AN INVESTIGATION INTO THE EFFECT OF RUMINAL AND POST-RUMINAL ADMINISTRATION OF CASEIN ON MILK YIELD AND COMPOSITION

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REVIEW OF LITERATURE

Introduction

The metabolism of protein in the digestive tract of the ruminant and its subsequent effect on production involves many reactions. In the rumen, proteolysis occurs, forming amino acids, volatile fatty acids and ammonia. The rumen micro-organisms can utilise the products of protein breakdown for growth.

Undigested feed protein-N and microbial-N pass to the lower digestive tract where digestion and absorption of amino acids occurs. Once absorbed, the amino acids may be metabolised at the liver, where the end products vary according to the glucogenic or ketogenic nature of the amino acids. The amino acids may also be utilised in protein production.

The following review encompasses the fate of dietary protein, and its subsequent effect on dairy production.

Proteolysis

Amino acids and peptides are products of the first step in protein digestion in the rumen (el Shazly, 1952). When toluene, which inhibits bacterial deaminases, is added to casein and incubated with rumen contents, amino acids and peptides accumulate (Warner, 1956). Further evidence of proteolysis has been provided by Liebholz (1965, 1969),

who measured both L-amino nitrogen and individual amino acids in rumen fluids immediately before, and for varying periods after, feeding. Annison (1956) reached similar conclusions with the demonstration of measurable quantities of amino acids and peptides in rumen contents.

Casein, arachin and soybean were extensively degraded by rumen micro-organisms, whereas bovine albumin, wheat gluten and zein were only slightly attacked (Annison, 1956). Proteolytic breakdown of a protein has been shown to be related to its solubility in alkali and salt solutions (Blackburn and Hobson, 1960 c; Warner, 1956). Unlike the activity of deaminating bacteria, which varied with the presence or absence of readily attacked protein in the diet (el Shazly, 1952), proteolytic activity of rumen contents was high before feeding and changed little immediately after feeding (Blackburn and Hobson, 1960 c, d) or with diet (Warner, 1956; Annison, 1956; Blackburn and Hobson 1960 a, 1962).

The studies of Schlotke (1936) and Sym (1938) established that rumen bacteria and protozoa possess proteolytic activity in the rumen. Sym followed the disappearance of casein during incubation with rumen contents. Within three hours, 44% of the casein (0.5% w/w) was no longer acid precipitable. Suspensions of bacteria and protozoa as well as extracts of microbial cells showed this proteolytic activity. Warner (1956) confirmed Sym's findings and

further concluded that aerobic conditions had little effect on proteolytic activity, even though, under the strongly reduced conditions provided by ferrous ion and cysteine, proteolysis was stimulated.

Early attempts to isolate the organisms involved in proteolysis were not particularly successful, since the only proteolytic organisms identified were facultative anaerobes (Appleby, 1955; Hunt and Moore 1958; Blackburn and Hobson, 1960b). With improvements in culturing techniques, Blackburn and Hobson (1962) and Abou Akkada and Blackburn (1963), successfully obtained from rumen liquor of two sheep on different diets six main isolates, which were obligate anaerobes and possessed proteolytic activity. Fulghum, King and Moore (1958) and Fulghum and Moore (1963) found that 30% and 38% respectively of the total rumen bacterial counts appearing on an isolation media showed proteolytic activity. Little free proteinase occurs in rumen fluid (Blackburn and Hobson, 1960) although Hunt and Moore (1958) isolated an organism which was capable of excreting an active proteinase. Blackburn (1968) studied the proteinase produced by Bacteriodes amylophilus strain H 18. The proteinase was mostly intracellular, associated with the cell wall fraction, and was neither induced nor repressed by a wide range of nutrients. Approximately 20% was released into the medium, but it is not known whether the proteinase in the medium was truly extracellular, or intracellular

material which was released when the micro-organisms lysed.

The lower rumen ammonia concentration in defaunated animals has been taken to indicate that protozoa have a role in proteolysis and deamination (Klopfenstein, Purser and Tyznik, 1966). Recent evidence from Males and Purser (1970) indicated that the above assumption was not necessarily correct, as the lower rumen ammonia concentration in defaunated animals could be explained by a greater bacterial concentration, resulting in greater ammonia utilization.

However protozoa may still have an important function in proteolysis. Warner (1956) noted that the proteolytic activity of rumen liquor which had protozoa removed was reduced below that of rumen liquor containing protozoa. Warner also found that acetone powder extracts of holotrich and oligotrich protozoa had proteolytic activity.

Separation of the rumen micro-organisms by centrifugation into protozoa, large bacteria and small bacteria (Blackburn and Hobson, 1960a) demonstrated that the highest percentage of proteinase activity was associated with the protozoal fraction. The authors did however, point to the difficulty of obtaining a pure protozoal fraction, as some bacteria are closely associated with the protozoa. The same authors observed oligotrich protozoa ingesting stained casein particles. A more detailed study on the proteolytic activity of protozoa carried out by Abou Akkada and Howard (1962), found that within four hours, 80% of the casein incubated with Entodinia was soluble in trichloracetic acid.

Peptide nitrogen, α-amino nitrogen, ammonia-N and amide-N accounted for 40%, 22%, 14% and 3% respectively of the non-protein nitrogen in solution at the end of the incubation. A cell free extract of Entodinia was shown to contain a "peptidase", capable of hydrolysing a wide range of peptides, as well as a "proteinase".

The importance of proteolysis to the ruminant depends on the subsequent fate of the amino acids released during proteolysis. The amino acids may be absorbed (Demaux, Bars, Molle, Rerat and Simonnet 1961) from the rumen, deaminated by the rumen bacteria, with much of the nitrogen lost in the urine, (el Shazly, 1952) or utilised by the rumen micro-organisms for synthesis of cellular material (Allison, 1969). If feed protein of low biological value is converted to microbial protein having a higher biological value than the feed, then there would be a net gain to the animal. If, however, the feed protein is rapidly deaminated, giving rise to high levels of rumen ammonia, then much loss of nitrogen could occur.

Metabolism of Amino Acids and Peptides

Blackburn (1965) and Hungate (1966) have reviewed the metabolism of individual amino acids by rumen micro-organisms. The products of metabolism are predominantly ammonia, volatile fatty acids, and carbon dioxide. The failure of orally administered amino acids to affect nitrogen

utilization has usually been attributed to their being degraded in the rumen. However, Lewis (1955) and Lewis and Emery (1962) presented evidence which suggested that amino acids differ in their rates of deamination. Recently Chalmers and Hughes (1969) reported that when glycine, alanine and lysine were fed to sheep in large daily doses for fourteen days, substantial amounts of the amino acids passed out of the rumen undegraded, as was indicated by an increase in plasma lysine from 0.9 mg. per 100 mls. plasma, four hours after feeding. Although absorption of amino acids from the rumen has been demonstrated by Demaux et al (1961), Lewis and Emery (1962) and Cook, Brown and Davis (1965), the magnitude of its importance was only recently demonstrated with the use of radioactive labelled amino acids. Label from D,L-Tryptophan, a slowly deaminated amino acid, (Lewis and Emery, 1962), poorly utilized by rumen micro-organisms, (Candlish, Devlin and La Croix, 1970) was found in urine within ten minutes of being injected into the rumen of sheep (Candlish, Stanger, Devlin and La Croix, 1970). It was calculated that 25 - 70% of the free tryptophan in the rumen was absorbed into portal blood within three hours.

Following the introduction of methionine-methyl-14C into the rumen of a nine month old calf, two distinct time periods were observed when significant quantities of radioactive methionine were absorbed into the blood (McCarthy, Patton and Griel, 1970). The first peak occurred

thirty minutes after the introduction of tracer into the rumen, with the amount of radioactivity in the blood at this time being equivalent of 10% of the total quantity given. The second period of absorption occurred nine hours after the injection, which most likely represents the absorption of amino acids released by digestion of the micro-organisms in the small intestine, releasing methionine incorporated into their structure. This recent data suggested that absorption can be of significant magnitude if the quantity of free amino acids was sufficiently high in the rumen, or if the amino acids present were only slowly metabolised by the rumen micro-organisms. These results contrast with findings of Annison (1956), based on arterial-portal differences, that the absorption of amino acids from the rumen did not occur following the addition of casein digest to the rumen. However, changes in blood flow, as well as the difficulty in detecting micromolar amounts of the amino acids, could easily have masked any changes in concentration. The α-amino nitrogen content of portal blood was less than the content in jugular blood, this could be taken as indicating increased splanchnic blood flow, resulting in a dilution of any increased amounts of amino acids absorbed from the rumen. Further work in which both blood flow and amino acid concentration in portal blood are measured under normal feeding conditions is required to quantitate the importance of ruminal absorption of amino acids.

Fate of Ammonia

The absorption of ammonia from the rumen and its subsequent conversion to urea, to be lost in the urine or recycled back to the rumen, has been adequately covered in reviews by Waldo (1968), Smith (1969) and Tillman and Sidhu (1969). The absorption of ammonia is affected by the pH of the rumen contents. As pH increases the ammonium ion will be converted to NH3 and rapidly absorbed. Using an isolated rumen, Mooney and O'Donovan (1970) found that at equilibrium, the concentration of ammonia was greater on the side with low pH. This phenomena occurred irrespective of the direction of ammonia gradient initially. These findings confirmed the hypothesis that rumen tissue was more permeable to ammonia than to the ammonium ion and that transport across the rumen was passive. This was in contrast with the active transport of urea which was observed by Houpt (1970). Little ammonia appears in peripheral blood, indicating the efficiency with which it is converted to urea at the liver. Smith (1969) has suggested that ammonia might be used for the synthesis of non-essential amino acids at the liver, as has been shown to occur in monogastrics, (Wrong, 1967).

Recycling of nitrogen, as urea, to the rumen via saliva, and by diffusion across the rumen wall, appears to increase on low nitrogen diets, with a concomitant decrease in urinary urea, (Houpt, 1970). In some cases a substantial

increase in nitrogen reaching the duodenum, over the level of nitrogen ingested, has been observed (Clark, Ellinger and Phillipson, 1966).

Pilgrim, Gray and Belling (1969) showed that on a diet of pelleted lucerne hay, or lucerne hay chaff, 59 - 66% of the ammonia-N was absorbed from the rumen, and the remainder passed to the abomasum. Although not specifically stated as such, the ammonia-N passing to the abomasum would include the ammonia-N incorporated by micro-organisms into amino acids and proteins.

Biosynthesis of Amino Acids by Ruminal Micro-organisms

The concentration of amino acids in ruminal fluid has been shown to be relatively low (Wright and Hungate, 1967 a), and some of the free amino acids are rapidly catabolised (Wright and Hungate, 1967b; Portugal, 1963). Bryant and Robinson (1962) found that 82% of the strains of rumen micro-organisms isolated on a non-selective medium grew with ammonia as the principal source of nitrogen. That *de novo* synthesis and the biosynthetic mechanisms involved in amino acid synthesis are important has been ably demonstrated by Virtanen (1966) in an experiment in which cows produced milk protein when fed on a diet entirely devoid of amino acids. A most detailed and comprehensive review of the biosynthesis of amino acids by rumen micro-organisms has been provided by Allison (1969). In general there are

three main requirements for amino acid synthesis; ammonia, carbon skeleton, and energy. Glutamic dehydrogenase appears to be the major enzyme system in the ammonia fixation mechanism in ruminal bacteria, (Chalupa, Clark, Opliger and Lavker (1970)) while other dehydrogenases, glutamate-oxaloacetate transaminase, and glutamate-pyruvate transaminase could also be of importance. The carbon for amino acid biosynthesis probably comes mainly from intermediates of carbohydrate fermentation, fermentation end products, viz. carbon dioxide, acetate, and for some bacteria, branched chain fatty acids. The energy for biosynthesis is mainly derived from glycolytic reactions, with the amount of energy produced, limited by the anaerobic nature of rumen fermentation (Hungate, 1966).

Synthesis of Microbial Protein

Measurement of the amount of microbial protein produced has been difficult, because of the problems faced in distinguishing between feed-N and microbial-N. Methods of overcoming this have involved using feed-N with particular chemical characteristics. McDonald (1954) and Ely, Little, Woolfolk and Mitchell (1967) used the solubility of zein in alcohol, and McDonald and Hall (1957) used the phosphorus content of casein to distinguish feed protein from microbial protein in duodenal contents. McDonald (1954), Temmler-Kucharski and Gausseres (1965) and Ely et al (1967)

used the lysine content of feed and microbial protein to estimate microbial protein in digesta.

Weller, Pilgrim and Gray (1962) and el Shazly and Hungate (1966) have used the concentration of α-ε-diaminopimelic acid, an amino acid peculiar to bacteria, to estimate bacterial growth, although Synge (1953) showed that the ratio of diaminopimelic acid to total nitrogen varied considerably amongst different bacteria.

Temmler-Kucharski and Gausseres (1965) based estimates of microbial protein on DNA contents. Recently McAllan and Smith (1969) overcame the difficulties involved in determining RNA and DNA, and they have suggested that RNA content could be used as an indicator. They found that the DNA to total nitrogen ratio varies markedly between different organisms and particularly between bacteria and protozoa, part of this variation being due to changes in microbial composition and stage of growth of the organisms. However RNA was a much more constant proportion of total microbial nitrogen and was thus suggested as being a much more reliable microbial marker in digesta than either DNA or total nucleic acid.

McDonald (1954) found that when zein provided 82 - 94% of the total nitrogen, approximately 40 - 50% of the zein was converted to microbial protein. When urea supplied 50% of the nitrogen, and zein the other 50%, only 13% of the zein was degraded in the rumen (Hume 1970c). Weller,

Gray and Pilgrim (1958) estimated that, on a diet of wheaten hay, 63 - 82% of the plant-N was converted to microbial-N. Further work involving diets of wheaten hay plus lucerne, and lucerne, showed that 60 - 82% of the plant-N was converted to microbial-N, with the upper figure possible being more correct, since omasal contents exhibited this level of conversion.

Smith and McAllan (1970) found that for a range of diets, 55 - 80% of the non-ammonia nitrogen in rumen fluid of calves, and 40 - 50% in cows, was of microbial origin. They also observed that the addition of starch (450 g. per feed) to the rumen had little effect on nucleic acid concentration whereas the addition of sodium caseinate (350 grams per feed, as a paste) increased the nucleic acid concentration by 65 $\pm$ 10% after a single meal, while 5.5 hours after the last of three successive meals, nucleic acid concentration was increased by 165%. On a low nitrogen diet of wheaten hay, 68% of the plant-N was converted to microbial-N, while on a higher nitrogen diet of lucerne hay the conversion rate was 53 - 55% (Pilgrim, Gray, Weller and Belling, 1970). Pilgrim et al (1970) also concluded that synthesis of microbial protein was more dependent on ammonia as a starting point with the low nitrogen diet than with the higher nitrogen diet.

Thus it would appear that 50 - 80% of the plant-N is converted to microbial-N. Decorticated groundnut meal (Smith

and McAllan, 1970) and zein (McDonald, 1954; Hume, 1970c) appear to be more resistant to microbial breakdown, whereas casein (McDonald and Hall, 1957) is almost completely degraded in the rumen.

Thermodynamic Aspects of Microbial Protein Production

Limitations on microbial protein synthesis in the anaerobic conditions of the rumen have been reviewed by Walker (1965), Hungate (1966) and Hogan and Weston (1970). While aerobic micro-organisms can synthesise 60-70% of the carbohydrate substrate into protoplasm, anaerobic organisms usually utilise 10%, and rarely more than 20%. Ruminococcus albus in pure culture, with cellobiose as the substrate, gave cell yields of 22% and 26.4% (Hungate, 1966).

Using a method involving measurement of S35 incorporation into protein, Walker and Nader (1968, 1970) estimated that on a diet of lucerne, 14.4 g of microbial protein was synthesised per 100 g of organic matter digested in the rumen. This estimate was in reasonable agreement with a value obtained by Hogan and Weston (1967) under in vivo conditions, and was therefore taken to confirm the validity of the method. However, Hume (1970 a,b,c,d) found 9.1 - 23.3 g of protein synthesised per 100 g organic matter digested in the rumen. Walker and Nader (1970) based their estimates on a situation in which energy was limiting. In Hume's work the nitrogen was supplied by urea or by urea plus gelatin,

and the amount of protein synthesised agreed with a personal communication estimate by Walker, of about 17 g of protein per 100 g of organic matter digested. However, when the nitrogen was supplied by urea plus casein or by urea plus zein, the protein synthesised was far above the theoretical estimate, viz. 23.3 and 22.5 g of protein per 100 g of organic matter digested, respectively. Hume thus concluded that energy was not limiting, as the intakes of organic matter and thus the potential ATP production, on all four diets were the same. The possibility therefore remains that some protein derivative, such as an amino acid or peptide, may have been limiting the urea and the urea plus gelatin diets.

Hume's findings would conflict with present theories on microbial synthesis and fermentative activity (Walker, 1965; Hungate, 1966). The synthesis of storage compounds may partly explain the differences observed in growth efficiency, since the amount of energy stored as polysaccharide in rumen bacteria can be as much as 80% of the energy available (Walker, 1968). Similarly Walker and Nader (1970) found decreasing utilisation of energy for protein synthesis correlated with an increase in polysaccharide content of of the microbial cells, while the return of cellular polysaccharide levels to pre-feeding values was accompanied by increased usage of energy for protein synthesis. These observations could explain the lack of increase in nucleic

acid concentration of rumen fluid 5.5 hours after starch was added to the rumen, (Smith and McAllan, 1970) whereas when casein was added there was a marked increase in nucleic acid concentration. There seems to be little doubt that production of microbial protein is limited to approximately 6.0 - 6.6 g per mole of ATP (Forrest, 1969) under energy limiting conditions. What is not known is whether, under some conditions, "growth factors" other than energy are limiting. It would appear from the work of Walker and Nader, (1970), Smith and McAllan (1970) and Hume (1970c) that if these other "growth factors" are provided in large amounts, then energy, instead of being stored, would be utilised for microbial protein production. Under these conditions, where there has been reduced cost of amino acid synthesis, the utilisation of energy may be more efficient.

Quality of Microbial Protein

McNaught, Owen, Henry and Kon, (1954), investigated the nutritive value of dried preparations of rumen bacteria and protozoa when fed to rats. They found the true digestibility, biological value, and net protein utilisation for bacteria to be 74, 81 and 60, and for protozoa 91, 80 and 73 respectively. In many respects little progress has been made in this field, since no difference in protein quality of micro-organisms has been demonstrated as a result of different experimental conditions. Purser (1970a) provides

an excellent review on this topic from two viewpoints:

1) the amino acid composition of the material presented for digestion, and

2) the availability of the amino acids from this material.

In general, the amino acid composition of bulk bacterial and protozoal preparations has been shown to be similar for a wide range of diets and conditions, although Hoeller and Harmeyer (1964) have shown differences in amino acid composition among various species of protozoa. Unfortunately, none of the data on the biological value of microbial protein have been obtained in ruminants, and as such, conclusions on the limiting amino acid of bacterial or protozoal fractions need to be considered with regard to this point.

The improvements in animal production obtained by abomasal infusion of methionine, cysteine and casein (Reis and Schinckel 1963, 1964 and Reis 1969) in sheep, and lysine (Devlin, 1966; Schelling and Hatfield, 1968) in cattle and sheep, would seem to indicate that under some dietary conditions the total amount of a particular amino acid absorbed was less than that required for optimum animal production. Bergen, Purser and Cline (1967) examined the free amino acids in solution after a three hour digestion by pepsin followed by a twenty-four hour digestion with pancreatin of eighteen strains of rumen bacteria. Although

there was similarity in the amino acid composition of acid hydrolysates of individual rumen bacteria, marked differences occurred between the bacteria with respect to their protein quality. These differences were due not only to digestibility differences, but also to the pattern in which the amino acids were released from bacterial protein. It would therefore seem reasonable to assume that changes in the composition of the rumen population must be considered as a possible explanation for changes in nitrogen utilisation or amino acid patterns as a result of dietary modifications.

The protein presented for digestion in the small intestine can thus affect the amino acid nutrition of the animal in two ways;

1) via variation in rates of release, and therefore in the total quantity of an amino acid available, and

2) by the interaction between amino acids which causes variation in absorption rates. For example, Robinson (1968), found that low concentrations of L-methionine stimulated L-leucine uptake, but at high concentrations of L-methionine, inhibition occurred. Similarly, L-arginine uptake is stimulated by L-citrulline and inhibited by L-isoleucine. Wiseman (1968) has comprehensively reviewed the absorption of amino acids from the gastro-intestinal tract. It has been assumed that absorption of amino acids in ruminants was similar to absorption in monogastrics,

although neither evidence to verify this assumption, nor to disprove it, has been found.

Purser (1970a) recalculated some of the data of Clark, Ellinger and Phillipson (1966) which enabled one to examine whether amino acids were absorbed in proportion to their concentration in digesta, from the small intestine. The data showed that except for threonine, the essential amino acids (Downes, 1961) were lower in concentration in ileal than in duodenal contents, while except for glutamic acid, and possible aspartic acid, the converse was true for the non-essential amino acids. Recalculation of data of MacRae, Pearson and Hendtlass (pers comm), in which three diets were used (dried grass, dried grass plus treated casein and dried grass plus untreated casein), gave virtually identical results to those obtained by Purser (1970).

These results are important in that they demonstrate the greater value of the nitrogen disappearing from the small intestine over that remaining. This is assuming that there is no limit to the synthesis of non-essential amino acids within the ruminant.

Although these results could be taken to indicate preferential absorption of essential amino acids, Purser pointed out that they may reflect the type of protein presented for digestion. A relationship between amino acid disappearance from the gut and the release of amino acids

from pure culture ($r = 0.74$ $p<0.05$) would tend to support this hypothesis. Hungate (1966), Øltjen (1969) and Smith (1969) have also discussed the quality of microbial protein in their reviews of nitrogen metabolism.

There appear to be two main difficulties associated with estimation of the quality and quantity of rumen microbial protein. The first has been the lack of a suitable method for distinguishing feed-N from microbial-N, although Smith and McAllan (1970) consider RNA measurements to be reliable. The second difficulty has been in measuring the amounts of individual amino acids absorbed from the gastro-intestinal tract. With improvements in catheterisation techniques and methods for measuring blood flow, the amounts of amino acids absorbed may soon be measured with some degree of accuracy, thus enabling the collection of data which would considerably expand our knowledge of the protein nutrition of the ruminant.

The amino Acid Requirements of the Lactating Ruminant

The dearth of information on the amino acid requirements for the growing or lacting ruminant has recently been the subject of reviews by Hatfield (1970), Jacobson, Van Horn and Sniffen, (1970), McCarthy, Patton and Griel (1970) and Purser (1970b). Barry (1961) reviewed the early data from arterio-venous differences and tracer studies, concerning the possible precursors of milk protein. Although

it was once considered that milk proteins were absorbed from the blood, preformed, it would now appear that casein and β-lactoglobulin, at least, are synthesised within the mammary gland from plasma free amino acids.

An examination of the arterio-venous differences of individual amino acids (required for milk protein synthesis) in cows by Verbeke and Peeters (1965) revealed that sufficient quantities of arginine, glutamine, iso-leucine, leucine, lysine, valine, threonine and histidine were taken up by the mammary gland to account for all their respective amino acid residues in milk protein. The uptake of asparagine, glutamic acid, serine and proline was insufficient to account for their respective residues, while ornithine, a non-casein amino acid showed an arterio-venous difference of up to 60% of arterial plasma concentration. Similarly, Shimbayashi, Ide and Sono(1967) found the uptake of aspartic acid, serine, glycine and glutamic acid, by the lactating cow mammary gland, to be insufficient to account for their respective residues in casein.

The possibility of ornithine being converted to proline was examined by Verbeke, Peeters, Massart-Leen and Cocqyt (1968). In sheep they found that nearly 25% of the proline in casein came from ornithine and arginine. Mepham and Linzell (1966) observed that in the goat, the uptake of arginine was three to four times in excess of requirements. Using labelled arginine, Mepham and Linzell

(1967), demonstrated that the arginine was being converted to proline. It was also apparent from this work that the uptake of the non-essential amino acids was more variable than the uptake of the essential amino acids, which would suggest that the mammary gland was not completely dependent on plasma percursors for the non-essential amino acids. Isotopic evidence showing that serine, alanine, glutamate and aspartate can be formed from glucose and serine, and proline, glutamate and aspartate from acetate (Linzell, 1968) is consistent with this hypothesis. This work gave some lead as to which are the essential amino acids for milk protein production.

More detailed amino acid requirements have come from in vitro work. Larson (1965, 1969) in his review of milk protein biosynthesis, concluded that there was no reason to believe that milk protein synthesis differed from protein synthesis in other tissues. Using a cell free system from lactating bovine mammary tissue, Beitz, Mohrenweiser, Thomas and Wood (1969) have demonstrated fairly conclusively that synthesis of milk protein conforms to the mechanism of protein synthesis found in other biological systems.

Schingoethe, Hageman and Larson (1967) have utilised cell cultures prepared from lactating bovine and rat mammary gland. The bovine cultures did not have a requirement for glutamine or tyrosine. The presence of glutamine synthetase adequately explained the lack of

a glutamine requirement, while the demonstration of phenylalanine hydroxylase activity in the bovine secretory cells, capable of converting phenylalanine to tyrosine, explained the lack of a tyrosine requirement. None of the other bovine tissues possessed this latter enzyme. Of great interest was the finding that synthesis of β-casein and β-lactoglobulin was not significantly depressed when the essential amino acids only, were added to the medium. Thus the mammary cells appear to be able to synthesise the usual non-essential amino acids, which is in contrast to the conclusion reached by Mepham and Linzell (1967) based on arterio-venous difference of urea. Elevated levels of amino acids above physiological levels resulted in a stimulation of β-lactoglobulin but not of α-casein in bovine cells which led Schingoethe et al, to suggest that the secretory cell may have no direct control other than substrate availability. Based on these observations, the authors also felt that the mammary cells were capable of producing more protein than is normally observed.

Whole animal studies

(i) Lysine.

The use of amino acid balances to determine limiting amino acids entails the measurement of amino acid concentration in feed, faeces and milk. A negative balance

of an essential amino acid indicates net synthesis in the rumen, while a positive balance indicates no net synthesis in the rumen. This method may indicate little or no net synthesis, but because of amino acid degradation or requirement for tissue protein synthesis, considerable synthesis could be occurring in the rumen.

Bigwood (1964) used a rough approximation of this method when he measured the amino acid content of feed and digesta at the omasal-abomasal junction. Based on changes in amino acid composition in digesta and the high lysine content of milk protein, Bigwood concluded that lysine was probably the first limiting amino acid. Purser, Klopfenstein and Cline (1966) have also suggested that lysine might be the first limiting amino acid for ruminants.

(ii) Methionine

Using the complete amino acid balance approach, Jacobson, Soewardi, Barnett, Hatton and Carr (1969) found glutamic acid and lysine to be in negative balance while tyrosine and the sulphur containing amino acids were essentially in balance. The authors thus considered there was little net synthesis of the sulphur amino acids and they might be the first limiting amino acids. The data might also be interpreted as indicating the high requirement for lysine and glutamic acid, and thus any interference to the synthesis of these amino acids could result in an

amino acid insufficiency, and a limitation to production.

Conrad, Hibbs and Pratt (1967) and Conrad, Miles and Butdorf (1967) have estimated the methionine synthesised in the rumen on an alfalfa hay concentrate diet. Their results indicated that sufficient methionine was synthesised for a lactating animal. The amount of methionine degraded in the rumen was assessed from the degradation of fish meal placed in the rumen in a nylon bag; they estimated that only 10% would be degraded, whereas Smith (1969) obtained 60-70% degradation.

Teichman, Caruolo and Mochrie (1969) and Fisher (1969) found intravenous infusion of methionine had no effect on milk yield and composition. Fisher also found no effect with DL-methionine plus L-lysine. In both cases the treatment period lasted only four days, and this may have been insufficient time to allow changes to occur. Broderick, Kowalczyk and Satter (1970) and Williams, Martz and Hilderbrand (1970) fed an encapsulated DL-methionine which will pass through the rumen intact and become available for absorption in the intestinal tract (Sibbald, Lougheed and Linton, 1968). Doses of 5, 12, 15 or 45 gm. of DL-methionine per day failed to give any beneficial effect on milk yield and composition.

The report of Griel, Patton, McCarthy and Chandler (1968) was the first published in which a response to the dietary addition of an amino acid had been obtained in a

lactating ruminant. In this case, methionine, fed as the hydroxy analogue, was used. A significant increase in the yield of fat corrected milk (FCM) was obtained, with evidence that not all breeds gave a similar response, as was shown by significant breed x treatment interaction. Examination of the fatty acid composition of milk fat showed that methionine hydroxy analogue (MHA) supplemented cows also secreted greater quantities of 18 carbon fatty acids than cows receiving no supplement (Patton, McCarthy and Griel 1970b). Since this report, Polan, Chandler and Miller (1970) have obtained evidence to suggest that levels greater than 25 grams per day of MHA depress FCM yield and milk yield. Similarly, Jacobson, Van Horn and Sniffen (1970) found that milk yield was depressed, fat percent increased, and yield of FCM unaffected, when 80 grams per day of MHA was fed to four high producing (40 kg milk per day) cows in a double reversal design.

It appears that the effect of MHA may be mediated via rumen lipid metabolism. Incubation of methionine with rumen contents in vitro has resulted in a stimulation of lipid synthesis (Patton, McCarthy and Friel, 1968), and in particular a stimulation of the production of polar lipid (Patton, McCarthy and Friel, 1970a). When rumen fluid from cows supplemented with 80 grams of MHA daily was incubated with labelled acetate and glucose, there was rapid incorporation

of label into complex lipids, 90% of which was polar lipid (Patton et al, 1970a). The increase in lipid was associated with the protozoa (McCarthy, Patton and Griel, 1970) and it has been suggested that methionine may be limiting the growth of some rumen micro-organisms, particularly some protozoa. These findings would agree with those of Keeney, Katz and Allison (1962), in which it was shown that the major proportion of the lipids in the rumen are associated with the protozoa.

Evidence that the effect of MHA is not only in the rumen was shown by the increase in total quantity of blood serum lipids (Patton, McCarthy and Griel, 1970b) and absence of triglyceride in the β-lipoprotein fraction of the serum of cows receiving MHA.

(iii) Glutamic acid

Amino acids are specifically required for protein synthesis, but many also play an important role as glucogenic precorsors. In a 450 kg lactating cow, producing 20 kg of milk per day, Black, Egan, Anand and Chapman (1968) have calculated that the glucose requirement for lactose production and oxidation is 1360 grams per day. The role that glucogenic precursors play is of some importance. Glutamic acid comprises 19.8% of all milk protein, and is a glucogenic amino acid. Egan and Black (1968) and Egan, Moller and Black (1970) have studied the metabolism of

glutamic acid in lactating cows and goats and found that only 12-15% of an injected dose of labelled glutamate was recovered in milk after 48 hours. Lactose accounted for more of the carbon-14 recovered than did casein or albumin. It would therefore seem that more of the glutamate carbon was utilised for gluconeogenesis than was incorporated into milk protein.

Halfpenny, Rook and Smith (1969) have examined the effect of an increased plane of nutrition, and intraruminal infusion of propionic acid on plasma and amino acid concentration. The main effect of the three treatments was a decrease in the plasma concentration of those amino acids whose carbon skeletons cannot be derived from the general metabolic pool, and which are in highest concentration in milk protein, namely valine, iso-leucine, leucine and lysine, whereas there was an increase in the concentration of the non-essential amino acids (NEAA). The decreased level of the essential amino acids (EAA) was taken to indicate increased uptake for milk protein secretion. Halfpenny et al suggest that the improvement in milk protein production resulting from their treatment was due to the provision of more NEAA, and in particular glutamic acid, and possible proline. They further suggest that there was some restriction in the mammary gland on the synthesis of NEAA's. The possibility of lack of interconversion among the NEAA to

glutamic acid, has been borne out by the results of Verbeke et al (1968) in which almost negligible amounts of ornithine or arginine were converted to glutamic acid while at the same time these two substrates provided almost 25% of the proline in casein.

Other Amino Acids

A recent report from Yousef, Huber and Emery (1969) indicated that protein production of cows could be increased by improving the supply of substrate. When 50 g of acid hydrolysed casein was infused intravenously, daily, protein secretion increased by 14%.

More recently, Broderick, Kowalczyk and Satter (1970) obtained a 6.2% increase in milk protein content, and a 11.4% increase in protein production when 800 g of sodium caseinate plus 24 g of methionine were infused daily into the abomasum.

Effect of Dietary Protein on Milk Yield and Composition

While it has been established that increases in dietary energy will result in increased solid not fat (SNF) and protein content in milk (Rook and Line, 1961), marked changes in the protein content of the ration have little effect on milk constituents (Rook and Line, 1962).

Burt (1957), Rook (1961), Huber and Boman (1966) and Kirchgessner, Friesecke and Koch (1967) have reviewed various

aspects of the effect of dietary protein on milk yield and composition. Rook and Line (1962) obtained no effect on level of fat, SNF, casein, α-lactalbumin, serum albumin or β-lactoglobulin in milk, by feeding diets providing 70-165% of the Woodman standards. They did, however raise the concentration of non-protein nitrogen in the milk, particularly with high levels of dietary protein.

Gordon and Forbes (1970) have confirmed these earlier findings, protein fed at 80% or 120% of requirements increased the non-protein nitrogen content of milk as well as increasing the milk energy output. Of interest was the observation that the response to additional energy was reduced on low protein diets. This data suggests that on a low protein diet there was insufficient ammonia or amino acids for maximum microbial growth, with the extra energy being stored as microbial polysaccharide, as observed by Walker and Nader (1970). The results of these experiments were hardly surprising in view of the observation of Hogan and Weston (1967) that the same amount of non-ammonia nitrogen entered the duodenum when sheep were fed diets containing 7.8% and 19.8% crude protein. Their results indicate the tremendous tempering effect the rumen has on the composition of dietary protein. It would be expected that, except in the case of very low levels of dietary protein, the only situation in which dietary protein might affect milk yield and composition would be when a large amount of

dietary protein containing the limiting amino acid(s) for milk production escaped degradation in the rumen.

AIM OF THE EXPERIMENT

Casein is the major milk protein produced by the cow, it was therefore considered that, if an amino acid was limiting milk protein production, feeding casein would ensure that the particular amino acid was supplied. The degradation of protein in the rumen indicated a need to by-pass the rumen with the additional dietary protein. The experiment was thus aimed at determining the effect of by-passing the rumen with additional protein. The control was addition of casein directly to the rumen. Evidence from other work indicated that casein added to the rumen would result in high levels of rumen ammonia, which in turn would result in an inefficient use of nitrogen by the animal.

In the experiment, the main rumen characteristics measured were ammonia level and volatile fatty acid (VFA) proportions and concentrations. Any differences occurring between treatments could possibly be partly explained by differences in these rumen characteristics. A nitrogen balance, which would indicate the overall efficiency of total N use, was also conducted.

If differences in milk yield and composition occurred between the treatments, supply of substrate to the mammary gland may be altered. To examine this aspect, blood samples were taken, and levels of some blood metabolites were measured.

Thus while the experiment was a comparison of the effect of casein administed post-ruminally or ruminally on milk yield and composition, it was considered that by taking the supplementary measurements mentioned above, an explanation for the results obtained could be offered.

SECTION II

MATERIALS AND METHODS

II : 1. ANIMALS AND EXPERIMENTAL DESIGN

The experiment was conducted using 3 pairs of monozygotic twins and incorporating 4 periods:-

Acclimatisation Period	Preliminary Period	Experimental Period	Post-experimental Period
18th October - 4th November	5th November - 15th November	18th November - 8th December	8th December - 18th December
18 days	10 days	20 days	10 days

The animals used were numbered 7 & 8, 77 & 78, and 105 & 106, and they were 4, 5, and 3 years old respectively. At the beginning of the acclimatisation period the cows had all been calved from 65 to 95 days. To avoid cows coming into season during the experiment, all cows were mated before the trial commenced. Two cows (numbers 77 and 106) did not hold to the first service, and they were mated during the trial period. Mating dates for cows were:-

7	19.9.69	77	15.11.69	105	28. 9.69
8	26.9.69	78	14. 9.69	106	5.12.69

The cows were rumen fistulated 4 months before the trial started.

Within a twin-set, cows were allocated at random to one or other of the two treatments, casein added to the rumen (treatment R) or casein added post-ruminally (treatment PR), as follows:-

	Treatment R	Treatment PR
	7	8
Cow	77	78
	106	105

Acclimatisation period. During this period the animals became accustomed to their new environment and routine. Each day commenced at 7.00 a.m. with feeding, and finished at 7.00 a.m. the following day immediately prior to feeding.

The cows were milked at 5.30 a.m. and 5.00 p.m. each day using a milking system designed by Alfa-Laval. A resume of all samplings is presented in Table I.

Preliminary period. During this period faeces and urine were collected from all cows except 77, which had swollen leg joints. The cows were weighed before the a.m. feeding on the 1st and 16th of November. Intravenous catheters were inserted in the jugular veins of all cows on the 6th day of the period, with blood samples being taken on the 8th and 9th days of the period at 0, 4 and 8 h after the a.m. feeding. The data obtained from these blood samples were assumed to represent normal blood metabolite concentrations under pre-treatment conditions.

Experimental period. Following each milking, and immediately prior to feeding, all cows received 200 g Casilan (Glaxo Laboratories) dissolved in 1.5l warm water, either into the

TABLE I

SAMPLING DAYS FOR EACH OF THE PARAMETERS MEASURED

SAMPLE	PERIOD		
	Preliminary	Experimental	Post-Experimental
Milk Samples	Days 1-10 (Pre)	Days 1- 5 (Ex1) 6-10 (Ex2) 11-15 (Ex3) 16-20 (Ex4)	Days 1- 5 (Post 1) 6-10 (Post 2)
Nitrogen Balance	Days 1-10 (Pre) all cows except 77	Days 11-20 (Ex)	Days 4-10 (Post)
Blood Samples	Days 8 & 9 (Pre)	Days 10&11 (Ex1) 19&20 (Ex2)	Days 9&10 (Post)
Rumen Total VFA Concentration		Days 16&17 (Ex)	
Rumen VFA Proportions and individual acid concentrations pH		Days 16 (Ex)	
Rumen NH_3 concentration		Days 16&17 (Ex)	Days 4&5 (Post)
Milk fatty acid yields		Days 9&10 (Ex1) Days 19&20 (Ex2)	

rumen or post-ruminally. On the 7th, 8th and 18th days of the experimental period intravenous catheters were placed in the jugular veins of all cows. Blood samples were taken at 0, 4 and 8 h after the a.m. feeding on the 10th, 11th, 19th and 20th days. Rumen liquor samples for VFA and ammonia determination were taken on the 16th and 17th days. Collection of faeces and urine for all cows began on the 11th day of the period and continued for 10 days. After the a.m. milking and before feeding on the 20th day all cows were weighed.

Post-experimental. A nitrogen balance was carried out on all cows for the last 7 days of this period. Following catheterisation on the 8th day, blood samples were taken on the 9th and 10th days at 0, 4 and 8 h after the a.m. feeding. Rumen liquor samples for VFA and ammonia determination were obtained on days 4 and 5 at 0, 2, 4, 6, and 8 h after the a.m. feeding.

ADMINISTRATION OF TREATMENT

Administration of casein post-ruminally during the experimental period was as follows:-

a stomach tube was passed through the reticulo-omasal orifice for a distance of approximately 40 cm. Access to the orifice was via the rumen fistula. Once the tube was in position the casein solution was infused, the normal time for infusion being about 5 minutes.

The cows receiving casein to the rumen were given 1.5 l of warm water post-ruminally, infused as above. At the same time, the cows which received casein post-ruminally were given an infusion of 1.5 l of water into the rumen.

Approximately 6-8 weeks prior to commencement of the trial an attempt was made at establishing partial exteriorisation of the abomasum. If partial exteriorisation of the abomasum could be established, it was thought that access to the abomasum for subsequent infusion of protein slurries could be obtained.

The operation involved suturing a portion of the abomasum to the abdominal muscle layers, similar to the partial exteriorisations of the rumen (Reid and Titchen, 1959; Reid, 1963). However, after preparation of the animals with the exteriorisations, attempts to gain entry to the abomasum with a 3 inch needle were unsuccessful. Five months after the trial one of the cows, number 7, died. On post-mortem examination it was found that the sutures between the abomasum and the abdominal wall had broken and no adhesion of abomasum to abdominal wall had occurred. The lack of adhesion of the abomasum would explain the failure to gain access to the abomasum by needle puncture, as the abomasum would most probably have moved from the site of the puncture.

HOUSING AND FEEDING

During the experiment the animals were housed indoors in individual stalls except for some periods during the acclimatisation period when the cows were allowed out onto a sawdust loafing pad from 8.00 p.m. to 5.00 a.m. The aim of this was to allow the cows' legs time to become accustomed to the hard rubber surface in the stalls. Despite precautions taken, some swelling occurred in the leg joints of the cows, and this neccessitated the spreading of wood shavings in all the stalls.

During the acclimatisation period the cows were fed ad-lib, and their intake finally levelled out at approximately 13 kg/day. From this data it was decided to feed 6.5 kg of dried grass at 7.00 a.m. and 6.00 p.m. daily. The feed refusals were measured every morning, but they consisted almost entirely of fine dust-like material and as such could not be considered as true refusals.

When the dried grass was being weighed into 6.5 kg lots, sub-samples were taken and bulked from the 70 - 110 kg bales. Dry matter content was determined on a portion of this bulked sample by drying at 105^{o}C for 24 h. Another portion of the grass was ground to pass through a 1 mm sieve and subsequently used for nitrogen determination.

II : 2. MILK YIELD AND COMPOSITION

The milk yield of the cows was measured at each

milking on scales (W & T Avery Ltd., England) allowing measurement to the nearest g. The milk was mixed thoroughly but carefully, to avoid churning. A sample was taken for analysis of composition using a Mk I Grubb Parsons Infra Red Milk Analyser, calibrated to standard chemical methods (Munford, 1968). Milk samples which were not being analysed within three to five hours were stored at 4°C. The longest period of storage occurred after milking on Friday afternoon, when milk was stored until the following Monday morning. No difficulties were encountered in analysing these samples, and there was no apparent deterioration of the sample.

Separation of milk fat. A 25 ml aliquot of milk was taken and shaken thoroughly with 20 ml of absolute ethanol to precipitate the protein. The fat was then extracted by shaking with 2 x 20 ml additions of peroxide-free diethyl ether. When the phases had separated the upper etherial layers were decanted and bulked. The ether was evaporated under vacuum, and the residue (comprising fat, ethanol and water) transferred to a separating funnel with washes of petroleum ether (bp 40-60°C). This was mixed gently to avoid emulsion formation and the ether layer was separated and evaporated to near dryness. The fat was washed into polyethylene tubes with a small quantity of petroleum ether, and stored at 4°C until analysis.

Milk fat fatty acid composition. Total milk fats were analysed as methyl esters of the fatty acids prepared by the boron-trifluoride transesterification method of Metcalfe, Schmitz and Pelka (1966), as modified by van Wijngaarden (1967). The methyl esters in n-heptane were dried over anhydrous sodium sulphate before analysis on a Varian Aerograph series 1200 gas-liquid chromatograph.

Separation of the fatty acid methyl esters was carried out on an 8' x 1/8" stainless steel column packed with 12.5% diethylene-glycol succinate (Max. temperature 240°C Analab) on Chromosorb W (DCMS) 60/80 (Varian), using nitrogen as the carrier gas and an hydrogen flame ionisation detector.

Operational conditions.

Injection	210°C
Oven initial	60°C
Detector	210°C
Nitrogen	25 ml/min
Hydrogen	25 ml/min
Linear programme	4°/min
Maximum	175°C

Measurement. Peak areas were measured by a model 207 disc integrator (Disc Instruments Inc., U.S.A.). Composition was determined on a molar basis with no adjustment for the different acids, or for the small differences in molecular weights between the methyl esters and the free acids. The

above procedure was considered satisfactory for the purpose of the experiment.

Column preparation. The solid support was coated by the funnel coating method of McNair and Bonelli (1967). The columns were packed using a vibro-tool (Burgers Products Co. Ltd., England) to aid consolidation, and under a gas pressure of approximately 5 lbs/in^2. Fibre glass yarn was used for plugging the columns at either end.

II : 3. NITROGEN BALANCE

Milk. A bulked p.m. - a.m. sample was used for the nitrogen determination.

Faeces and urine. Faeces and urine were collected for 10 days in the preliminary and experimental periods, and for 7 days in the post-experimental period. Excreta was collected using a modification of the faeces and urine shute of Hughes (1963) as designed by Hughes (pers. comm.). Urine was collected into buckets containing 200 ml of 50% (v/v) hydrochloric acid, giving conditions sufficient to lower the pH of the urine to approximately 3.0. Each morning the urine was weighed, the specific gravity measured, and a 300 ml aliquot taken for nitrogen determination. The sample was stored at -5.0^{o}C until analysis, when a bulked composite was taken. The faeces were weighed, thoroughly mixed, and two samples taken, one for dry matter determination, and the other for later analysis was frozen and stored at -5.0^{o}C.

Nitrogen was determined on a bulked composite sample of wet faeces for each period.

Nitrogen determination. Nitrogen was determined induplicate according to the A.O.A.C. (1960) method. Five ml urine, 10 ml milk, 5-6 g wet faeces and approximately 1 g of feed was used to determine the nitrogen in each substance.

II : 4. BLOOD METABOLITES

Sampling. (a) Blood was sampled from an indwelling jugular catheter immediately prior to the morning feeding, and at 4 and 8 hours after feeding.

(b) Catheterisation. The jugular vein was punctured with a 14 gauge needle, and a 7-9" piece of polythene tubing introduced into the vein toward the head of the animal. The needle was withdrawn leaving the polythene tubing in the vein. A small piece of rubber tubing placed on the end of the catheter enabled clamping of the catheter without damage to the polythene portion. The catheter was flushed with heparinised saline (100 units/ml saline) and plugged with a nylon plug. The catheter was then attached to the skin of the animal by a single suture.

Blood preparation. Fifty ml of blood was withdrawn from each cow at sampling, into a syringe containing 500 units of heparin. The blood was immediately placed in a polypropylene centrifuge tube in an ice-bath. The time which elapsed between sampling and centrifugation was variable, but rarely

exceeded 45 minutes. Plasma was obtained by centrifuging the blood for 20 minutes at 3150 x g. Aliquots containing approximately 7 ml plasma were put into polypropylene centrifuge tubes and stored at -5.0°C. Storing the plasma in small aliquots avoided the repeated freezing and thawing which would have occurred if the plasma samples had been stored as a single sample, with small amounts being withdrawn for the various analyses. A sample of plasma was also deproteinised with sulphosalicyclic acid at this time. Two deproteinisation techniques were tried:

(i) 1 ml plasma : 30 mg solid sulphosalicyclic acid

(ii) 1 ml plasma : 0.5 ml of ice-cold 20% (w/v) solution of sulphosalicyclic acid.

The second method was preferred for the majority of the samples as it resulted in a more distinct precipitate. The deproteinised plasma was stored in polypropylene tubes at -5.0°C until analysed.

Blood urea nitrogen. Plasma urea nitrogen was determined using a Technicon Autoanalyser. (Technicon method file N-IC).

Plasma glucose. Plasma glucose was determined by the glucose oxidase-o-dianisidine dihydrochloride method of Saifer and Robin (1965). Difficulty was encountered in dissolving the o-dianisidine dihydrochloride in the buffer, however this

was easily overcome by dissolving the o-dianisidine dihydrochloride in a small quantity (5-10 ml) of water before adding it to the buffer.

Two different glucose exidase preparations were used during the analysis:

(i) Fermcozyme 653 AM

(ii) Koch-light

Five mg of peroxidase (Sigma) was added to 10 ml of the glucose exidase solution.

Plasma amino nitrogen. Plasma amino nitrogen was determined on a Technicon Autoanalyser using the method of Palmer and Peters (1969). Deproteinised plasma was used and therefore the dialyser was replaced with a single mixing coil in a 37^{o}C water bath. The standard amino acid solution was according to Goodwin (1968), and contained L-glutamic acid and L-glycine.

II : 5. RUMEN PARAMETERS

Rumen liquor sampling. Rumen digesta samples from the centre of the reticulo-rumen have been shown to be representative of the whole rumen contents (Bryant, 1964; Davey, 1965), therefore digesta was sampled from this region immediately prior to feeding and then at 2 hourly intervals until 8 h after feeding.

Determination of VFA concentration. The volatile fatty acid concentration in rumen liquor was determined by steam

distillation in a Markham still (Bryant, 1964). Following titration the distillates were evaporated to dryness and stored at -5.0°C for later analysis of individual VFA's by gas liquid chromatography.

Determination of individual VFA proportions. Each vial containing VFA salts had 0.2 ml of deionised water added. A 1.5 µl sample was injected into the chromatograph. Separation of the VFA's was carried out on a Varian Aerograph, series 1200, gas-liquid chromatograph, using an 8' x 1/8" stainless steel column packed with 15% FFAP + 4% phosphoric acid on Chromosorb W (DCMS) 60/80 (Varian). The carrier gas was nitrogen, saturated with formic acid vapour after passing through a formic acid bubbler (Ackman and Burgher, 1963). Detection was by hydrogen flame ionisation.

Working conditions.

Injection port	190°C
Oven temperature	145°C
Detector	190°C
Gas flow	
nitrogen	25 ml/min
hydrogen	25 ml/min
air	360 ml/min

Some difficulty was encountered at first with "ghosting" when using a column packed with 20% FFAP. This was largely overcome by including 4% phosphoric acid (Kabbot and Ettre, 1963; Metcalfe, 1963) with the FFAP, using glass inserts in

the injection port, and inserting a formic acid bubbler in the carrier gas line. Since this work was carried out the source of the "ghosting" has been traced to sample decomposition in the injection port and absorption onto the glass wool plugs (Geddes and Gilmour, 1970; Dressman, 1970). On column injection, regular changing of the glass inserts or use of teflon tape as a plug for the columns results in the absence of "ghosting". When the carrier gas is saturated with formic acid, salts of the VFA's can be injected into the chromatograph; rather than acidification of the salts prior to injection which might result in some volatile losses of the VFA's.

Coating the support. The funnel coating method of Bonelli and McNair (1967) was used to coat the support. The phosphoric acid was dissolved in 100 ml of alcohol and applied to 10 g of support, which was then dried for 3 hours at 105^{o}C before coating with FFAP.

Separation of VFA's. The separation of isobutyric from propionic acid was not sufficiently clear for individual measurement of the peaks. Thus the propionic acid peak, as measured, contained isobutyric acid.

After the trial samples had been analysed, the liquid support of Dehority, Scott and Kowaluk (1967) was tried. This consisted of 3% phosphoric acid + 5% stearic acid, and gave excellent separation of all acids. Unfortunately, due to the shortage of time, the trial samples were not reanalysed

on this column.

Quantitation of the VFA peaks. The area under each peak was measured by a Model 207 disc integrator (Disc Instruments Inc., U.S.A.). Correction factors for each peak, relative to butyric acid, were calculated from the following formula:

$$\text{Correction factor. VFA X} = \frac{\text{area of butyric acid peak x molar \% X}}{\text{area of VFA X peak x molar \% butyric}}$$

The correction factors were calculated from the tracing of a standard solution of the VFA's of known molar proportions.

II : 6. STATISTICAL METHODS

Analysis of covariance models were employed as the basis for statistical analysis of the data wherever possible. When such models were not applicable, because there was no consistent relationship of treatment or post-treatment observations to corresponding observations in the preliminary period, analysis of variance models formed the basis of statistical treatments of the data. Models A, B, D, E, F, G were according to Snedecore and Cochran 1967, and model C, R.E. Munford (personal communication).

Blood metabolites. Statistical analyses were carried out on mean values for samples taken on two consecutive days in four "periods", as defined in Table I, Section II.

The data for each period were analysed according to one of the following models:

$$X_{ijk} = M + T_i + S_j + (TS)_{ij} + E_{ijk} \quad \ldots\ldots \text{ Model A}$$

Model A, an analysis of variance model, was the basis of analyses of all four periods.

$Y_{ijk} = M + T_i + S_j + (TS)_{ij} + BX_{ijk} + E_{ijk}$ ..... Model B

Model B, an analysis of covariance model, was the basis of analyses of three periods (Ex I, Ex 2, Post) with data from the first period (Pre) employed as the covariant (the term BX_{ijk}).

In the models:

M represents the overall mean,

T_i the effect of the i^{th} treatment (i = I, 2),

S_j the effect of the j^{th} time (j = 1, 2, 3) of sampling after feeding,

$(TS)_{ij}$ the effect of the interaction of the i^{th} treatment and the j^{th} time of sampling,

E_{ijk} the random experimental error (k = 1, 2, 3 cows within treatments and sampling times),

B the regression coefficient of level of metabolite in the period being examined on the level of the metabolite in the preliminary period (Pre).

In both models "Treatments" and "Time of Sampling" were assumed to be fixed effects.

Milk Yield and Composition. Statistical analyses for yields of milk and milk components and concentrations of milk components were carried out on mean values for ten or five consecutive days. Periods were as defined in Table I, Section II.

The data for the six subdivisions of the treatment and post-treatment periods were analysed according to the following model:

$Y_{ijk} = M + T_i + P_j + (TP)_{ij} + BX_{i.k} + E_{ijk}$ ..... Model C

where M, T_i, and E_{ijk} are defined as in model B,

P_j represents the effect of the j^{th} "period" (j = 1 to 6; Ex 1 to 4; and Post 1 and 2);

$(TP)_{ij}$ the effect of the interaction of the i^{th} treatment and the j^{th} "period".

B represents the common regression coefficient for the values of all j "periods" on the value for the pre-treatment period, $X_{i.k}$.

This form of analysis of covariance model requires that the regression coefficients for individual i x j subclasses of treatments and period should not differ from the common regression over all i x j subclasses. The period means are not adjusted in the analysis based on this model. Adjustment is restricted to the treatment means. In the case of the treatment by period interaction means, adjustment is restricted to the comparison between treatments.

The assumption was made that both "treatments" and "periods" were fixed effects.

Nitrogen Balance. The data were analysed according to the following one-way analysis of variance model in each of the preliminary, treatment and post-treatment periods:

$X_{ij} = M + T_i + E_{ik}$ ..... Model D

where M and T_i are defined as in model A,

and E_{ik} represents the random experimental error (k = 1, 2, 3 cows within treatments).

Rumen Metabolites. The data were analysed according to model E or model F. The choice of model for a given variable was determined by the availability of data from either two consecutive days, or a single day.

Model E specifies a "split plot" analysis of variance with twin pairs corresponding to the main plots.

$X_{ijkl} = M + T_i + B_k + (TB)_{ik} + S_j + (TS)_{ij} + E_{ijkl}$ ..... Model E

where M, T_i, S_j and $(TS)_{ij}$ are defined as in model A.

B_k represents the effect of the k^{th} twin pair (k = 1, 2, 3) "Main Plot"

$(TB)_{ik}$ represents the effect of the interaction of the i^{th} treatment and the k^{th} twin pair - "Main Plot Error".

E_{ijkl} the random experimental error - "Sub-plot Error" (l = 1, 2 days).

Model F specifies a randomised block analysis of variance, with twin pairs corresponding to blocks.

$X_{ijk} = M + T_i + B_k + (TS)_{ij} + E_{ijk}$ ..... Model F

where E_{ijk} represents the random experimental error and other parameters are defined as in model E. In both models "Treatments" and "Time of Sampling" were assumed to be fixed

effects. Cows and Days were assumed to be random effects. The Periods are as defined in Table I, Section II.

Milk Fatty Acid Yields. Statistical analyses were carried out on mean values for samples taken on two consecutive days in 2 periods as defined in Table I, Section II. Data were analysed according to the following model:

$$X_{ijk} = M + T_i + P_j + (TP)_{ij} + E_{ijk} \quad \ldots\ldots \text{ Model G}$$

where P_j represents the effect of the j^{th} period ($j = 1$ or 2).

$(TP)_{ij}$ the effect of the interaction of the i^{th} treatment and the j^{th} period.

Other parameters are defined as in Model A. Treatments and Periods were assumed to be fixed effects.

SECTION III

RESULTS

(a) MILK YIELD

Statistical analysis was carried out according to model C, section II. Mean squares for analysis of variance of Y after fitting the regression on X for the yields of milk, fat corrected milk (TCM), fat., protein and lactose are presented in Appendix A-1 with the treatment and period means. The treatment-period means are presented in figs. 1, 2, 3, 4 & 5, respectively.

Treatment R resulted in significantly greater yields of milk, ($P<0.01$), FCM ($P<0.05$), protein ($P<0.01$) and lactose ($P<0.01$) than treatment PR. However, the yield of milk fat did not differ significantly ($P<0.10$) between treatments. This observation is somewhat surprising when it is considered that milk yields and fat content ($P<0.10$) were significantly different between treatments. It would appear that the lack of a significant difference in fat yield is largely a reflection of the relatively large unaccountable variation in fat yield. Covariance analysis resulted in a considerable reduction in the error variance for yields of milk, FCM, protein and lactose (83%, 45%, 73% and 81% respectively), but for fat yield the reduction in error variance was only 16%.

A significant difference in the yields of all components occurred between periods. The period effect appeared to be largely due to a diminished yield in the 2nd 5-day period of the post-experimental period for the

yield of milk, FCM, protein and lactose. For the above mentioned yields, no significant differences occurred between any other periods ($P<0.05$).

The milk fat yields in all periods, except post-experimental 2, did not differ from one another ($P<0.05$); the yields in experimental periods 2 and 3 only were significantly ($P<0.05$) greater than the yields in post-experimental 2. The yields in the other periods did not differ significantly from the yields in post-experimental 2 ($P<0.05$).

(b) MILK COMPOSITION

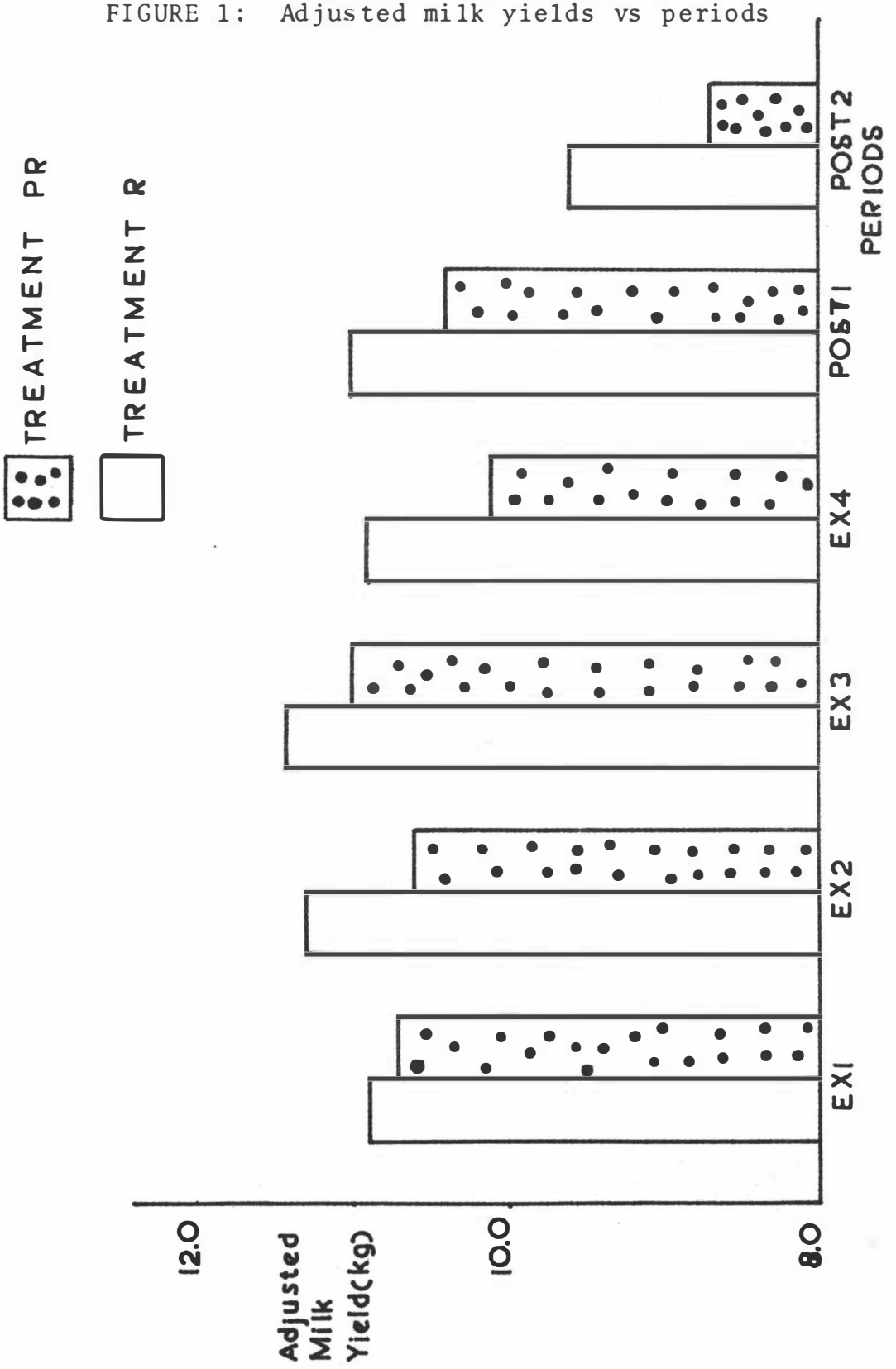
Analysis of milk composition data were according to model C, section II. Mean squares for analysis of variance, treatment means and period means are presented in Appendix Table A-2.

The treatment-period means are presented diagrammatically in figs. 6, 7 & 8.

The only significant difference between treatments was with fat percent ($P<0.10$) in which treatment R had a higher fat percentage than treatment PR. None of the other differences between treatments were significant ($P>0.20$).

Significant differences between periods were observed with protein percent and protein percent on a fat free serum basis ($P<0.05$, and $P<0.01$ respectively). In both cases the maximum protein percent occurred in the 4th

FIGURE 1: Adjusted milk yields vs periods



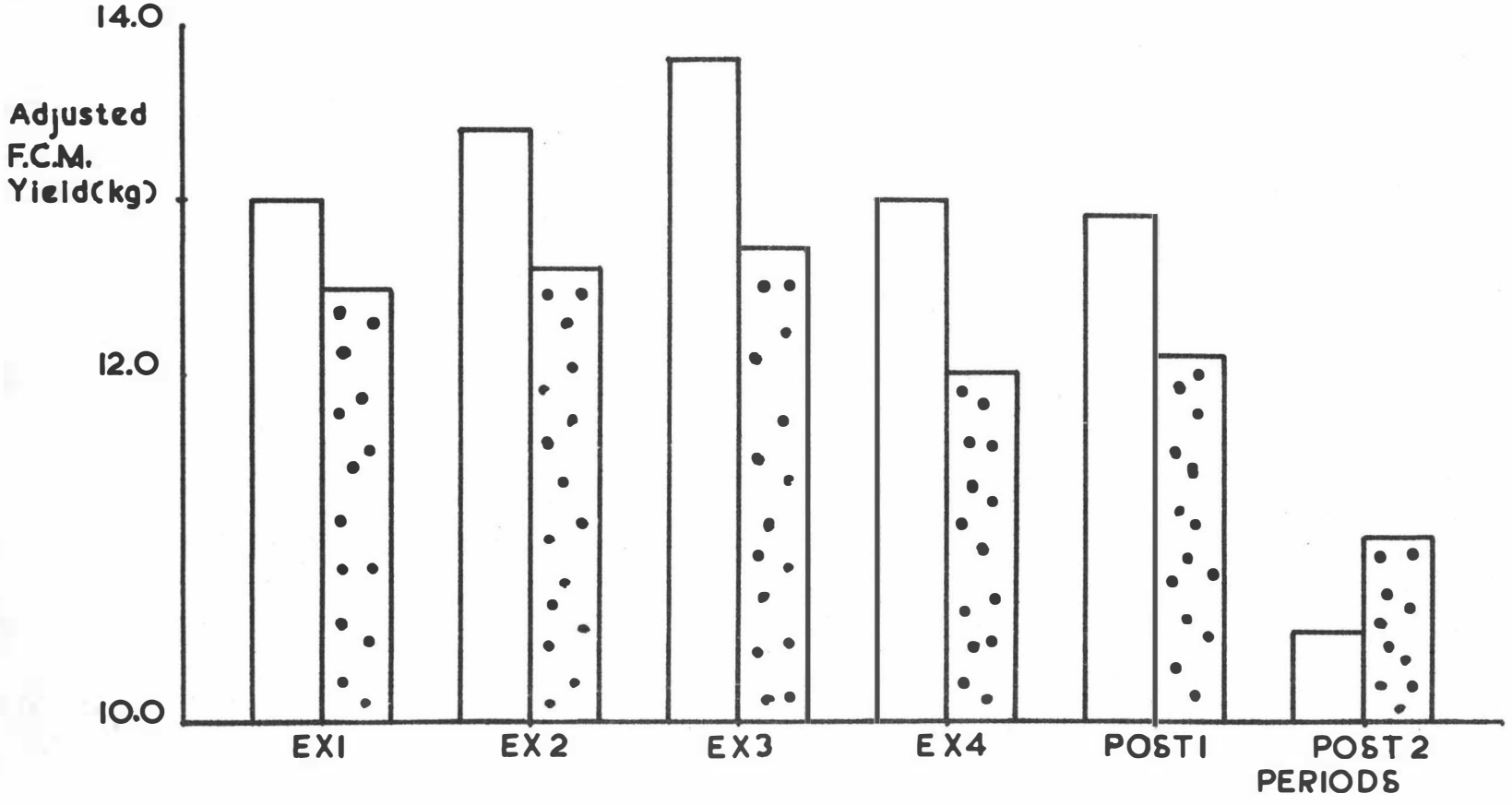


FIGURE 2: Adjusted FCM yield vs periods
Symbols as for Figure 1.

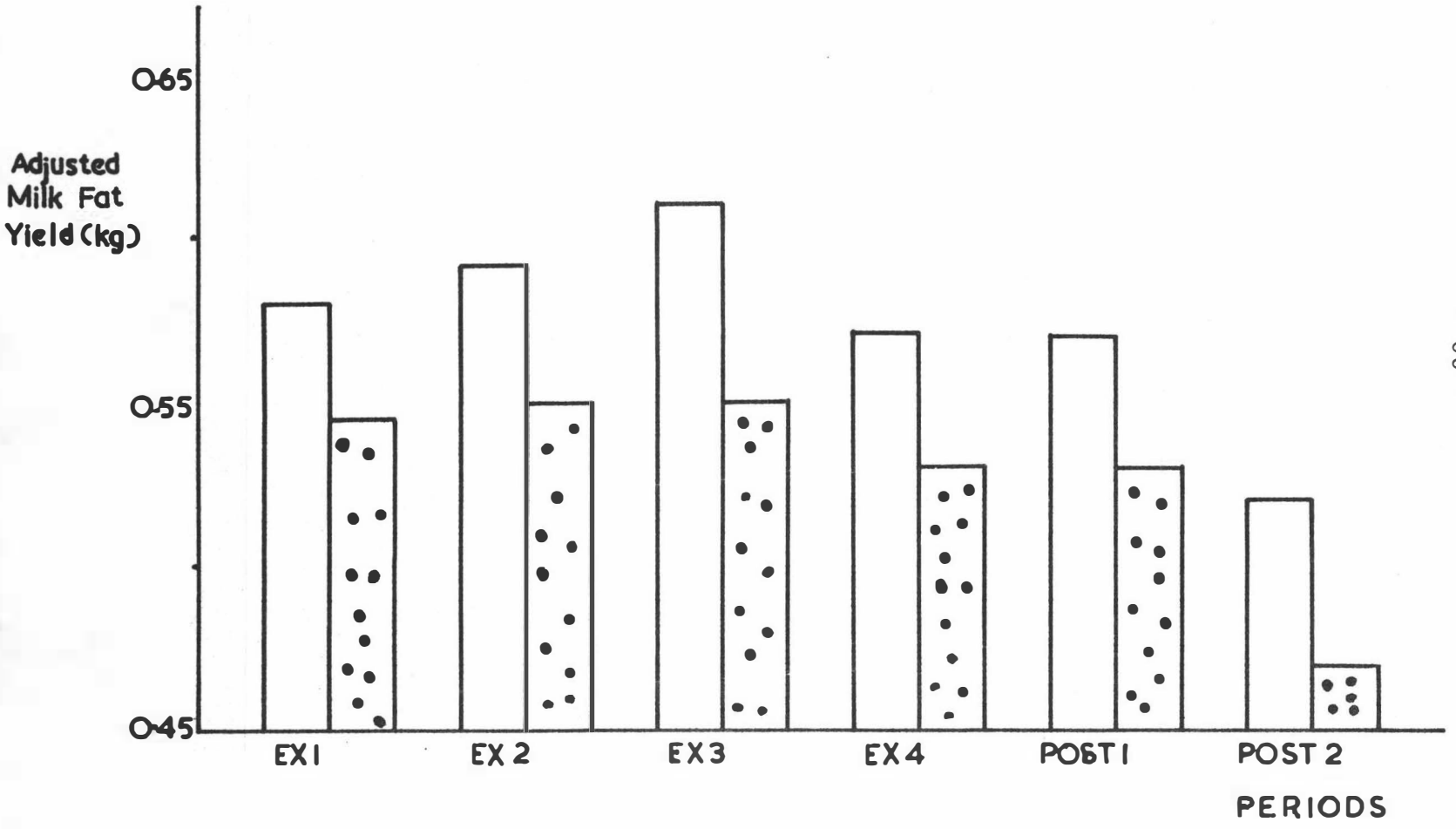


FIGURE 3: Adjusted Milk Fat Yield vs Periods
Symbols as for Figure 1.

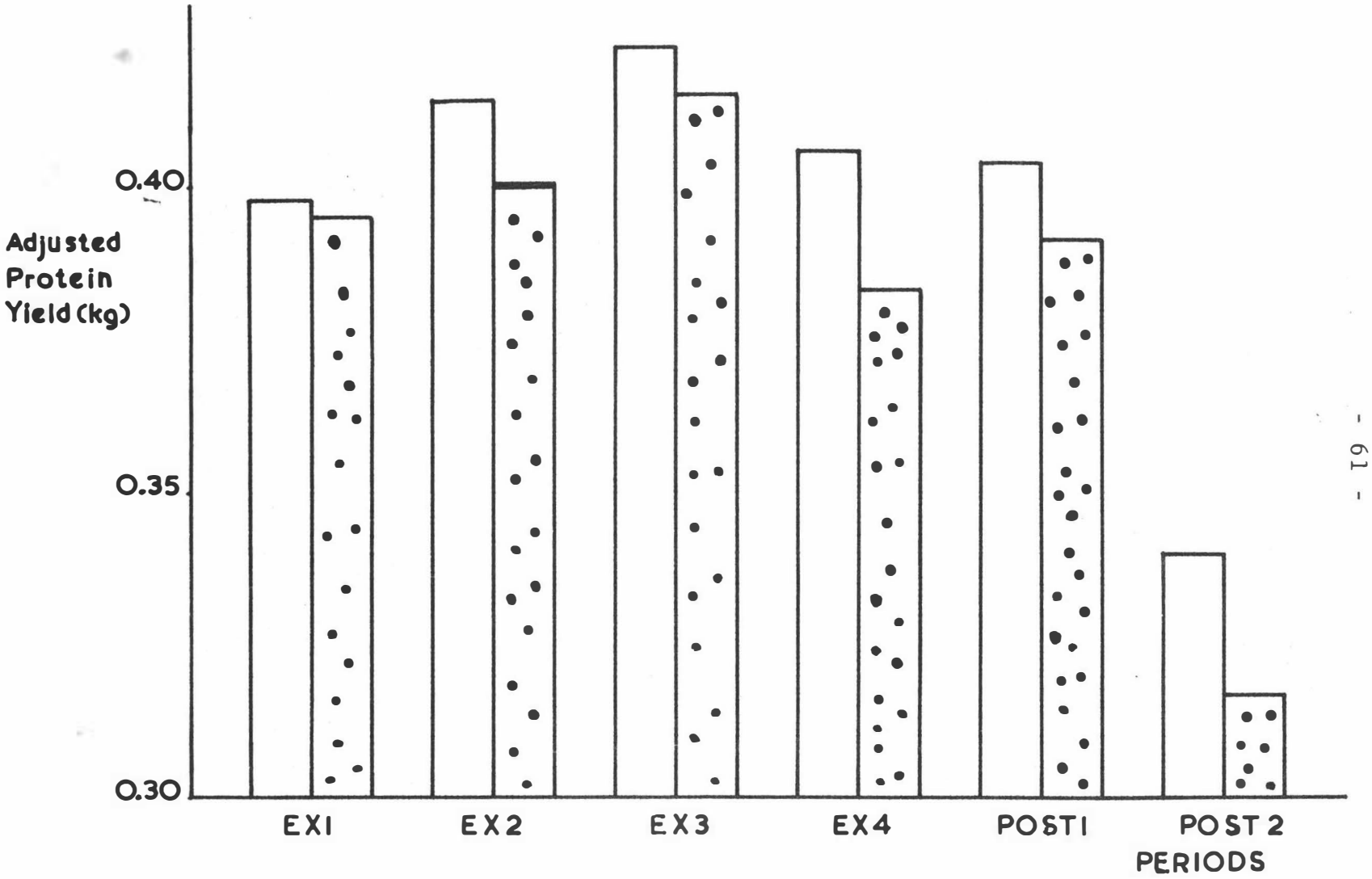


FIGURE 4: Adjusted protein yield vs Periods
Symbols as for Figure 1.

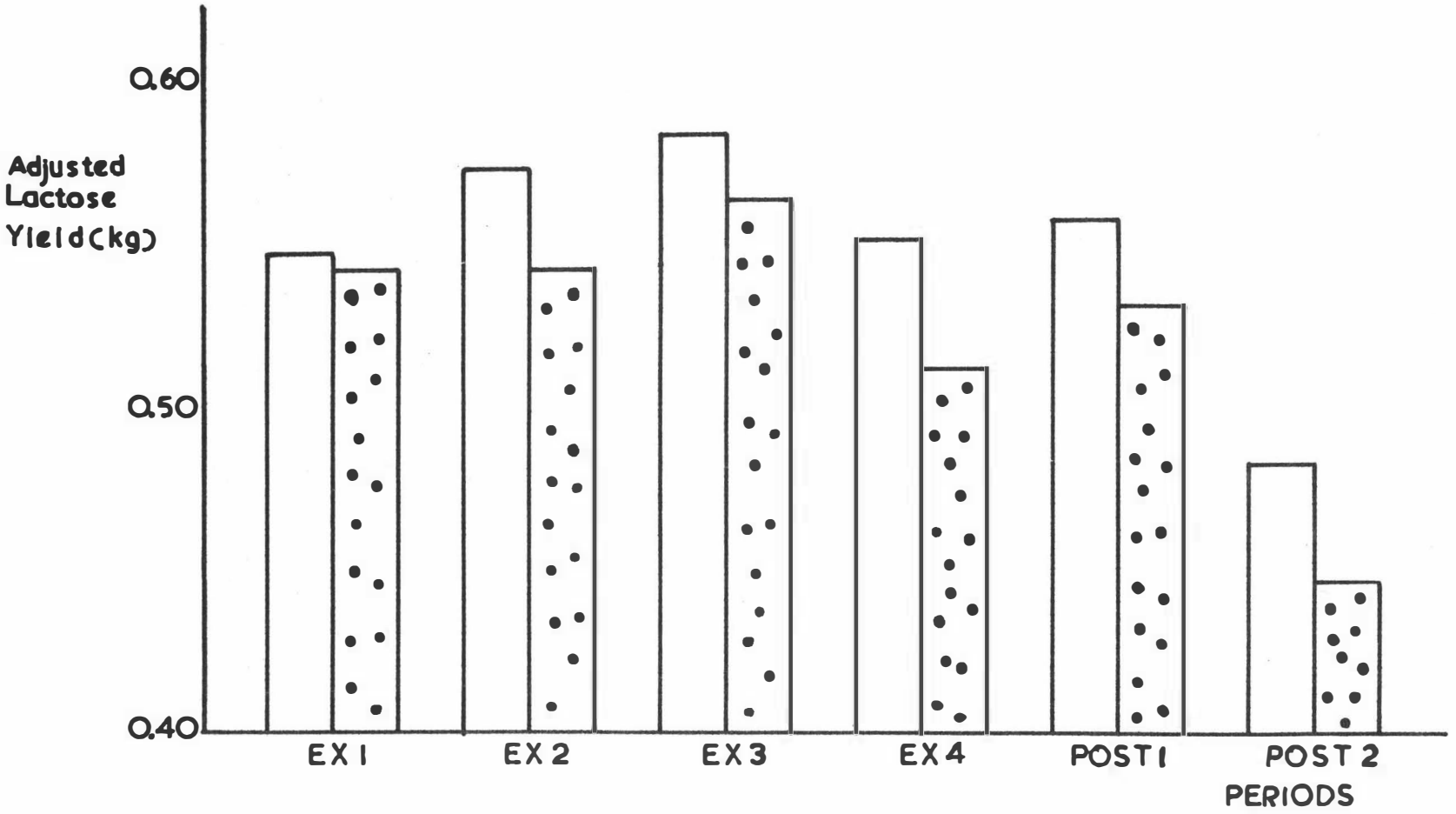


FIGURE 5: Adjusted lactose yields vs periods
Symbols as for Figure 1.

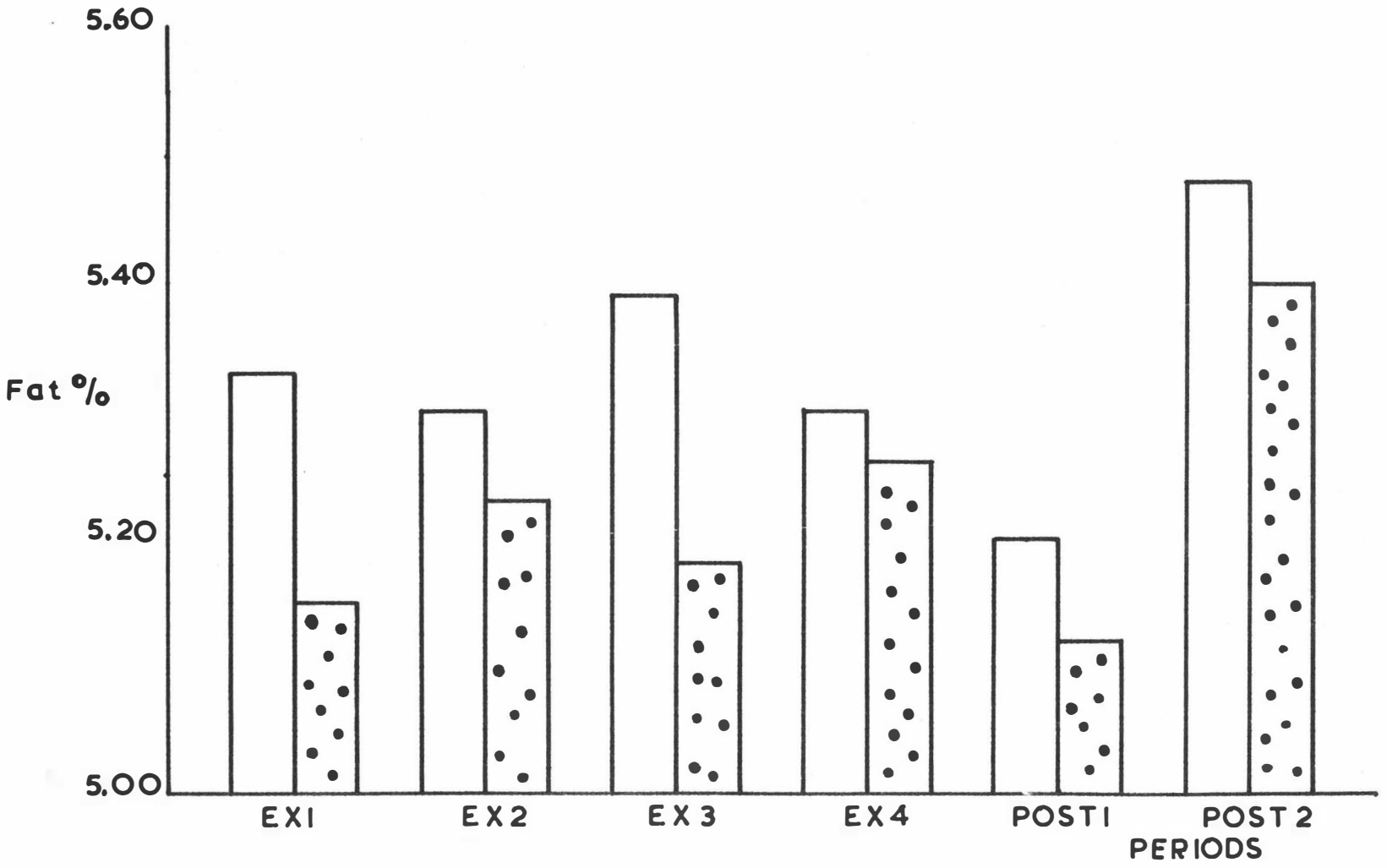


FIGURE 6: Adjusted fat percent vs periods
Symbols as for Figure 1.

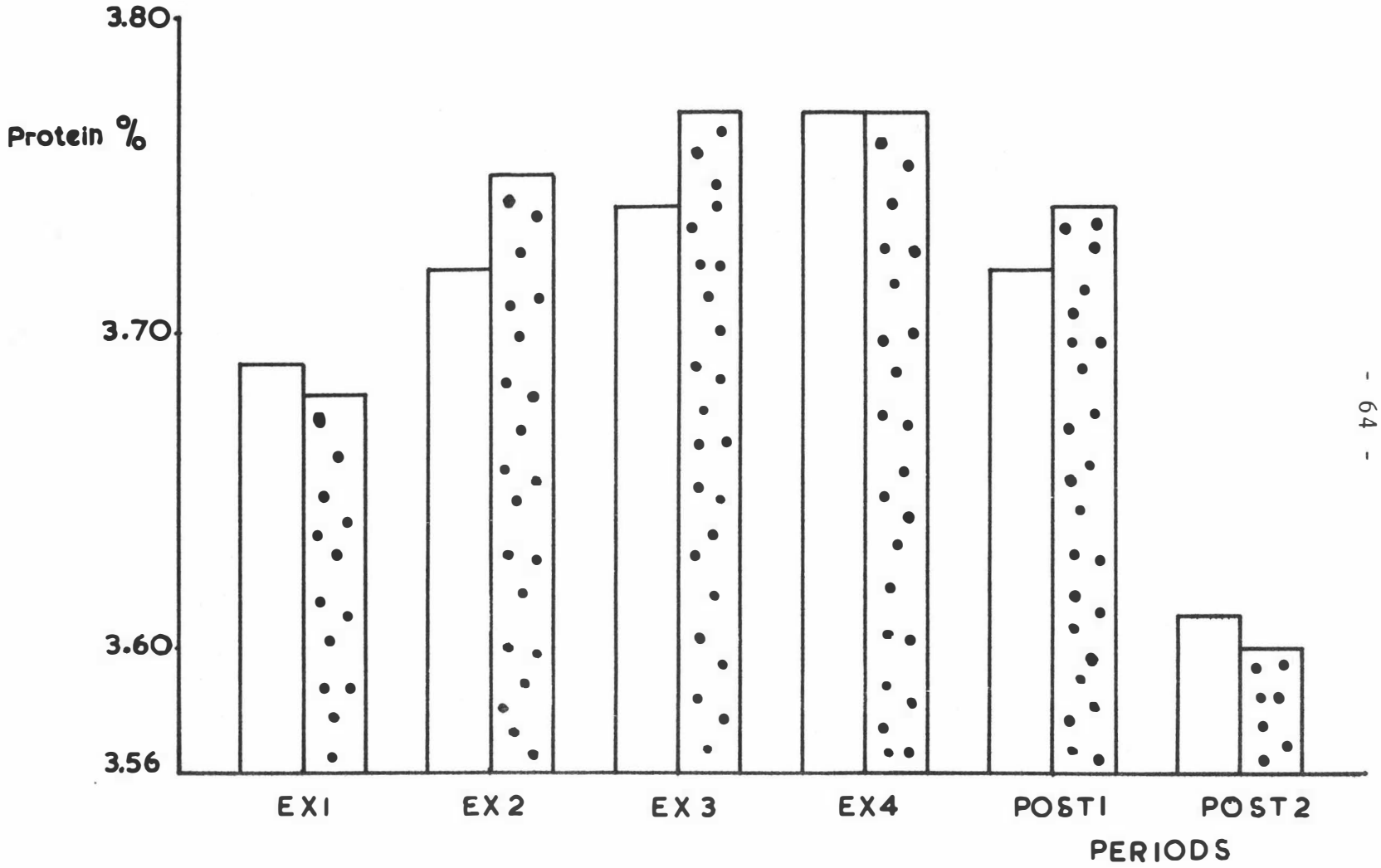


FIGURE 7: Adjusted protein percent vs periods
Symbols as for Figure 1.

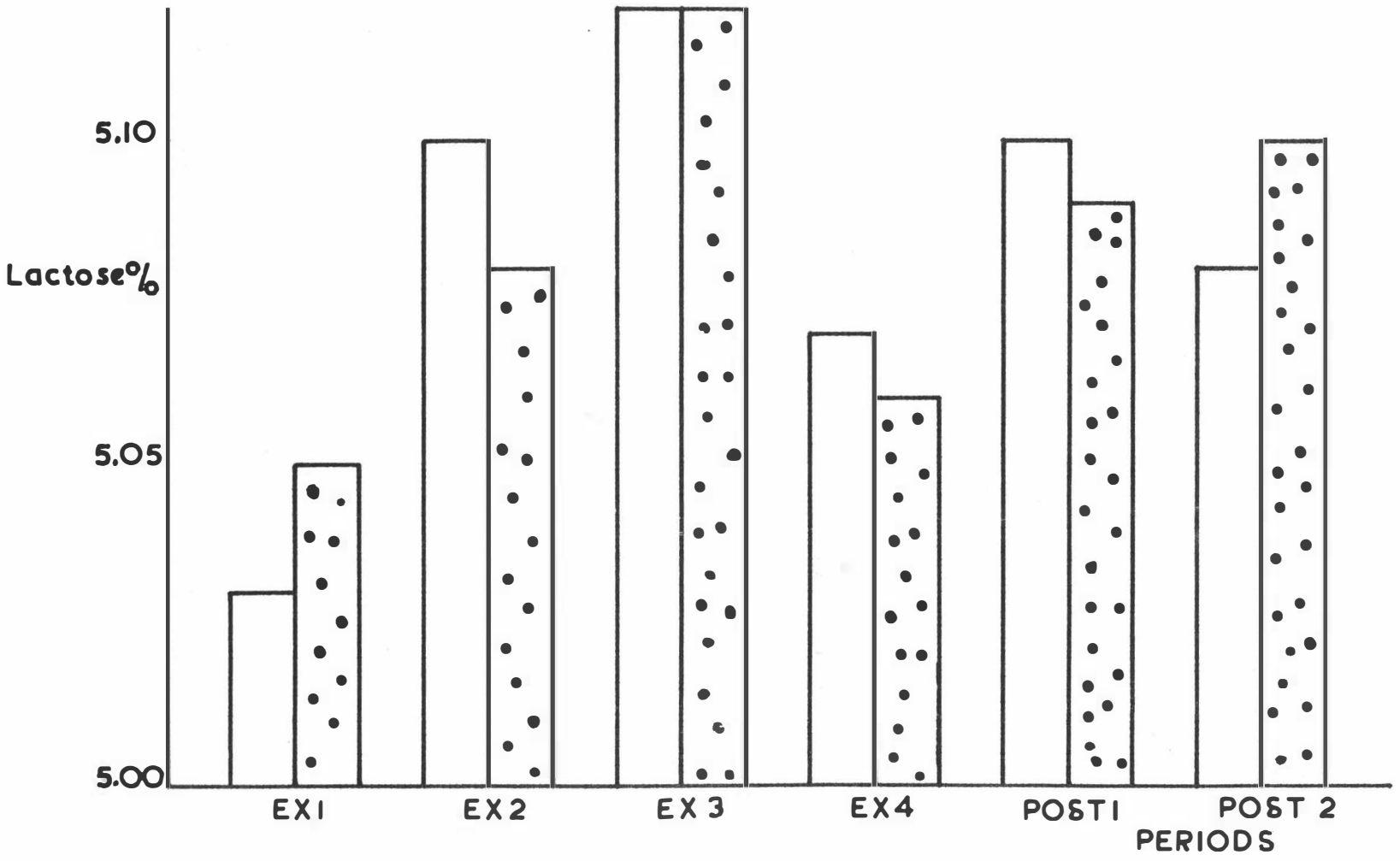


FIGURE 8: Adjusted lactose percent vs periods
Symbols as for Figure 1.

experimental period.

(c) NITROGEN BALANCE

The analysis of variance (model D, Section II) and treatment means for the nitrogen balance, faecal N, and urinary nitrogen excretion are present in Table 2. Crude data for the nitrogen balance are presented in Appendix Table A-13. The results from the one-way analysis of variance show that significant differences in nitrogen retention between treatments occurred only during the experimental period ($P<0.10$). The nitrogen retention of the cows on treatment PR was greater than that of the cows on treatment R. Similarly, the only period in which significant differences in faecal N excretion occurred ($P<0.05$) between treatments was the experimental period. During the experimental period the faecal nitrogen excretion of the cows receiving treatment R was greater than for those on treatment PR ($P<0.05$). The differences in urinary nitrogen excretion for each period were not significant; $P<0.50$, $P<0.25$, $P<0.20$, for periods I, II and III respectively.

An analysis of variance incorporating both treatment and periods as main effects revealed that differences in nitrogen retention between periods reached the 5% level of significance. The retention for period II was greater ($P<0.05$) than nitrogen retention in period III, but was not

significantly different from nitrogen retention in period I ($P>0.05$). The retention in period I was not significantly different ($P>0.05$) from the retention in period III.

The difference in faecal nitrogen excretion between periods, was significant ($P<0.10$), with the excretion during period II being significantly greater ($P<0.05$) than excretion during period I, but not significantly different ($P>0.05$) from excretion during period III.

There was a significant ($P<0.01$) treatment x period interaction in the analysis of faecal nitrogen excretion. Examination of the treatment x period means showed that the level of excretion in the treatment PR cows was greater ($P<0.05$) in period I than in periods II or III, however this level was not significantly different ($P>0.05$) from the level of excretion of treatment R cows during periods II and III.

(d) RUMEN CHARACTERISTICS

(i) Volatile Fatty Acids

Mean squares for the analysis of variance (model F, section II) of individual VFA concentrations and proportions are presented in Appendix Tables A-3 and A-4 respectively along with treatment means. Treatment-periods means are shown graphically in figures 9 & 10. The concentration of acetate in the rumen did not differ between treatments ($P>0.25$). However, the molar percentage

TABLE 2

ANALYSIS OF VARIANCE - NITROGEN BALANCE

PERIOD		DF	N retention	Faeces N	Urine N
Preliminary:	between treatments	1	265.00	657.306	103.34
	within treatments	3	454.56	148.502	3903.33
Experimental:	between treatments	1	732.62	517.08*	509.682
	within treatments	4	122.50	59.148	383.748
Post-experimental:	between treatments	1	105.00	21.66	126.960
	within treatments	4	723.280	44.990	60.88

* $P<0.05$

TABLE 2 (Continued)

Treatment means in gN/day

PERIOD		N retention	Faeces N	Urine N
Preliminary	Rumen	82.3	96.7	211.9
	Post-rumen	68.9	117.7	203.6
S.E. of mean		12.3	7.0	36.1
Experimental	Rumen	78.5	130.2*	258.2
	Post-rumen	100.6	111.5	239.7
S.E. of mean		6.39	4.44	11.3
Post-experimental:	Rumen	54.6	114.4	191.4
	Post-rumen	46.2	110.6	182.2
S.E. of mean		15.53	3.9	4.5

* $P<0.05$

of acetate associated with treatment PR was significantly greater ($P<0.05$) than the level in treatment R. Propionic acid concentration differed between treatments, ($P<0.05$) with treatment R greater than treatment PR; but the molar percentage of propionate did not differ significantly between treatments ($0.10<P>0.05$).

The treatment x time interaction in the analysis of propionate concentration was significant ($P<0.05$), with the concentration at 2, 4, and 6 h post-feeding being greater with treatment R cows than with treatment PR cows. Neither butyric acid concentration nor molar percentage differed significantly between treatments ($P>0.50$). Both the concentration and molar percentage of isovaleric and valeric acid differed between treatments ($P<0.001$), with the level of each acid being greater in the cows receiving treatment R.

With the exception of the molar proportion of propionic acid, the level of all other rumen parameters differed significantly ($P<0.05$) depending on the time of sampling relative to feeding.

(ii) Total VFA concentration (Table A-5, Fig. 11)

Data were analysed according to model E, Section II. The difference between treatment means was not statistically significant ($P>0.10$). The variation associated with time of

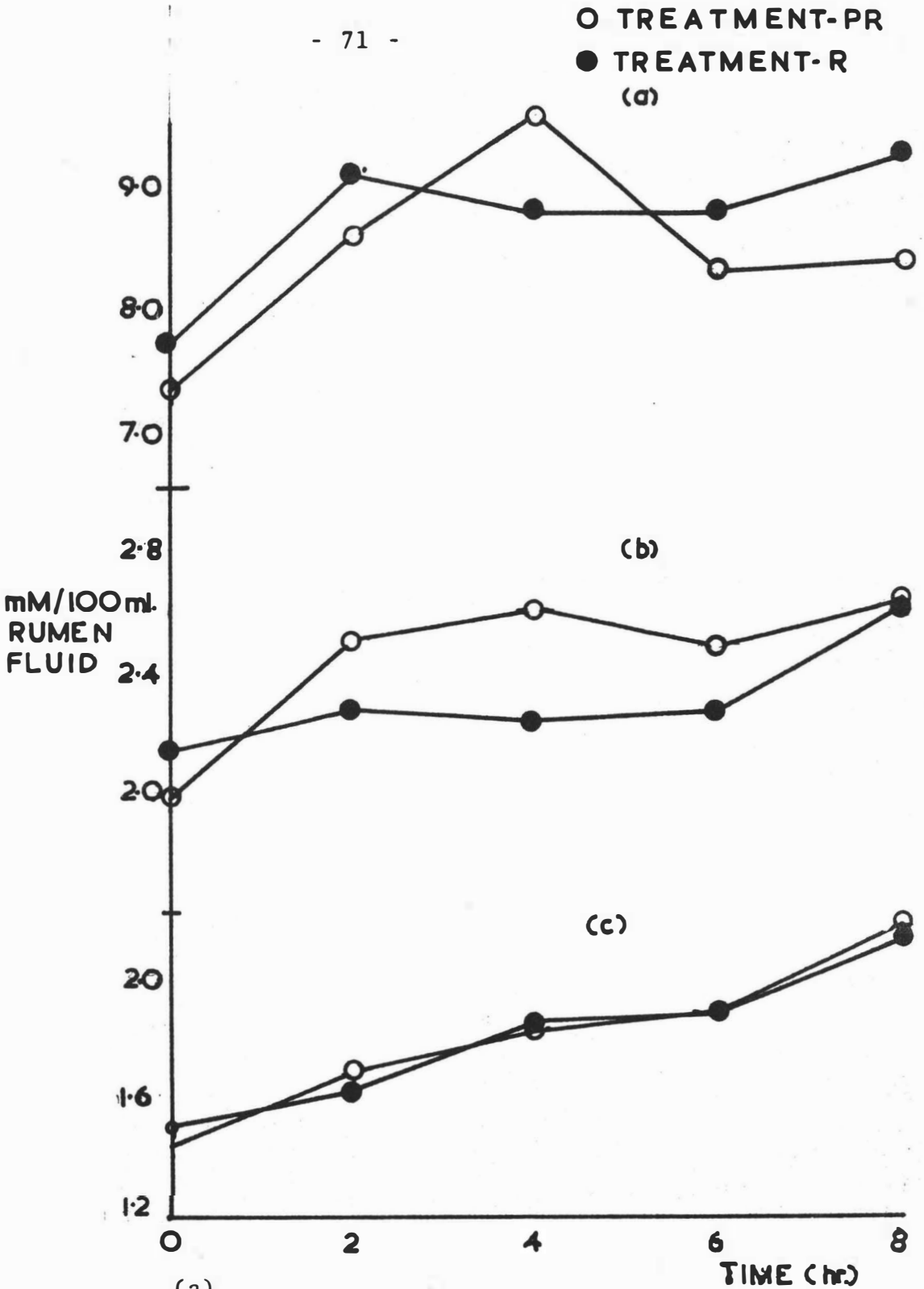


FIGURE 9: (a) Acetic acid concentration vs time after feeding
(b) Propionic acid concentration vs time after feeding
(c) Butyric acid concentration vs time after feeding

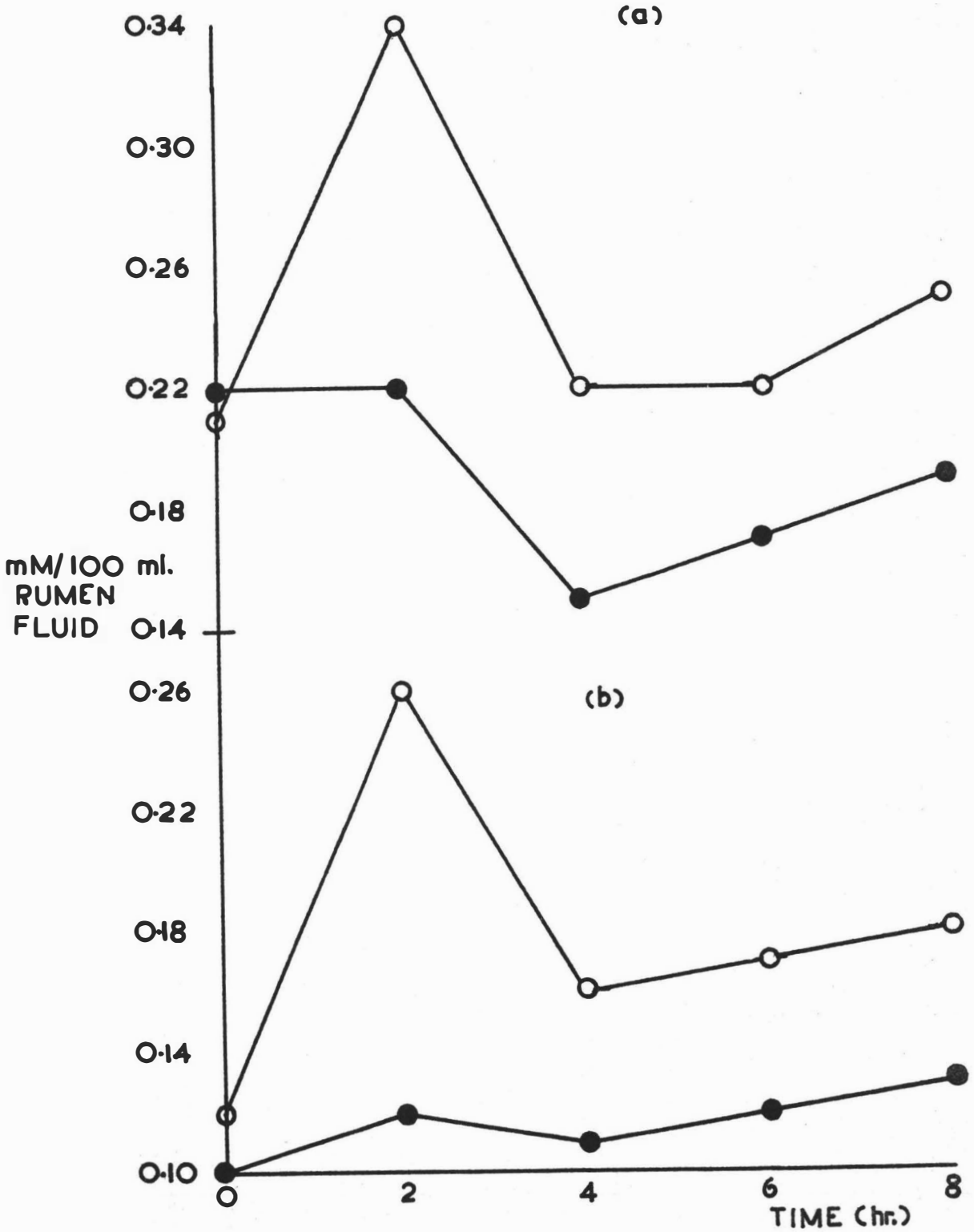


FIGURE 10: (a) Isovaleric acid concentration vs time after feeding
(b) Valeric acid concentration vs time after feeding

Symbols as for Figure 9.

sampling after feeding was statistically significant ($P<0.001$), with the VFA concentration greatest at 2 h and 8 h post-feeding.

(iii) Rumen Ammonia Concentration

Data were analysed according to model E, Section II. The mean squares for the analysis of variance and means for treatment, time, and treatment x time interaction are presented in Appendix Table A-5. The treatment x time means are presented in Fig. 12. The difference between treatments was statistically significant ($P<0.001$); the level in treatment R being greater than in treatment PR.

The maximum value for rumen ammonia concentration occurred 2 h post-feeding with both treatments, however the concentration of ammonia within treatment R was significantly greater than within treatment PR at both 2 h and 4 h post-feeding. ($P<0.05$).

Analysis of variance of ammonia concentration in the post-experimental period showed that there were no differences between treatments ($P>0.20$).

(iv) pH

(Day 16 of experimental period only). Mean squares for analysis of variance (Model F, Section II) and means are presented in Appendix Table A-6. The treatment-time means are shown in Fig. 13. The between treatment difference was not significant ($P>0.20$). There was a significant difference between sampling times ($P<0.05$), with the highest pH occurring 8 h post-feeding. The treatment x

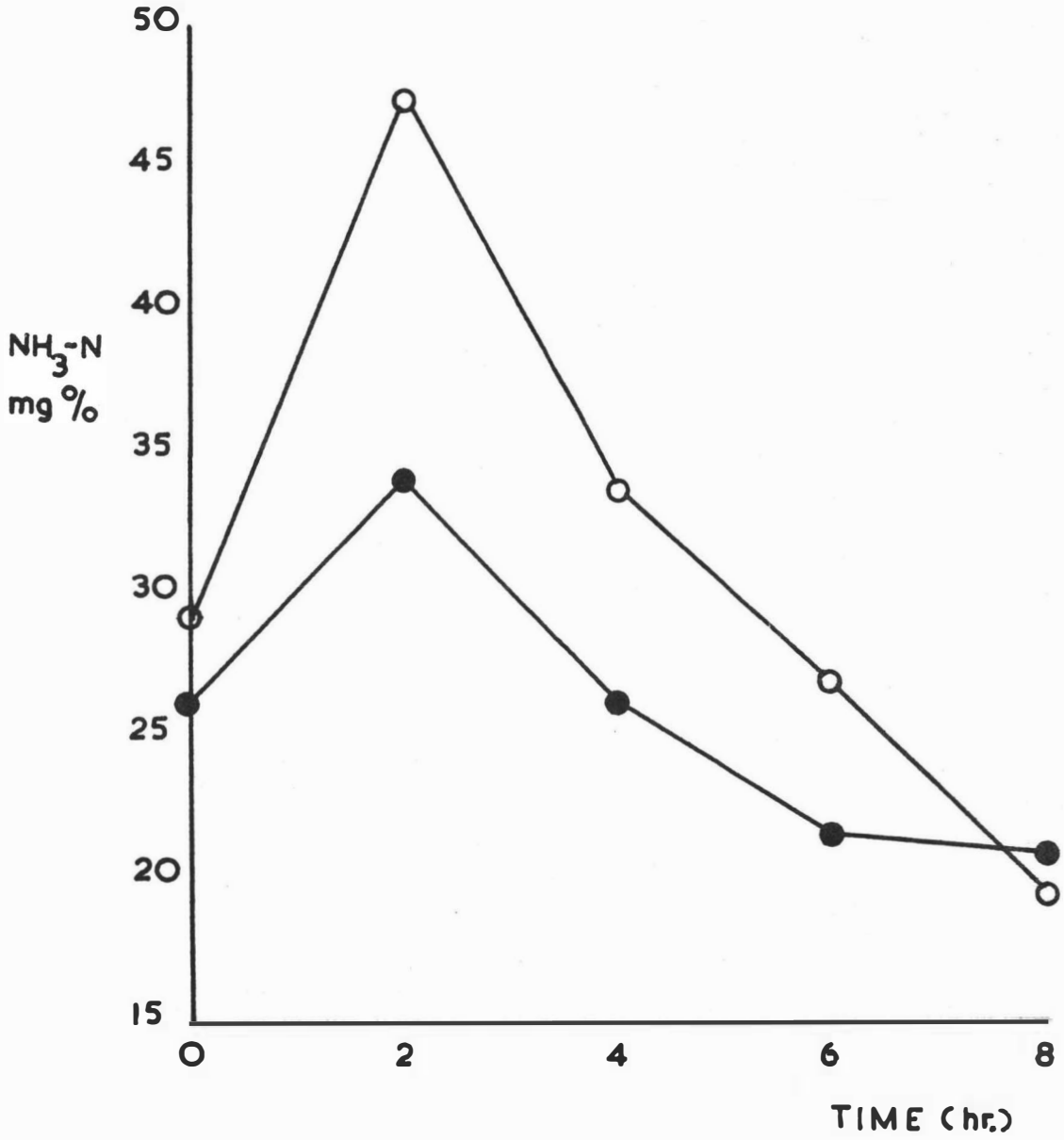


FIGURE 11: Rumen ammonia-nitrogen concentration vs time after feeding.

Symbols as for Figure 9.

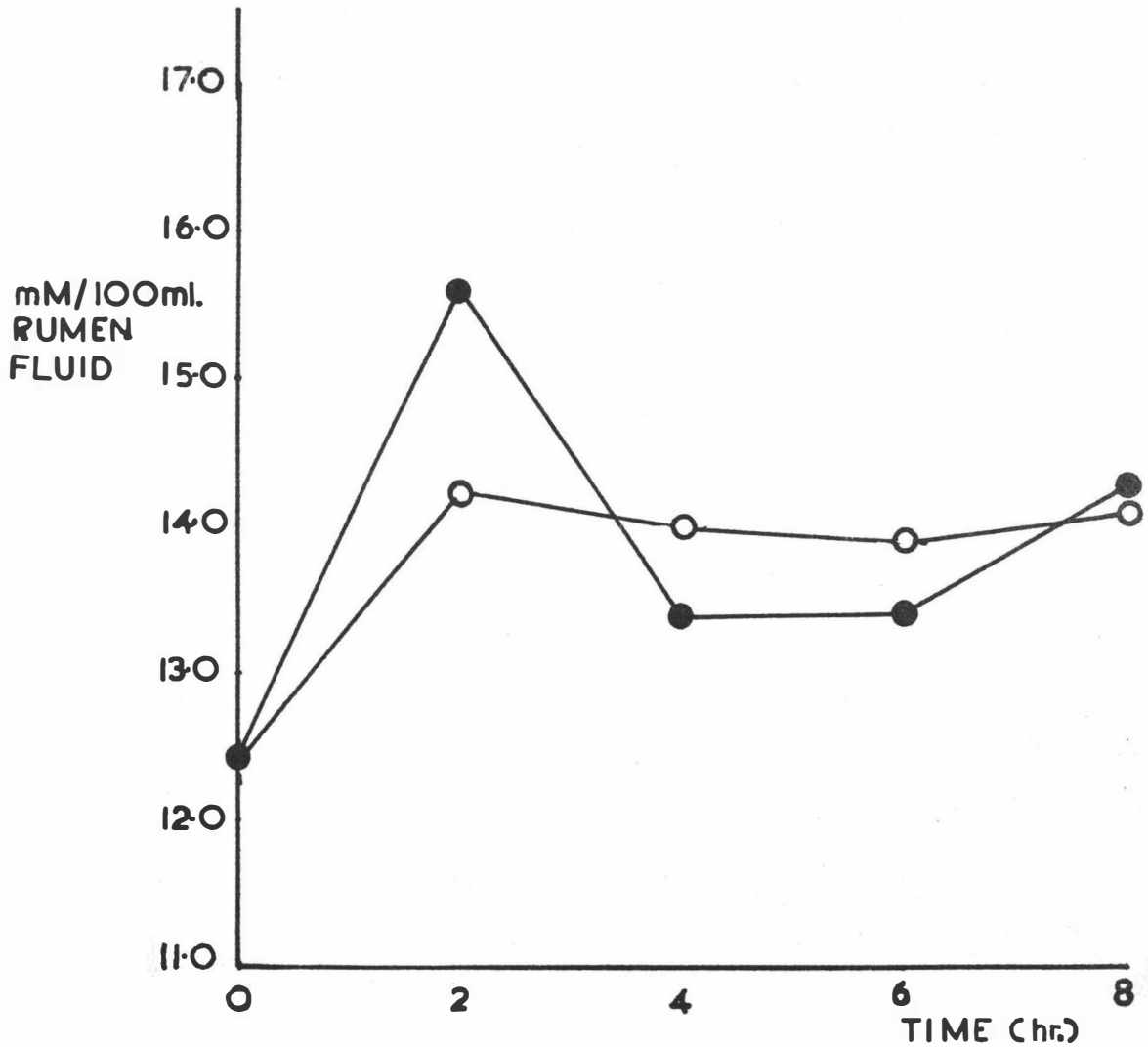


FIGURE 12: Total VFA concentration vs time after feeding

Symbols as for Figure 9.

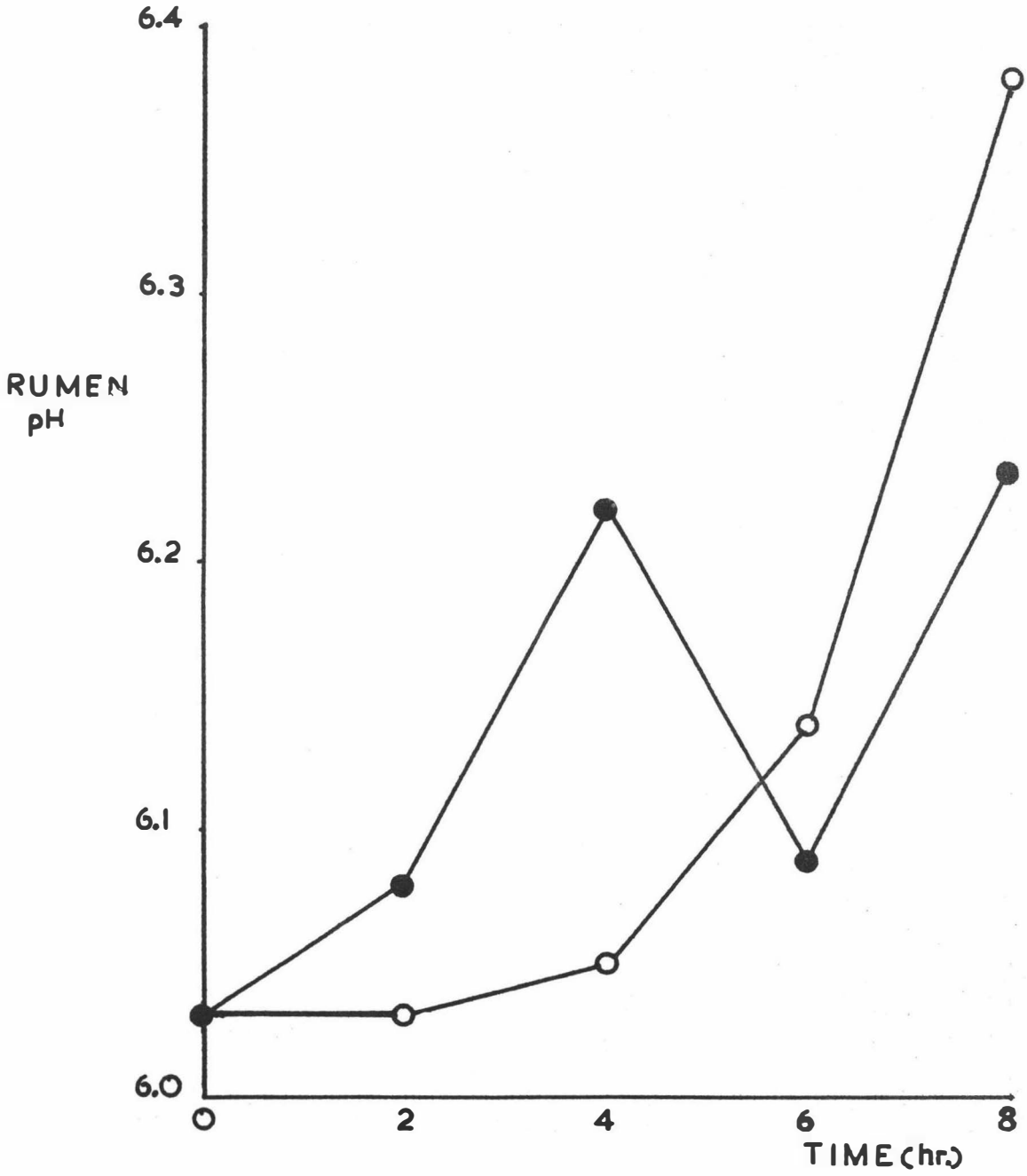


FIGURE 13: pH vs time after feeding

Symbols as for Figure 9.

time interaction was also statistically significant ($P<0.01$). The treatment PR resulted in a much more rapid rise in pH, with a maxima at 4 h, compared to an 8 h maxima for the treatment R cows.

(e) BLOOD METABOLITES

(i) Plasma glucose

The mean squares are presented in Table A-8 and the adjusted treatment means for the two-way analysis of covariance (Model B, Section II) are presented in Table 3, with the treatment period means shown in Figure 14. Only in the second experimental period was there a significant treatment effect. Treatment PR had a higher plasma glucose level than treatment R. It appears that the glucose levels during the post-experimental period were markedly lower than the levels in either experimental period.

Statistically significant differences ($P<0.05$) arose between sampling times in both experimental periods, but not in the post-experimental period. In the 1st experimental period the 0 and 4 h samples were not significantly different ($P>0.05$), but both were greater than the 8 h sample ($P<0.05$). In the second experimental period, the reverse was true, with the 8 h sample being significantly greater ($P<0.05$) than either the 0 or the 4 h sample.

(ii) Plasma urea

A substantial reduction in the error variance was

obtained by analysing the data as a two-way analysis of covariance (Model B, Section II). The percent reduction was 95.3, 95.9 and 96.2 for the 1st experimental, 2nd experimental and post-experimental periods respectively. The mean squares for the analysis of covariance are presented in Table A-9 and the adjusted treatment means are presented in Table 4. Adjusted treatment-period means are illustrated in Figure 15. In both the experimental periods and the post-experimental period, treatment R resulted in significantly greater levels of plasma urea-N ($P<0.001$) than treatment PR.

In each period the variance associated with time of sampling was significantly greater than zero. In both experimental periods the maximum level of plasma urea-N occurred at 4 h post-feeding. However, in the post-experimental period the zero h sample was significantly greater than the 4 and 8 h samples ($P<0.05$).

(iii) Amino-nitrogen (N)

When analysed using a two-way analysis of covariance (Model B, Section II), there was no reduction in the error variance. The F ratios for the error regression were non-significant ($P>0.10$) for each of the periods. The data were then subjected to a two-way analysis of variance (Model A, Section II). Mean squares are presented in Appendix Table A-10 and treatment means in Table 5. Treatment-period means are illustrated in Figure 16.

TABLE 3

PLASMA GLUCOSE CONCENTRATION

Adjusted Treatment means. Plasma glucose. (mg./100ml. plasma)

TREATMENT	P E R I O D		
	Ex 1	Ex 2	Post
Rumen	69.3	69.4	63.3
Post-rumen	70.6	71.4*	63.0
S.E. of mean	0.8	0.65	1.1

* $P<0.05$

Adjusted Time means. (mg/100 ml. plasma)

TIME (hours postfeeding)	P E R I O D		
	Ex 1	Ex 2	Post
0	70.6a	69.4ab	64.0
4	72.0a	69.0b	62.4
8	67.2b	72.7a	63.1
S.E. of mean	1.0	0.8	1.4

means within a period having different letter are significantly different from one another ($P<0.05$)

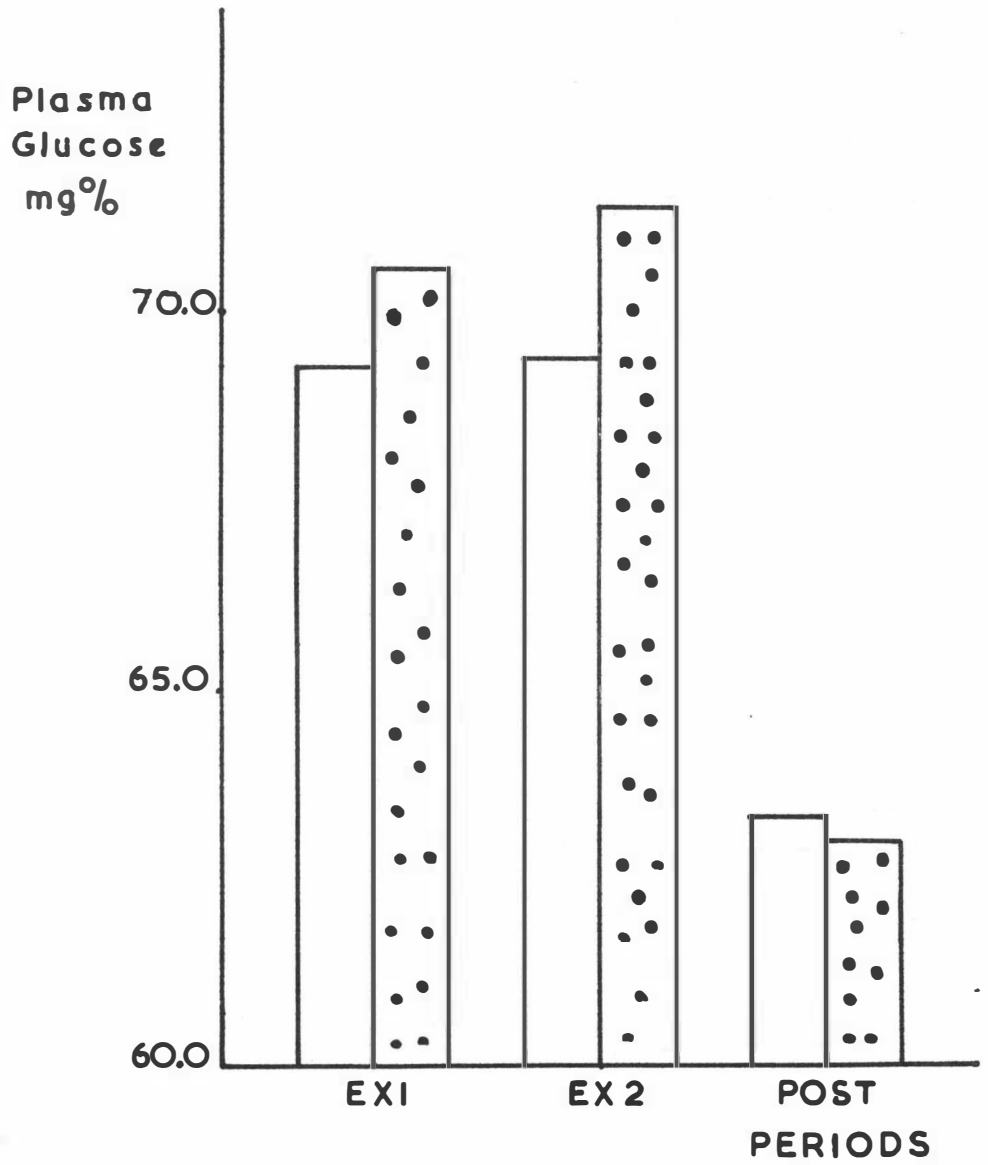


FIGURE 14: Plasma glucose vs periods

Symbols as for Figure 1.

TABLE 4

PLASMA UREA CONCENTRATION

Treatment means. (mg. Urea/100 ml. plasma)

		PERIOD		
		Ex 1	Ex 2	Post
Treatment	R	24.63***	23.67***	17.77***
	PR	21.53	20.79	16.27
S.E. of mean		0.33	0.29	0.21

*** P<0.001

Time means. (mg. Urea/100 ml. plasma)

		PERIOD		
		Ex 1	Ex 2	Post
Time	0	22.18b	21.06b	17.84a
	4	23.78a	23.14a	16.91ab
	8	23.29ab	22.49ab	16.31b
S.E. of mean		0.39	0.34	0.25

Means within a period not having the same letter are significantly different P<0.05.

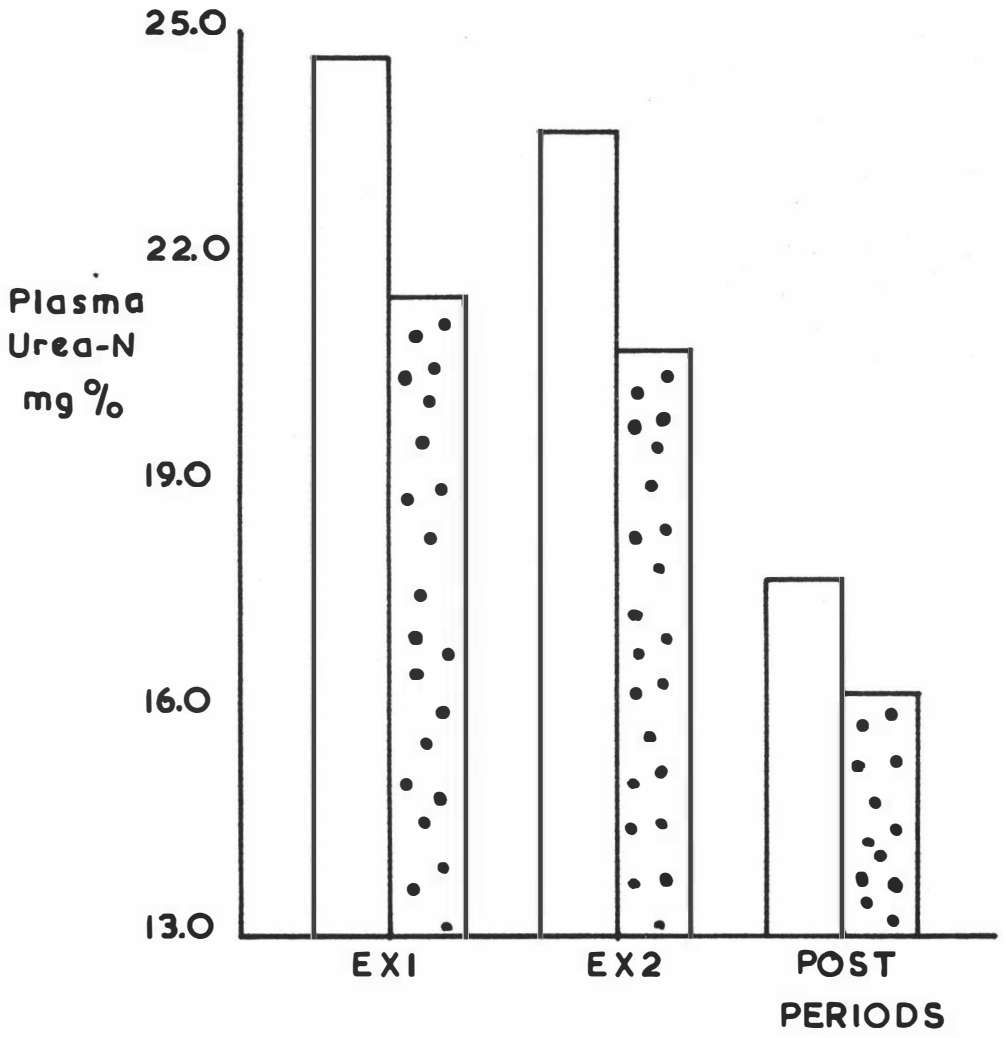


FIGURE 15: Plasma urea nitrogen vs periods

Symbols as for Figure 1.

A significant difference existed between the treatment groups before the experimental period commenced ($P<0.01$), treatment R being greater than treatment PR. This difference is unexpected as the animals were monozygous twins.

During both experimental periods, treatment PR was significantly greater than treatment R, $P<0.10$ and $P<0.05$ for the first and second experimental periods respectively. From Figure 16 it can be seen that a cross-over effect was obtained, treatment PR rising from a low preliminary value and treatment R falling from a relatively high value. One would have expected that the covariance analysis would have given a reduction in error variance, resulting in highly significant differences between treatments during both experimental periods. This did not occur, indicating that a large source of random variation was not accounted for.

In the post-experimental period the level of amino nitrogen fell quite markedly in the treatment PR group, resulting in a significantly ($P<0.10$) higher level of amino nitrogen in the treatment R group.

In the first experimental period there was a significant ($P<0.01$) treatment x time interaction. An examination of the means, using Duncan's Multiple Range Test

(Duncan, 1955), revealed that the treatment PR cows had significantly higher ($P<0.05$) levels of amino-N at 4 h post-feeding than at any other time, and that this level was greater than the level reached at any time in the treatment R group. The treatment x time interaction was also significant ($P<0.10$) in period Ex 2.

(f) MILK FAT FATTY ACID YIELDS

The data from the two experimental periods were analysed according to Model G, Section II. Mean squares for the analysis of variance are presented in Appendix Table A-11 and treatment means and standards errors in Table 6.

The only difference between treatments which approached significance was in the yield of oleic acid (18:1) ($P<0.10$), where the yield of oleic acid was greater for treatment PR than treatment R.

TABLE 5

PLASMA AMINO NITROGEN

Treatment means. Amino N (mg./100 ml. plasma)

PERIODS		PRE	Ex 1	Ex 2	POST
Treatment	R	4.71**	4.33 (10%)	4.11*	4.06 (10%)
	PR	3.91	4.63	4.45	3.69
S.E. of mean		0.15	0.10	0.10	0.14
Time	0	3.91b	4.49	3.85b	3.99
	4	4.02b	4.66	4.52a	3.96
	8	4.99a	4.29	4.48a	3.69
S.E. of mean		0.19	0.13	0.13	0.17

** P<0.01 * P<0.05 10% P<0.10

Treatment-time means	Ex 1		Ex 2	
	R	PR	R	PR
0	4.47ab	4.51ab	3.74b	3.97b
4	4.10b	5.21a	4.11ab	4.92a
8	4.40ab	4.17b	4.49ab	4.47ab
S.E. of mean	0.18	0.18	0.18	0.18

Means within a period having different letters are significantly different, P<0.05.

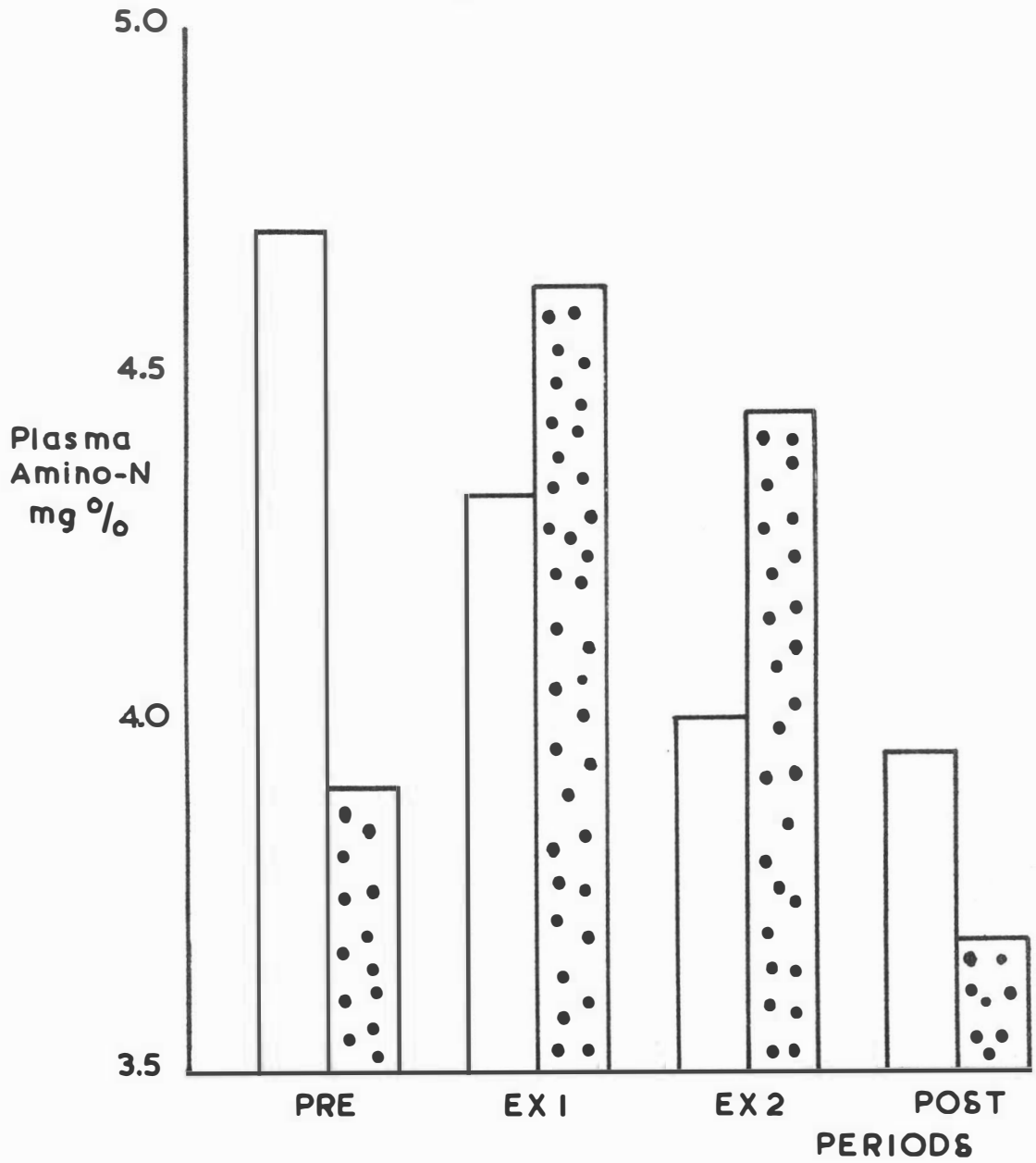


FIGURE 16: Plasma amino nitrogen vs periods

Symbols as for Figure 1.

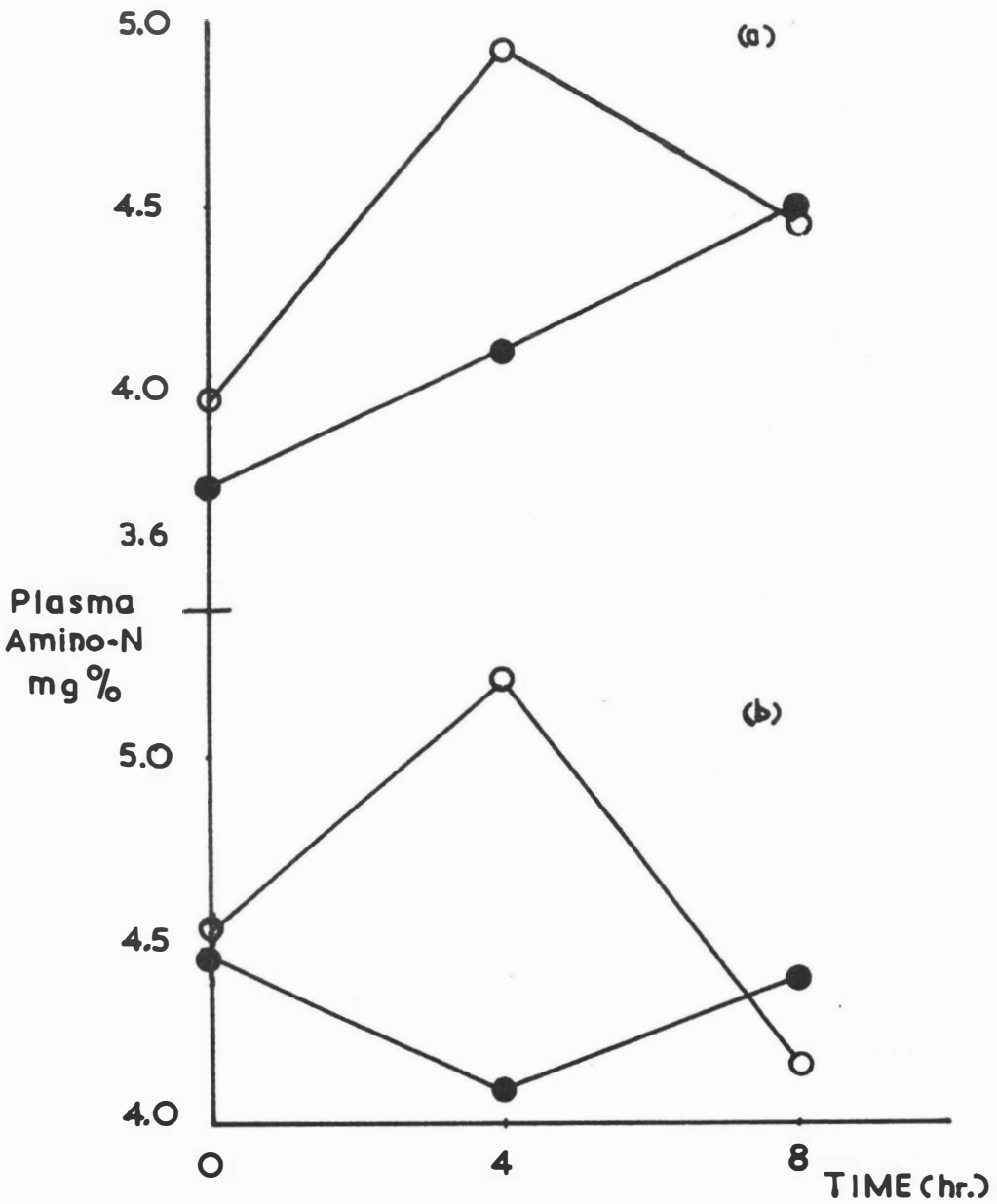


FIGURE 17: Treatment vs time for plasma amino nitrogen
(a) 2nd experimental period
(b) 1st experimental period

Symbols as for Figure 9.

TABLE 6

MEAN YIELDS OF INDIVIDUAL FATTY ACIDS (g/day) DURING THE EXPERIMENTAL PERIODS

TREATMENT	FATTY ACID*			
	4:0	6:0	8:0	10:0
Rumen	13.78	12.72	8.30	20.16
Post-rumen	15.44	14.26	9.32	22.44
S.E. of mean	0.72	0.70	0.44	1.08

TREATMENT	FATTY ACID*				
	12:0	14:0	16:0	18:0	18:1
Rumen	22.58	64.46	136.32	98.90	153.72
Post-rumen	24.76	64.00	139.30	101.70	164.04
S.E. of mean	1.16	2.42	5.60	3.90	4.18

* Number of carbon atoms and number of double bonds (Farquhar, Insull, Rosen, Stoffel and Ahrens, 1959)

SECTION IV

DISCUSSION

(i) Volatile Fatty Acid Proportions and Concentrations

Although the concentration of acetate did not differ between treatments, the molar percent of acetate in treatment R was significantly reduced ($P<0.05$) below the level in treatment PR. Hume (1970 (b)) found an inverse relationship between microbial protein produced in the rumen and the molar proportion of acetic acid. Ørskov and Øltjen (1967), Lewis (1962) and Menahan and Schultz (1964) have also noted a fall in molar percent acetic with an increase in rumen ammonia level. The treatment R cows had a significantly higher rumen NH_3 concentration than the treatment PR cows. Hume has interpreted his results to indicate that, as the proportion of acetic acid increases, the efficiency with which the energy from the fermentation is used for microbial growth is diminished. However, since carbon for amino acid biosynthesis within rumen bacteria is probably from fermentation end-products, carbon dioxide and acetate (Allison, 1969), and as acetate stimulates several groups of rumen bacteria, especially when ammonia is the main source of nitrogen (Hungate, Bryant and Mak, 1964), the decreased molar percent of acetate observed in the trial along with the raised levels of rumen NH_3 indicate that the growth of rumen bacteria had been stimulated. El-Shazly (1952) demonstrated that the higher volatile fatty acids, namely isobutyric, isovaleric and valeric acids, arose from the breakdown of amino acids. The present work would confirm these

observations in as much as treatment R cows had a higher concentration and molar percentage of valeric and isovaleric acids than treatment PR. As isobutyric acid could not be separated from propionic acid it is not know whether the level of isobutyric differed between the treatments. A difference in isobutyric level could account for part or all of the difference measured as propionic acid concentration, which differed significantly ($P<0.05$) between treatments; the level in treatment R being greater in treatment PR.

Annison (1954) noted a relationship between the amount of protein fed and the level of branched chain volatile fatty acids in the rumen fluid. Cline, Garrigus and Hatfield (1966) were unable to detect isobutyric or isovaleric acid in rumen fluid of sheep fed purified diets containing non-protein nitrogen. The addition of leucine or valine to the rumen of goats (Menahan and Schultz, 1964) increased the level of isovaleric and isobutyric acids significantly.

Allison, Bryant and Doetseh (1962) and Dehority, Scott and Kowaluk (1967) have demonstrated a branched chain volatile fatty acid requirement for some species of rumen bacteria. They classified the bacteria into three groups:-

(i) those that required valeric acid or higher straight chain acid plus isobutyric or 2-methylbutyric;

(ii) those requiring two or more branched chain acids

(iii) a group requiring only one branched chain acid. Allison, Bryant and Doetseh (1962) found that with

R flavefaciens, there was a limited ability to utilise exogenous amino acids which they suggested was due to a poor amino acid transport system. The authors also note that the concentration of isovaleric acid in rumen fluid is 20 to 200 times that of leucine and thus the poor transport system may be an evolutionary adaptation: Lassiter, Emery and Duncan (1958) concluded that valeric plus isovaleric acid added to a ground corn cob plus soybean meal diet, increased weight gain of dairy heifers by some mechanism other than appetite.

Hume (1970 (b)) obtained an increased in microbial protein production when a mixture of isobutyric, 2-methylbutyric, isovaleric and n-valeric acids was added to a basal diet in which the majority of the nitrogen was supplied as urea. Of interest was the observation (Hume, 1970 (c)) that urea plus the volatile fatty acid mixture enabled as great a microbial protein production as when gelatin provided most of the nitrogen in the diet. Of particular relevance here was the observation of McDonald and Hall (1957) that 90% of the casein nitrogen in the diet they fed, was converted to microbial nitrogen.

It would therefore seem reasonable to suggest that in the present experiment the addition of casein to the rumen may have increased the microbial protein production in the rumen.

TOTAL VFA CONCENTRATION

The significant difference between sample times after feeding has been observed by Davey (1964). The maxima 2 h post feeding is indicative of the rapid fermentation that occurs immediately following ingestion of a meal.

(2) Rumen Ammonia Concentration

A number of workers (Chalmers, Cuthbertson and Synge, 1954, Annison 1956, Chalmers and Synge, 1954) have shown that when casein is added to the rumen the level of rumen ammonia is greater than for the basal diet alone. The high level of ammonia results from proteolysis (Annison, 1956) and deamination (Warner, 1956) of the casein. The results from the present trial are in agreement with the earlier work, thus demonstrating that the casein was rapidly attacked by the rumen micro-organisms. It would appear from the results obtained, that the method of administering the casein post-ruminally was successful. This conclusion is based on the observation that there was no abnormal increase in rumen ammonia level in the treatment PR cows. There was thus no evidence that the casein had been returned to the reticulo-rumen by a reverse peristaltic contraction.

The pattern of rumen ammonia concentration, relative to the time of feeding in the treatment PR cows was similar to the pattern observed in the post-experimental period on both treatments. This observation would further support

the idea that little casein was returned to the rumen after being administered post-ruminally.

(3) Nitrogen Balance

Chalmers, Cuthbertson and Synge (1954) demonstrated that the nitrogen in a casein supplement was utilised more efficiently when administered directly into the duodenum of a sheep than when administered to the rumen. Many workers have since confirmed these earlier observations. (Reis and Schinckel, 1961; Schelling and Hatfield, 1967; Little and Mitchell, 1967; and Egan 1965 a, b, c, d). An improvement in nitrogen retention in lambs was also noted with abomasally infused soybean protein, relative to when the protein was administered orally (Little and Mitchell, 1967). The treatment PR cows had a significantly ($P<0.10$) greater nitrogen retention than the treatment R cows and this is in agreement with the observations of Reis and Schinckel (1961); Schelling and Hatfield (1967) and Little and Mitchell (1967).

Of particular importance is the observation that the faecal nitrogen excretion of the treatment R cows was significantly ($P<0.05$) greater than treatment PR. McNaught et al (1954) and Bergen, Purser and Cline, 1968, have demonstrated that bacteria are not as digestible as protozoa. Thus if the casein administered to the rumen resulted in an increase in the number of bacteria and not the protozoa, then

most likely there would be an increase in faecal nitrogen excretion as was observed in the present experiment. Similarly, Blaxter (1964) observed that 6.5% of the casein-N infused into the rumen appeared in the faeces, compared with 0.2% for abomasally infused casein.

Ammonia that is absorbed from the rumen is converted to urea at the liver and is largely excreted in urine (McDonald, 1968) since treatment R resulted in significantly higher levels of rumen ammonia than treatment PR one might expect a greater loss of nitrogen in urine with treatment R cows. Although the difference (18.5 g nitrogen per day) in urinary nitrogen secretion was not significant ($P>0.25$) this would appear to be largely a reflection of the large within treatment variation in urinary nitrogen excretion since a difference in faecal nitrogen excretion of 18.7 g per day was significantly different ($P<0.05$).

(4) Blood Metabolites

(a) Plasma Glucose

Although appreciable amounts of water soluble carbohydrates are found in grasses (MacKenzie and Wylan, 1957) they are almost completely fermented (Waite, Johnston and Armstrong, 1964). When frozen grass was fed to sheep, 98.2% of the total water soluble sugars ingested had disappeared before reaching the pylorus; with the same grass fed dried, the comparable value was 95.5% (Armstrong and Beever, 1969).

Therefore very little glucose would be absorbed from the digestive tract of a ruminant fed grass and gluconeogenesis is of importance in providing glucose for the animals metabolism.

Propionate has been believed to be a major substrate for gluconeogensis, although estimates of the contribution of propionate carbon to glucose carbon have varied from 27%, (Judson, Anderson, Luick and Leng, 1968; Bergman, Roe and Kon, 1966) to 62% (Leng, Steel and Luick, 1967).

Metabolised protein is also a major substrate for gluconeogenesis and could account for up to two thirds of the glucose carbon, (Bergman, Roe and Kon, 1966). Ford (1965) fed diets supplying 126, 197 and 460 g crude protein per day and found a greater glucose utilisation rate with the high protein diet. This he suggested was due to greater amino acid absorption and subsequent gluconeogenesis. The significantly higher level of plasma glucose observed in the PR cows could be explained on the basis of a greater supply of gluconeogenic material at the liver since it is unlikely that the amino acids would be degraded in the digestive tract post-ruminally, unless they reached the caecum. There was no significant difference between treatments in Ex 1 in glucose concentration, the reason for this is difficult to establish. The lower level of plasma glucose in the Post Period in both treatments is surprising since the animals were being fed the same basal ration. The fall may

have reflected a general overall drop in the level of nutrition due to cessation ofcasein infusion.

(b) Plasma Urea

The absorption of ammonia across the rumen wall was first reported by McDonald (1948). The rate of absorption is influenced both by concentration gradient (Hogan, 1961; Lewis, Hill and Annison, 1957) and pH (Hogan, 1961). Absorbed ammonia is carried via the portal circulation to the liver where it is converted to urea (Waldo, 1968; Smith, 1969; Tillman and Sidhu, 1969). Lewis et al. (1957) reported that the liver was able to convert all of the absorbed ammonia into urea until the level of ammonia in rumen fluid reached approximately 84 mg%.

McIntyre (1970) has demonstrated that plasma urea nitrogen and rumen ammonia levels increased linearly to about 30 mg % as nitrogen intake increased. A correlation between protein intake and the concentration of urea in blood plasma has been observed by Lewis (1957); Leibholz and Cook, (1967) and Van Horn, Jacobson and Erader (1969).

In the present experiment, the significantly ($P<0.01$) higher level of plasma urea nitrogen in the treatment R cows appears to reflect the higher protein intake in the rumen, and the significantly ($P<0.01$) higher level of rumen ammonia of the treatment R cows.

Although blood samples were not taken at the time that corresponded to maximum rumen ammonia concentration the

higher level of plasma urea at four hours post-feeding would be a reflection of the high rumen ammonia plasma levels at two hours post-feeding.

The continued high level of urea in the treatment R group in the post experimental period is difficult to explain. Both groups of cows in this period were receiving similar nitrogen intakes and there were no significant differences in rumen ammonia concentration.

(c) Plasma Amino Nitrogen

Low concentrations of free amino acid have been demonstrated in the rumen (Annison, 1956; Wright and Hungate, 1967; Leibholz, 1965 and Leibholz, 1969). Although there is conflicting evidence on the absorption of amino acids from the rumen, Annison (1956) found no evidence to indicate absorption while Demaux et al. (1961); Cook, Brown and Davis, 1965 and Leibholz (1971) using the technique of placing amino acid solutions in washed rumens, found evidence for absorption of some amino acids although this depended on the initial concentration and the concentration of other amino acids present (Leibholz, 1971).

The use of isotopically labelled D, L-Tryptophan (Candlish, Stanger, Devlin and LaCroiz, 1970) and methionine (McCarthy, Patton and Griel, 1970) has indicated that substantial amounts of these amino acids can be absorbed from the rumen.

The significant difference between treatments ($P<0.01$) for amino-N in the preliminary period is difficult to account for. The results obtained during the experimental periods are what one would expect if there was increased absorption of amino acids from the lower gut as a result of increased amounts of microbial and casein protein being digested. That differences between treatments were only significant at the 10% level of probability in the first experimental period and at the 5% level in the second experimental period would suggest that there was an adaptation over a period of time to the dietary change imposed by the treatment. From Figure 16 it can be seen that treatment R cows had a high level of amino-N which progressively decreased, while the treatment PR cows had a low level in the preliminary period which increased during treatment.

The significant treatment X time interactions in the first experimental period ($P<0.01$) and the second experimental period ($P<0.10$) are interesting, the peak level of α-amino nitrogen occurred 4 h post feeding in treatment PR while it was a minimum in treatment R at this time during the first experimental period. However during the second experimental period while treatment PR still resulted in a 4 h maxima, treatment R had an ever increasing level at both 4 and 8 h post feeding.

If substantial absorption of amino acids did occur

from the rumen, one might expect the results obtained in the first experimental period to be similar to those in the second experimental period, since the steadily increasing level of ∝-amino nitrogen would indicate absorption of amino acids from the rumen followed by increased absorption of amino acids from digested microbial protein in the lower gut at a later time viz eight hours post-feeding.

Although the data may indicate increased absorption of ∝-amino nitrogen in the treatment PR cows, one needs to bear in mind the unexplained significant difference between the treatment groups in the preliminary period. The observation in the preliminary period would seem to indicate that factors other than dietary supply of ∝-amino nitrogen are important in influencing the plasma level of ∝-amino nitrogen, since all cows received identical diets in this period.

(5) Yield of Milk and Milk Components

All yields were greater on treatment R than on treatment PR. The effect on fat yield was the least significant ($P>0.10$), despite a significantly greater content of milk fat in the treatment R group. The lack of a significant difference in fat yield reflects the greater variance in milk fat yield, and milk fat percent differences didnot give significant differences in milk fat yield.

The content of lactose ($P>0.10$), protein ($P>0.10$), protein in fat free serum ($P>0.10$) and lactose in fat free serum ($P>0.10$) were not significantly different between treatments. The content of milk fat in the treatment R group was significantly different ($P<0.10$) from the treatment PR group.

Although the greater variability in milk fat yield explains in part the lack of significant differences between the treatment groups, one should not overlook possible nutritional factors.

Generally an increase in milk yield can be attributed to an increase in energy intake (Rook and Line, 1961). Since the cows in this experiment would have been receiving similar energy intakes, as all animals were fed the same amount of food, one can only conclude that extra energy via the rumen was more beneficial than extra energy post-ruminally.

One needs also to note that the energy obtained from casein via the rumen would differ from energy obtained from casein post-ruminally. With ruminal administration the energy would be largely via volatile fatty acids; since the casein is rapidly deaminated, as shown by the significant differences in rumen ammonia concentration. The post-ruminal administration would supply energy in the form of amino acids.

It is difficult to determine the effect of higher proportions and concentrations of isovaleric and valeric acid on production. Lassiter, Emery and Duncan (1958a) found no effect of valeric or isovaleric on fat corrected milk yield although there was a tendency to depress milk fat percent. With dry dairy heifers valeric plus isovaleric acid added to a ground corn cob plus soybean meal diet resulted in increased weight gains by some mechanism, other than an increased appetite (Lassiter, Emery and Duncan, 1958b). The addition of a mixture of isovaleric and valeric acids to a diet has been shown to increase microbial protein production (Hume, 1970b). Hume (1970b) has also noted a negative correlation between molar percent acetic acid and microbial protein production. Since acetate provides a large proportion of the carbon for amino acid biosynthesis in the rumen bacteria (Allison, 1969) and as branched and long chain volatile fatty acids are required for growth of some species of rumen bacteria (Allison, Bryant and Doetsch, 1962) it is conceivable that in the present experiment there was a twofold advantage from adding casein to the rumen:-

(i) increased absorption of propionic, isovaleric and valeric acid from the rumen, and

(ii) increased digestion of microbial protein in the intestines due to stimulation of microbial growth in the rumen. The greater N excretion in the faeces of the treatment

R cows would support the suggestion that there was increased rumen microbial growth in the cows receiving casein to the rumen. Blaxter (1964) has pointed out the high obligatory loss of faecal nitrogen in ruminants - 6.5% of the casein-N infused into the rumen vs 0.2% of the casein infused into the abomasum recoverable in the faeces - due to the trapping of nitrogen in the indigestible cell walls of the micro-organisms.

(b) Milk Composition

As mentioned in Section III the only difference in composition was with milk fat percent ($P<0.10$). This is somewhat surprising in view of the increased protein content obtained with treated casein by Wilson (1970). However in Wilson's experiment there was no significant difference between treated casein and non-treated casein when given orally as suspensions. However when the caseins were incorporated into a meal mixture, untreated casein resulted in significantly greater contents of fat and protein in the milk. As there may have been interactions between the energy and protein these results are not strictly comparable with the present experiment. Similarly the results of Broderick, Kowalczyk and Salter (1970) are not comparable since the comparison was between the abomasal administration, of casein and the equivalent amount of nitrogen and energy as urea and glucose. Broderick et al. (1970) obtained a 6.2% increase in protein content ($P<0.10$) and an 11.6% increase in

protein production ($P<0.05$), in response to casein infusion into the abomasum.

Plasma free amino acids are the precursors of at least 90% of the protein nitrogen in cows milk (Barry, 1961). It is therefore somewhat surprising that the significant increase in concentration of plasma amino nitrogen observed in the PR cows did not result in an increased content of protein in the milk. Similarly, isotopic studies have shown that both halves of the lactose molecule are derived from blood glucose (Linzell, 1968), however in the present experiment, the significantly higher plasma glucose ($P<0.05$) level observed in the PR cows did not result in an increased secretion of lactose in the milk.

6. YIELD OF MILK FAT FATTY ACIDS

Milk fat short chain acids (4 to 10 carbon atoms) are derived almost entirely by synthesis within the mammary gland. The long chain acids (18 carbon atoms) are derived from the triglycerides of the low density β-lipoproteins of plasma and the intermediate acids (12 to 16 carbon atoms) from both these sources (Barry, 1964). Oleic acid may be derived directly from oleic acid or indirectly by intramammary dehydrogenation of stearic acid (Lauryssens, Verbeke and Peeters, 1961; Annison, Lenzell, Fazakerley and Nichols, 1967). The yield of oleic acid was significantly ($P<0.10$) greater for the treatment PR cows. This could

indicate greater absorption of oleic acid from the digestive tract, or increased metabolism of adipose tissue in the treatment PR group. It is conceivable that if changes in bacterial numbers did occur in the treatment R group, some changes in rumen hydrogenation might occur. However as the difference in yield is only at the 10% level of significance one needs to be cautious in drawing conclusions as to cause and effect. At this stage the yields of the milk fat fatty acids would not appear to offer any explanation for the observed effects on milk yield and composition.

CONCLUSIONS

This thesis describes an experiment designed to investigate efficiency of utilisation of extra protein, as casein, on milk production of a lactating cow when administered ruminally or post-ruminally. During the course of the experiment the cows were housed indoors and fed a constant diet of dried grass.

The fate of the administered nitrogen was examined by a nitrogen balance. Rumen ammonia and VFA measurements were taken to give an indication of the type of fermentation occurring in the rumen. Blood samples were also taken, for analysis of glucose, urea and amino-nitrogen.

Except for milk fat, the yield of all milk components were significantly greater in the treatment R group. The concentration of rumen ammonia was greater in the treatment

R cows, in accordance with observations made in other investigations. There was greater retention of nitrogen in treatment PR cows, with treatment R having a significantly greater excretion of nitrogen in the faeces. The rumen ammonia and nitrogen balance data are indicative of inefficient use of nitrogen in the treatment R group and a general conclusion along this line would be in agreement with observations by other workers. In spite of the apparent inefficient use of nitrogen in the treatment R group, the yield of milk and its components was greater than in the treatment PR group.

A depressed level of acetic acid concentration, and an elevated level of propionic, valeric and isovaleric acid concentrations were observed. At present the observations on the rumen parameters seem to explain the effect on milk yield best. The elevated levels of valeric and isovaleric acids are associated with fermentation of amino acids, while the depressed level of acetic acid is considered by several authors to indicate greater utilisation for rumen microbial growth.

The measurements of blood metabolites indicated a significantly greater level of α-amino nitrogen and a lower level of plasma urea in the treatment PR group. No differences occurred in the level of plasma glucose between treatment groups.

A further investigation into the relationship between "excess" dietary protein and rumen microbial growth

seems to be indicated by the present experiment. The general argument for an amino acid to be limiting in milk production is that the amino acid composition of rumen microbial matter alters very little with changes in bacterial and protozoal species, and that the amino acid levels supplied are unlikely to meet the demands of a high yielding cow. However, if the total amount of rumen microbial matter can be increased, there may be some benefit to the animal. It should be noted that in the investigation reported in this thesis the cows used were not high yielding, daily milk production being approximately 10 kg.

An alternative approach to administering an amino acid mix, would be to administer individual amino acids via the abomasum. Data available on the requirements for milk protein and glucogenesis indicate that the abomasal infusion of glutamic acid would be worthy of investigation.

APPENDICES

APPENDIX TABLE A-1

MEAN SQUARES FOR ANALYSIS OF VARIANCE OF Y, AFTER FITTING THE REGRESSION ON X.

(b) Yields

Source	DF	MY	FCMY	FY	PY	LY
Treatment (TR)	1	2.24649**	2.91065*	0.32235	0.12133*	0.44356**
Periods (P)	5	3.20085***	3.35400***	0.55891*	0.60374**	0.83836***
Tr x P	5	0.08414	0.07201	0.01739	0.01000	0.02273
Error	23	0.23478	0.53259	0.14011	0.02513	0.05522

* P<0.05 ** P<0.01 *** P<0.001

Adjusted treatment means (kg/day)

(1) Treatments	MY	FCMY	FY	PY	LY
TR	10.823	12.943	0.574	0.398	0.584
TPR	10.242	12.041	0.530	0.383	0.522
S.E. of mean	0.124	0.228	0.016	0.004	0.006

Period Means (not adjusted) (kg/day)

(2) Periods	MY	FCMY	FY	PY	LY
Ex 1	10.783a	12.719a	0.560ab	0.396a	0.543a
2	10.937a	12.978a	0.573a	0.407a	0.556a
3	11.196a	13.207a	0.582a	0.419a	0.572a
4	10.479a	12.471a	0.552ab	0.394a	0.530a
Post-Ex 1	10.673	12.498a	0.549ab	0.397a	0.543a
2	9.126b	11.079b	0.495b	0.329b	0.464b
S.E. of mean	0.198	0.298	0.015	0.006	0.010

Means within any one parameter having different letters are significantly different.

APPENDIX TABLE A-2

MEAN SQUARES FOR ANALYSIS OF VARIANCE OF Y, AFTER FITTING THE REGRESSION ON X.

(a) Milk Composition

Source		Fat %	Protein %	Lactose %	Protein(FFS)	Lactose(FFS)
Treatment (Tr)	1	0.13937	0.00333	0.0000035	0.00737	0.0000279
Periods (P)	5	0.05428	0.02213*	0.0041501	0.02472**	0.0054125
TR x P	5	0.01532	0.00435	0.0002020	0.00226	0.0006550
Error	23	0.03798	0.00574	0.003875	0.00427	0.0049264

* P<0.05 ** P<0.01

Adjusted Treatment means

Source	Fat %	Protein %	Lactose %	Protein(FFS)	Lactose(FFS)
TR	5.33	3.71	5.08	3.91	5.35
TPR	5.21	3.72	5.08	3.91	5.35
S.E. of mean	0.05	0.02	0.01	0.02	0.02

Period means (not adjusted)

Source		Fat %	Protein %	Lactose %	Protein(FFS)	Lactose(FFS)
Experiment	1		3.69ab		3.88ab	
	2		3.73ab		3.94a	
	3		3.75a		3.96a	
	4		3.77a		3.97a	
Post-Ex.	1		3.73ab		3.93a	
	2		3.60b		3.79b	
S.E. of mean			0.03		0.03	

Mean within a parameter not having the same letter are significantly different P<0.05.

APPENDIX TABLE A-3
VFA ANALYSIS

(2) Individual acid concentration

Mean squares for the analysis of variance

Source	DF	Acetate	Propionate	Butyrate	Isovalerate	Valerate
Pairs	2	1.8573	0.177	0.1790	0.0001	0.00005
Treatment	1	0.6021	0.1267*	0.0120	0.0264***	0.0288***
Time	4	2.4984	0.2400***	0.3846**	0.0078**	0.0054*
Tr x time	4	0.6339	0.0602*	0.0066	0.0031	0.0026
Error	18	0.4525	0.0201	0.0435	0.0017	0.0015

* P<0.05 ** P<0.01 *** P<0.001

Treatment means (mM/100 ml. Rumen Liquor)

Treatment	Acetate	Propionate	Buryrate	Isovalerate	Valerate
Rumen	8.45	2.44	1.80	0.25	0.18
Post-rumen	8.73	2.31	1.76	0.19	0.12
S.E. of mean	0.17	0.04	0.06	0.01	0.01

APPENDIX TABLE A-3 (cont.)

Time means (mM/100 ml. Rumen Liquor)

Time	Acetate	Propionate	Butyrate	Isovalerate	Valerate
0	7.57b	2.07c	1.47d	0.22b	0.11c
2	8.85a	2.39b	1.65c	0.28a	0.19a
4	9.17a	2.43b	1.80b	0.18c	0.14b
6	8.53a	2.38b	1.84b	0.20b	0.15b
8	8.88a	2.63a	2.16a	0.22b	0.16ab
S.E. of mean	0.27	0.06	0.08	0.02	0.02

Treatment-time means. Propionic acid concentration (mM/100 ml. Rumen Liquor)

Time	Rumen	Post-rumen
0	1.99c	2.15c
2	2.50a	2.28b
4	2.61a	2.25b
6	2.48a	2.28b
8	2.64a	2.62a
S.E. of mean	0.08	0.08

Means having different letters are significantly different ($P<0.05$).

APPENDIX TABLE A-4
RUMEN VFA ANALYSIS

(1) Molar percent

Mean squares for the analysis of variance

Source	DF	Acetate	Propionate	Butyrate	Isovalerate	Valerate
Pairs	2	0.225	0.28	1.01	0.06	0.033
Treatment	1	23.23*	2.82(10%)	0.26	1.45***	1.634***
Time	4	11.31*	0.98	8.11**	0.62***	0.220*
Tr x time	4	3.66	0.85	0.56	0.13	0.142
Error	18	3.59	0.812	1.403	0.076	0.068

* P<0.05 ** P<0.01 *** P<0.001
10% P<0.10

Treatment means

Treatment	Acetate	Propionate	Butyrate	Isovalerate	Valerate
Rumen	64.8	18.4	13.56	1.87	1.35
Post-rumen	66.6	17.8	13.37	1.43	0.88
S.E. of mean	0.49	0.23	0.31	0.07	0.07

APPENDIX A-4 (continued)

Time means

Time	Acetate	Propionate	Butyrate	Isovalerate	Valerate
0	66.1a	18.23	13.0b	1.9ab	0.97b
2	66.2a	18.00	12.3b	2.1a	1.43a
4	66.7a	17.93	13.1b	1.3c	0.97b
6	66.2a	17.68	13.7b	1.4b	1.08a
8	63.2b	18.75	15.4a	1.6bc	1.12a
S.E. of mean	0.77	0.37	0.48	0.11	0.11

Means having different letters are significantly different $P<0.05$.

APPENDIX TABLE A-5

ANALYSIS OF VARIANCE OF RUMEN AMMONIA AND TOTAL VFA CONCENTRATION

Source	DF	Experimental period		Post-experimental period
		NH3	VFA	NH3
Pairs	2	51.10	6.325	112.8
Treatment	1	366.45***	1.44	18.92
Time	4	768.43***	5.678***	131.23**
Tr x time	4	98.21**	0.518	26.2
Within pairs	18	19.79**	0.6488	21.36*
Error (Days within subclasses)	30	7.02	1.190	8.7

P<0.05 = * P<0.01 = ** P<0.001 = ***

Means	Mg. NH3-N/100 ml RF	mM/100 mls RF	
(1) Treatment			
rumen	30.98	13.73	
post-rumen	26.04	13.42	
S.E. of mean	0.81	0.15	

APPENDIX A-5 (continued)

Time Means	Experimental Period		Post-Experimental Period
	NH3	VFA	NH3
(2) Time 0	27.89b	12.41a	25.37a
2	40.98a	13.89bc	26.80a
4	30.25b	13.71b	23.35a
6	23.26c	13.67b	21.25b
8	20.16d	14.21c	18.47b
S.E. of mean	1.28	0.33	1.33

(3) Treatment-time means

Time	Rumen	Post-rumen
0	29.26c	26.53c
2	47.68a	34.28b
4	34.02b	26.47c
6	24.56d	21.97d
8	19.39e	20.94e
S.E. of mean	1.82	1.82

R2>R4; PR2>RO; PRO, & PR4>R6; PR6>R8, & PR8

Means having different letters are significantly different $P<0.05$.

APPENDIX TABLE A-7

ANALYSIS OF VARIANCE OF RUMEN pH (DAY 16 OF EXPERIMENTAL PERIOD)

Mean squares

Source	DF	
Pairs	2	0.039399
Treatment	1	0.000083
Time	4	0.071295*
Treatment x time	4	0.092553**
Error	18	0.016112

* P<0.05 ** P<0.01

Time means

Time	
0	6.03b
2	6.05b
4	6.14ab
6	6.12b
8	6.31a
S.E. of mean	0.05

Treatment-time means

Rumen	Post-rumen
6.03b	6.03b
6.03b	6.08b
6.05b	6.22ab
6.14ab	6.09b
6.38a	6.23ab
0.07 S.E. of mean	0.07

Means having different letters are significantly different (P<0.05).

APPENDIX TABLE A-8

PLASMA GLUCOSE
ANALYSIS OF COVARIANCE

Mean squares

Source	DF	Ex 1	Ex 2	Post
Treatment	1	6.63	18.65*	0.30
Time	2	36.40*	24.30*	3.88
Tr x time	2	4.00	7.29	2.59
Error	11	5.54	3.85	11.59

* P<0.05

APPENDIX TABLE A-9

PLASMA UREA
ANALYSIS OF COVARIANCE

Mean squares

Source	DF	Ex 1	Ex 2	Post
Treatment	1	36.24***	31.31***	8.56***
Time	2	3.89*	6.56**	3.56**
Tr x time	2	0.12	0.85	0.45
Error	11	0.89	0.70	0.37

* P<0.05 ** P<0.01 *** P<0.001

APPENDIX TABLE A-10

PLASMA AMINO NITROGEN
ANALYSIS OF VARIANCE

Mean squares

Source	DF	Pre	Ex 1	Ex 2	Post
Treatment	1	2.884**	0.417 10%	0.5067*	0.6160 10%
Time	2	2.091**	0.206	0.8267**	0.1600
Tr x time	2	0.208	0.743**	0.2761 10%	0.0003
Error	12	0.208	0.095	0.0983	0.1751

* P<0.05 ** P<0.01 10% P<0.10

APPENDIX TABLE A-11

MEAN SQUARES FOR ANALYSIS OF VARIANCE OF FATTY ACID YIELDS

Source	DF	4:0	6:0	8:0
Treatment (TR)	1	4.125	3.534	1.54
Period (P)	1	0.567	0.840	0.190
Tr x P	1	0.456	0.071	0.019
Error	20	1.595	1.433	0.602

Source	DF	10:0	12:0	14:0	16:0
Tr	1	7.911	7.095	0.309	13.22
P	1	6.201	12.14	30.532	195.790
Tr x P	1	0.023	0.026	19.495	1.650
Error	20	3.541	4.063	17.540	94.171

Source	DF	18:0	18:1
Tr	1	11.886	160.160 (10%)
P	1	49.220	298.350
Tr x P	1	136.274	18.590
Error	20	45.835	52.615

(10%) $P<0.10$

APPENDIX TABLE A-12

DIGESTIBILITY DATA

A. Preliminary period*

Cow	D.M. Intake (kg)	Faecal D.M. Excretion (kg)	D.M. digested (kg)	Digestibility
7	125.03	30.483	94.542	75.6
8	128.94	32.876	96.066	74.5
77	------	------	------	----
78	129.75	31.06	98.69	76.1
105	124.01	31.47	92.54	74.6
106	124.61	29.97	94.65	76.0

* All data is based on twelve days collection.

B. Experimental period*

Cow	D.M. Intake (kg)	Faecal D.M. Excretion (kg)	D.M. digested (kg)	Digestibility
77	109.47	26.51	82.96	75.8
78	101.38	25.08	76.29	75.3
105	101.17	23.60	77.57	76.7
106	103.15	27.74	75.42	73.1
7	110.54	25.91	84.63	76.6
8	110.96	25.13	85.83	77.4

* All data are based on a ten day collection period, except for data for 78 which is based on a nine day period.

APPENDIX TABLE A-12 (cont.)

C. Post-experimental period*

Cow	D.M. Intake (kg)	Faecal D.M. excretion (kg)	D.M. digested (kg)	Digestibility
77	74.70	18.44	56.26	75.3
78	62.33	16.38	45.95	73.7
105	71.20	17.61	53.59	75.3
106	72.56	18.51	54.05	74.5
7	76.18	19.16	57.02	74.8
8	77.64	18.47	59.17	76.2

* All data is based on a seven day collection period.

APPENDIX TABLE A-13
CRUDE NITROGEN BALANCE DATA

A. Preliminary Period*

Cow N sources	77	78	105	106	7	8
Faecal-N	-	107.0	120.0	95.6	108.6	126.1
Urine-N	-	238.3	185.9	182.4	206.8	186.7
Milk-N	-	58.5	62.6	57.3	63.4	68.2
Feed-N	-	459.0	442.3	444.3	448.1	458.8
Retention	-	55.2	73.8	109.0	69.3	77.8

* All data are from a 10 day collection period and are expressed as gN/day for each source of nitrogen.

APPENDIX TABLE A-13 (Continued)

B. Treatment (Experimental) Period.*

Cow N-source	77	78	105	106	7	8
Faecal-N	131.2	101.6	113.5	135.4	123.8	119.6
Urine-N	268.0	252.2	217.4	235.3	271.2	249.6
Milk-N	65.5	64.0	68.2	62.6	67.3	74.0
Feed-N	545.8	509.0	516.2	507.9	554.7	550.0
Retention	81.1	91.2	117.1	74.6	92.4	106.8

* All data are from a 10 day collection period except for cow 78, which had a 9 day collection period. Data is expressed as gN/day for each source of nitrogen.

C. Post-treatment Period.*

Cow N-source	77	78	105	106	7	8
Faecal-N	112.0	107.2	108.9	107.6	123.7	115.8
Urine-N	192.4	180.6	193.1	186.7	195.2	173.0
Milk-N	54.2	52.3	58.0	53.0	57.3	58.1
Feed-N	413.9	355.4	394.9	405.3	426.6	435.3
Retention	55.3	15.3	34.9	58.0	50.4	88.4

* All data expressed as gN/day are based on a 7 day collection period for all cows.

APPENDIX TABLE A-14
COW WEIGHTS

COW	D A T E		
	1.11.69	16.11.69	8.12.69
7	718	741	754
8	711	723	751
77	691	694	728
78	796	798	782
105	684	686	709
106	668	693	707

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