth rate in the different diets leading to varying measures of compensatory growth patterns. Nevertheless it was thought interesting to assess the magnitude of such possible differences in daily liveweight gain. The sequence of dietary changes were as follows:-

Calf group No.	1	2	3
Period			
I	A	B	C
II	B	C	A
III	C	A	B

There were no palatability problems and all diets were readily consumed. The mean results from the entire trial are detailed in Table 26. The overall growth rate of 1.94 lbs/day for the unsupplemented group was increased to 2.39 lbs by the inclusion of vegetable protein and to 2.33 lbs by the inclusion of urea in the sugar beet pulp. Mean food conversion ratios for the two supplemented groups were very comparable (4.65 and 4.66) and were much better than that for the unsupplemented Group C. of 6.13 lbs/lb L.W.G. However, the differences in growth rate were found not to be significant.

On the other hand the mean results obtained during the first 4 weeks of the trial (Table 26.) showed a clear benefit from the inclusion of either vegetable protein or urea and much superior food conversion. The growth rate found in this first 4-week period is rather less than that averaged over the whole 12 weeks partly because during this first 4 weeks the calves were still being accustomed to a full ad-lib dietary regime.

During the second 4-week period, the calves which had previously received no protein supplement increased their daily L.W.G. from 1.0 to 3.0 lbs/day when vegetable protein was included and this must have included a large element of "compensatory growth".

This and other anomolous growth patterns have contributed to the large experimental error shown for the overall mean results in Table 26. Nevertheless if the dietary regimes fed in the initial 4-week period had been continued it would have been reasonable to assume that the inclusion of urea

Table 26.

Fattening Cattle Experiment 4. 1971

Mean results obtained from period I (29/12-26/1)

Diet	L.W.G./day (lbs)	F.C.R.
A. Barley/S.B.P./Veg. protein	1.59*	4.9
B. Barley/S.B.P. urea	1.81 **	4.3
C. Barley/S.B.P.	1.06	8.0

S.E. of treatment mean ± 0.19 lbs.

Mean results obtained from complete experiment

Diet	L.W.G./day (lbs)	F.C.R.
A. Barley/S.B.P./Veg. protein	2.39	4.65
B. Barley/S.B.P. + urea	2.33	4.66
C. Barley/S.B.P.	1.94	6.13
S.E. of treatment mean	± 0.184	± 0.483
Least Sig. diff. between P = 0.05	0.714	1.89
2 treatment means P = 0.01	1.820	3.14

in the S.B.P. cube would have given improved growth rates comparable to that following the inclusion of vegetable protein.

It does not seem practical to use such a Latin Square Design in experiments with young fattening cattle. It is well known that animals of all species which have been subjected to a period of 'under nutrition' subsequently exhibit "compensatory growth" during the period of realimentation (Wilson & Osbourn I960). The work of Winchester & Howe (I955) and Tayler, Alder & Rudman (I957) showed that while part of the increased gain could be ascribed to increased gut content, cattle exhibiting compensatory growth also laid down tissue at a faster rate than unrestricted controls. Winchester & Howe (I955) and Winchester,Hiner & Scarborough found that animals could recover from restriction of either energy or protein to the extent that feed conversion on a life-time basis was unaffected.

FATTENING CATTLE EXPERIMENT 5. 1969-70

This experiment commenced on 27th October 1969 and was completed on 4th March 1970. Ten Hereford x Friesian steers were divided at random into two groups of mean liveweight 630 lbs. The animals were housed in separate covered pens allowing 40 square feet of bedded area per head. Each animal had 30" of trough space allowed. The groups were fed twice daily.

Two concentrate diets consisting of 50 % Barley,+50 % Sugar beet pulp, one diet having urea included in the sugar beet cubes, were fed on a limited roughage/concentrate scheme. During the first two months of the trial the steers were fed up to ad libitum concentrate intakes. Thereafter the concentrate intakes were limited to 20 lbs/head/day and the hay allowance suitably increased.

Results

No differences existed between the groups in liveweight gain or food conversion ratios.

Table 27.

Fattening Cattle Experiment 5. 1969-70

Hereford x Friesian steers

Diet	CP %	Mean Liveweight (5) lbs. 27/10/69	4/3/70	Gain	lbs/ day	Food lbs	Overall F.C.R.
Hay + 50 Barley 50 S.B.P.+urea	13.6	635	883	248	2.00	1792	7.2
Hay + 50 Barley 50 S.B.P.	10.0	627	880	253	2.06	1881	7.4

FATTENING CATTLE EXPERIMENT 6. 1969-70

This experiment commenced on 5th November 1969 and was completed on 4th March 1970.

Twenty six Aberdeen Angus cross Galloway steers were divided at random into two groups of mean liveweight 530 lbs. The animals were housed in separate covered pens allowing 35 square feet of bedded area per head. The animals were fed twice a day. Two concentrate diets consisting of 50 % Barley,+50 % Sugar beet pulp were fed on a limited roughage, ad-lib concentrate system. Both diets contained urea in a proportion of the Sugar beet pulp cubes, one diet having twice the quantity urea containing cubes. An unfavourable factor in this trial was the poor quality of hay fed during the earlier stages. Some information was lost owing to there being more 'finished' steers in one of the groups before the end of the trial.

Table 28.

Fattening Cattle Experiment 6.

Aberdeen Angus cross Galloway steers

	Diet		CP %	Mean Liveweight lbs 5/11/69	4/3/70	Gain	lbs/ day	Food lbs	F.C.R.	Nos.
A	50	Barley	13.5							
	50	SBP+urea		526	730	204	1.7	1557	7.6	13
B	50	Barley								
	25	SBP+urea	11.7	526	750	224	1.9	1580	7.1	13
	25	SBP.								

Discussion.

The results of Experiments I and 2 indicate that in concentrate diets offered ad lib to growing cattle and containing about I3.5 or I2.0% crude protein the substitution of molassed sugar beet pulp nuts for one-half of the barley did not reduce the daily liveweight gain. Liveweight gains were essentially the same for diets containing either urea or groundnut as protein sources.

In both Experiments 3 and 4 where either urea or groundnut / cottonseed mixtures were included to increase the crude protein concentration from about I0 to about I3.5%, each produced broadly comparable and significant increases in daily liveweight gain and improvements in the food conversion ratio.

In each of Experiments I, 2 and 3 where direct comparisons were made between (a) the normal "barley beef" type diets of either 85% barley/I5% vegetable protein/mineral/vitamin supplement or over 95% barley plus a urea/mineral/vitamin supplement (Experiments I and 2) with (b) a mixture of 50% barley with 50% of the Triple Nut SBP urea product, entirely comparable liveweight gains were produced. However, in each case there was a I0 - I2% reduction in food conversion ratios as a result on the SBP inclusion. Very comparable reductions in food conversion ratios have been previously reported by other workers. (e.g. Kercher et al, I966; Connolly et al, I967).

Hurst and Thompson (I7I) have recently reported the results of a comparable trial to the experiments described in this section using the same SBP urea product. Four groups, each of I2 Friesian steers with an initial liveweight of I50 Kg were offered ad lib four diets each containing about I3.5% crude protein for a period of I69 days. The mean daily liveweight gain (LWG) and food conversion ratios (FCR) of the cattle growing from I50 to about 390 Kg were;

	LWG (Kg/day)	FCR
(a). 50% barley/50% SBP with urea	I.44	5.0
(b). 50% barley incl. urea/50% SBP.	I.40	4.9
(c). I00% barley incl. urea.	I.30	4.6
(d). 85% barley/I5% veg. protein conc.	I.40	4.8

The inclusion of sugar beet pulp at 50% of an otherwise predominantly barley-based diet appeared to marginally improve the daily liveweight gain relative to the more normal 85% barley/I5% vegetable protein supplement. The food conversion ratio was perhaps reduced marginally by 2-4%.

The results obtained in this present series of experiments are in accord with the general findings that urea-supplemented sugar beet products, suitably formulated, may be used as carriers of non-protein nitrogen, minerals and vitamins and be used in substantial amounts in diets for intensively-fed beef cattle.

SECTION VI.

Experimental work with pregnant and lactating ewes involving Sugar Beet Pulp products.

a). Introduction.

b). Experimental.

Housed Ewe Experiment I. (1969).

Housed Ewe Experiment 2. (1970).

Housed Ewe Experiment 3. (1971).

Discussion.

c). Changes in blood urea and free fatty acid concentrations as affected by the diet in Greyface ewes during late pregnancy and early lactation during Housed Ewe Experiments I - 3.

Experimental,

a). Urea concentrations in the blood of ewes during late pregnancy and early lactation when fed diets containing SBP $\pm$ urea.

b). The variation in blood urea concentrations in late pregnant Greyface ewes, a).fed diets containing SBP $\pm$ urea during a normal twenty-four hour day, b). deprived of food over a twenty-four hour period.

c). Concentrations of FFA observed in Greyface ewes fed diets containing SBP $\pm$ urea during late pregnancy and early lactation. (Housed Ewe Expt. 2. 1970).

(cont.)

d). Digestibility and Nitrogen Retention Trials involving the Sugar Beet Pulp products fed during Housed Ewe Experiments I - 3.

e). The fattening of Blackface lambs on different urea-containing diets and some relationships between blood urea concentration and other production factors.

Experimental Work with Pregnant and Lactating Ewes Involving Sugar Beet Pulp Products

Introduction

Many previous investigations involving the nutrition of the ewe recorded in the literature have mainly been concerned with feeding either a) in pregnancy to assess effects on birthweight or b) in early lactation to determine the effects on lamb growth. The experimental work described in this thesis has been mainly concerned with the protein intake of ewes during the period from mid-pregnancy to three weeks after parturition and the progress of the ewes and lambs has been followed until some nine weeks after lambing. A short review of the literature concerned with the nutrient requirements of the ewe during pregnancy and lactation follows.

Nutrient Requirements

Precise information on the nutrient requirements of non-pregnant, late pregnant and lactating ewes is not available. There is a fairly wide range of recommendations for both protein and energy requirements of ewes at different stages of gestation and lactation. For example investigations into responses to additional protein during late gestation and early lactation have given variable results. Some of the diversity in recommendations (Table 29) is likely to arise from differences in the amount and composition of the milk produced and the extent to which ewe body reserves are altered during pregnancy and lactation.

TABLE 29.

Protein Requirement for pregnancy: Composition of some requirements suggested by different authorities.

Source		g. D.C.P. required for 70 Kg ewe bearing twins
Phillipson (1959)	Early pregnancy	57
	Last 6 weeks of pregnancy	114
A.R.C. (1965) Shortwool	Months 1 and 2	45
	3	54
	4	75
	5	115

(cont.)

Source		g. D.C.P. required for 70 Kg ewe bearing twins
Robinson (1966)	Diet given throughout pregnancy	
	Range given	40-96
	Optimum for maximum nitrogen retention	81
Lowman (1970)	Diet given throughout pregnancy	
	Range given	24-68
	Minimum requirement suggested	31

Other difficulties confronting research on this topic have been the large number of different breeds of sheep, the diversity in systems of management, a lack of adequate recording systems, and often failure to analyse all foods for protein and energy content. Another basic difficulty is to assess changes in the general body condition of the ewe during the course of the investigation.

Nutrient requirements during pregnancy

The morphological growth data of foetal lambs obtained by Cloete (1939) and Wallace (1948) showed that the nutrient requirements for the products of conception were negligible up to mid-pregnancy. Over 70 % of the lamb birthweight is laid down over the last six weeks of pregnancy (Curson & Malan, 1935; Winters& Feuffel, 1936) and Wallace (1947) showed that poor feeding during that period could severely reduce the birthweight of the lamb. Birthweight is commonly used as a criterion to assess the relative success of contrasting nutrition of the pregnant ewe. The majority of evidence indicates an advantage from additional feeding in late pregnancy. Only a few reports e.g. Whiting & Slen (1958), record no increase in lamb birthweight by extra feeding in late pregnancy.

Recommendations for energy requirements for ewes for maintenance, late pregnancy and lactation are varied.. The energy requirement of a 70 Kg ewe for maintenance is given by Evans (1960) as 759 g SE/day and the A.R.C. (1965) recommended the equivalent of 474 g SE/day. (i.e. 1.81 - 2.03 M.cal ME.) After a most extensive review of the literature the A.R.C. (1965) were unable to give any proposals for the energy requirments of the ewe during pregnancy. In a review of experiments with late-pregnant

ewes, Lowman (1970) extracted separate energy requirement estimates. These estimates ranged from 144-278 % (mean 196 %) of the maintenance requirements. Doney & Reid (1967) used the method of maintaining constant and adequate plasma free fatty acid concentrations as an index of adequate nutrition and estimated the requirement of Scottish Blackface sheep to be 240 and 312 % of maintenance for single and twin lambs bearing ewes respectively. Their calculations however included experimental periods immediately before and after lambing and this may have substantially increased these estimates.

Russel (1967) used a regression of the amounts of food supplied to ewes on the birthweight of their lambs which provided an estimate of additional nutrient requirements in late pregnancy. This amounted to 100 g D.O.M./Kg foetus. If a maternal maintenance requirement of 8-9 g D.O.M./Kg is assumed then 900 g D.O.M./head/50 Kg ewe with a single foetus would be adequate. If twins were carried a further 350 g D.O.M./day was estimated to be required to avoid a degree of ketosis.

Thomson & Aitken (1959) used the absolute gain or loss of body weight of the ewes as an indirect criterion of the plans of nutrition in an attempt to find a common denominator on which to base positive recommendations. A relationship was found between the degree of energy under-nourishment and the concentration of plasma free fatty acids, which could in turn be related to the foetal load carried by the individual ewe.

Owen (1970) reviewed recent literature in an attempt to assess the liveweight gain necessary during late pregnancy to yield lambs of normal birthweight. He concluded that when the liveweight of ewes remained constant during the last 7 weeks of pregnancy the birthweight of lambs was lower than that of those born to ewes which had gained some weight during this period. This, together with experiments by Treacher (1967), suggests that a gain of 15-20 % of the liveweight during the six to eight weeks before lambing is consistent with the production of twin lambs of normal birthweight. For single lambs, gains of 5-10 % in ewe liveweight

would appear to be adequate.

The recommended standards for protein intake during pregnancy are also somewhat varied. In a review of previous experimental work Forbes & Robinson (1967) have shown that metabolic faecal nitrogen varies with feed intake and accordingly there is no unique protein requirement in terms of apparently digestible protein. However, a daily intake of about 120 g digestible crude protein (DCP) has been widely accepted as the standard protein requirement of the 68 Kg (150 lb) breeding ewe. (Thomson & Aitken, 1959; Morrison, 1959; Phillipson, 1959). Other workers (e.g. Klosterman, Bolin, Buchanan, Bolin, Dinusson 1951, 1953) have reported satisfactory results with as little as 50 g DCP/day. Slen & Whiting (1952) found that 160 lbs (72 Kg) ewes receiving 0.13 lbs (59 g) DCP/head/day over the last six weeks of gestation produced significantly lighter lambs than those receiving 0.23 lb (104 g) DCP. (There was evidence also that the milk production during the first six weeks of lactation was adversely affected by the lower protein intake in pregnancy). The National Research Council (1946) recommendations concluded that 0.25 lb (112 g)/day was adequate for ewes weighing 150 lbs (68 Kg) and the Agricultural Research Council (1965) calculating from their estimates of the available protein (AP) requirement based on the factorial method indicated a requirement of approximately 0,30 lb (136 g) DCP/day/150 lb ewe during the last six weeks of pregnancy.

Other factors which affect nitrogen utilization in sheep include the energy content of the diet and the proportion of fibre present. (Chalmers, 1961; Armstrong & Blaxter, 1957; Annison & Lewis, 1959; Elliot & Topps, 1964). Few experiments have involved treatments with the feeding of diets of standardised fibre content but with differing energy and protein intakes.

Nutrient requirements during lactation

British standards for energy requirements during lactation for ewes (Evans, 1960) are based on the requirements of cows adjusted for the

high content of fat in ewe's milk. The estimates range from 4-6 lbs SE/gallon.

The recommended standards for protein intake during early lactation are also somewhat varied. The National Research Council (1968) indicated that 115 g DCP/68 Kg ewe to be satisfactory. Morrison, (1959) showed 150 g DCP/68 Kg ewe to be fully adequate and the Agricultural Research Council (1965) from factorial estimates gives a requirement for a 68 Kg ewe of 165 g DCP/day. Hogue (1967) recommended a daily crude protein intake of 230 g and 265 g for single and twin suckling ewes respectively. Assuming 70 % digestibility these are equivalent to 161 and 185 g DCP/day.

Urea in diets for breeding ewes.

Most of the literature on the use of urea in rations for pregnant and lactating ewes indicates a definite improvement in lamb birthweight, and liveweight gain, ewe liveweight gain in pregnancy and fleece weight when urea is added to a basal ration of lower protein content. Differences which have been found in these production factors when a urea-containing diet is compared with a vegetable protein containing diet have generally been quite small. e.g. Pope, Whitehair, Bell, Tidwell, Bonner & Gallup, 1951; Jordan, 1952; Klosterman, Bolin, Buchanan, Bolin & Dinusson, 1953; Mason, Stratton & Hilston, 1953; Kurelec, 1959; Paliam & Markotic, 1962; Sattarov, 1965.

Experiments with Housed Ewes during Pregnancy and early Lactation

Three principal experiments (1, 2, 3) were conducted during the winter/spring period of each of the seasons 1968/69, 1969/70 and 1970/71. Each followed the same general experiment pattern and the following information summarises the features common to all three trials; more precise experimental details are given in the sections detailing each particular experiment.

A group of about 150 Greyface ewes were mated with Suffolk tups at grass in early October of each year. The tups were fitted with colour marking devices and service dates were recorded twice weekly. In early January about 100 ewes were housed, selection from the larger flock being made on a basis of a restricted range of tupping dates.

The ewes were housed on slats in a well ventilated building in groups of about 15 allowing about 15 sq ft of floor space per head and about 15 inches of trough space. Water was freely available. At housing the ewes were dosed with an efficient anthelmintic ("Thibenzole") and routine precautions were taken against foot disorders.

Because of the somewhat restricted numbers of animals involved and the complications of ewes giving birth of single, twin and triplet lambs, the differential diets fed to the ewes have been such that the total dry matter and energy intakes have been substantially similar for the various groups of ewes in each of the three years. Comparisons have essentially been made between (a) 2 lbs/day (908 g) of sugar beet pulp nuts (c. 10 % crude protein) and (b) 2 lbs sugar beet pulp + urea (c. 16.5 % crude protein) fed during pregnancy and/or early lactation. These were invariably fed on a single occasion each morning. In addition, the ewes received 2 lbs (908 g) hay/head fed once per day in the late afternoon from Norwegian-type hay racks.

In each year the allocation of ewes to each nutritional treatment was made in the following general manner, variations in each year being described in detail later. About two thirds to three quarters of the ewes

were group-fed sugar beet pulp nuts with no protein supplement in pregnancy. After lambing a proportion continued to receive no protein supplement, and a proportion commenced on the day of lambing to receive either urea or vegetable protein (groundnut) supplementation. In this way it was possible to allocate ewes with single or multiple births to even-sized groups after lambing. This was done on a random basis but giving an even spread of lambing date and age of ewe. The remaining one-third to one-quarter of ewes received sugar beet nuts with no nitrogen additive throughout late pregnancy and early lactation.

At lambing the ewes and their individual lambs were separately penned for 24-48 hours and subsequently grouped together according to nutritional treatment. Ewes giving birth to triplets had one lamb removed. These were either twinned on to a suitable ewe with a single lamb or alternatively were reared separately on milk powder; i.e. normal animal husbandry practices were adopted.

The ewes and lambs continued to be housed for three weeks when the lambs were docked and castrated. As individual ewes and lambs attained three weeks of age they were turned out to grass. At grass, all treatment groups were grazed together and concentrate supplementation (sugar beet nuts with no protein additive) were fed as appropriate (generally for 2-3 weeks depending on the season). Ewes and lambs were weighed at birth, at three weeks, six weeks and nine weeks after lambing. In the tabulated results lamb weights have been adjusted to exactly three weeks, six weeks and nine weeks based on interpolation from more frequent weighings.

During each of the three years digestibility and nitrogen balance trials were conducted with ewes from each nutritional group towards the end of pregnancy. The form of metabolic cage used was that described by Duthie (1959) which allows for the mechanical separation of the urine and faeces from ewes.

Individual liveweight records of the ewes and lambs involved in Experiments 1, 2, and 3 number many hundreds and are too voluminous to be

presented here. The full records are deposited in the Department of Animal Husbandry, Glasgow University.

Housed Ewe Experiment 1, 1969

Ninety three Greyface ewes were housed on 13th January, 1969. Four ewes subsequently were found not to be in-lamb and a total of eighty nine ewes were accordingly used in the main experiments. The contrasting diets fed during housing were:

During Pregnancy		For three weeks after lambing
38 ewes.	Sugar beet alone	Sugar beet alone. 14 ewes with single lambs 24 ewes with twin lambs
26 ewes.	Sugar beet alone	Sugar beet + urea 9 ewes with single lambs 17 ewes with twin lambs
25 ewes.	Sugar beet + urea	Sugar beet + urea 9 ewes with single lambs 16 ewes with twin lambs

Both types of sugar beet pulp cubes were fed at 2 lbs (908 g)/day during pregnancy and at 2½ lbs (1130 g)/day during the first three weeks after lambing. In addition the ewes received 2 lbs (908 g) hay/day throughout the whole period. The amounts of crude and digestible crude protein given were as shown in Table 30.

Calculation shows that 35.0 % of the total crude protein intake was supplied by urea in the urea-supplemented group. During late pregnancy and lactation a noteable observation made during this particular experiment was that those ewes given sugar beet + urea consumed their hay very much more rapidly than those given sugar beet alone.

TABLE 30.

Experiment 1, 1969. The composition and amounts of crude and digestible protein in the sugar beet pulp and hay fed to ewes during pregnancy and lactation.

Table 30.

	Sugar Beet alone	Sugar Beet + urea	Hay
Crude protein %	9.5	18.0	6.8
Digestible crude protein %	5.9	14.1	1.5
2 lbs/day in pregnancy			
Crude protein g.	77.2	145.0	48.7
Digestible protein g.	47.8	120.0	10.7
2½ lbs/day in early lactation			
Crude protein g.	96.5	181.0	-
Digestible crude protein g.	59.7	150.0	-

Lambing occurred between 4th March and the 2nd April 1969. There were no particular difficulties associated with lambing but subsequently many of the lambs developed severe orf. Some of the ewes were similarly affected on their udders and this undoubtedly had an adverse effect on the subsequent liveweight gain of the lambs. Observations indicated that the group given no urea throughout were perhaps the worst affected. The initial appearance and growth of all the lambs for the first week was quite satisfactory but during pregnancy and early lactation was apparently severely inadequate. The twin lambs born to such ewes were particularly adversely affected. Five ewes in this group lost a total of ten lambs between 2 and 3 weeks after lambing and it became essential to offer creep-feed to the surviving lambs in this particular group of ewes.

The ewes and lambs were transferred to grass as they reached three weeks of age and thereafter grazed as one large group. The weather was very cold and wet. Supplementary feeding with both hay and concentrates (sugar beet nuts with no urea) continued for a further five weeks.

Tables 31 and 32 detail the changes in mean ewe and lamb liveweights respectively throughout the course of the epxeriment. It should be noted that the mean liveweights recorded for the twin lambs in the group of ewes fed no urea during both pregnancy and early lactation refers to the 38 survivors (of the total of 48 lambs potentially in that group) and which

Table 3I.

Experiment 1, 1969. Changes in Mean Liveweight (lbs) of Greyface Ewes

a) Single lambing ewes

Protein before lambing	Supplement after lambing	Number of Ewes	Tupping	Mid Pregnancy (16/1/69)	Late Pregnancy	Three weeks after lambing
0	0	14	127	128	145	119
0	urea	9	136	135	153	125
urea	urea	9	123	124	155	141*
		L.S.D. (P = 0.05)	14	13	15	13

b) Twin lambing ewes

0	0	20	141	140	163	130
0	urea	20	134	135	158	130
urea	urea	18	140	141	170	130
		L.S.D. (P = 0.05)	16	16	15	12

Table 32.

Experiment 1, 1969. Changes in mean liveweight (lbs) of Cross lambs born to Greyface ewes.

Ewe diet	Pregnancy Lactation	0 urea 0 urea	0 urea + urea	+ urea + urea	L.S.D. (P=0.05)* (P=0.01)**
Single lambs	No. of lambs	14	9	9	
	No single lambs died				
	Birth	10.4	10.6	13.2*	2.4
	3 weeks	20.4	24.1	26.0*	4.9
	6 weeks	29.6	35.3	39.0*	7.0
	9 weeks	38.1	46.5	51.2**	9.1
Twin lambs	No. of lambs	38	34	32	
	Additional lambs which died	10(21%)	6(15%)	4(11%)	
	Birth	9.4	9.4	9.9	0.8
	3 weeks	16.0	18.0	19.2*	3.0
	6 weeks	24.0	26.2	28.4*	4.1
	9 weeks	31.1	35.0	38.1*	6.8

necessarily received creep-feed. Ewes bearing single lambs which were not fed urea in pregnancy had an overall mean liveweight gain from 132 to 149 lbs whereas those which were fed urea in pregnancy gained from 123 to 155 lbs. i.e. + 17 lbs compared with + 32 lbs and this differences was significant (P = 0.05). By three weeks after lambing the ewes which were fed urea throughout had a significantly higher liveweight (141 lbs) than those which were not fed urea in pregnancy (122 lbs).

The mean liveweight increase during pregnancy for all those ewes bearing twins which did not receive urea during that period was from 137 to 160 lbs (i.e. + 23 lbs). Inclusion of urea in pregnancy increased the liveweight gain to 30 lbs (i.e. 140 to 170 lbs).

This difference was not quite significant at P = 0.05. The mean liveweight three weeks after lambing was 130 lbs irrespective of urea inclusion.

It is somewhat surprising that a greater bodyweight gain was observed in the single lambing ewes fed urea than in those bearing twins fed urea. There werehowever rather limited numbers in the former groups. (9), for any real significanceto be interpreted. The single lambs produced from ewes fed SBP+urea during both pregnancy and lactation were significantly (P = 0.05) heavier at birth (13.2 lbs compared with 10.5 lbs) than those born to ewes fed SBP alone. These lambs continued to grow significantly faster until at 9 weeks of age they had a mean liveweight of 51.2 lbs compared with 38.1 lbs for those lambs born to ewes given SBP alone during both pregnancy and lactation. The single lambs born to ewes fed SBP+urea in lactation only grew somewhat better than those from ewes fed SBP alone, and at 9 weeks of age had a mean liveweight of 46.5 lbs compared with 38.1 lbs. This difference is almost significant (P = 0.05). None of the single lambs died. Of the twin lambs, those born to ewes fed SBP+urea during both pregnancy and lactation had mean liveweights significantly greater than those born to ewes fed SBP alone throughout the experiment. For example, the mean liveweight at 9 weeks of age was 38.1 lbs

compared with 31.1 lbs. Twin lambs born to ewes fed SBP + urea only after parturition grew somewhat better (although not significantly) than those born to ewes fed SBP alone throughout. For example, 35.0 lbs at 9 weeks of age.

Some 21 % of lambs born to ewes fed the SBP alone (10 out of a total of 48) died because of shortage of milk and the survivors were necessarily given additional creep food as a supplement. Only 4 of a total of 36 lambs (11 %) died which had been born to ewes fed SBP + urea during both pregnancy and lactation.

Housed Ewe Experiment 2, 1970

Eighty seven Greyface ewes were housed on 15th January, 1970. The trial was essentially a repeat of that conducted in 1969 but with the inclusion of a vegetable protein (groundnut) supplement to give the same crude protein concentration as that with the urea supplement. A total of fifty nine ewes had twins and only twenty two had single lambs. Six ewes did not lamb. Six treatments were applied post lambing and thus some of the groups with single lambs contained only two or three ewes. Accordingly, discussion is confined to the results for those 59 ewes with twins.

The contrasting diets fed during housing were:-

During Pregnancy		For three weeks after lambing	
35 ewes.	Sugar beet alone.	13 ewes.	Sugar beet alone
		12 ewes.	Sugar beet + urea
		10 ewes.	Sugar beet + Groundnut
24 ewes.	Sugar beet + urea.	9 ewes.	Sugar beet alone
		8 ewes.	Sugar beet + urea
		7 ewes.	Sugar beet + Groundnut

All types of concentrates were fed at 2 lbs (908 g)/day during both pregnancy and during the first three weeks after lambing. In addition the ewes received 2 lbs (908 g) hay/day. The amount of crude and digestible protein given were as shown in Table 33. Calculation shows that 28.0 % of the crude protein intake was supplied by urea in the urea-supplemented group. The principal mean liveweight changes are given in Table 34.

All the ewes were in very good condition throughout the whole trial and were generally superior to those used in the previous season. The hay was of better quality and was more readily eaten than that fed in the previous year. There was no difference between the rate of consumption of the hay between the variously fed groups of ewes.

Table 33.

Experiment 2, 1970. The compositions and amounts of crude and digestible protein in the sugar beet pulp, groundnut concentrate and hay fed to ewes during pregnancy and lactation in 1970.

	Sugar Beet alone	Sugar Beet + urea	Sugar Beet + Groundnut	Hay
C.P. % D.M.	10.9	17.9	18.0	8.6
D.C.P. % D.M.	7.7	14.5	(15.1)*	3.9
2 lbs/day as fed				
C.P. g.	82.8	139.6	140.4	65.9
D.C.P. g.	58.5	110.2	(117.8)*	29.6

* Estimate as no digestibility trial using sugar beet pulp plus groundnut was performed.

The ewes and lambs were transferred to grass as the lambs reached three weeks of age. All the groups grazed together and supplementary sugar beet nuts (no urea) were fed for 2-3 weeks. The weather and grazing conditions were markedly better than they had been during the previous trial.

There were no significant differences in the mean liveweights of the ewes bearing twin lambs in the different treatment groups at any time during the experiment. Ewes fed urea in pregnancy had a mean gain in liveweight from tupping to late pregnancy of 29 lbs compared with 23 lbs for those ewes fed SBP alone. The weight lost from parturition to some three weeks after lambing was similar in all treatment groups. (i.e. Range of 25-34 lbs).

There were no significant differences between the mean liveweights of the twin lambs in each of the treatment groups during the course of the experiment. Lambs born to ewes fed urea in pregnancy were slightly heavier than those born to ewes fed SBP alone (i.e. mean of 9.8 lbs compared with 9.4 lbs) and continued to grow at a slightly better rate.

Table 34.

Experiment 2, 1970. Changes in Mean Liveweight (lbs) of Greyface Ewes with Twin Lambs

Protein Supplement		Number of ewes	Tupping	Mid Pregnancy	Late Pregnancy	Three weeks after lambing
Before Lambing	After Lambing					
0	0	13	147	150	167	142
0	Urea	12	146	149	172	138
0	Groundnut	10	146	152	167	145
Urea	0	9	152	156	183	149
Urea	Urea	8	143	146	167	143
Urea	Groundnut	7	151	153	182	157
		L.S.D. (P = 0.05)	15	16	16	15
Overall Means						
No urea in pregnancy		35	146	150	169	142
With urea in pregnancy		24	149	152	178	150

Table 35.

Experiment 2. 1970. Changes in Mean Liveweight (lbs) of Twin Lambs born to Greyface Ewes.

Protein Supplement		Number of lambs	Birth 0	Weeks 3	after 6	lambing 9
Before Lambing	After Lambing					
0	0	26	9.2	17.8	27.8	37.0
0	Urea	24	9.0	19.4	29.5	40.2
0	Groundnut	20	10.0	19.9	29.5	39.7
Urea	0	18	10.5	19.3	28.8	39.5
Urea	Urea	16	9.2	17.9	29.1	40.4
Urea	Groundnut	14	9.5	19.8	30.2	42.5
		L.S.D. (P = 0.05)	0.8	2.7	3.4	5.9
Overall Means						
No urea in pregnancy		70	9.4	18.9	28.7	38.9
With urea in pregnancy		48	9.8	19.0	29.3	40.7

Those lambs born to ewes fed either urea throughout pregnancy and lactation or urea for pregnancy and groundnut for lactation were somewhat (but not significantly) heavier at 9 weeks of age than those lambs born to ewes fed SBP alone. (i.e. 42.5 and 40.4 lbs compared with 37.0 lbs). Feeding either urea or groundnut during lactation resulted in improved liveweight gains in the lambs over that of the unsupplemented group. (i.e. 40.2 and 39.7 lbs compared with 37.0 lbs). Removal of additional protein in the form of urea by feeding SBP alone in lactation after feeding SBP + urea during pregnancy had the effect of reducing the growth rate of the lambs. The mean liveweight gain from birth to 9 weeks of age for these lambs was 29 lbs compared with 31.2 lbs for those in the urea fed treatment and 33.0 lbs for the groundnut treatment.

Housed Ewe Experiment 3, 1970

One hundred and six ewes were housed on 19th January, 1971. Only those ewes with twin lambs which lambed within a 10-day period were used for the main experiment. A total of sixty two ewes had twins within this period. The contrasting diets fed during housing were:-

During Pregnancy		For three weeks after lambing	
47 ewes.	Sugar beet alone	15 ewes.	Sugar beet alone
		15 ewes.	Sugar beet + urea
		17 ewes.	Sugar beet + groundnut
15 ewes.	Sugar beet + urea	15 ewes.	Sugar beet + urea

Each of the concentrate diets was fed at 2 lbs (908 g)/day during pregnancy and during the first three weeks after lambing. In addition the ewes received 2 lb (908 g) hay/day. The amounts of crude and digestible protein given were as shown in Table 36. Calculation shows that 28.0 % of the total crude protein intake was supplied by urea in the urea-supplemented group.

Table 36.

Experiment 3, 1971. The composition and amounts of crude and digestible protein in the sugar beet pulp, groundnut concentrate and hay fed to ewes during pregnancy and lactation.

	SBP only	SBP + urea	G. Nut conc.	Hay
C.P. % D.M.	10.8	17.6	17.5	7.3
D.C.P. % D.M.	7.9	13.6	13.5*	2.3
2 lbs/day, as fed				
C.P. g.	85.5	139.3	136.0	52.9
D.C.P. g.	62.8	107.9	105.0*	16.8

* Estimates only as no digestibility trial conducted

Tables 37 and 38 detail the changes in mean ewe and lamb live-weights. No particular difficulties were encountered during the period of the experiment. The ewes and lambs were transferred to grass as the

Table 37.

Experiment 3. 1971. Changes in Mean Liveweight (lbs) of Greyface Ewes with Twin Lambs

Protein Supplement		Number of ewes	Tupping	Mid Pregnancy	Late Pregnancy	Three weeks after lambing
Before Lambing	After Lambing					
0	0.	15	153	159	168	135
0	Urea	15	156	168	186	142
0	Groundnut	17	155	164	181	140
Urea	Urea	15	148	157	177	140
		L.S.D. (P = 0.05)	14	15	15	14
Overall Means						
No urea in pregnancy		47	155	163	178	139
With urea in pregnancy		15	148	157	177	140

Table 38.

Experiment 3. 1971. Changes in Mean Liveweight (lbs) of Twin Lambs born to Greyface Ewes

Protein Supplement		Number of Lambs	Birth 0	Weeks 3	after 6	lambing 9
Before Lambing	After Lambing					
0	0	30	9.2	18.6	29.0	44.5
0	Urea	30	9.0	20.0	31.0	48.6
0	Groundnut	34	9.7	20.8	31.8	47.6
Urea	Urea	30	10.0*	21.5*	32.6	48.9
		L.S.D. (P = 0.05)	0.6	2.4	3.7	5.6
Overall Means						
No urea in pregnancy		94	9.3	19.8	30.6	46.9
With urea in pregnancy		30	10.0*	21.5	32.6	48.9

lambs reached three weeks of age. Spring grazing conditions were good and all the ewes continued to recieve about 1 lb/day of sugar beet nuts (no urea)for about two weeks.

There were no significant differences between the mean live-weights of the ewes in each of the treatments throughout the experiment. The liveweight gain from tupping to late pregnancy for ewes fed no urea in pregnancy was 23 lbs and 29 lbs for ewes fed SBP + urea in pregnancy. The birth weight of lambs born to ewes fed SBP + urea during pregnancy were significantly heavier (i.e. 10.0 lbs compared with 9.3 lbs) than those born to ewes fed SBP alone. There were no significant differences in the mean liveweight of the lambs in the various treatments at any time, but lambs born to ewes fed urea in pregnancy and in lactation continued to grow at a rather better rate than lambs from ewes fed SBP alone during pregnancy and lactation. (e.g. 48.9 lbs mean liveweight compared with 44.5 lbs at 9 weeks of age). Lambs from ewes fed either urea or groundnut during lactation also had a better liveweight gain than those in the unsupplemented group. (i.e. 48.6 and 47.6 lbs compared with 44.5 lbs mean liveweight at 9 weeks of age).

Discussion

Experiments 1-3 compare the performance of Greyface ewes, and subsequently their lambs, fed during pregnancy and early lactation with diets supplying similar amounts of energy but of varying protein content, where the additional protein was supplied by either urea or groundnut cake. It was considered important that the energy supplied should not be a limiting factor at any stage of the experiments. The maintenance requirement for energy for a 70 Kg ewe is given as c. 2.0 M. cal. Metabolisable Energy. (ME) per day. (A.R.C., 1965). Lowman (1970) gave the mean estimate for the energy requirement of ewes in late pregnancy as 196 % of the maintenance requirement. i.e. c. 4.0 M. cal. ME. The energy requirement estimates for 70 Kg ewes in lactation range from 3.0-6.0 M. cal. ME/day. (A.R.C., 1965; Evans, 1960). The ration of 2 lbs (908 g) sugar beet pulp cubes and 2 lbs (908 g) hay was calculated to supply c. 4.0 M. cal ME/day. When an additional $\frac{1}{2}$ lb (226 g) cubes was fed this value increased to 4.6 M. cal ME.

Table 39 gives the total amount of crude protein and digestible crude protein supplied by the various diets during the three experiments. Table 40 details the mean weight gain of ewes during pregnancy.

In all experiments the mean increase in liveweight over the pregnancy period for ewes bearing twin lambs fed pulp and urea-supplemented pulp corresponded to liveweight gains of c. 15 % and 20 % of their mean tupping weights respectively. Lambs born to the ewes fed urea in pregnancy had a mean birthweight which was greater than the mean birthweight of lambs born to the unsupplemented ewes in every experiment. All the birthweights were within the normal range for this class of sheep. There is a large difference to be expected in the weight gain of the conception products between different breeds of sheep; and this clearly prevents accurate definition of desirable weight gains. However within a single breed type other results may afford some guidance on desirable liveweight gains. A comparison of the present experimental results with those estimates of

Table. 39.

Mean total weight gain (lbs) of Greyface ewes during pregnancy.

(Expts. I,2 & 3).

Diet.	Lambs.	Expt.I.	Expt.2.	Expt.3.
No urea	twins	23.0	23.0	22.0
+ urea	twins	29.0	29.0	29.0
No urea	single	I7.5	I7.0	-
+ urea	single	3I.0	2I.0	-

Table. 40.

Total crude and digestible protein (g/day) fed to Greyface ewes.

(Expts. I,2 & 3).

	Diet.	Total C.P.		Total D.C.P.	
Expt. I.	SBP	I25.9	(I45.2)*	58.5	(70.4)*
	SBP urea	I93.7	(229.7)*	I30.7	(I60.7)*
Expt. 2.	SBP	I48.7		88.I	
	SBP urea	205.5		I39.8	
	Groundnut	206.3		I47.4	
Expt.3.	SBP	I38.4		79.6	
	SBP urea	I92.2		I24.7	
	Groundnut	I88.9		I2I.8	

( )* Amounts supplied when $2\frac{1}{2}$ lbs concentrated fed/day.

liveweight gain of sheep reproducing on 'good planes' of nutrition is of interest. Corriedale ewes (130 lbs at tupping) kept on 'high and 'low' planes of nutrition gained 27.5 lbs and 6.5 lbs in pregnancy respectively, (Coop, 1950). For Border Leicester x Cheviot ewes mated with a Suffolk ram and producing single foetuses weighing about 13 lbs after 140 days of gestation the increase in weight of the genital tract and udder between the 28th and 140th day of gestation was 25 lbs of which 18 lbs was gained in the last month (Wallace, 1948). Guyer & Dyer, (1954) showed the lower limit of acceptable weight gain in the last 6-8 weeks of pregnancy to be about 4 lbs for ewes of 160 kg bearing single lambs. The estimated weight of products of conception (weight loss at parturition) was 25 lbs for ewes with single lambs and 39 lbs for ewes with twins. (Papadopoulos & Robinson, 1957). Generally, liveweight gains in late pregnancy, smaller than the expected gains of gravid uterus and udder, are normally considered to be consistent with good reproductive and subsequent lactation performance if supported with adequate nutritional intakes during lactation. The results from Experiments 1-3 are very consistent with regard to the total ewe liveweight gain during pregnancy. The difference in weight gains between the urea-supplemented groups and the non supplemented groups bearing twins are also remarkably similar in each year. i.e. 29 and 23 lbs respectively, (Table 39). Additional protein in the form of urea was undoubtedly beneficial in improving weight gain of the ewes. This was particularly noticable in the last month of gestation. Differences in weight gains between single and twin bearing ewes are also consistent with previous experimental observations (e.g. Thomson & Aitken, 1959).

It is the performance of the lambs in each of the treatment groups which must remain as the main index of the relative degree of success of the various experimental diets. Table 40 details the D.C.P. supplied/day (calculated from the digestibility experiments subsequently described) from each of the diets in Experiments 1-3. The range of D.C.P. supplied in g./day was from 58.5 - 147.4. The quality of the hay fed was

markedly different between experiments but in all treatments the ration of 2 lbs of a SBP product and 2 lbs hay was calculated to supply an adequate energy intake. There are clear differences to be seen in the performance of the lambs between experiments. Lambs grew much faster in Experiment 3 than in either Experiments 1 or 2. Some of these differences must partly be attributed to one or more of the following reasons. Winter conditions were considerably milder during both Experiments 2 and 3 than in Experiment 1. In all three experiments ewes and lambs were put out to grass when the lambs had reached 3 weeks of age. However, owing to the lack of suitable grazing due to the late spring in Experiment 1, supplementary feeding had to be continued outside for several weeks. In contrast, there was good grass growth and excellent weather at the appropriate time in Experiment 3. The ewes used during Experiment 1 were somewhat inferior to those used subsequently in Experiments 2 and 3.

Within each particular experiment the differences in liveweight of the lambs at different stages of growth are comparable. In Experiment 1, twin lambs born to ewes fed SBP alone in both pregnancy and lactation grew very poorly since many ewes yielded little milk. Many lambs (10) died. The total daily D.C.P. intake during pregnancy and lactation was 58.5 and 70.4g/day respectively (Table 40). In contrast, twin lambs born to ewes fed SBP + urea in both pregnancy and lactation (supplying 130.7 and 160.7 g D.C.P./day respectively) grew in an entirely satisfactory manner and only 4 out of a total of 36 lambs died. This result agrees with that obtained by Slen & Whiting (1952) and would indicate that the minimum requirement in pregnancy of 31 g. D.C.P./day suggested by Lowman (1970) to be a gross underestimation of the protein requirement, and that the generally accepted value of about 120 g. D.C.P./day e.g. A.R.C. (1965); N.R.C. (1946); Thomson & Aitken, (1959), is fully adequate. In all three experimentslambs produced from ewes fed additional crude protein in the form of urea contained in SBP cubes were heavier at birth and had higher

liveweight gainsthan those lambs produced from ewes fed the unsupplemented SBP. Increasing the D.C.P. intake after lambing by feeding either SBP + urea or SBP alone + groundnut also improved the growth rate of the lambs indicating the benefit of feeding supplementary protein during early lactation. It is perhaps unfortunate that the experiments did not include a diet fed in pregnancy where groundnut was the source of additional protein. This was impracticable since it would have involved too many different treatments in any one experiment. The experiments have clearly shown the undoubted improvement in performance of both ewes and lambs by the addition of either urea or groundnut to the basal diet of SBP. The experiments give no reason to suppose that there is any difference in the degree of utilization of the additional crude protein whether it be supplied from vegetable protein or urea contained in the SBP cube.

Changes in blood urea and free fatty acid concentrations as affected by the diet in Greyface ewes during late pregnancy and early lactation during Housed Ewe Experiments 1-3.

There are no generally accepted determinations on biological fluids which give a reflection of the energy or protein intake of ewes during late pregnancy or early lactation. Protein adequacy in ruminant animals (provided the energy component of the diet is adequate) is generally assessed by one or more factors related to growth. (Preston, 1961). For several reasons these estimates are not precise (Preston, Schnakenberg & Pfander, 1965). Criteria based on specific nitrogen compounds in the body fluids (e.g. blood urea concentration) may perhaps be more accurate measures of the protein adequacy of the diet being currently fed. In the ruminant animal there are two major sites of nitrogen absorption, namely the reticulo-rumen and the small intestine. Lewis (1957) demonstrated that the amount of this absorbed nitrogen (including NH_3 - N) is reflected in the concentration of urea in the circulating blood. Work by Preston et al (1965) conducted with growing lambs showed a close relationship ($r = 0.986$) between blood urea concentration and protein intake. (Studies presented elsewhere in this thesis confirm these findings). Blood urea concentration however, is known to vary over a twenty-four hour period and its variation is broadly related to the time of feeding and the nature of the protein source. This present investigation examined

a) the apparently normal variation in blood urea concentration in ewes during late pregnancy fed diets containing SBP ± urea over twenty-four hour periods.

b) the concentrations of blood urea of Greyface ewes during late pregnancy and early lactation fed the various diets containing SBP ± urea and/or groundnut during the Housed Ewe Experiments 1-3 conducted from 1969-71.

With regard to the biochemical evaluation of the adequacy of the energy intake much work has been devoted to the study of free fatty acid (FFA) concentrations in the blood (e.g. Annison, 1960; Reid & Hinks, 1962 a and b). Blood serum non-esterified fatty acids are mainly derived from the breakdown of body fat deposits. Circulating FFA concentrations are a reflection on the amount of fat being mobilized for oxidation. Examination of FFA concentrations under various conditions of nutrition in sheep has shown a relationship to the degree of energy intake (e.g. Slee & Halliday, 1968). Concentrations are high in sheep during fasting (Annison, 1960), and during pregnancy with 'undernutrition' (Reid & Hinks, 1962 a and b). During the course of Housed Ewe Experiment 2 FFA concentrations were examined at intervals in the blood of those sheep fed diets containing SBP $\pm$ urea, and an attempt was made to correlate these concentrations to the liveweight gain and diet.

Experimental

a) Urea concentrations in the blood of ewes during late pregnancy and early lactation when fed diets containing SBP $\pm$ urea.

During the course of the Housed Ewe Experiments 1-3, blood samples were taken from the jugular vein of the ewes at intervals throughout the period of housing and were examined for blood urea concentration. The samples were obtained between 10.00 and 11.00 hours, some 2-3 hours after morning feeding when 2 lbs SBP $\pm$ urea was given. The results for those ewes bearing twin lambs are presented separately in Fig 8 . The blood urea concentrations for ewes bearing single lambs were at all stages of the investigations greater than those recorded for the ewes bearing twin lambs. This may well be a reflection upon the lower nitrogen requirement of the ewes concerned. The same essential changes in blood urea concentrations during late pregnancy and early lactation were observed. Fig 8 shows

Figure 8.

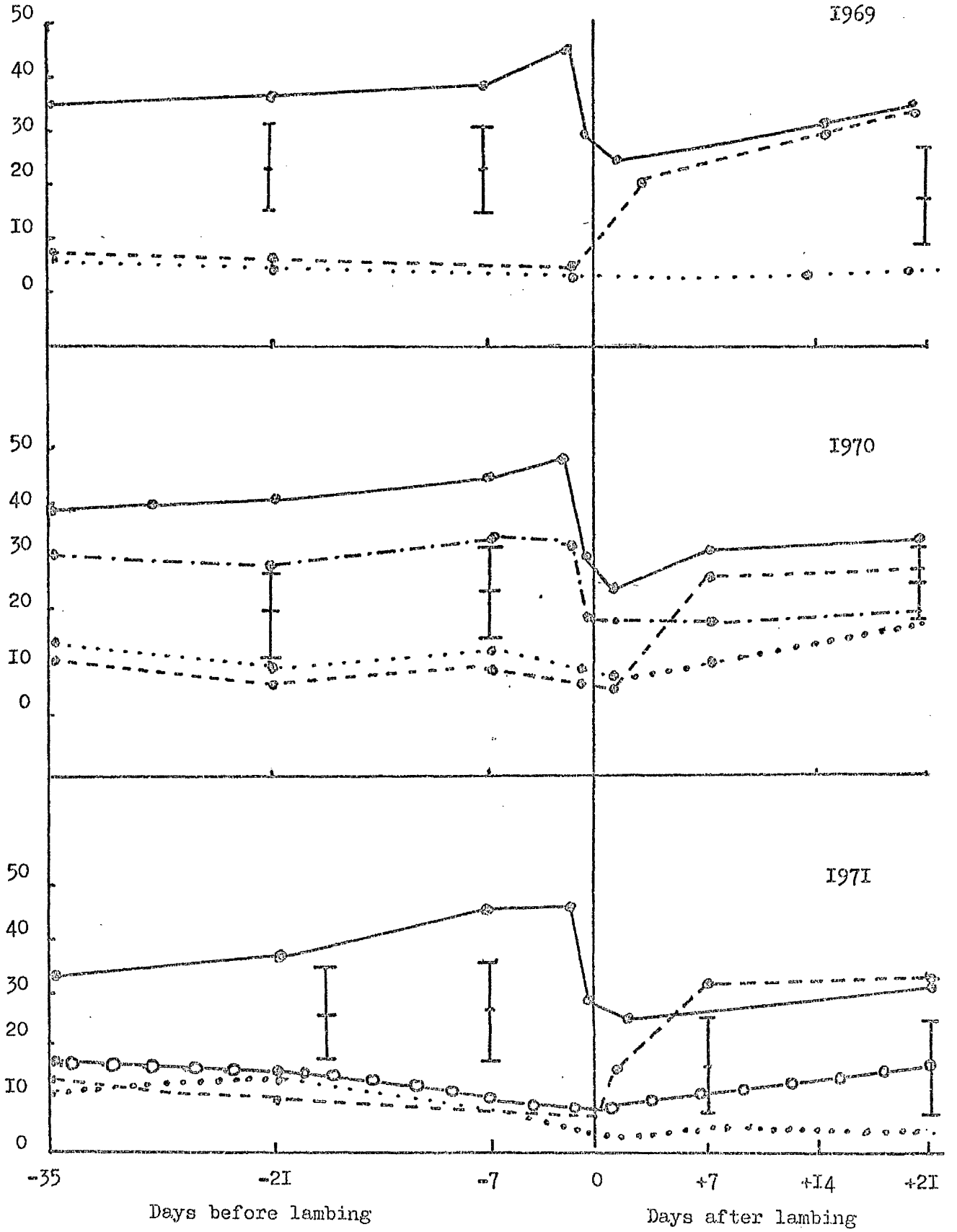


Figure 8. Urea concentration (mg/100 ml) in the blood of ewes bearing twin lambs during late pregnancy and early lactation when fed diets containing SBP+ urea.

Pregnancy	Lactation
urea	urea
urea	0
0	urea
0	groundnut
0	0

. . P = 0.01

there to be significant differences ($P = 0.01$) in the blood urea concentrations between the ewes fed SBP + urea and those fed SBP alone. In 1969 those ewes bearing twin lambs fed SBP alone were recorded as having blood urea concentrations in the range 5-18 mg %, whilst those fed the urea supplemented diet were about 30-40 mg % . At parturition certain of the dietary treatments were changed. (see Housed Ewe Experiments 1-3). It was noted that those ewes which first received supplementary protein in the form of urea or groundnut after lambing exhibited a marked increase in blood urea concentration which was evident 4-7 days after supplementary feeding commenced. Blood urea concentrations tended to fall prior (from about 7 days) to lambing. This was most noticeable in those ewes fed SBP + urea. At this time a marked decrease in appetite was noted in the individual ewes (also noted by Reid & Hinks, 1962 a.) and it would appear that this decrease in protein intake accounts for the reduction in blood urea concentration at this time.

Results from the main experiments (Housed Ewe Experiments 1-3) have demonstrated the benefit from additional protein feeding in the form of urea in that lamb birthweights and the general bodily condition of the ewes together with the subsequent growth of the lambs were superior to those obtained when no protein supplement was fed. Accordingly, some indication of protein adequacy may be interpreted from the concentrations of blood urea recorded during these experiments, in that concentrations under about 15 mg % may indicate there to be an inadequate protein intake, and that satisfactory protein intakes are reflected in blood urea concentrations of 25 mg % or more.

b). The variation in blood urea concentrations in late pregnant Greyface ewes a) fed diets containing SBP ± urea during a normal twenty-four hour day. b) deprived of food over a twenty-four hour period

Blood urea concentrations in twenty four Greyface ewes in late pregnancy were examined at intervals, throughout the day. Two diets were fed:

1) SBP alone (2 lbs) a.m. and hay (2 lbs) p.m. to twelve of the ewes.

2) SBP + urea (2 lbs) a.m. and hay (2 lbs) p.m. to twelve of the ewes.

This experiment was performed on three separate occassions. The results are shown graphically in Fig 9a. A comparable group of ewes were examined for blood urea over the same period when no food was offered. The results are presented in Fig 9 b.

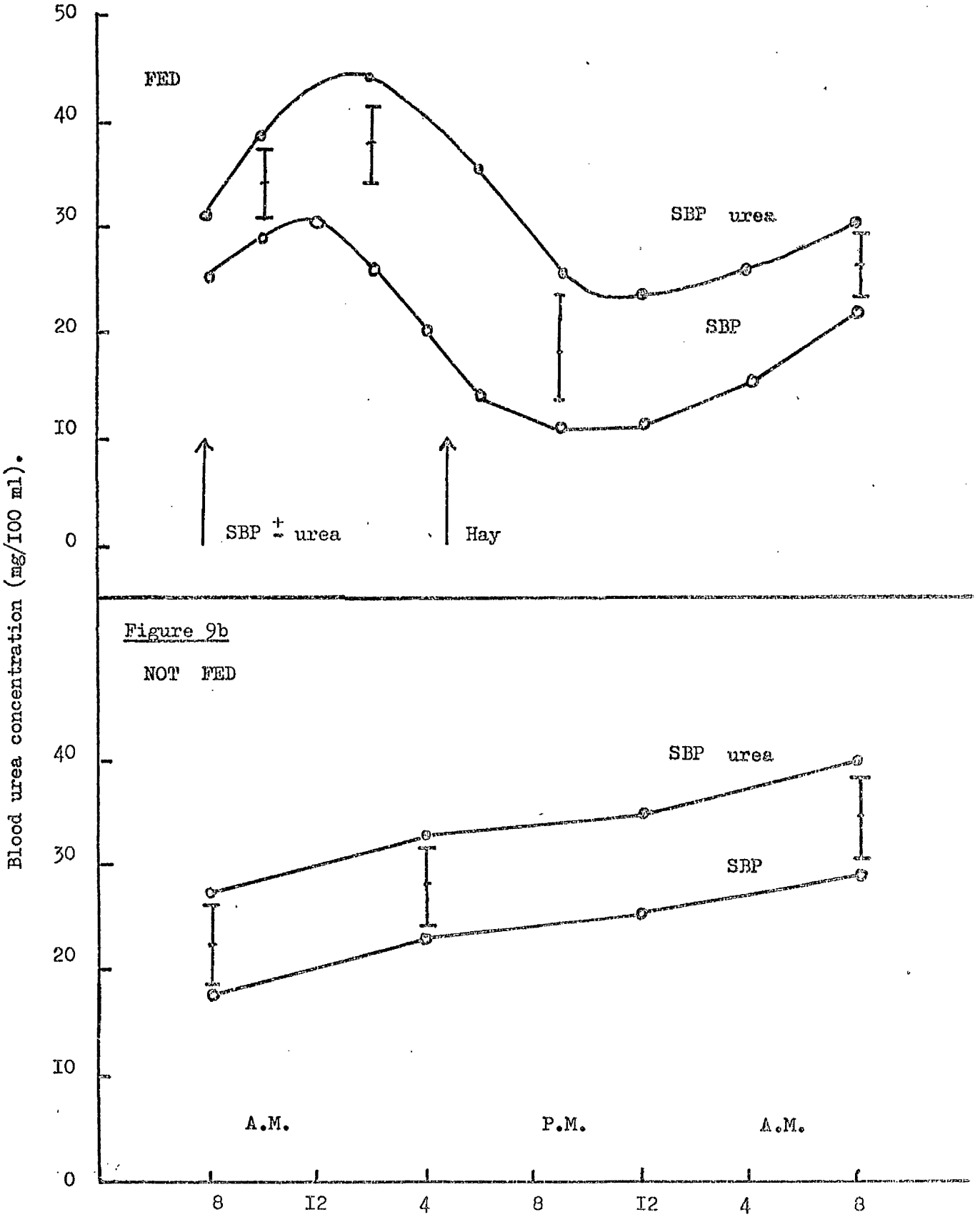
Results

There would appear to be a distinct diurnal variation in the blood urea concentration. Blood urea concentrations rose after the morning feed of sugar beet pulp both in the urea-supplemented group and the unsupplemented group. Concentration of urea was at a maximum some 4 hours after feeding.

Thereafter the concentration of urea fell until some 4-5 hours after the evening feed which consisted of hay alone. Overnight from approx. 21.00 hours until 08.00 hours, where/naturally imposed period of 'starvation' occurred there was a steady rise in the urea concentration. At all times there was a significant difference ($\hat{P}$ = 0.05) between the mean blood urea concentrations of the ewes in the different feeding groups.

In those ewes in both feeding groups deprived of food over the same period there was a steady increase in the blood urea concentration. Ewes normally fed SBP + urea showed an increase in blood urea

Figure 9a



The variation in blood urea concentrations in late pregnant Greyface ewes a) fed diets containing SBP ± urea during a normal twenty-four hour day. b) deprived of food over a twenty-four hour period.

| . . P = 0.05.

concentration over the 24 hours from 27.5 mg% to 39.5 mg %. Similarly ewes normally fed SBPalone increased from I7.4 to 29.0 mg %. This is possibly a transient phenomenon, where in late pregnancy protein requirements are high and continual and perhaps some mobilisation of ' labile protein reserve ' occurs from the liver or gut viscera (see Munro, I964) resulting in an increase in blood urea concentration. Similar rises in blood urea concentration in adult wethers fed restricted amounts of protein have been noted by Parkins, Holmes & Bremner (I972) and Berry & Dargie (I972). (Personal communication).

Because of this diurnal variation in blood urea concentration observed in animals fed concentrates on one occasion per day the data concerned with the concentrations of blood urea observed in other parts of this thesis are, unless otherwise stated, obtained from analyses of blood samples collected from the jugular vein at I0.00 - II.00 hours some 2-3 hours after the morning feed. Observations with animals fed on an ad-lib basis have shown there to be a relatively constant blood urea concentration over the normal twenty-four hour day.

c). Concentrations of FFA observed in Greyface ewes fed diets containing SBP $\pm$ urea during late pregnancy and early lactation. Housed Ewe Experiment No 2, I970.

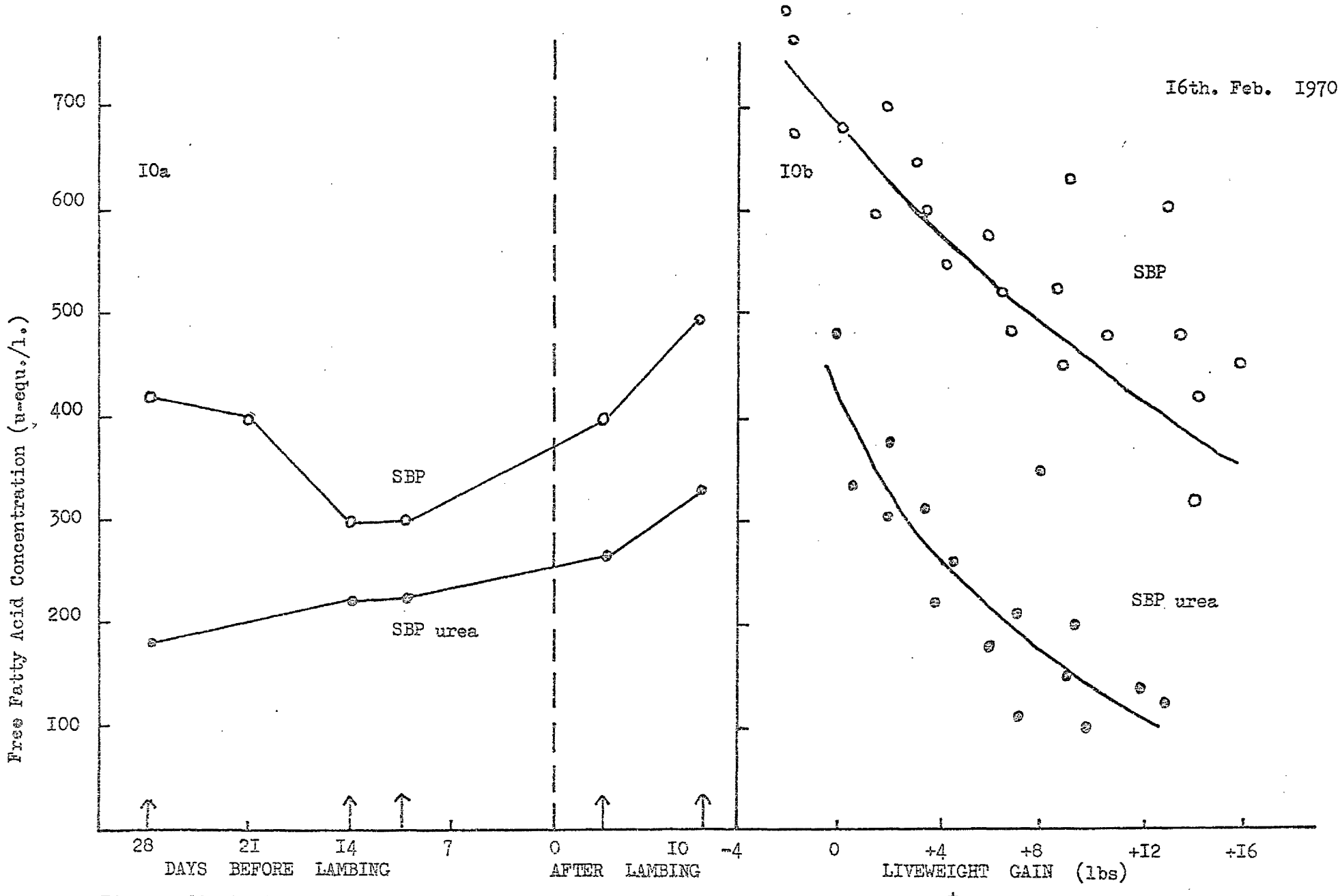
This experiment was undertaken partly in order to assess the possible value of serum FFA concentration analysis as an index of the adequacy of the energy intake of ewes during late pregnancy and early lactation, and partly in order to examine the possible relationships which might exist between the individual liveweight gain of the ewe during pregnancy and its FFA concentration. Slee & Halliday (I968) reported an increase in FFA concentration due to the effects of cold exposure and experimental handling on sheep. During this present experiment blood

samples were obtained before the morning feed and the sheep were handled quietly and competently and at no time were there any apparent signs of distress in the ewes arising from the blood sampling procedure employed. On five occasions during the course of Housed Ewe Experiment 2, 1970, blood samples were obtained two hours after the morning feed from 18 ewes in each feeding group (i.e. concentrate ration consisting of either 2 lbs SBP + urea/day or 2 lbs SBP alone/day) and analysed for serum FFA by the method described by Itaya & Ui (1965). The results in Fig 10a are presented only for those 12 ewes in each group which subsequently gave birth to twin or triplet lambs.

On one separate occasion, 16th Feb. 1970, 25 ewes in each feeding group were bled immediately before the morning feed in order to determine if any relationship existed between weight gain during pregnancy and the corresponding increase in FFA concentration. The results are presented graphically in Fig 10 b.

Figs 10 a and 10 b show a clear difference between the FFA concentrations in ewes fed SBP and SBP + urea. This is somewhat surprising in view of the fact that the total energy intake provided by each diet is essentially the same. It would tend to suggest that an interaction may exist between the varying protein intakes acting on the rate of mobilisation of body fat. In both groups, ewes which had gained 8-16 lbs weight during the time between tupping and 16th Feb. had lower FFA concentrations than those whose gain was in the order of only - 2 to + 4 lbs. Three ewes fed SBP alone which had lost weight over this period had FFA concentrations as high as 850 μ - equ/l, whereas ewes fed the urea-supplement SBP which had gained no weight had concentrations in the order of 400 μ - equ/l. In both groups the concentrations of free fatty acids subsequently rose markedly during early lactation. Similar observations have been reported by Reid & Hinks, (1962 b).

It is now generally accepted that the FFA concentration is a more sensitive criterion of undernutrition in pregnancy than either



Figures 10a & 10b. Concentration of FFA in Greyface ewes bearing twins fed SBP± urea during late pregnancy and early lactation and the relationship to ewe liveweight gain.

blood glucose or total ketones. In this laboratory, analytical error on triplicate samples was rather high ($\pm$ 10.0 %) and significant variations in blood FFA concentration in individual animals was noted from day to day. Some of the variation may perhaps be related to the time of feeding. (e.g. Annison, 1960) and climatic conditions (e.g. Alexander, 1962). These factors cause technical difficulties in the use of FFA as nutritional criteria. For example, ewes in the different feeding groups latterly known to have produced good twin lambs with comparable growth rates were recorded as having mean FFA concentrations in pregnancy in the wide range between 200-400 μ - equ/l. Lamb growth might, of course, have been much worse if the dietary intake of these ewes during lactation had been reduced.

The results obtained under the specified conditions of this particular experiments suggest that the addition to urea to the diet lowered the mean serum FFA concentration as a consequence of a reduction in fat mobilisation. This may partly be attributed to a higher energy utilization of the hay component of the diet. A state of 'undernutrition' was not indicated unless FFA concentrations were substantially in excess of 400 μ - equ/l.

In combination, a state of 'undernutrition' might be interpreted in a group of late pregnant ewes if blood analysis revealed a urea concentration below 10 mg %and a serum FFA concentration markedly above 400 μ - equ/l.

d). Digestibility and Nitrogen Retention Trials involving the Sugar Beet pulp products fed during Housed Ewe Experiments I-3.

During each of Experiments I,2 and 3 digestibility and nitrogen balance studies were conducted using the diets fed to the ewes in each of the treatment groups during pregnancy. In a preliminary trial in each year the digestibility of the hays fed was determined using wether sheep. Digestibility of the complete hay and S.B.P. ($\pm$ urea) diets were undertaken with late pregnant ewes. The ewes were ascertained as bearing one or more lambs by both radiographic and Doppler probe techniques.

In Experiment I (I969), six late pregnant gimmers (I9 weeks of gestation) each bearing a single lamb were selected for trial. In Experiment 2 ewes bearing twins (I7 weeks gestation) were selected and in Experiment 3 each treatment group was composed of 2 ewes bearing triplet lambs and one ewe bearing twins (I6 weeks gestation). In each of the three years the ration offered to the caged sheep was I$\frac{1}{2}$ lbs. hay and 2lbs. S.B.P. ($\pm$ urea). The pulp was fed at 08.00 hours and the hay at I6.30 hours. These experiments involved many pages of recorded values of individual daily intakes of food, faecal and urinary outputs and laboratory analyses. They are too voluminous to be presented in detail here and have been housed in the Department of Animal Husbandry, Glasgow University Veterinary School.

Materials & Methods.

The sheep were confined in standard metabolism cages (Duthie, I959) and given strictly controlled amounts of experimental diets at least one week before the start of the investigation. They had, of course, been group-fed the particular diets for several weeks previously. Individual daily feed intakes, and faecal and urinary outputs were recorded throughout the experiments. Ten percent of this daily output was retained (stored at 0-4^{o}C), and combined at the end of each collecting week for sampling prior to laboratory analysis. A portion of the fresh faecal matter was analysed for moisture content by drying. Crude fibre, ether extracts and ash

determinations were performed using this dried material. Nitrogen determinations were performed using a slurry (C.A.B., Hurley, 1961) prepared directly from a sample of the fresh faeces. Urine was collected in vessels which had previously been acidified with 100 ml of 5 N sulphuric acid (Martin, 1966). Total nitrogen analysis was performed on the faecal slurry and urine samples by semi-micro and micro-kjeldahl techniques respectively. Samples of each day's feed were bulked each week for appropriate analyses.

Results

Details of the nitrogen balance studies are given in Table 41 and are also presented as a histogram in Fig II. Digestibility results are given in Table 42.

Table 41. Nitrogen balance of the complete Diet. (g/day).
Mean of 3 ewes per group.

		1969 Singles		1970 Twins		1971 1 Twin + 2 Triplets	
Urea inclusion		0	+	0	+	0	+
Period of gestation		19 weeks		17.5 weeks		16 weeks	
N input	Hay	3.0	4.6	7.0	5.6	6.2	5.3
	SBP	12.5	23.4	13.5	22.4	13.8	21.7
	Total	15.5	28.0	20.5	28.0	20.0	27.0
N output	Faeces	7.3	7.6	9.8	8.8	10.0	8.2
	Urine	3.0	11.4	6.2	12.8	6.4	14.2
	Total	10.3	19.0	16.0	21.6	16.4	22.4
N retained		5.2	9.0	4.5	6.4	3.6	4.3
Effect of added urea							
Increase in intake		12.5		7.5		7.0	
Increase in urine		8.4**		6.6**		7.8**	
Increase in retention		3.8*		1.9		0.7	

* P = 0.05

** P = 0.01

Figure 11. Nitrogen Retention Trials involving the Sugar Beet Pulp products fed during Housed Ewe Experiments 1 - 3.

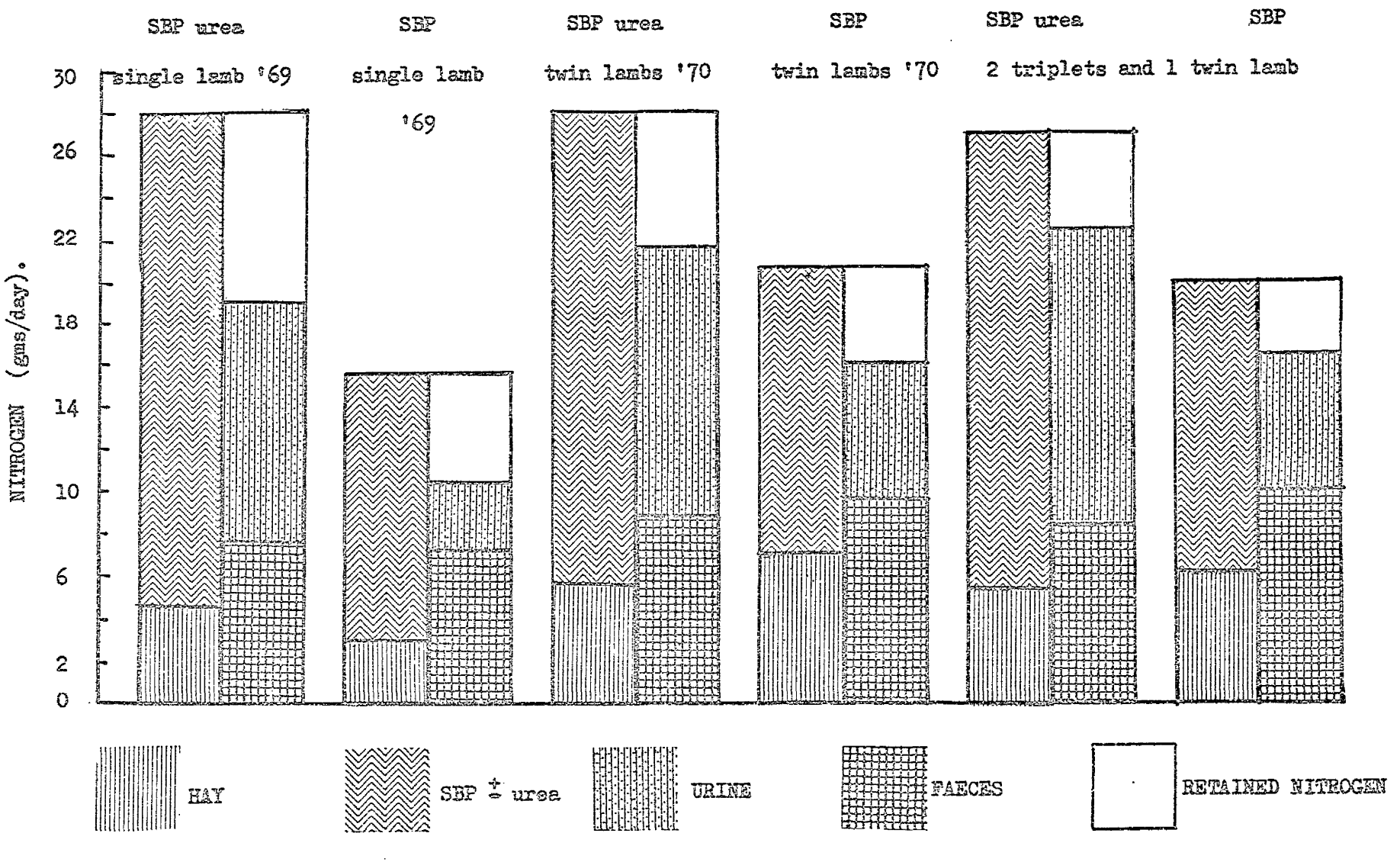


Table 42. Mean Apparent Digestibility Coefficients (%) of entire Diets offered to pregnant ewes in Experiments 1-3. (3 ewes/group)

	1969 Singles		1970 Twins		1971 1 Twin + 2 Triplets	
Urea inclusion	0	+	0	+	0	+
D.M.	73.9	75.3	74.8	76.0	71.7	75.2
O.M.	77.0	77.8	75.0	77.6	73.6	77.2
C.P.	54.5	72.7*	52.0	70.4*	49.3	68.6*
C.F.	81.9	81.7	71.3	74.2	55.4	60.7
N.F.E.	78.5	82.3	72.1	84.6	82.9	84.4

Discussion of results

The diets used in this investigation were intended to supply two different intakes of crude protein of the same energy content, where the difference in crude protein was supplied from urea contained in sugar beet pulp. Elliot & Topps (1964) have shown that the dietary ratio of roughage to concentrate has a significant effect on nitrogen utilization, and for this reason only 1.5 lbs hay (680 g) was offered to these experimental animals whilst in metabolism cages so that they might reasonably be expected to consume it all, thereby standardising the fibre intake. In 1969, sheep fed a concentrate ration consisting of SBP alone consumed only 66 % of that amount eaten by sheep fed the urea-supplemented pulp cubes. It is of interest that there was a similar observation on the hay consumption on the ewes in the main experiment (Housed Ewe Experiment 1, 1969). Ewes fed SBP alone ate only 1.25 - 1.5 lbs hay compared with 2 - 2.5 lbs for those sheep fed SBP + urea. However in 1970 and 1971 sheep fed the unsupplemented cube ate some 20 % more hay than those fed SBP + urea. No similar observation was made from the sheep on the main feeding experiments where both groups consumed about 2 lbs/day. This difference in roughage intake was not considered important. There were no refusals of the different sugar beet pulp materials and all of the offered ration of 2 lbs (908 g) was readily consumed.

In all three balance trials there was an increase in N retention when SBP + urea was fed compared with SBP alone. (e.g. 9.0 g compared with 5.2 g N retained/day in 1969). The increase in N intake (g/day) when urea was included in the diet for all three experiments was 7.0-12.5 and significant increases ($P = 0.01$) in urinary nitrogen were observed (6.6-8.4). There were large differences in the urea N fraction of the total Nitrogen content of the urines. For example, in 1969 63 % of the total nitrogen in the urine of sheep fed SBP + urea was apparently

derived from urea compared with 48 % in sheep fed SBP alone. In 1970 these values were 59 % and 43 % for the non-urea and urea groups respectively. There were no real differences in the ammonia content of the urines and less than 4.0 % of the total nitrogen was calculated to be derived from ammonia.

Robinson & Forbes (1967) have shown that N retention is not affected at any stage of gestation by the number of foetuses carried suggesting that retention may not be entirely governed by demand, but that N retention increases as gestation approaches term. They also demonstrated a decrease in the N requirement as pregnancy proceeds to establish a zero balance. This may also indicate urea recycling in late pregnancy. (Houpt, 1959; Somers, 1961; Packet & Groves, 1965). It is interesting to examine some of the findings obtained from the balance experiments performed in 1969,'70 and'71,in view of these and other experimental results reported in the literature.

The sheep used for the nitrogen balance and digestibility experiments were all of similar weight (c. 68 kg) but differed between experiments in that a) they carried differing foetal loads i.e. single lambs in 1969, twin lambs in 1970 and 2 ewes of each group bore triplets in 1971. b) they were at different stages of late pregnancy i.e. 16-17 weeks in 1971, 17-18 weeks in 1970 and 19-20 weeks in 1969.

Apparently, N retention increased as gestation approached term. e.g. 3.6 and 4.3 g N retention/day for those ewes fed SBP and SBP + urea in 1971 at 16-17 weeks compared with 4.5 and 6.4 and 5.2 and 9.0 in 1970 and 1971. Also the nitrogen excreted in the urine decreased over the same period i.e. 6.4 and 14.2 g N excreted in urine in 1971, compared with 6.2 and 12.8 in 1970 and 3.0 and 11.4 in 1971. The increase in N retention accompanied by a corresponding decrease in urinary N output would suggest that increased demand is met by an increase in the utilization of absorbed N rather than by an increase in the amount of N absorbed. These findings agree with those of Graham (1964) and of those of

Robinson & Forbes (1967).

Digestibility

There were no significant differences in the apparent digestibility coefficients of the complete diets between treatments (Table 42) except in that the crude protein digestibility was about 70 % for the diet containing urea compared with 50 % for the diet containing SBP alone. This is a direct result of the increased crude protein content of the SBP + urea diet. (120g C.P. cf c. 70 g C.P.). There was some increase in the apparent digestibility of the dry matter and organic matter fractions of the urea-containing diet. For example dry matter digestibilities for sheep fed urea in 1971 and 1969 were recorded as 75.2 and 75.3 respectively compared with 71.7 and 73.9 for sheep fed the non-supplemented diet. Organic matter digestibilities for the supplemented groups in 1971 and 1970 were 77.2 and 77.6 compared with 73.6 and 75.0 for those sheep fed SBP alone. Burroughs, Gall, Gerlaugh & Bethke (1950) and Williams, Nottle, Moir & Underwood (1953) found a significant reduction in dry matter digestibility with decreasing protein intake. The experimental results presented here show no difference in digestibility between different stages of late gestation. This agrees with the findings of Head (1953) and supports the view that the apparently increased efficiency of the pregnant animal is not likely to be the results of increased efficiency in digestibility (Thomson & Aitken, 1959).

e). The fattening of Blackface lambs on different urea-containing diets and some relationships between blood urea concentration and other production factors.

It is often indicated in the literature that the physical form and the nature of the "carrier" of dietary urea is of considerable importance. The carrier should both ideally provide carbohydrate as a quickly available energy source and should disintegrate in the rumen at such a rate as to allow a reasonable release rate for the contained urea. Section 3 of this thesis describes some relevant studies performed with SBP materials in vitro and in vivo. This experiment examines the fattening of lambs where additional nitrogen in the form of urea was included in a) a barley cube, b) a sugar beet cube, c) a composite cube of both ground barley and ground sugar beet pulp.

Three levels of crude protein were employed 9.6 %, 11.5 % and 13.0 %. All the dietary constituents were cubed and all the additional nitrogen provided was from urea. The diets were all of equal energy content being comprised of sugar beet pulp and barley in equal proportions. The diets fed were as follows:-

% Inclusion in Diets

	SBP no urea (9.6% CP)	SBP urea (17.0%CP)	Barley (9.6% CP)	Barley urea (17.0%CP)	urea (288% CP)	% CP of complete diet
Group						
1*	50	-	50	-	-	9.6
2	25	25	50	-	-	11.5
3	-	50	50	-	-	13.0
1*	50	-	50	-	-	9.6
4	50	-	25	25	-	11.5
5	50	-	-	50	-	13.0

% Inclusion in Diets (cont.)

Group	SBP no urea (9.6% CP)	SBP urea (17.0% CP)	Barley (9.6% CP)	Barley urea (17.0% CP)	Urea (288% CP)	% CP of complete diet
6	50	-	50	-	-	9.6
7	50	-	50	-	1.5	11.5
8	50	-	50	-	3.0	13.0

* i.e. The same group

Sixty-four Blackface lambs were allocated at random to eight pens with eight lambs in each pen. The pens had an even distribution of individual liveweights and the total liveweight of each pen was very similar at the commencement of the trial. The experimental period began on the 12th December and ended on the 6th March, 1970.

The diets were fed ad lib. In the initial stages the lambs rejected a proportion of the sugar beet nuts of both types. Gradually, however, consumption increased and by the end of the trial there were no difficulties.

Results

The main results are presented in Table 43.

Table 43.

Experiment. Blackface Lamb fattening Trial

Diet	Pen	Urea% in diet	Mean Wts.lbs 12/Dec Start	6/Mar End	Gain	Total Overall F.C.R.	Intake lbs.	Marketed** at end of trial
Sugar Beet/ urea + Barley cube	1*	0	51.3	70.5	19.2	9.0	172	3
	2	1½	51.1	75.7	24.6	7.1	174	4
	3	3	50.1	76.9	26.8+	6.6	176	5
Barley/urea cube+Sugar beet cube	1*	0	51.3	70.5	19.2	9.0	172	3
	4	1½	50.8	77.8	27.1+	6.9	186	7
	5	3	50.0	75.0	25.0°	8.0	199	5

Experiment Blackface Lamb fattening Trial (cont.)

Diet	Pen	Urea% in diet	Mean Wts. lbs 12/Dec Start	6/Mar End	Gain	Total Overall F.C.R.	Intake lbs.	Marketed** at end of trial
All ground and cubed together.	6	0	48.8	66.6	17.8	9.9	175	1
	7	1½	50.5	72.5	22.0	7.8	175	4
	8	3	50.4	74.8	24.4+	7.3	176	4

\+ Significant at P = 0.05

o Just misses at P = 0.05

i.e. The same group

** Maximum possible = 8

Within each dietary treatment those lambs which received either 1½ or 3 % of added urea had a greater overall weight gain and a better food conversion ratio than those lambs which received no urea. For example lambs in pen 3 fed 3 % urea contained in the barley cube had a mean liveweight gain of 26.8 lbs and a food conversion of 6.6 compared with lambs in pen 1 fed no urea which had a mean liveweight gain of 19.2 lbs and a food conversion of 9.0. A comparison of the results obtained between different dietary treatment groups shows that the poorest growth and F.C.R. responses were obtained in the group fed the composite cube of barley and SBP. Results obtained from the other two treatment groups were essentially comparable except that lambs in pen 5 fed 3 % urea contained in a barley cube had poorer responses than those fed only 1½ % urea. (i.e. Mean liveweight gains of 25.0 and 27.1 lbs respectively). In any event the results obtained from lambs fed SBP+urea containing diets were entirely satisfactory when it is considered that the animals used were initially poor, undersized Blackface lambs.

Blood Urea Concentrations in Blackface lambs

Blood samples were collected from all lambs at the end of the experiment, and analysed for blood urea content. Some of the relationships between the mean blood urea concentrations and other production factors are shown in Table44 and in Fig. I2

Results

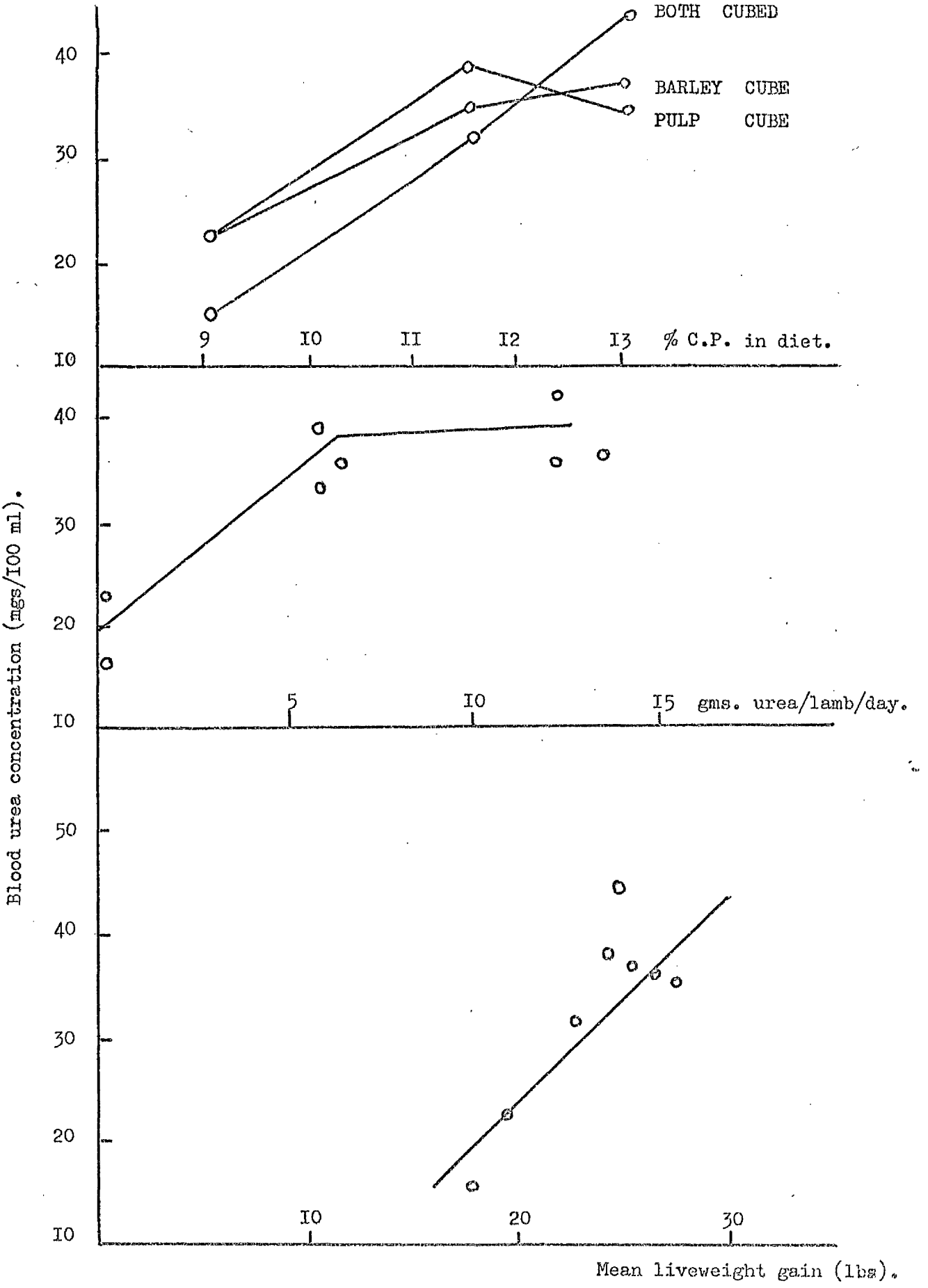
Table 44.

Diet	CP %	Mean Blood urea mgs %	Total Mean Wt. gain lbs.	Gms.urea/ head/ day
1	9.0	22.7	19.2	0
2	11.5	39.3	24.6	42.8
3	13.0	36.0	26.8	86.5
4	11.5	35.6	27.0	45.5
5	13.0	37.2	25.0	97.5
6	9.0	15.7	17.8	0
7	11.5	33.3	22.0	43.0
8	13.0	45.0	24.4	85.5

Discussion.

The results presented from this particular experument show a positive relationship to exist between crude protein intake and blood urea concentration. Since those lambs fed 11.5 and 13.0 % C.P. containing diets grew rather better those fed a diet containing only 9.0% C.P. There was also a relationship between mean liveweight gain and blood urea concentration. These results agree with those of Preston, Schnakenberg & Pfander (1965) who demonstrated a close relationship ($r = 0.986$) between crude protein intake and blood urea concentration in growing lambs. It is likely therefore that concentrations of blood urea may possibly be used to partially assess the adequacy of protein of growing lambs fed ad lib.

Figure I2. The fattening of Blackface lambs on different urea-containing diets. Some relationships between blood urea concentration and other production factors.



SECTION VII.

Studies in Ammonia Toxicity in Sheep.

a). Introduction.

b). Experimental.

Experiment I. Concentration of ammonia in blood of fed and starved sheep resulting from the oral administration of urea.

Experiment 2. The protective and curative effects of certain amino acid mixtures on ammonia toxicosis in sheep.

2A. Protective effects.

2B. Curative effects.

Discussion.

Experiment 3. An examination into the effect of experimental liver damage induced by either I). Carbon tetrachloride or 2). chronic copper poisoning on the rate of ammonia detoxification in sheep given oral doses of urea.

I). Carbon tetrachloride poisoning.

2). Chronic Copper poisoning.

Studies in Ammonia Toxicity in Sheep

Introduction — Detoxification of Ammonia in Sheep.

Some consideration of the metabolism and toxicity of urea in the ruminant and has been presented in the General Introduction of this thesis. It is the intention here to discuss certain aspects of these topics in greater detail as an introduction to the experimental work performed.

Urea is far the most widely used non-protein nitrogen source presently incorporated in diets for ruminant animals. Despite a worldwide research effort, problems in the efficient utilization of urea still exist. (Chalupa, 1968). The reduced performance of animals fed has been attributed to a number of causes, (see Oltjen, 1969; Tillman & Sidhu, 1969). Some important recent studies have examined the effects of urea feeding upon intermediary metabolism in ruminants (e.g. Prior, Clifford, Hogue & Visek, 1969; Chalupa, Clark, Opliger & Lavker, 1969 ab).

The toxic signs and symptoms of ammonia intoxication have been presented elsewhere in this thesis. The biochemical aspects of ammonia toxicity have not been fully elucidated. It is of value to note here some aspects of the topic which are well documented.

If a large quantity of urea is presented to the rumen, urea hydrolysis, by the enzyme urease, occurs at a faster rate than the incorporation of the liberated ammonia into cellular protein of the rumen microorganisms, resulting in large increases in rumen ammonia concentrations (Bloomfield, Garner & Muhrer, 1960; Tillman & Sidhu, 1969). The rumen ammonia concentration is observed to reach a maximum some 1-2 hours after feeding (or dosing). (e.g. Chalupa, Evans & Stillion, 1964; Oltjen & Putnam, 1966; Oltjen, Slyter, Kozak & Williams, 1968). It has been recognized for some time that part of the ammonia produced in the rumen is absorbed via the portal system and passes to the liver.

Normally the greater part of this ammonia is removed in the liver and converted to urea. (McDonald, 1948; Lewis , 1960). Under certain circumstances, when the quantity of ammonia absorbed by this route exceeds a certain level, the liver appears to be unable to detoxify it completely. As a consequence the peripheral blood ammonia concentration markedly increases. (Head & Rook, 1955; Lewis, Hill & Annison, 1957). Acute toxicity is generally reported to occur when peripheral blood ammonia concentrations exceed 1,000 ug NH_3 - N %. (see Austin, 1967; Chalupa, 1968). Such high values are reported to occur when the rumen ammonia concentration exceeds 80 mg NH_3 - N %. (e.g. Lewis et al, 1957; Coombe & Tribe, 1958). The rate of ammonia transfer across the rumen wall is dependent on both the concentration gradient and the pH of the rumen liquor. Most membranes are more permeable to the NH_3 moiety than the NH_4^+ ion, and ammonia is potentially most toxic in conditions of alkaline pH where unionized NH_3 predominates. (see Visek, 1968; Warren, 1962; Coombe et al, 1960). It is apparent that urea toxicity is synonymous with ammonia poisoning (Morris & Payne, 1970). It is the opinion of most researchers that ammonia, either directly or indirectly is the toxic agent involved in urea poisoning. An exception to this view was that of Hale & King (1955 ab) who reported experiments to demonstrate that the toxicity was caused by the carbamic acid ion. This view has been subsequently largely discounted. Lewis (1960) considered ammonia toxicity was due principally to the circulating NH_4^+ ion, and at present this is generally agreed, even considering the results of Clark, Oyaert & Quin (1951) who failed to simulate urea toxicity by injecting ammonia intravenously into sheep. Another suggestion that ammonia poisoning may be attributed to hypomagnesaemia (Head & Rook, 1955, 1957; Voisin, 1963) has also been discounted in view of the experiments performed by Care, (1965); Ross, 1961; Toothill, 1963; Rys, 1959 and Wilson, (1963).

Warren (1962) noted that ammonia intoxication could be correlated with potassium (but not magnesium) insufficiency and Dukes (1955) had previously described urea poisoning as causing an electrolyte and water imbalance. Lewis (1961) agreed with this opinion and thought a disturbance of the acid-base relationship, accompanied by a potassium leak from the cells could possibly explain the toxicity of ammonia.

As the primary signs of acute ammonia intoxication are neurological some important work has been concerned with the possibility of cerebral metabolism derangement. McIlwain (1959) has demonstrated that increased cerebral ammonia concentration lead to increased muscular and nervous activity. Heinz (1959) made similar observations in subjects where high blood ammonia concentrations ultimately resulted in coma. It was noticed that original administration sometimes alleviated the condition. Bessman & Bessman (1955) postulated the depletion of α-ketoglutarate from the tricarboxylic acid cycle but forwarded no experimental evidence to support this opinion. Other views include the impairment of pyruvate and α-ketoglutarate decarboxylation (McKhann & Tower, 1961), a decrease in NADH availability (Worcel & Erecinska, 1962), and an increased loss of ATP resulting from an enhanced requirement for the synthesis of glutamine from ammonia (Weil-Malherbe, 1967). Evidence that toxic doses of ammonia deplete phosphocreatine in basilar areas of the brain in mice and that a reduction in ATP occurred, indicating that the energy metabolism of the brain is affected, has been presented by Schenker, McCandless, Brophy & Lewis, (1967) and by Schenker & Mendelson (1964). Ammonia-poisoned dogs (Stevenson & Wilson, 1963) exhibited a syndrome similar to human hepatic coma, characterized by nervous disorder, convulsions and coma. Austin (1967) remarked "It is pertinent to reflect that urea toxicity has been likened to poisoning by strychnine, itself a nerve poison of notoriety". Since ammonia can cause aberrations in cellular energy metabolism (Visek, Prior & Clifford, 1968) the apparently

poorer performance of ruminants fed urea-containing diets may possibly be partly due to biochemical derangements produced by ammonia. (Chalupa et al, 1970). Also of note in this connection is the report that the normal functioning of the tricarboxylic acid cycle of rat liver mitochondria was inhibited by ammonia which was associated with a depletion of reduced pyridine nucleotides. (Katanuma, Okada & Nishii, 1966). Feeding urea as the sole nitrogen source to sheep caused a depression in certain urea-cycle enzyme systems and the metabolism of the additional ammonia involved an increase in liver ornithine content. (Chalupa et al, 1970). Prior et al, (1969) concluded from their experiments that "urea in ruminant diets can cause marked changes in intermediary carbohydrate and nitrogen metabolism. The possibility exists that there is increased need for precursors of pyridine nucleotides to partially overcome the decreased performance of urea-fed animals".

Experimental work described in this thesis examines several aspects of urea toxicity in sheep. There is much information in the literature on the importance of the role of the liver enzyme systems in detoxifying ammonia (see Morris & Payne 1970). Experiments are described where liver function has been impaired by carbon tetrachloride poisoning, and by chronic copper poisoning. The rate of detoxification of formed ammonia when oral drenches of urea have been administered to sheep so treated has been studied in sheep during progressive degrees of induced liver damage.

In more recent years some interesting work has been carried out in which ammonia intoxication has been ameliorated by the stimulation of the Krebs ornithine cycle. Experimental work is described here where

various amino acid mixtures have been used in attempts to both examine the potential of such mixtures as a 'cure' for urea toxicity, and also to examine the "protective effect" such mixtures afford when administered prior to the oral administration of potentially toxic amounts of urea. The experiments subsequently described have been concerned with various amino acid treatments in the prevention and alleviation of clinical experimental ammonia toxicity in sheep.

Greenstein, Winitz, Gullino, Birnbaum & Otey (1956) studied the toxicity, in rats kept without food for 24 hours, of intraperitoneal injections of most of the individual amino acids. It was observed that rats injected with lethal doses of most, but not all, of the individual amino acids died with elevated blood ammonia concentrations. Intraperitoneally injected mixtures of amino acids containing L-Arginine were much less toxic than those containing no arginine and caused much less rise in blood ammonia concentration. Harper, Najarian & Silen (1956, ab), using intravenous injections, also demonstrated that arginine afforded protection against ammonia toxicity and the toxicity of injected amino acids. Arginine has also been found to reduce blood ammonia concentration in dogs which had been administered with large amounts of glycine (Harper et al, 1956 b), and it protected rats effectively against the toxicity of injections of ammonium salts (Ingle & Williams - Ashman, 1962; Wergedal & Harper, 1964). Arginine is thought to stimulate urea production from ammonia in the liver. This has been demonstrated by Goldsworthy, Middleton, Kelly, Banbeck, Aoki & Nyhus, (1968) with isolated perfused bovine liver. Clearance of ammonia added to the perfusate was greatly accelerated by arginine, slightly by aspartate and not by glutamate. Concentrations

of arginine, citrulline and ornithine were all increased in the perfusate together with the increased production of urea. Ammonia may also be taken up by keto acids, especially by α-ketoglutarate (see Harper, Benevenga & Wohlhueter, 1970). Studies on ammonia distribution after an ammonia load suggest that muscle initially takes up ammonia, which is followed by glutamine synthesis in several tissues, probably in liver, with urea synthesis proceeding throughout. (Rosado, Flores, Mora & Soberon, 1962). Experiments in which arginine has proven particularly effective have been performed with animals deprived of food for 24 hours, (Greenstein et al, 1956). Such animals are much less resistant to ammonia toxicity than animals in the fed state (Winitz, Gullino, Greenstein & Birnbaum, 1956). This is probably because during starvation the liver is depleted of intermediates of the urea cycle that are ordinarily present in low concentration (Schimke, 1962; 1963). Since urea synthesis occurs almost exclusively in the liver but ammonia production does not, susceptibility to ammonia toxicity should be greater if circulation to or through the liver is impaired or if the amount of functional liver tissue is small (Ingle et al, 1962). Studies will be presented here to indicate that impaired liver function in sheep induced by the administration of carbon tetrachloride or copper salts in quantity resulted in greater increases in blood concentration following oral doses of urea than in healthy control animals.

Animals fed high-protein (i.e. 14 % C.P. or more) undergo a variety of metabolic adaptations, amongst which is the substantial increase in activity of many of the enzymes of amino acid catabolism, which affect their individual susceptibility to injections of ammonium salts. Wergedal & Harper, 1964). In the fed state animals adapted to a high protein intake were less susceptible to the toxicity of ammonium salts than those not adapted, and in animals in the fasting state arginine was more effective in preventing toxicity in those adapted to a high protein

intake. This may be due to an increase in urea-cycle enzyme activities (Schimke, 1962; 1963).

Experiment 1. Concentration of ammonia in blood of fed and starved sheep resulting from the oral administration of urea.

A total of 12 Blackface lambs (mean liveweight 26 kg) which were being fed a concentrate ration containing 13.5 % C.P. on an ad lib basis indoors were divided into two comparable groups each composed of 6 animals. One group was deprived of food for 24 hours and the other group allowed to feed as normal. An aqueous solution of urea (c. 50-100 ml) at the rate of 0.3 g urea/kg. bodyweight was administered to each lamb as an oral drench. Blood samples were taken periodically and analysed for blood ammonia concentration. The experiment was repeated one week later when the feeding treatments were interchanged.

Results: Sheep fed normally exhibited only relatively small increases in blood ammonia concentration (i.e. 120 µg NH_3 - N %) at the time of maximum concentration (45-60 mins) compared with those lambs which had been deprived of food for 24 hours. (i.e. 280 µg NH_3 - N %).

As a consequence of these findings it was decided that in all subsequent experiments, in order to ensure adequate increases in blood ammonia concentration for comparative purposes, the experimental animals be deprived of food (but not water) from 12 noon on the day before the experiment.

Experiment 2. The protective and curative effects of certain amino acid mixtures on ammonia toxicosis in sheep.

In all the described experiments the experimental animals used were Blackface lambs housed indoors and fed on a restricted concentrate and ad lib hay diet. Food was withdrawn at least 12 hours before the experiment was performed. At least 7 days was allowed between successive experiments where the same animals were used.

2A Protective Effects

(a) Four Blackface lambs (mean liveweight 26 kg) were injected in the jugular vein with a 0.9 % NaCl solution containing a mixture of arginine and aspartic acid. The dose administered was 1.0 m. mole/kg liveweight. A further two lambs were treated with aspartate alone. An oral aqueous drench of 0.5 g urea/kg liveweight was administered 30 minutes later. Four untreated control lambs of similar liveweight were drenched with urea at the same time. Blood samples were taken from the other jugular vein at intervals and analysed for blood ammonia concentration. The mean results are presented graphically in Fig 13. All the non-injected control lambs exhibited a marked increase in blood ammonia concentration, reaching a maximum of about 300 µg/100 ml about 45 minutes after the urea solution had been administered. No deaths occurred. The two lambs treated with aspartate alone exhibited similar increases in blood ammonia concentrations. (Peak concentration, 240 µg/100 ml). In striking contrast, those lambs which had been injected with the mixture of aspartate and arginine prior to the urea drench demonstrated very little elevation in blood ammonia concentration (i.e. generally below a mean of 50 µg/100 ml).

(b) The effects of L-Ornithine and L-Aspartic acid injections

Four lambs of mean liveweight 28 kg were injected intravenously with a 0.9 % NaCl solution containing a mixture of ornithine and aspartic acid. The dose administered was 2.0 m. moles/kg

liveweight. A further three lambs were injected with ornithine alone. An oral dose of 0.8 g urea/kg liveweight was administered 30 minutes later. Four non-injected control lambs were similarly drenched with urea solution. Blood samples were taken periodically and analysed for ammonia, urea and potassium concentrations.

The mean results are presented graphically in Figs I3, I4 and I5. The non-injected control lambs exhibited marked and rapid increases in blood ammonia (to 700 µg/100 ml), and potassium but not urea concentrations. They were recumbent and exhibited tetany some 45 minutes after the urea drench had been administered. Those lambs which had been injected with ornithine alone and the mixture of ornithine and aspartic acid exhibited much lower increases in blood ammonia (300-400 µg/100 ml) and plasma potassium concentration, but much greater increases in blood urea concentration.

(c) The effects of L-arginine and α-ketoglutaric acid injections

Three lambs of mean liveweight 27 kg were injected intravenously with a mixture of arginine and α-ketoglutarate in a 0.9 % NaCl solution. The solution was neutralized. The dose administered was 1.0 m. mole/kg liveweight. Two lambs of mean liveweight 26 kg were injected with 1.0 m. moles α-ketoglutaric acid. Thirty minutes after injection an oral drench of 0.8 g urea/kg was administered. Four non-injected control lambs were similarly drenched with urea. Blood samples were collected periodically and analysed for ammonia, urea and potassium concentrations. The results are presented graphically in Fig I3. The non-injected control lambs showed typical signs of ammonia toxicity after 30 minutes and two died. The clincially affected lambs, which were treated had much reduced increases in blood ammonia and potassium concentration. The increase in blood urea concentrations of those treated lambs however were marked. The two lambs (treated) given a prior injection of α-ketoglutarate alone exhibited large increases in blood ammonia and one died after

60 minutes.

(d) The effects of L-Arginine and L-Ornithine injections in normal saline

(i) Three Blackface lambs were injected intravenously with a 0.9 % NaCl solution containing arginine and ornithine at the rate of 2.0 m. moles/kg liveweight 30 minutes prior to the administration of an aqueous drench of 0.8 g urea/kg liveweight. A further three lambs were injected with 2.0 m. moles of arginine alone prior to drenching with urea, and six non-injected control lambs were similarly drenched with 0.8 g/kg of urea. The results are shown in Fig 13.

All the control sheep exhibited typical symptoms of ammonia toxicity and three died. In direct contrast only relatively small increases in blood ammonia were observed in those animals given the pre-treatment injections. (Max. 350 µg NH_3 - N %). A considerable increase in mean blood urea concentration was noted.

(e) The effects of L-Arginine and L-Ornithine injections in calcium borogluconate solution.

Six lambs were injected intravenously with a solution of 40 % Calcium Borogluconate containing arginine and ornithine at the rate of 2.5 m. moles/kg liveweight, 30 minutes prior to drenching with 0.8 g urea/kg liveweight. Six untreated lambs were similarly drenched with 0.8 g urea/kg liveweight and acted as controls. The results are shown in Fig 13.

Five of the control sheep exhibited marked signs of ammonia toxicosis. Two of these control lambs subsequently died with extremely high blood ammonia concentrations. The Potassium levels in one individual had risen from 3.96 to 10.2 m. equ/l at the time of death.

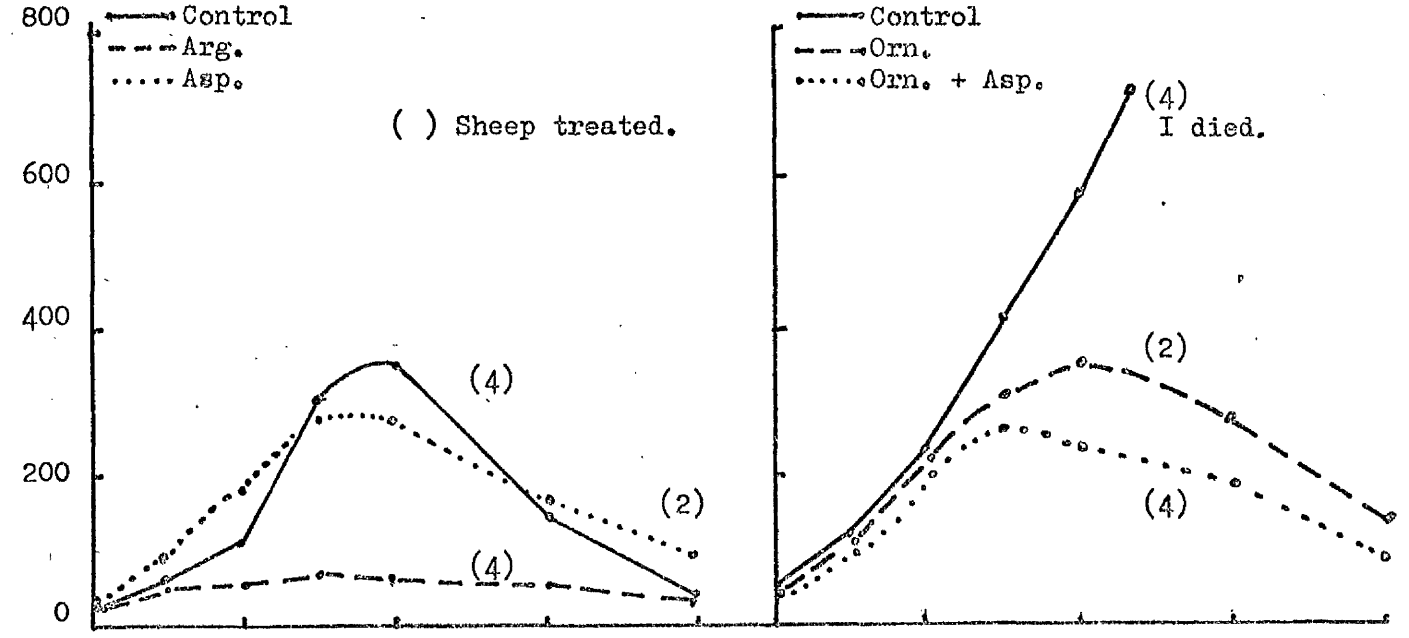
All the sheep treated with the arginine and ornithine mixture in calcium borogluconate prior to the administration of urea survived and exhibited only relatively small increases in blood ammonia concentration. (under 100 µg/100ml). Blood urea concentrations increased markedly.

Figure I3.

Experiment 2. Blood ammonia concentrations (µg/I00 ml. whole blood) following oral doses of urea.

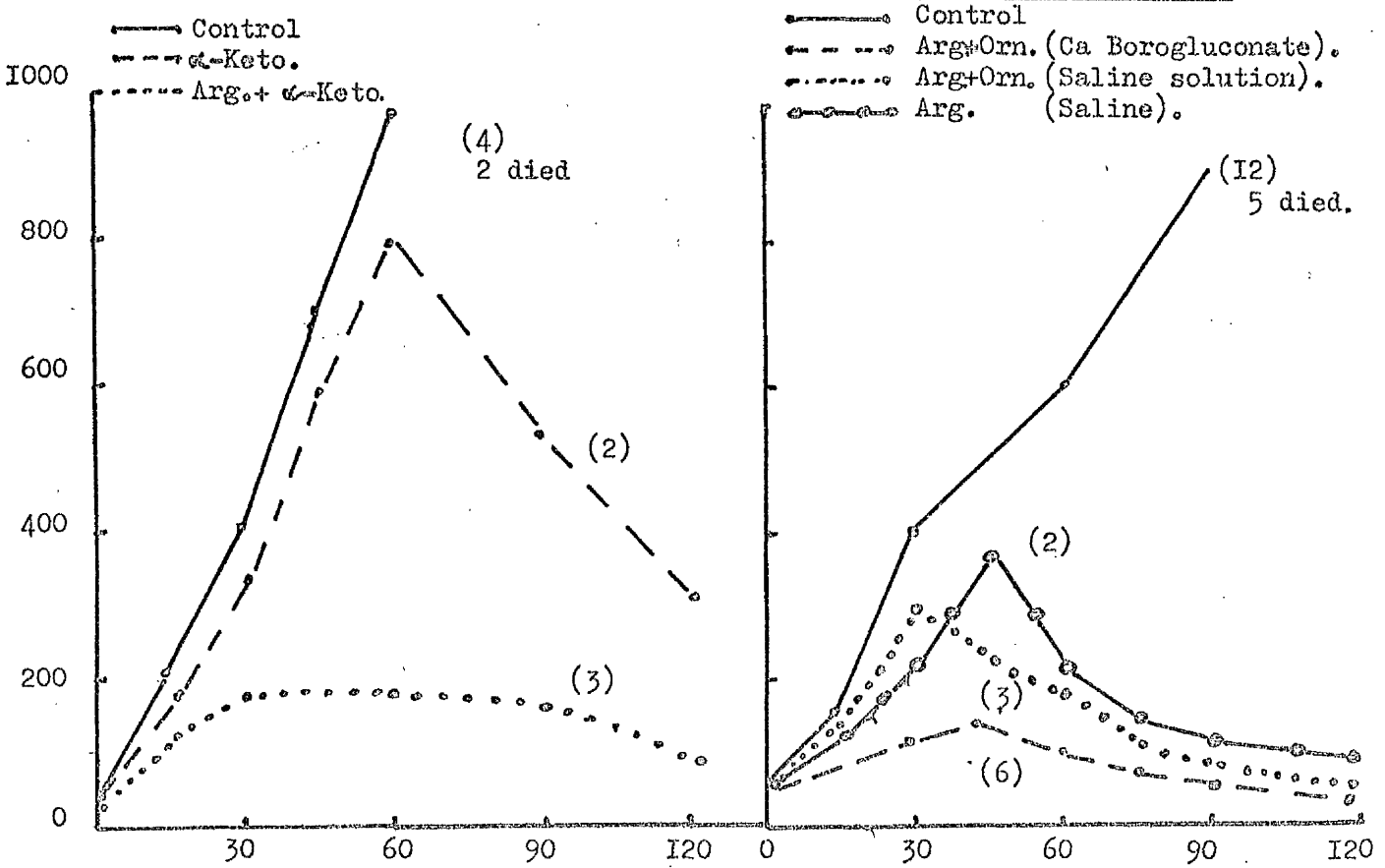
a). Arginine & Aspartic.
Urea dose 0.5 g/Kg.

b). Ornithine & Aspartic.
Urea dose 0.8 g/Kg.



c). Arginine & α-Ketoglutaric acid.

d)& e). Arginine & Ornithine.



Minutes after the administration of urea.

Figure I4.

Experiment 2. Blood urea concentrations (mg/I00 ml) following oral doses of urea.

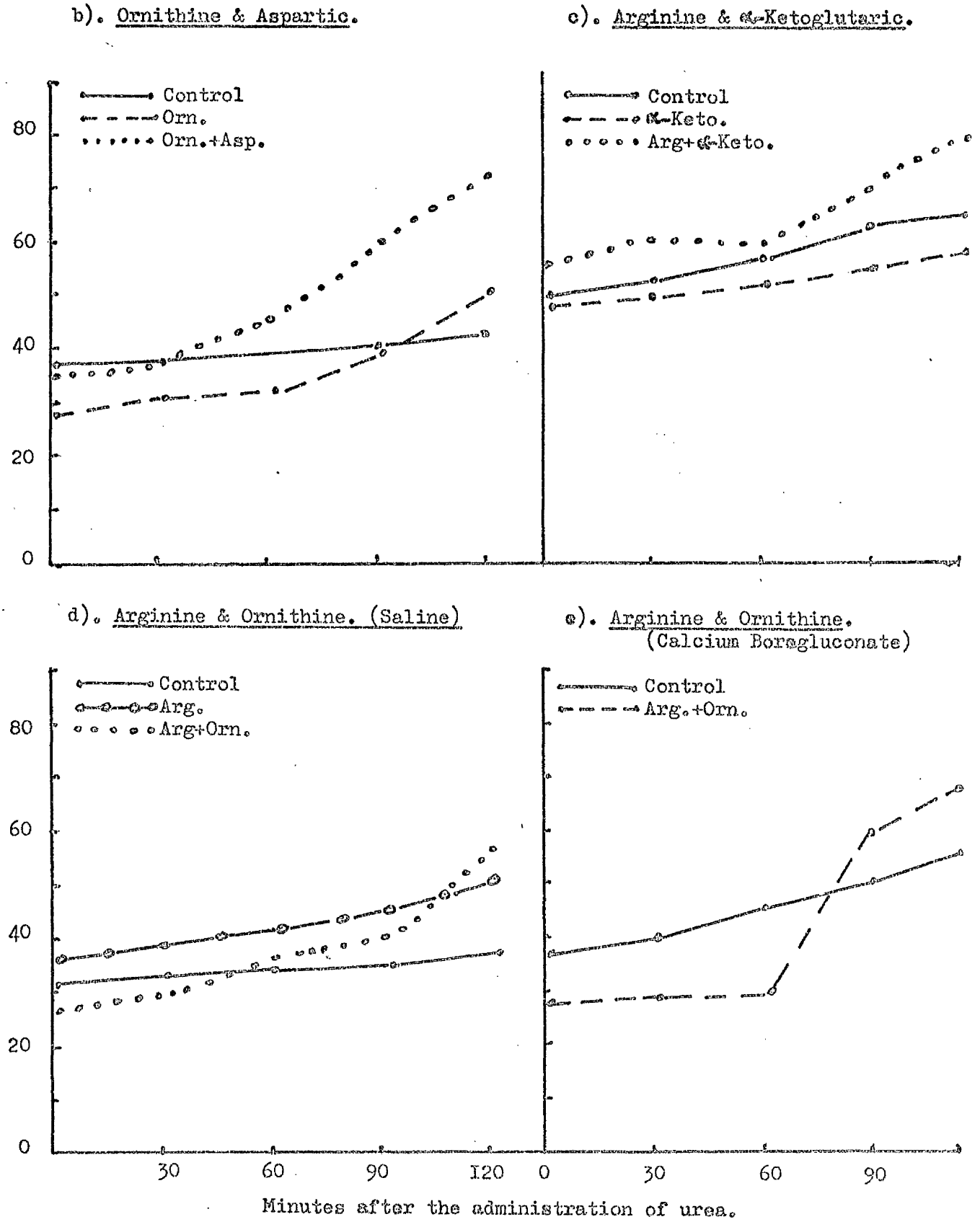
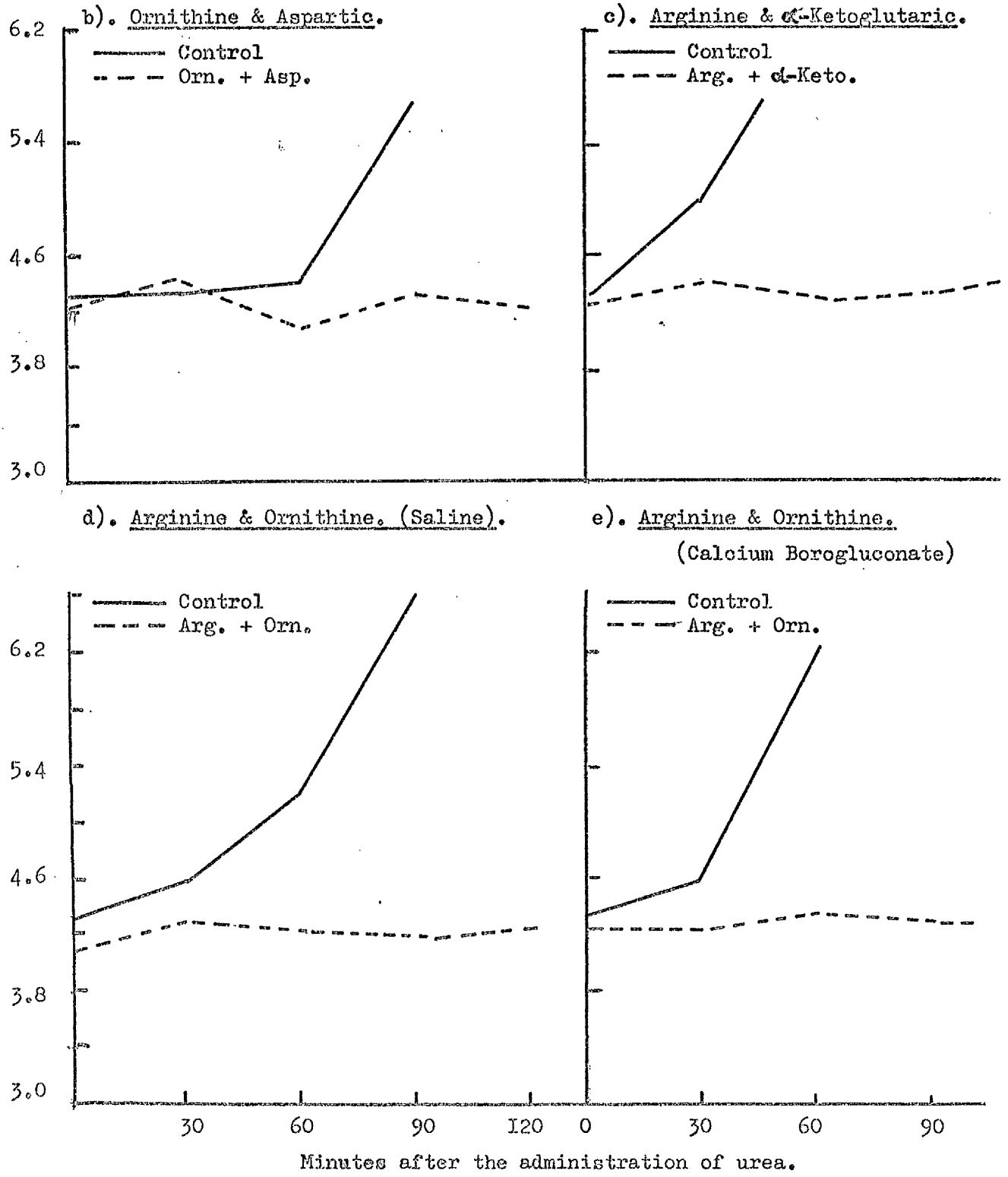


Figure I5.

Experiment 2. Blood potassium concentrations (m. equ. /l) following oral doses of urea.



2B. Curative effects

Some of the mixtures examined in Experiment 2A for their protective effects against ammonia intoxication were also examined as to their curative properties for the control of sheep experiencing ammonia toxicity. During the course of Experiment 2A (a-c) the appropriate amino acid(s) were administered to one half of those control sheep in each experiment when the sheep were observed to be recumbent and were in tetany or apparently rapidly approaching this stage. Blood samples were taken when possible and analysed for blood ammonia, urea and potassium concentrations. Careful note was taken of the individual animals clinical condition during the period after the 'therapeutic' intravenous injection had been administered. The various mixtures administered to sheep in or approaching tetany in the experiments and the effects observed were as follows.

(a) Arginine and Aspartic acid (2.0 m. mole/kg) was administered to three sheep 45 minutes after having been dosed with an aqueous solution of 0.8 g urea/kg liveweight, and which showed obvious clinical signs of hyper-ammonaemia. (i.e. Recumbent, twitching and some frothing).

Two of the sheep showed no sign of recovery at any stage and died 15 minutes after administration with very high (over 1000 μg/100 ml) blood ammonia concentrations. The severity of the muscular spasms diminished appreciably in the remaining lamb and the blood ammonia concentration had fallen from 850 ug to 450 μg NH_3 - N %, some 20 minutes after administration. Recovery was complete in 60 minutes. Blood urea concentration rose from 40 mg to 68 mg % during the period from the administration of the mixture to apparent complete recovery.

(b) Ornithine and Aspartic acid (20 m. moles/kg) was injected intravenously into two control lambs in early tetany after they had received an oral drench of 0.8 g urea/kg liveweight during the course of Experiment 2A (b). No apparent recovery occurred and death ensued some 25 & 40 minutes after administration of the amino acid solution.

(c) Arginine and α-ketoglutaric acid (1.0 m. mole/kg) was injected intravenously into two of the control lambs experiencing tetany during the course of experiment 2A (c). The severity of the symptoms of ammonia intoxication were quickly alleviated and recovery was complete within 90 minutes. The mean ammonia concentration was reduced from c. 800 ug to 400 ug NH_3 - N % in 10 minutes and remained steady for a further 30 minutes before falling to normal.

(d) Arginine and Ornithine (2.0 m. moles/kg in a solution of physiological saline) was administered to three control lambs obviously approaching tetany during the course of Experiment 2A (d). The severity of the symptoms of two of the lambs were quickly ameliovated and the mean blood ammonia concentration fell from 660 to 420 µg NH_3 - N. The lambs remained recumbent for 20 minutes before tetany reoccurred. A further 1.0 m. mole/kg of the amino-acids was injected. Symptoms were again alleviated in 15 minutes, blood ammonia concentration fell from 580 to 315 µg NH_3 - N % and complete recovery was eventually effected 2 hours after the first therapeutic injection. The remaining lamb showed no sign of recovery at any stage and died with a blood ammonia concentration of 2,300 µg NH_3 - N %.

(e) Arginine and Ornithine (2.5 m. moles/kg contained in a solution of 40 % Calcium borogluconate) was administered to three lambs in early tetany during the course of Experiment 2A (e). Symptoms of hyperammonaemia were quickly alleviated, the mean blood ammonia concentration fell from 720 µg to 395 µg NH_3 - N % after 30minutes. Recovery was complete in 75 minutes. The two control lambs in tetany not so treated died.

Discussion of Results

Greenstein et al, (1956) noted the beneficial action of L-Arginine in experimental hyperammonaemia produced in rats following intraperitoneal injections of ammonium salts. L-Arginine may involve the mobilization or 'enhancement' (Salvatore & Bocchini, 1961) of the Krebs-Henseleit urea synthesizing cycle. Appreciable increases in blood urea concentration would support this view. (Greenstein et al, 1956; Najarian & Harper, 1956). Work performed by Salvatore & Bocchini (1961) described experiments where protection against ammonia intoxication in rats was afforded by injections of L-Ornithine and L-Aspartic acid. Blood ammonia concentrations were lowered and marked increases in blood urea concentrations were noted. A recent study performed with sheep examining inter alia the improvement in tolerance to ammonium acetate following an injection of a 'primer' dose of L-Arginine has been reported by Morris & Payne (1970). Lewis (1960) however reported that the intravenous injection of L-Arginine did not ameliorate ammonia toxicity in the sheep, and Morris et al, (1970) summarised that the intravenous administration of arginine did not appear to be of practical value in preventing urea poisoning.

The results from the various experiments presented here demonstrate that primer injections of the separate amino acids, L-Arginine & L-Ornithine appear to afford a greatly enhanced tolerance to otherwise toxic doses of urea. Aspartic acid alone appears to have little effect, agreeing with the results of Salvatore & Bocchini (1961). The pre-injection of α-ketoglutaric acid alone (suggested as a possible rate-limiting moiety in ammonia fixation by Balaguna-Baruch, Shurland & Welbourne, 1970) also had no effect. The most successful results in this present work were obtained by injections of arginine together with ornithine in solutions of either physiological saline or 40 % calcium borogluconate. Bullington (1958) had suggested the use of Calcium chloride in glucose solution as a therapy for urea toxicity.

As regards the curative effect of the administration of these mixtures to clinically affected sheep. The results present a rather uncertain picture. The most striking recovery of lambs from impending death due to ammonia toxicity was noted in lambs treated with the mixture of both arginine and ornithine either in saline or calcium borogluconate solution. It should be stressed however that these lambs were treated as soon as the first signs of tetany were noted. Experiments performed subsequently to the work presented in this thesis involving Greyface ewes in a state of hyperammonaemia have shown varied responses to therapeutic treatment by intravenous injection of large quantities of arginine and ornithine.

It is of note that blood urea concentration of those sheep either treated before or after urea drenching increased markedly over that of the untreated controls, thus supporting the hypothesis that enhancing the efficiency of the removal of incoming ammonia in the liver will result in the increased production of urea, the end product of ammonia detoxification. Plasma potassium concentrations of sheep in a state of hyperammonaemia apparently increased very quickly and thereby partly supporting the view of Lewis (1961) who postulated that an electrolyte imbalance accompanied by a potassium leak from the cells could explain the toxicity of urea.

In conclusion there is much doubt at present as to whether the various amino acid solutions examined here would be of any real value under field conditions of urea toxicity, where afflicted animals may not be noticed until such time that any applied therapeutic measure is simply too late.

Experiment 3. An examination into the effect of experimental liver damage induced by either 1) carbon tetrachloride or 2) chronic copper poisoning on the rate of ammonia detoxification in sheep given oral doses of urea.

The object of this experiment was to examine the ability of the liver to detoxify large quantities of ammonia resulting from oral challenge doses of urea at intervals during courses of either carbon tetrachloride or chronic copper poisoning and also to assess the potential value of blood ammonia concentration determination following such an urea challenge as a test for hepatic function.

Both carbon tetrachloride and copper poisoning in sheep cause severe liver dysfunction. Boyd (1962) examined the normal levels of various enzymes in the tissues and blood of sheep. He also recorded the changes in enzyme activities, notably those of serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT), during carbon tetrachloride induced liver necrosis. The rate of clearance of bromosulphalein (BSP) from circulating blood has long been employed as a measure of liver function(e.g. Cornelius, Holm & Jasper, 1958). In sheep suffering from liver malfunction there is an increase in the length of time taken for the injected dyestuff to be eliminated. The increase in clearance time has been related to the extent of the liver damage. MacPherson & Hemingway (1969) examined the relative merit of various blood analyses and liver function tests in allowing an early diagnosis of chronic copper poisoning in sheep. Several different biochemical determinations were performed to assess blood copper status and liver function at intervals on sheep which had been dosed regularly with copper sulphate. The determinations performed were, blood copper, SGOT, SGPT, BSP and packed cell volume (PCV). They concluded that SGOT determination was the best determination which gave adequate warning of the approach of the haemolytic crisis incurred in

chronic copper poisoning. There have been an increasing number of reports of chronic copper poisoning of sheep in more recent years particularly among intensively housed lambs fed on high concentrate diets. This study partly attempts to assess the possible consequence of feeding urea-containing diets to housed sheep in circumstances where liver copper accumulation has occurred. Parallel studies were also performed with sheep experimentally poisoned with carbon tetrachloride.

The experiments examined the concentrations of blood ammonia observed in the peripheral circulating blood in sheep following an oral challenge dose of 0.35g urea/kg liveweight at intervals during courses of (a) dosing with carbon tetrachloride. (b) feeding an all concentrate diet containing a high copper content. Changes in SGOT, and blood copper concentrations together with BSP clearance were used as indicators of the extent of liver damage at any one time.

Methods

During the course of the experiments described here analyses were performed for the determination of blood copper concentration (Brown & Hemingway, 1962). SGOT concentration (Reitman & Frankel, 1957) was determined according to the procedure described by the Sigma Chemical Company (Technical Bulletin No. 505, 1963). Clearance of the dyestuff BSP was carried out according to the suggested procedure detailed by Cornelius and Kaneko (1963).

1) Carbon tetrachloride poisoning

Six Greyface wethers (mean liveweight 45 kg) were individually penned indoors. The diet fed consisted of 2 lbs concentrate (13.5 % C.P.) and 2 lbs of hay/day. Initial BSP clearances, SGOT activity and blood ammonia concentrations following an oral aqueous dose of 0.35 g urea/kg liveweight were measured on the day before the commencement of the

experiment. On this and each subsequent occassion the BSP clearance test was performed at least one hour before the urea was administered.

Four sheep, in two pairs, chosen at random (Nos. 2 & 4 and Nos. 1 &5) were thereafter orally dosed each day (at 10.00 hours) with a capsule containing 1 ml carbon tetrachloride (CCl_4). The pairs of sheep were again dosed with 0.35 g urea/kg liveweight on either days 8 and 22 or on days 6, 15 and 20 of the experiment. SGOT activity, BSP clearances and blood ammonia concentrations were measured.

Results

Tables45 and46/(pp.202,203) detail the progressive changes in BSP clearance and SGOT activity for the individual sheep at intervals over the 22 days of the experiment. Owing to the extremely rapid clearance of BSP from the plasma the use of a percentage dye retention record was adopted. (Cornelius et al, 1958; Arendarcik, 1959). Injected BSP disappears from the plasma exponentially for only 7 minutes after administration. $T_{\frac{1}{2}}$ values for BSP removal from the plasma between 15 and 30 minutes after injection together with percentage retention after 10 minutes are also recorded in Table 45. Table47 details the blood ammonia concentrations immediately following the oral administration of urea on particular days during the 22 days of the experiment.

BSP clearances (Table45) for all six sheep were in the order of 97-100 % with a mean $T_{\frac{1}{2}}$ time of 2.1 minutes before the experiment commenced. Similar values were recorded for the two untreated control sheep (Nos. 3 and 6) over the whole experimental period. Clearance of BSP became progressively poorer for those sheep given 1 ml carbon tetrachloride per day. After 6 days of administration the clearances had fallen to about 70 % (sheep Nos. 1 and 5) and after 8 days to about 50 % (sheep Nos. 2 and 4). After 20-22 days clearances were in the order of only 20-35 % for all four treated sheep. The $T_{\frac{1}{2}}$ values rose rapidly from 2.1 to about 7 minutes after 6-8 days and finally to 25-44 minutes

after 20-22 days.

Parallel changes were observed in SGOT activities. Typical individual values of about 100 units were recorded initially and over the whole period for the untreated control sheep. In contrast, the values for sheep Nos. 2 and 4 increased to about 150-430 units after 8 days and were in the order of 1000-2000 units on Day 22 when both sheep died following oral dosing with urea. Similarly for sheep Nos. 1 and 5 the SGOT values increased markedly to 250-300 units by Day 6 and to over 1500 by Day 20 when both sheep died as a result of urea toxicity.

Concentrations of ammonia nitrogen (Table 47) were generally greatest some 60 minutes following the administration of urea. Typical values of about 180-270 µg/100 ml were recorded of all the sheep at the commencement of the experiment and for the two control sheep (Nos. 3 and 6) throughout the experimental period. In contrast, administration of carbon tetrachloride led to progressively greater increases in blood ammonia concentrations. e.g. values of 217-402 were recorded 60 minutes after oral dosing with urea on days 6, 8 and 15. On Day 20 when sheep Nos. 1 and 5 died some 90 minutes following urea administration, blood ammonia concentrations had increased to 500 and 900 µg/100 ml respectively. Similarly, sheep Nos. 2 and 4 died on Day 22 with values of about 1000 µg/100 ml.

This experiment indicates that where the efficiency of liver function is progressively impaired following administration of carbon tetrachloride (as inidcated by increased SGOT activity and inefficient clearance of BSP) then the liver becomes less able to deal with an increase in circulating blood ammonia resulting from the oral administration of urea. No signs of acute toxicity were however observed until BSP clearances were as low as 20-35 % ($T_{\frac{1}{2}}$ being 25-45 minutes) or until SGOT

activity increased to above 500 units.

It is concluded from this experimental situation that oral intake of urea is likely to be more toxic for sheep which have an impaired liver function than for normal, healthy animals.

Table 45. BSP clearances (%) and $T_{\frac{1}{2}}$ values (15-30 mins) from the blood plasma of sheep following continued daily dosage with 1 ml carbon tetrachloride.

CCl_4 ml		Control Sheep 3	Control Sheep 6	Treated Sheep 2	Treated Sheep 4	Treated Sheep 1	Treated Sheep 5
Pretreatment	Clearance %	97.0	99.6	98.0	98.0	100	99.0
	$T_{\frac{1}{2}}$ mins	2.2	1.9	2.1	2.1	2.0	2.1
6	Clearance %	96.8	97.2	-	-	75	65
6	$T_{\frac{1}{2}}$ mins	2.3	2.1	-	-	6.0	7.1
8	Clearance %	99.0	97.6	56	45	-	-
	$T_{\frac{1}{2}}$ mins	2.0	2.2	7.1	12.0	-	-
15	Clearance %	98.0	98.0	-	-	70	59
	$T_{\frac{1}{2}}$ mins	2.1	2.1	-	-	8.0	11.4
20	Clearance %	96.0	98.0	-	-	20	32
	$T_{\frac{1}{2}}$ mins	2.3	2.1	-	-	44.0	34.5
22	Clearance %	99.0	99.0	34	30	-	-
	$T_{\frac{1}{2}}$ mins	2.0	2.0	26.4	31.0	-	-

Table 46. SGOT enzyme concentrations (S-F units/ml at 37° C) in the blood of sheep following continued daily dosage with 1 ml carbon tetrachloride.

CCl_4 ml	Mins	Control Sheep 3	Control Sheep 6	Treated Sheep 2	Treated Sheep 4	Treated Sheep 1	Treated Sheep 5
Pretreatment	0	102	98	117	110	87	90
	30	115	110	220	113	110	125
	60	100	100	114	113	95	100
6	0	102	102	-	-	310	240
	30	100	130	-	-	340	250
	60	108	110	-	-	360	251
8	0	98	80	158	375	-	-
	30	95	110	168	430	-	-
	60	95	120	160	430	-	-
15	0	100	88	-	-	380	280
	30	100	90	-	-	425	310
	60	98	96	-	-	410	315
20	0	100	98	-	-	800	1000
	30	106	106	-	-	1300	1500
	60	107	105	-	-	died	died
22	0	96	100	895	1000	-	-
	30	100	98	1200	2000	-	-
	60	101	106	1500	-	-	-

Table 47 Concentrations of Ammonia nitrogen (µg NH_3 - N/100 ml) in the blood of sheep following continued daily dosage with 1 ml carbon tetrachloride.

CCl_4 ml	Mins after urea dose	Control Sheep 3	Control Sheep 6	Treated Sheep 2	Treated Sheep 4	Treated Sheep 1	Treated Sheep 5
Pretreatment	30	132	80	97	160	242	140
	60	180	217	272	242	277	242
	90	60	40	120	140	115	160
6	30	120	80	-	-	175	230
	60	135	95	-	-	337	217
	90	70	60	-	-	250	170
8	30	160	180	330	345	-	-
	60	138	157	402	295	-	-
	90	87	84	335	221	-	-
15	30	125	105	-	-	260	205
	60	165	145	-	-	245	308
	90	70	90	-	-	230	390
20	30	110	130	-	-	320	450
	60	130	160	-	-	500	900
	90	90	70	-	-	died	died
22	30	142	120	490	1000	-	-
	60	140	130	980	died	-	-
	90	90	87	died		-	-

Experiment 3.

2) Chronic Copper poisoning.

In a preliminary experiment 11 Blackface lambs (initial liveweight 20 kg) were fattened indoors on an ad lib diet containing 17.2 % crude protein and 187 p.p.m. Cu. They were fattened over a period of 8 months. At slaughter liver copper concentration ranged from 948-2247 p.p.m. (dry matter basis). At intervals during this period an oral challenge dose of 0.35 urea/kg liveweight was administered to each lamb. Analyses were performed for blood copper, plasma SGOT and blood ammonia concentration. On two separate occassions a BSP dye clearance was performed. No significant increase in dye retention, blood ammonia concentration following the urea dose, plasma SGOT concentration or blood copper concentration was observed at any time during the trial. The lambs grew steadily (but somewhat slowly) and only one carcass was refused at meat inspection. No sign of copper poisoning was noted at any stage during the trial.

The experiment was repeated with eight Blackface lambs (mean initial liveweight 22 kg) with the exception that the concentrate fed contained only 10.25 % crude protein and 223 p.p.m. Cu. The high crude protein content of the diet fed during the preliminary experiment may well have afforded a 'protective effect' against chronic copper poisoning (MacPherson & Hemingway, 1965). The experiment commenced on 3/11/70 and was terminated on 17/2/71 (i.e. a period of 106 days). Urea drenches were performed on five separate occassions at intervals during the period 57 - 106 days. The results are shown in Tables 48,49 and 50. (pp.207,209,210).

Results.

The lambs grew quite slowly averaging only 1.5 lbs/week increase during the course of the experiment. Only one of the sheep (183) showed the markedly elevated concentration of copper in the blood which has been associated with signs of copper toxicity (Table 48). Sheep 71 also showed an abnormal concentration after 106 days. There were no other clinical signs of approaching copper poisoning (such as inappetance or jaundice) for any of the sheep.

At the commencement of the trial all the sheep exhibited normal concentrations of blood copper and SGOT concentrations (Table 48). All the sheep also exhibited the normal, rapid clearance of BSP (Table 49).

During the course of the experiment blood copper concentrations were not observed to rise markedly except for one individual (183) which had a concentration of 6.1 p.p.m. on the day of death following an oral challenge with 0.35 g urea/Kg liveweight after 80 days of copper administration (Table 48).

SGOT concentrations increased progressively in 4 of the sheep (nos. 7,40,23 and 183) and these were four of the total of five sheep which died (Table 48). Sheep no. 7 died before a BSP clearance test was undertaken on Day 64, but on that date nos. 157,40 and 23 were showing reduced BSP clearances of 52%, 45 and 78% respectively (Table 49). By Day 80 the BSP clearance of no. 183 had fallen to 22%.

At about this critical period of 57-64 days of feeding a diet containing high levels of copper sulphate changes were apparent in the increases in blood ammonia concentrations following the oral challenge with 0.35 g urea/Kg liveweight. Considerably elevated concentrations of about 400 ug/100 ml were noted some 30-60 minutes after drenching for sheep nos. 157,7 and 40 after 57 days, for no.23 after 64 days and for no. 183 after 73 days (Table 49).

Table 48. Experiment 3. Copper Poisoning

Concentration of SGOT (S.F. units/ml) and blood copper (p.p.m.) during 106 days of feeding supplementary copper to Blackface lambs.

No.		0+	44	49	57*	64*+	73*	80*+	106*	Remarks
157	SGOT	132	122	104	116	118	-	-	-	Died 7/1
	Cu.	0.98	1.14	1.04	-	1.14	-	-	-	
57	SGOT	128	100	112	106	104	110	100	115	
	Cu.	1.15	1.19	1.12	-	1.11	1.03	1.18	1.22	
71	SGOT	102	102	104	102	110	98	116	228	
	Cu.	0.98	1.04	0.91	-	1.08	1.10	1.16	1.50	
7	SGOT	105	110	180	600	-	-	-	-	Died 3/1
	Cu.	1.10	1.05	1.12	-	-	-	-	-	
40	SGOT	108	120	110	380	900	-	-	-	Died 6/1
	Cu.	1.05	1.07	1.00	-	1.00	-	-	-	
23	SGOT	98	98	110	105	410	230	-	-	Died 16/1
	Cu.	1.06	1.02	1.04	-	1.13	1.14	-	-	
183	SGOT	106	104	110	126	100	280	800	-	Died 22/1
	Cu.	0.89	1.12	0.89	-	0.97	0.94	6.1	-	
NT	SGOT	98	116	100	106	106	98	120	104	
	Cu.	1.19	1.28	1.11	-	1.08	0.96	1.08	1.00	

\+ BSP clearance performed.

* Urea administered 0.35g/kg liveweight.

Table 49 . Experiment 3. Copper poisoning.

Concentration of blood ammonia (µg/I00 ml.) following oral challenges of urea (0.35 g/Kg liveweight) at intervals during I06 days of feeding supplementary copper to Blackface lambs.

	Days of feeding								
	57			64			73		
	minutes after dosing								
No.	0	30	60	0	30	60	0	30	60
I57	70	4I5	360	30	400	356	-	-	-
57	0	60	0	55	50	I06	0	42	50
7I	I0	80	20	22	32	22	25	I22	I05
7	60	380	420	-	-	-	-	-	-
40	80	460	395	I20	800	I400*	-	-	-
23	25	205	I42	50	387	280	80	450	342
I83	I5	I60	8I	25	I00	40	42	2I2	442
NT	I5	I2	20	I5	I5	32	I6	50	62

	80			I06		
57	I0	II9	I30	20	I30	I20
7I	I0	2I7	I94	nd.	395	365
I83	62	450	8I2*	-	-	-
NT	30	35	30	25	65	40

* died

n.d. not determined.

B.S.P. clearance after I5 minutes (normal value 95-I00%)

Days.	No.	0	64	80
	I57	98	52	-
	57	95	97	97
	7I	99	98	66
	40	97	45	-
	23	97	78	-
	I83	96	96	22
	NT	99	98	98

The results summarised in Table 50 indicate that two of the sheep (40 and 183) died as a direct result of the oral urea challenge dose. Both exhibited the usual signs of ammonia intoxication. Two further sheep (157 and 23) died one day after being given an oral dose of urea. Of the three sheep that did not die, neither no. 57 or NT showed any increase in either blood copper or SGOT concentrations nor any progressive increase in blood ammonia resulting from the urea challenges at any time during the course of the experiment. Sheep 71 which also survived was exhibiting abnormal SGOT and blood copper concentrations by day 106 and some elevation in blood ammonia from urea dosage on that day, but the experiment was terminated at that stage.

It must be concluded that the prolonged feeding of a diet containing high levels of copper sulphate (as was the case for carbon tetrachloride) causes a progressive impairment in liver function which, inter alia, puts the sheep at greater risk from ammonia intoxication following an oral dose of urea. The possibility of impaired liver function must therefore be taken into account in assessing the potential toxicity of urea in livestock feeding.

At the same time it appears that the degree of elevation of blood ammonia following the oral administration of urea has some potential as a diagnostic measure for assessing the efficiency of liver function. Such elevations occured at about the same time as SGOT concentrations were found to be increased.

Table 50. The relationship between the time (days) of the first observation of abnormal concentrations of blood copper and SGOT, delay in BSP clearances, elevation of blood ammonia concentration resulting from urea administration and death in sheep associated with urea toxicity.

Elevated blood copper	>	1.3 p.p.m.
Elevated SGOT	>	200 units
Reduced BSP clearances	<	95 %
Elevated blood NH_3 N.	>	200 µg/100 ml

Sheep	Blood Cu	SGOT	BSP Clearance	Elevated NH_3.N	Died following urea challenge
157	-	-	52	57	65. 1 day after
57	-	-	-	-	Did not die
71	106	106	80	80	Did not die
7	-	57	nd	57	61. No urea given
40	-	57	64	57	64. Same day
23	-	64	64	64	74. 1 day after
183	80	73	80	73	80. Same day
NT.	-	-	-	-	Did not die

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