

THE EFFECT OF FORMALDEHYDE TREATMENT
OF DIETARY PROTEIN SUPPLEMENTS ON
CATTLE GROWTH

by

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DEDICATION

The author wishes to dedicate this thesis to his beloved parents, The Honourable The Right Reverend Brimah Turay and Mrs. Alice Turay for the inspiration they gave him to study agriculture.

ABSTRACT

M.Sc.

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Animal Science
(Nutrition)

THE EFFECT OF FORMALDEHYDE TREATMENT OF DIETARY PROTEIN SUPPLEMENTS ON CATTLE GROWTH

A corn silage (CS) and high-moisture corn (grain) ration supplemented with either soybean meal (SBM) or rapeseed meal (RSM) significantly increased ($P < .05$) average daily gains (ADG) of cattle for the former, 1.13 vs. 1.02 kg, respectively (experiment I). A hay and barley ration supplemented with RSM or SBM resulted in non-significant differences ($P > .05$) in ADG of young cattle, 0.82 vs. 0.78 kg, respectively, with no significant differences due to formaldehyde (FA) treatment (1 g FA/100 g protein) (experiment II). Supplementing a CS and molasses ration with RSM (treated or untreated) in combination with urea, resulted in non-significant differences ($P > .05$) in ADG of steers (0.57 vs. 0.52 kg) due to FA-treatment, but a significant decrease ($P < .01$) in ADG due to urea supplementation (0.66 vs. 0.51 vs. 0.48 kg) (experiment III). A haylage-molasses ration supplemented with protein supplement (as experiment III) resulted in non-significant differences ($P > .05$) in ADG of steers (0.73 vs. 0.78 kg), either due to FA-treatment or due to urea supplementation (experiment IV). With sheep fed a CS and molasses ration supplemented with RSM (treated or untreated) with or without urea, nutrient digestibility was not significantly increased ($P > .05$) but nitrogen retention was significantly increased ($P < .05$) due to FA-treatment (experiment V). Lack of effect due to FA-treatment in these experiments might possibly be due to limitations in dietary energy.

RESUME

M.Sc.

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Zootecnie
(Nutrition)

EFFETS DU TRAITEMENT AU FORMALDEHYDE DES COMPLEMENTES PROTEIQUES ALIMENTAIRES SUR LE CROIT DES BOVINS

La complémentation d'une ration faite d'ensilage de maïs (EM) et de maïs grain humide par du tourteau de soja (TS) a donné un gain moyen quotidien (GMQ) significativement meilleur ($P < 0.05$) chez des bovins que la complémentation au tourteau de colza (TC): 1.13 contre 1.02 kg (exp. I). Par ailleurs, ces deux tourteaux n'ont pas provoqué de différences de GMQ significatives ($P > 0.05$): 0.82 contre 0.78 kg chez des jeunes bovins recevant une ration de vase foin-orge, pas plus que le traitement de ces compléments protéiques au formaldéhyde (FA), à raison de 1 g/100 g de protéine (exp. II). De même, le traitement au FA n'a pas accru significativement ($P > 0.05$) le GMQ (0.57 contre 0.52 kg) de bouvillons nourris d'un régime EM-mélasse complémenté de TC avec ou sans urée. D'autre part, l'apport d'urée a déclenché une baisse significative ($P < 0.01$): 0.66 contre 0.51 contre 0.48 kg (exp. III). Ni le traitement au FA, ni l'apport d'urée n'ont entraîné de différences significatives ($P > 0.05$) de GMQ (0.73 contre 0.78 kg) chez des bouvillons recevant une ration ensilage mi-fané et mélasse, complémentée comme à l'essai III (exp. IV). Chez des moutons soumis à un régime EM-mélasse avec TC, le traitement par FA a causé un accroissement non significatif ($P > 0.05$) de la digestibilité des nutriments, mais un accroissement significatif ($P < 0.05$) du taux de rétention de N (exp. V). L'absence d'effet de FA dans ces essais pourrait s'expliquer par la teneur énergétique restreinte des rations.

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I. INTRODUCTION

The prevention of malnutrition throughout the world through the provision of adequate diets for a rapidly expanding population is a task of staggering proportions. Solution of this problem is perhaps the greatest challenge facing nations of the world today. In the developing countries, it was estimated by FAO (1968) that at least 20 per cent of the population was undernourished and about 60 per cent received diets inadequate in nutritional quality. Protein malnutrition could therefore be a widespread nutritional problem.

One of the most complex aspects of the world food problem is the provision of sufficient quantities of proteins of adequate nutritional quality to foster good health and prevent protein malnutrition. Man traditionally has balanced his diet consisting largely of plant materials with foods of animal origin which contain essential amino acids, as well as fats, minerals, and vitamins. The unique and important contribution that animals can make to the world food supply is often underestimated or even overlooked by world food planners, presumably because of the belief that increases in animal production can be made only by diverting to animals, foods that otherwise would be eaten by people. This approach ignores the fact that livestock consume great quantities of feed such as forages, wastes, and by-products that cannot be eaten by man. Whether or not grains are fed to livestock

generally is a question of economics and when it is profitable to do so, livestock producers feed grains; when it is not, they utilize other feedstuffs. Animals can use a wide range of agricultural and industrial wastes and by-products. Unfortunately, many of these feedstuffs are not now being utilized. Residues from the production of food grains such as corn, wheat, rice, sorghums, oats, barley, make useful feeds. Vegetable wastes, sugar beet by-products, rapeseed meal, soybean meal, cottonseed meal, gin wastes, extracted sugar cane, spent brewery grains, molasses, are suitable for animal feed.

Rapeseed is widely grown in Canada, and although primarily used for oil production, a high protein meal is produced as a by-product. It is being used in Canada as a protein supplement for livestock and has also been fed elsewhere in the world for many years. The fact that there has been less research done on the feeding of rapeseed meal to ruminants than to non-ruminants is probably due to the fact that fewer problems have arisen in the case of the former. Since there appears to be no report of thyroid disorders and no indication of significant changes in milk produced by cattle fed rapeseed meal supplemented diets, it can be assumed that the glucosinolates present in rapeseed meal are effectively destroyed during ruminant digestion. A limitation on the use of rapeseed meal for beef cattle feeding can be its palatability. The flavor of rapeseed meal is apparently associated with its glucosinolate content but can be offset by using molasses or silage in the diets.

Protein supplements in beef rations usually form a very significant portion of the total feed costs. This awareness in the cost of protein supplements has led research workers in Australia, England and North America to find ways of efficiently utilizing these proteins in cattle rations. Several methods have been developed to increase rumen "by-pass" of these proteins in order to minimize protein wastage in this organ. Among these methods is the use of formaldehyde treatment which was first developed by Australian research workers. This compound will form cross-linkages with the epsilon amino group of the amino acids, thus rendering them insoluble in the rumen. The protection should usually be such that the post-ruminal digestibility and nutritive value of the proteins is not affected. So far, the experimental data reported in the literature have not indicated a consistent animal response in terms of improved weight gains due to protein treatment.

The experiments reported herein were conducted in order to determine the value of rapeseed meal as a protein source for growing cattle, and to examine the effect of formaldehyde treatment of protein meals on the growth response of cattle.

II. LITERATURE REVIEW

A. Nitrogen Metabolism in the Rumen

1. General

Many aspects of nitrogen metabolism in the rumen have received considerable attention and the topic has been reviewed several times (Chalupa 1968, 1970; Blackburn 1965; Allison 1969).

Based on the early findings of Cuthbertson and Chalmers (1950) that casein added to the duodenum was more efficiently utilized than casein added to the rumen, the concept was developed that the digestion in the rumen of proteins of a high Biological Value (proteins of animal origin) could be an essentially wasteful process because these proteins were lowered in protein quality due to conversion to microbial protein as a result of the rumen fermentation.

On the other hand, the rumen fermentation process can be useful in upgrading the value of proteins with a lower Biological Value (those of plant origin) through production of microbial protein. In both cases, because rumen microorganisms need a source of nitrogen for growth and production of microbial protein, it is necessary that nitrogen be supplied in the host ruminant's ration. The fact that these rumen microorganisms are able to synthesize various amino acids and incorporate them into microbial protein gives them great value to

the ruminant because amino acid deficiencies of dietary origin can be corrected. Furthermore, the ability of the rumen microorganisms to use nitrogen from non-protein nitrogen sources like urea for microbial protein synthesis is of great advantage to the ruminant animal.

2. Amino acid requirements of ruminants

The specific amino acid requirements of ruminants have been reported to be similar to those of non-ruminants and the list includes histidine, phenylalanine, valine, threonine, lysine, methionine, arginine, leucine, isoleucine and tryptophane (Black et al. 1957; Downes 1961). Amino acid requirements of ruminants should ultimately be examined in terms of amino acids absorbed from the gastro-intestinal tract in relation to the amino acids required for productive purposes (Chalupa 1972). This will mean that a pattern of essential amino acids similar to that required for productive purposes must become available by absorption from the small intestines. The amino acids available for absorption are supplied by microbial protein synthesized in the rumen, undegraded feed proteins which bypass the rumen and endogenous secretions. Quantitatively, the precise requirements for each individual essential amino acid will depend on the physiological and nutritional status of the animal, and be determined by the nature and amount of protein being synthesized. With these concepts in mind, it becomes important to emphasize that due to the extensive degradation of dietary proteins in the rumen, specific amino acid requirements cannot be directly determined by feeding experiments.

Numerous factors can influence the absorption of amino acids from the gastro-intestinal tract of the ruminant animal. Among them is the portion of the protein in the ration that is degraded and converted to microbial protein. This portion of amino acid supply is important because Weller (1957) was able to show that for a wide range of diets, bacterial protein had almost a constant amino acid composition, and protozoal protein was richer than bacterial protein in certain essential amino acids, especially lysine, and also had a higher true digestibility (Johnson et al. 1944; McNaught et al. 1954; Bergen et al. 1968). Other factors will include the presence of undegraded feed protein which bypasses the rumen and endogenous secretions.

The fact that deficiencies of one or more amino acids may limit productivity in the ruminant animal is indicated by alterations in plasma amino acid profiles associated with urea feeding. For example, low plasma concentrations of valine, leucine, isoleucine, phenylalanine and methionine and increased levels of serine and glycine have been reported by Chalupa (1968, 1970), Virtanen (1966), Oltjen (1969) and Jacobson et al. (1970). In addition, positive responses have been obtained from post ruminal administration of amino acids and proteins (Broderick et al. 1970; Hartfield 1970; Nimrick et al. 1970).

Determination of amino acid requirements and of possible amino acid deficiencies or imbalances is complicated in ruminants by the

varying degrees of microbial modifications of dietary proteins and amino acids. In general, dietary supplements of amino acids have not produced consistent positive responses in terms of weight gains or retention of dietary nitrogen (Chalupa 1968; Fontenot 1970; Moore et al. 1970; Nelson 1970). Direct abomasal administration of proteins or amino acids thus provides information about amino acids that may be limiting animal productive response. The requirements are usually better considered from the point of view that muscle growth, reproduction and wool growth will have varying amino acid requirements and that responses to post ruminal administration of a particular amino acid may vary depending on the physiological function required by the animal. Chandler (1970) calculated the amino acid balance for production of body proteins in steers fed high silage rations and found that none of the amino acids was calculated to be in negative balance, but he suggested the following rank in order of importance in the limitation of growth: isoleucine, lysine, methionine, arginine, threonine, valine, histidine, phenylalanine, tryptophane and leucine. Oltjen et al. (1970) reported that the abomasal infusion of a mixture of valine, isoleucine and phenylalanine increased the plasma concentration of these amino acids and increased the nitrogen retention of urea fed cattle to essentially the amount retained by cattle fed a soy protein purified diet. Just one gram of nitrogen from the infused amino acids improved the utilization of other absorbed amino acids. These results indicate that ruminants fed large quantities of non-protein nitrogen may retain less dietary nitrogen not only because of

a deficiency of certain essential amino acids but also because of an excess of certain non-essential amino acids (Chalupa 1972).

The protein requirements of lactating dairy cows are much higher than those of lactating beef cows of comparable size because of higher rates of milk production in the dairy cows (Muhrer et al. 1964; NRC 1970). Therefore, lactating dairy cows may be more likely to encounter a shortage of amino acids than other ruminants. Chandler (1970) calculated an amino acid balance considering only the amino acids required for milk protein production and ranked them in order of limiting milk production as follows: methionine, valine, iso-leucine, tryptophane and lysine. Other workers have reported lowered plasma levels of methionine and histidine to be associated with reduced milk production in lactating dairy cows (Jacobson et al. 1970; Virtanen 1966).

3. Degradation of proteins, amino acids
and non protein nitrogen in the
reticulo-rumen

Research data have accumulated over the years on ruminant productive functions, intermediary metabolism and digestion, and the indication is that while ruminants are highly efficient in obtaining energy from the fibrous components of plant cell walls, they have a low efficiency in utilizing high protein diets (Church 1969). The nitrogenous compounds presented to the rumen are quite variable and will normally include different proteins which markedly differ in amino acid content and solubility (Church 1969; Chalupa 1970a).

The degradation of non-protein nitrogenous compounds, proteins and amino acids is brought about by rumen microorganisms as neither proteolytic nor ureolytic enzymes are secreted by the rumen epithelial cells (MacDonald 1968; Helmer and Bartley 1971). MacDonald (1952), Annison (1956) and Lewis (1961), have reported that the proteolytic activity of rumen bacteria and protozoa will depend on the solubility of dietary proteins. Deaminase activity in the rumen will increase as the protein content of the diet increases (Warner 1956), which means that despite the high proteolytic activity in the rumen, deaminases found in some species of rumen microorganisms help to keep the pool size of free extra-cellular amino acids in the rumen fluid low (Annison 1956).

El-shazly (1952) was the first to report that degradation of proteins by rumen microorganisms resulted in the production of branched-chain volatile fatty acids and higher fatty acids as indicated by a correlation between ammonia concentration and concentration of isobutyric acid and of the six-carbon acids. Reticulo-ruminal catabolism of nitrogenous compounds has been demonstrated to be affected by the pH conditions prevailing in reticulo-rumen. Church (1969) reported that the optimum pH for proteolysis and amino acid catabolism was between six and seven. The amino acids from proteins are either deaminated to yield ammonia and finally microbial protein or they may be ingested by protozoa. Deaminated amino acids give rise to volatile fatty acids, carbon dioxide and hydrogen (Church 1969), while

the hydrolysis of urea will yield ammonia and carbon dioxide (Chalupa 1972).

4. Production and fate of ammonia in the reticulo-rumen

The activity of rumen microorganisms on proteins, amino acids, urea and other non-protein nitrogenous compounds results in the production of ammonia and other by-products (Warner 1964; Blackburn 1965; MacDonald 1968). Ammonia production is dependent on the presence and activities of proper enzyme systems. Proteases, ureases, biuretases and uricases are probably present in many microbial species. Since deaminative activity is not as widespread as proteolytic activity (Annison 1956; Blackburn and Hobson 1960), proteins are more rapidly degraded than deaminated. Deamination, a property of some strains of rumen bacteria (Abou Akkada and Blackburn 1963) would also release ammonia from feedstuffs (Warner 1964). Ammonia is important because many rumen microorganisms use it as the primary nitrogenous nutrient for growth and protein synthesis (Bryant 1963; Hungate 1966). Its production seems to be correlated with active growth of rumen microorganisms, which agrees with the increase in ammonia production in the rumen after feeding when the increase in bacterial numbers is most rapid (Abou Akkada and Blackburn 1963).

Ammonia is primarily incorporated into bacterial cells and its nitrogen could also appear in protozoal cells as a consequence of ingestion of bacteria by protozoa (Allison 1969, 1970). Protein and

non-protein nitrogen sources could be used most effectively when they are utilized to produce an ammonia concentration in the rumen that would be optimal for microbial protein synthesis. A rapid ammonia production in the rumen often causes a net loss of nitrogen to the animal because the ammonia would be absorbed through the walls of the rumen, converted to urea in the liver, and excreted through the urine. Part of this urea could be recycled via the saliva or blood into the rumen and again become available for microbial protein synthesis. In the liver, ammonia is utilized in the synthesis of non-essential amino acids and urea (Chalupa 1968).

On the other hand, microbial growth may be limited by availability of nitrogen. A deficiency of ammonia in the rumen may limit microbial growth and consequently microbial protein production; whereas the elevation of rumen ammonia concentrations above 5-7 mg $\text{NH}_3\text{-N}/100$ ml of rumen fluid may result in losses of nitrogen and in some cases ammonia toxicity (Satter and Roffler 1973, 1975). The extent of ammonia absorption is dependent on the pH of the gastrointestinal tract components, which has an effect on the occurrence of the ionic or non-ionic forms of ammonia. The ionic form is more readily used for microbial protein synthesis, whereas the non-ionic form which is lipid-soluble and prevails under alkaline pH is preferably absorbed across the walls of the gastro-intestinal tract (Hogan 1961; Bloomfield et al. 1963). The ammonia concentration gradient also plays a part in the absorption (Lewis et al. 1957; Hogan 1961).

5. Synthesis of microbial protein in the reticulo-rumen

The synthesis of microbial protein is a principal function of the rumen and its microorganisms. Loosli et al. (1949) were the first to report the synthesis of the essential amino acids by rumen microorganisms. They reported that the rumen material from sheep and goats fed purified urea rations contained nine to 20 times more amino acids than found in the diet. Duncan et al. (1953) confirmed that the amino acids were synthesized in the rumen of calves fed urea-containing diets. They further observed that as the animal grows and productive functions commence, the microbial biosynthetic capacity increases. Hume (1970a, 1970b) concluded that there was an increased production of microbial protein when a ration containing sufficient energy and urea was supplemented with protein. The synthesis of proteins by rumen microorganisms was found to increase with an increase in dietary protein or nitrogen and energy (Conrad et al. 1967a; Hume et al. 1970). Even in animals fed rations containing sufficient quantities of protein, Allison (1970) reported that an appreciable amount of amino acids in microbial protein are synthesized de novo from intermediates or end-products of carbohydrate fermentation, or end products of amino acid metabolism.

The mechanisms involved in the biosynthesis of microbial protein in the rumen have been reviewed by Allison (1969). In general, amination and transamination reactions have been considered as the major pathways in which rumen microorganisms assimilate ammonia.

(Allison 1969; Tsubota and Hoshino 1969; Chalupa 1970; Chalupa et al. 1970). Even though there are indications that other enzyme systems are involved, glutamate dehydrogenase has been considered the most important enzyme system for the initial fixation of ammonia to carbon skeletons (Allison 1969; Chalupa et al. 1970; Tsubota and Hoshino 1969). Enzyme systems like glutamate-oxaloacetate and glutamate-pyruvate transaminase are also important in the transfer of ammonia to other carbon skeletons. Other dehydrogenase and transaminase enzyme systems have also been considered important in the assimilation of ammonia by rumen microorganisms (Tsubota and Hoshino 1969). The carbon skeletons that are usually required for microbial protein synthesis will be expected to come mainly from the intermediates of the glycolytic pathway like phosphoenol pyruvate or carbon dioxide and acetate that are all produced as a result of carbohydrate fermentation (Chalupa 1969; Allison 1969; Tillman and Sindu 1969; Chalupa 1970). Besides this requirement for a source of carbon skeletons, requirements for isobutyrate, phenylacetate, idole-3-acetate, isovalerate, and 2-methylbutyrate in the synthesis of branched chain amino acids like valine, phenylalanine, tryptophane, leucine and isoleucine respectively, indicates that specific carbon precursors are needed (Allison 1969). Branched-chain volatile organic acids are present in the rumen mainly as a result of the degradation of dietary protein and deamination of branched-chain amino acids. Other potential sources are the desquamation of the rumen epithelium (Phillipson 1964), salivary secretions (Phillipson and Magnan 1959), the death and lysis

of bacterial cells by bacteriophage (Broderick et al. 1970) and unidentified factors (Johnson et al. 1944). The feeding of protein-free, urea diets can cause depressions in these acids (Chalupa et al. 1970; Oltjen 1969; Oltjen and Putman 1966), and isovalerate and isobutyrate seem to be influenced the most. The addition of various combinations of these volatile organic acids to the diet of urea-fed animals has resulted in an increase in the synthesis of microbial protein (Hume 1970), in some instances has increased retention of dietary nitrogen (Cline et al. 1966), and in other cases had no effect on nitrogen retention (Chalupa 1970; Oltjen et al. 1971). When the sodium salts of these volatile organic acids were added to a soy protein purified diet and fed to cattle, Oltjen et al. (1971) obtained a significant increase in nitrogen retention. Many cellulolytic bacteria have also been reported to require higher volatile organic acids for growth (Hume 1970; Oltjen et al. 1971), but when Oltjen added them to a urea or soy protein purified diet, they could not find an increase in the numbers of such bacteria, nor was there an increase in the utilization of dietary cellulose. Thus, while a deficit of the higher volatile organic acids may be preventing maximal production of microbial proteins, other pit-holes could be apparently involved. For example, Chalupa (1968) reported that there was a requirement for a variety of minerals for growth and metabolism of rumen microorganisms. He based this report on the fact that if other dietary constituents remain constant, substituting urea or other non-protein nitrogen compounds for protein will remove a major source of calcium and

phosphorus from the diet. However, Oltjen et al. (1965), Clark et al. (1970) met with little success when trace minerals like manganese, zinc, iron and copper were added to urea containing diets and fed to cattle. The requirements for sulphur, especially in rations containing non-protein nitrogen, have been reported in the literature. This is because the sulphur is used in the synthesis of sulphur containing amino acids and possibly other compounds. For example, Gall et al. (1951) observed an increase in the microbial population when a low sulphur ration was supplemented with sulphate. The sulphate was first reduced to sulphide before being incorporated into the sulphur amino acids of bacterial protein in vivo (Block et al. 1951; Anderson 1956), in vitro (Anderson 1956; Henderickx 1961a, 1961b), and by pure cultures of rumen bacteria (Emery et al. 1957). Conrad et al. (1965) confirmed that methionine was synthesized in the rumen of dairy cows by incorporation of S^{35} into the amino acid molecule. They also found that methionine synthesis was increased when the dry matter and nitrogen intakes were increased or when protein degradation in the rumen was reduced to a minimum (Conrad et al. 1967a). Further evidence on the role of sulphur in increasing rumen microbial protein is provided from studies by Hume and Bird (1970) and Whanger (1972). They reported that microbial protein production was influenced by amount but not by the form in which the sulphur was supplemented (i.e., cystine vs. sulphate).

Many investigators have attempted to quantify the amount of microbial protein synthesized in the rumen, but this quantification may not be considered to be most accurate. Pitman and Bryant (1964), Blackburn (1965), MacLaren et al. (1965), Cline et al. (1966), Wright (1967), Allison (1969), Hume (1970a, 1970b), Huber and Thomas (1971), Oltjen et al. (1971), Van Horne and Jacobson (1971) have stated generally that the extent to which nitrogen of dietary origin is converted to microbial protein will depend on factors such as:

- (a) The plane of nutrition and the nature of dietary constituents.
- (b) The rate at which the dietary protein or the non-protein nitrogenous products are broken down to amino acids and ammonia.
- (c) The rate of rumen fermentation of organic matter and the rates of absorption of amino acids and ammonia through the rumen wall.
- (d) The rate of passage of digesta from the rumen to the abomasum.

Nitrogen utilization for microbial protein synthesis has been reported to be linearly related to digestible energy input (Conrad and Hibbs 1968). Chalupa (1968) reported that nitrogen utilization could be decreased in diets containing large quantities of readily fermented sugars such as molasses or large quantities of roughage carbohydrates. This is because the utilization of dietary protein may be influenced by the proportions of bacteria and protozoa. The magnitude of the protozoal population can vary depending on the type of diet fed. For example, in high roughage diets, the protozoal

biomass is approximately equal to that of bacteria, but feeding all concentrate, purified, or ground and pelleted diets can produce rumen conditions that will decrease or completely void the rumen of protozoa (Chalupa et al. 1965; Chalupa et al. 1970b; Oltjen 1969). Faunated animals have been reported to retain more dietary nitrogen than those without protozoa (Abou Akkada and El-Shazly 1965). Therefore the source of energy is very important in the efficient production of microbial protein in that readily fermented carbohydrates are needed. Traditionally starch is considered to be the best carbohydrate for ammonia assimilation by rumen microorganisms (Chalupa 1968).

Hungate (1966) has indicated that the extent of protoplasm synthesis depends not only on the amount and nature of dietary constituents which can be incorporated into cells, but also on the usable high energy compounds that can be derived from the substrate. Walker (1965) and Hungate (1965, 1966) stressed the fact that the rumen system is anaerobic which places, in theory at least, a limitation upon the maximum possible conversion of dietary nitrogenous material to microbial cellular material. Anaerobic fermentation would take place with, as Hungate has calculated, a maximum cell yield of 15 per cent of the substrate fermented which is equivalent to 9.84 per cent of protein yield. Assuming a protein yield of 9.84 per cent in material leaving the rumen, either by direct absorption or by passage, the animal has digestible protein and energy available in the ratio of 18.3 : 1 when protein is expressed in grams and energy in megacalories.

Similarly, Purser (1970) also indicated that 18.3 g of digestible microbial protein could be produced per digestible megacalorie, and the experiments which he reviewed have given indications of cell yields that are greater than those considered theoretically. Miller (1973), making some assumptions, estimated that a yield of 7 g of microbial nitrogen per megacalorie fed would be expected (i.e., an equivalent of 38.15 g protein per megacalorie fed, using a factor of 5.45 instead of 6.25 to calculate protein (Reichl and Baldwin 1975)). In soy, zein, casein and gelatin supplemented diets, Little et al. (1968) estimated that 97, 109, 89 and 85 per cent, respectively, of the dietary nitrogen passed through the abomasum of sheep, and of this, 60, 74, 63 and 60 per cent, respectively, was protein. While Potter et al. (1969) reported that approximately 20 per cent less protein reached the abomasum of steers fed a urea supplemented ration as compared with a soy supplemented ration. Even though analytical methods are used to distinguish and determine bacterial, protozoal and feed protein nitrogen, it still remains to be determined in the above reported data whether these differences in quantity of protein reaching the abomasum were due to rumen by-pass of unaltered dietary protein, decreased synthesis of microbial protein, or a combination of both. This is because the mixture of amino acids and/or nitrogen reaching the lower portions of the gastro-intestinal tract consists of protozoal, bacterial and undegraded feed proteins.

Purser (1970a) has reported that the amino acid composition of microbial protein differs slightly depending on whether it is bacterial

or protozoal. He further reported that protozoal protein contains slightly higher quantities of certain essential amino acids like lysine, leucine, phenylalanine and tyrosine than bacterial protein. Supporting this reported work is earlier work by Johnson et al. (1954), McNaught et al. (1954) and Bergen et al. (1968a) that the quality of microbial protein suggests that protozoal protein has a slightly higher biological value than bacterial protein because of its higher true digestibility and net utilization. Even though the type of diet fed to ruminants has been reported to affect the proportions of bacteria and protozoa in the rumen (Chalupa et al. 1965, 1970b; Oltjen 1969), there seems to be, however, no appreciable effect of diet on the amino acid composition of rumen bacteria or protozoa (Weller 1957). Nucleic acid nitrogen is also produced in substantial amounts during microbial protein synthesis and Smith (1969) reported that per unit of dietary nitrogen that is incorporated into microbial protein, 20 per cent is converted to nucleic acids while 80 per cent is converted to bacterial protein or amino acids. Smith (1969) further reported that duodenal ingesta will usually be comprised of 8 to 13 per cent nucleic acids of microbial origin, and approximately 40 to 50 per cent of the microbial nucleic acid nitrogen is either not absorbed from the gut but excreted as allantoin in the urine, or re-enters the urea pool. This suggests that nitrogen from nucleic acids is of little use to the ruminant animal. In general, even though the amino acid composition of rumen microorganisms is relatively constant (Purser and Buechler 1966; Williams and Dinusson 1973)

differences in the release and availability of specific amino acids has been reported to exist (Bergen et al. 1967; Purser 1970a).

B. Prevention of Protein and Amino Acid Degradation in the Reticulo-rumen

1. General

Many research workers have tried several methods to prevent the degradation of proteins in the reticulo-rumen. Among these methods are the reduction of the rate of microbial activity in the rumen (Harbes et al. 1962; Hoshino 1965), rumen bypass (Colebrook and Reis 1969; Amos et al. 1970a, 1970b; Downes et al. 1970; Moore et al. 1970; Oltjen et al. 1970), the encapsulation of proteins and amino acids (Neudorffer et al. 1971), the heat treatment of proteins and/or amino acids (Chalmers et al. 1964; Danke et al. 1966; Hudson et al. 1970) and the chemical treatment of proteins and/or amino acids in such a manner that rumen degradation is decreased but their post-ruminal digestibility or nutritive value is not impaired (Fergusson et al. 1967; Reis and Tunks 1969; Driedger and Hartfield 1970a, 1970b; Driedger 1970; Leroy and Zelter 1970; Peter 1970; Faichney 1971). In all of the above methods, the literature has so far presented contradicting results as far as significant improvement in animal growth performance is concerned. On the other hand, these methods have in general consistently reduced ammonia production in the rumen and increased the flow of proteins or amino acid nitrogen to the intestines and have increased nitrogen retention.

Ideally, diets containing protected proteins and/or amino acids should still generate enough rumen ammonia concentration to permit maximum microbial growth. Supplementation of the diets with urea may be required to achieve this end. The possibility also exists that some unprotected protein may be required as indicated by experimental data reported by Hume (1970), showing increased microbial protein production when the diet contained protein as compared with a diet with urea only, a situation which is comparable to a diet where all the protein is protected. This assumes that all other requirements of rumen micro-organisms like fermentable energy, sulfur, etc., will be met by diets containing protected proteins. The protected protein will then serve as an additional source of protein in "excess" of microbial protein reaching the abomasum and small intestines. This means that a larger total amount of protein will be available to the animal. If microbial protein production is not impaired and a significant amount of dietary protected protein escapes rumen proteolysis, the efficiency of the process in terms of the percentage of the nitrogen intake retained by the animal would still be expected to vary considerably depending on the biological value of the protected protein. This in turn will determine the final amino acid pattern of the absorbed protein, that is protected plus microbial protein. Therefore, the amino acid pattern of the absorbed protein will depend basically on how "diluted" the protected protein is by microbial protein because Purser et al. (1966) and Burris et al. (1974) have reported that microbial protein composition is quite constant.

2. Chemical methods

Various chemicals have been used to cause the reduction of degradation of dietary proteins by rumen microorganisms. Among these chemicals are tannins, glyoxal, acetaldehyde, butyraldehyde, glutaraldehyde, propionaldehyde and formaldehyde. The basic mechanism of action of these chemicals with the proteins involves the formation of Schiff's bases or methylene bridges and other cross-linkages between the epsilon amino groups of the proteins and the chemicals (Walker 1964). The protein complex formed is relatively stable at the near alkaline pH of the rumen but readily decomposes at the highly acid pH found in the abomasum.

a. Tannins

Evidence that tannins could be used in the protection of dietary proteins against rumen degradation was first demonstrated by Zelter and Leroy (1966). Using 13% and 23% tannin solutions to treat soybean meal and groundnut meal, they found this treatment to be effective in protecting the proteins during a 15-hour incubation period in an "artificial rumen." At a lower level of 6% tannin solution the tannin did not give complete protection to the protein. Delort-Laval and Virben (1969) observed a decrease in ammonia production in an "artificial rumen" when they treated skim milk and soybean meal with tannin or formaldehyde. They also further observed a reduced total and available lysine due to tannin treatment. With this information on the use of

tannins in the reduction of protein degradation in vitro, several workers carried out in vivo studies to measure the effect of tannin treatment of dietary proteins on actual animal performance. Delort-Laval and Zelter (1968), treating linseed meal and peanut meal with tannins, were able to increase slightly the efficiency of nitrogen utilization in experimental animals. In studies with 120 steers, Hartfield, Driedger and Garrigus (1972) showed that tannic acid treated soybean meal was a superior source of protein compared with untreated soybean meal and urea. Driedger and Hartfield (1970a, 1970b), in further studies with soybean, were able to confirm reports by earlier research workers that tannic acid treatment of the protein resulted in decreased ruminal degradation of the protein while post-ruminal degradation was not affected. Driedger (1970) further reported several trials on the effects of tannin-treatment of soybean meal. He reported better feed per gain ratio and increased nitrogen retention for treated over untreated soybean meal. Treating soybean meal with tannins, Driedger and Hartfield (1972) reported greater average daily gains, feed efficiencies, nitrogen utilization and nitrogen balance in lambs compared with lambs receiving the untreated soybean meal. Leroy and Zelter (1970) studied the effects of tannin treated proteins on the metabolism of the rumen. They fed sheep with soybean meal or groundnut meal that was treated with tannins with the treated material accounting for 70 per cent of the total protein ingested. They found tannins to protect significantly the protein from degradation but had little effect upon the digestibility of organic matter, cellulose and

nitrogen-free extract of the feed, with an overall decrease in soybean meal and groundnut meal protein digestibility of nine per cent.

In general, the data presented on tannin treatment of dietary proteins has indicated a consistent increase in nitrogen retention, a non-significant effect on dry matter digestibility and in some cases improved average daily gains and feed efficiency when compared with untreated protein supplements (Driedger 1970). The method, therefore, has a potential for decreasing rumen degradation of dietary proteins.

b. Formaldehyde

Extensive studies have been carried out in Australia and elsewhere on the use of formaldehyde to treat protein supplements in ruminant rations as a method of decreasing their rate of degradation in the rumen. This use of formaldehyde in the protection of dietary proteins from rumen degradation has been based on the fact that 0.6 to 2.0 per cent of the bound formaldehyde will make a significant reduction in the solubility of the proteins, thereby making them highly resistant to microbial degradation in the rumen, without a significant reduction in their post-ruminal digestibility (Annison 1972). Fergusson *et al.* (1967), treating casein with 10 volumes of a solution containing 4% formaldehyde, were able to show that none of the casein was degraded in a six-hour in vitro incubation period. Their further work with sheep fed with a ration supplemented with formaldehyde-treated casein demonstrated an increase of 70 per cent in wool growth. In general, most Australian workers and other workers

have used increased wool growth and nitrogen retention in sheep as an index of the nutritive value of formaldehyde-treated casein (Barry 1972; Faichney 1971; Hemsley et al. 1973; MacRae et al. 1972; Reis and Tunks 1969; Wright 1971). This apparent positive response in wool growth could be attributed to the high Biological Value of casein and the requirements of specific amino acids for wool growth. This observation contrasts to the rather inconsistent animal growth response (Colby and Tollert 1973; Faichney and Davids 1972; Ghosh et al. 1971; Hartfield 1973; Nimrick et al. 1972; Nishimura et al. 1972) when plant proteins treated with formaldehyde are fed, which could be partly attributed to their relatively lower Biological Value. Favourable effects of formaldehyde-treated proteins on other aspects of ruminant production have not been precisely reported, and the paucity of published comments in this area is less a reflection of lack of interest than a failure to achieve worthwhile responses.

The expectation under practical feeding conditions, has been that formaldehyde treatment of dietary proteins would result in an improvement in the efficiency of utilization of dietary proteins. This improvement in efficiency could come about either by allowing a similar level of production with less dietary protein or by increasing production with the same amount of protein. This expectation has not generally been fulfilled. In a few of the publications that have reported what could be considered a positive animal growth response, the following points should be noticed:

- (i) A basal diet without protein supplement (treated or untreated with formaldehyde) is not generally included among the treatments studied, thereby making it difficult to calculate the improvement in utilization of supplemental nitrogen attributable to formaldehyde treatment. Only work by Wright (1971) is an exception and from his data it was estimated that formaldehyde treatment of casein resulted in a significant improvement in the percentage of casein nitrogen apparently retained as body tissue (from 14.2 to 26.6 per cent for untreated compared with treated casein respectively).
- (ii) The basal diet is generally a concentrate.
- (iii) The proportion of dietary protein supplied by the formaldehyde-treated or untreated nitrogen source is high, usually 33 per cent or more.
- (iv) Growth response has also not been high. Wright (1971) fed lambs at an average daily protein intake of 190, 233, and 281 g for the control, casein untreated and casein treated groups respectively, and had small improvement in growth response. Similarly, Faichney (1971) fed 45 per cent more protein to lambs when compared with NRC (1968), requirements and calculations showed a much lower growth of lambs that could be expected from the tissue nitrogen retention figures presented (approximately only 23 per cent of the theoretical gain). This poor relationship between nitrogen retention and body growth has been observed by many research workers like Nimrick et al. (1972), Rettary and Joyce (1970).

Even though there are no clearly defined effects of formaldehyde treatment of dietary proteins on animal growth response, various parameters measured that have given consistent results include:

- (i) A decrease in the rate of ammonia release both in vitro and in vivo, which indicates adequate protection of the proteins from microbial degradation (Fergusson et al. 1967; Reis and Tunks 1969).
- (ii) A reduction in the level of blood urea nitrogen which could be interpreted to be an indication of efficient utilization of dietary protein. This is because Schmidt et al. (1974) and Faichney (1972) and other research workers have reported that elevated blood urea nitrogen does not only reflect rumen ammonia concentrations but also an inefficient extra-ruminal utilization of proteins of poor Biological Value.
- (iii) A reduction in the level of urinary nitrogen in animals fed protected diets. This is because of the relationship between blood urea nitrogen and kidney excretion of urea that is dependent upon plasma urea levels (Faichney 1974).
- (iv) Some researchers have reported an improvement in the total nitrogen retention and this generally occurs in spite of the decrease in protein or amino acid digestibility associated with formaldehyde treatment. This is simply because of the reduction in urinary nitrogen (Faichney 1974).
- (v) An increase in the amount of crude protein flowing to the intestines (Faichney and Weston 1972; Faichney 1972). This was

associated with the increased amounts of individual amino acids reaching the small intestines (Faichney 1974).

- (vi) An increase in voluntary feed intake (Weston 1971) and this was attributed to an improved protein status of the animal (Ørskov et al. 1973).

On the other hand, factors which have been consistent with formaldehyde treatment of dietary proteins but may not favour animal growth response include:

- (i) An increase in fecal nitrogen; a result of the overall reduction of protein and/or amino acid digestibility by formaldehyde treatment of proteins. The magnitude of the increase in fecal nitrogen appears to decrease with proteins of a high Biological Value like animal proteins (Chalupa 1975).
- (ii) The concentration of volatile fatty acids in the rumen has been reported to be lowered. This may be indicative of a general depression of bacterial activity and could result in reduced rumen digestion of non-protein dietary components. The reduction in volatile fatty acids concentration could result from lack of availability of substrate for rumen microorganisms of formaldehyde-treated proteins (Faichney 1972).
- (iii) Increased plasma concentration of the amino acid formyl-N-methyl-lysine (Faichney 1974; Reis and Tunks 1973). This increase in the amino acid has been associated with a decrease in the relative and often absolute amounts of plasma lysine,

which could possibly produce a relative deficiency of the latter amino acid (Faichney 1974). Wachira et al. (1974), however, concluded that reduced rat growth by 10 to 20 per cent observed with formaldehyde treatment of casein could not be attributed to reduced lysine availability.

- (iv) Abnormally high incidences of bloat have been apparently associated in some experiments with the formaldehyde treatment of the protein (Faichney and Davies 1973; Sharma et al. 1972) or diet (Faichney and Davies 1973).

Other aldehydes like gluteraldehyde, butyraldehyde, acetaldehyde, propionaldehyde, and glyoxal, have been reported at least in in vitro studies to protect the dietary proteins and decrease their solubility (Zelter et al. 1970; Peter et al. 1970b). Peter et al. (1970a) reported that glyoxal or formaldehyde treatment of soybean meal improved weight gains and feed conversion of lambs. Nimrick et al. (1972) reported increased nitrogen retention in growing lambs fed fish meal treated with glyoxal.

3. Heat treatment

The mechanism of action in this method involves the slight denaturing of the proteins which results in the decrease of their degradation in the rumen. Chalmers et al. (1954) reported that heat-treatment of casein decreased the rate of breakdown in the rumen, reduced ammonia production and increased nitrogen utilization by sheep. With the heat-treatment of groundnut meal, Whitelaw et al. (1961)

reported increased nitrogen retention and growth in calves. Tagari et al. (1962), working with soybean meal, and Sherrod and Tillman (1962), working with solvent extracted soybean meal and cottonseed meal, reported increased nitrogen retention by sheep. Feeding groundnuts (peanuts) to lactating goats and heat processed fish meal (which is usually resistant to ruminal proteolysis) to lactating sheep, Chalmers et al. (1954) and Chalmers and Marshall (1964) respectively found an increase in milk production and nitrogen retention. Danke et al. (1966) subjected cottonseed meal to heat for varied periods of time and their general conclusions were that there was a critical range at which positive responses could be seen. Beyond this critical range, the susceptibility of the protein to ruminal degradation was still reduced but there was a marked reduction in the digestibility of the product, which made the heated material inferior to the unprotected material. Working with sheep, Hudson et al. (1970) reported that heat-treatment of soybean meal resulted in better ruminal and post-ruminal nitrogen utilization by sheep. All of these workers were able to recognize the fact that the main effect on heat treatment was to reduce protein and/or amino acid degradation and thus ammonia formation in the rumen.

4. Rumen bypass studies

It has been shown with sheep that the rate of wool growth can be strongly influenced by the total feed intake but not by the variations in the protein content of the diet, especially when it

contains readily degraded proteins and/or amino acids (Fergusson 1959). This is because of the influence rumen microorganisms have in modifying feed proteins. With the infusion of casein into the abomasum, Reis and Schinckel (1963) were able to demonstrate an increase in wool growth. The results in the two research reports above were explained by Hogan and Weston (1967b) when they used two of Fergusson's diets containing 8% and 20% crude protein in sheep diets and had an approximately similar output of protein from the rumen, that is 8.8 and 8.1 g N/day respectively. From these experiments, it would seem that the output of microbial protein from the rumen is not determined by the input of feed protein, but chiefly by the solubility of the protein and the amount of energy available to the microorganisms from the fermentation of organic matter in the rumen.

Therefore, depending on the solubility and Biological Value of the protein, rumen bypass procedures could be useful techniques in the study of utilization of proteins for animal growth performance. Most research data have consistently reported an increase in nitrogen retention when the rumen is bypassed. Reis and Schinckel (1961) reported increased nitrogen retention when casein was administered through the abomasum in sheep. Chalmers et al. (1954), studying the effects of administering casein to pregnant ewes by duodenal and ruminal fistulas, were able to show an increase in nitrogen retention and a decrease in urinary nitrogen excretion in those ewes given casein through the duodenal fistula. Comparing abomasal to oral

supplementation of small amounts of casein and sulphur amino acids (both methionine and cystine), Reis and Schinckel (1964) observed a large increase in both wool sulphur content and growth. Devlin and Woods (1965) obtained an improvement in nitrogen retention of steers when lysine was infused into the abomasum, and Little and Mitchell (1967), administering soybean protein abomasally as compared with orally, showed higher nitrogen retention in sheep. Reis (1967), studying the effects of abomasal administration of sulphur containing amino acids on wool growth, found that administration of 0.5 to 2.0 g L-cysteine or equi-molar levels of D-L methionine increased wool growth. Abomasal compared with oral feeding of methionine hydroxy analog (MHA) to sheep was found to improve wool growth (Reis 1970). Working with genetically low and high wool producing sheep, increased wool growth with abomasal infusion of sulphur amino acids has been reported by Williams et al. (1972); while Chalupa et al. (1972) in several experiments have consistently observed improved nitrogen retention in growing beef steers when casein or casein hydrolysates were abomasally infused to supplement feedlot type rations. They failed to observe any consistent benefit to abomasally infused methionine or other essential amino acids. Ørskov et al. (1969, 1970) used the novel technique of oesophageal groove closure to obtain ruminal bypass of dietary proteins. They obtained enhanced weight gains and feed efficiencies as well as substantially improved nitrogen retention.

5. Encapsulation of amino acids

Theoretically it should be possible to encapsulate proteins and/or amino acids with a material that will remain insoluble in the rumen but which will easily dissolve in the abomasum. In the encapsulation of a mixture of D,L-methionine, kaolin and stearic acid, one of the protective agents used was hydrogenated animal fat. The capsules were able to escape microbial degradation in the rumen but were broken down easily in the duodenum by the action of lipases and bile, thereby releasing the methionine (Neudeorffer et al. 1971). The ideal density of these capsules should be in the range of 1.0 to 1.4 (Sibbald et al. 1968) and this can be achieved by including varying amounts of kaolin and other materials. This is important because if the capsules float or sink to the bottom of the rumen liquor, the rate of passage of the capsules could be slowed down. The ideal situation is to have capsules that are large enough to avoid being engulfed by rumen protozoa, but small enough to be conveniently mixed in feed.

Grass and Unangst (1972) combined methionine, oleic acid, calcium carbonate and tristearin and were able to recover 89 per cent of the methionine in the rumen after 17 hours, while 92 per cent of the methionine was released in the lower digestive tract. Feeding 40 g of the encapsulated mixture to sheep increased their plasma methionine to 23 µg/ml compared with 5 µg/ml in the unsupplemented sheep (Grass and Unangst 1972).

Growth studies with encapsulated methionine have been reported in the literature by several workers. Sheep diets supplemented with 0.4% encapsulated methionine have been reported to increase weight gains and feed efficiencies in sheep compared with those on the unsupplemented diets (Mowat and Deelstra 1972). Steinacker et al. (1970) could not obtain a constant growth response to the feeding of encapsulated methionine to growing Holstein steers. Grass and Unangst (1972), supplementing diets containing 10.5% or 14% crude protein with 0.1, 0.2 or 0.4% encapsulated methionine in one experiment, and in a second experiment supplemented 16% and 10% crude protein diets with 0.8% and 1.2% encapsulated methionine. They reported that only sheep fed the 10% crude protein diet and 0.8% encapsulated methionine had significantly increased weight gains. The difference in weight gains may be due to the fact that the sheep in the first experiment may have received insufficient quantities of methionine, while those in the second experiment may have received quantities of methionine in excess of their body requirements. This was reflected by the low and increased plasma concentrations of methionine for the sheep in the first and second experiments respectively. Sibbald et al. (1968) and Broderick et al. (1970) were able to demonstrate that feeding encapsulated methionine to steers elevated blood plasma methionine levels and increased the valine to methionine ratio. Non-significant increase in milk composition, milk production and feed intake of cows fed 12 g/day of encapsulated methionine were reported by William et al. (1970) and Martz et al. (1970).

6. Reduction of enzymatic activity in the rumen

The reduction of the proteolytic activity of rumen micro-organisms without completely disrupting all the functions of the rumen is possible from a theoretical point of view. Attempts to accomplish this have so far not met with success, and published data on the manipulation of ruminal protease and deaminase activity are scanty. The nature of the diet does not appear to affect proteolytic activity, but deaminase activity may be influenced. Ramirez (1972), in support of the foregoing statements, found diminished deaminative activities in rumen microbes obtained from animals fed low-protein, molasses-urea diets.

The attempts of Hogan and Weston (1968) to control ruminal and/or deaminase activities with antibiotics did not produce encouraging results. Only transient effects on levels of rumen ammonia were obtained with neomycin, oxytetracycline and streptomycin. Penicillin and erythromycin decreased rumen ammonia concentration by 15 per cent but net loss of dietary nitrogen in the stomach was not reduced and reduced food intakes were suspected to occur at ad libitum feeding levels. Using oxytetracycline, Schelling et al. (1972) found that 1 g per day of the antibiotic had no general overall effect on rumen metabolism in sheep, and when added in combination with methionine and lysine, resulted in increased levels of these amino acids in the abomasum and plasma. Hoshino (1965) attempted to inhibit deaminative enzymes of the microflora but had only marginal success. Urease inhibitors

like barbituric acid (Harbers et al. 1962) and acetohydroxamic acid (Brent and Adepoju 1967) which might improve urea utilization by reducing the rate of urea hydrolysis through inhibition of rumen urease, have been used but met with little success.

7. Amino acid analogs

Another potential method for the rumen by-pass of amino acids is the structural manipulation of these amino acids. In addition to being absorbable from the small intestines, such analogs should have biological potency at the tissue metabolism level. Many workers have investigated several of these analogs but the one most investigated is methionine hydroxy analog (MHA), since methionine could be limiting in most ruminants' diets. There are many reports in the literature that have indicated the possibility of methionine and other essential amino acids to limit the performance of ruminants, especially when they are fed urea-supplemented rations. MHA is the calcium salt of methionine hydroxy acid and at the tissue level of metabolism it has been shown to have methionine activity in ruminant animals (Reis 1970; Belasco 1972), similar to that known to exist in non-ruminants. The supplementation of diets with MHA has shown inconsistent results. Gossett et al. (1962) fed 5 g and 10 g of MHA daily to steers for 207 days. The steers received a high-urea supplement with ground shelled corn and corn silage. They found the 5 g level of MHA to be of no benefit in daily gain, feed efficiency, and carcass values, while the 10 g MHA level gave a significant depression in rates of gain and

reduced feed intake. Hale et al. (1970a), adding MHA to a milo ration containing 90 per cent concentrates, 5 per cent cottonseed hulls, and 5 per cent alfalfa hay, could not demonstrate an effect of MHA in increasing rate of gain or reducing feed requirements for steers. Lofgreen (1970) showed a depressing effect from MHA alone or MHA in combination with sulphur when added to a mixed grain ration with 10 per cent roughage and 0.5 or 1.0 per cent urea. Beeson et al. (1961) reported seven per cent less rapid gains in cattle receiving 3 g MHA as compared with the control animals. Feed intakes were similar for the two groups, resulting in decreased feed efficiency for cattle receiving MHA.

A few trials have, however, reported improved animal performance due to MHA supplementation. For example, in trials with heifers fed 3 g MHA per head daily with a ration supplemented with urea, Burroughs et al. (1969) found the MHA-supplemented heifers to gain 13 per cent faster and required 10 per cent less feed per hundredweight of gain, which contrasts to previous reports. In a follow-up trial, Burroughs et al. (1970) reported that MHA additions to a urea supplement improved the performance of steers but that the response was inferior to that of added sulphur.

In general, it could be indicated that depending on the composition of the rations fed to ruminants, more or less dietary protein will either be degraded or by-pass the rumen and become available for post-ruminal digestion. Similarly, depending on the

dietary composition of the ration, more or less rumen microbial protein will be synthesized and eventually will become available for post-ruminal digestion. So that depending on the energy available, the specific pattern of amino acids and the level of production, the capability of the ruminant to synthesize protein will vary. It could therefore be conceived that the feeding of a given supplement like an encapsulated amino acid, analog or protected protein may or may not be beneficial to the ruminant animal. A situation where this supplementation may be beneficial is where a deficit of the amino acid or protein existed and the other dietary conditions (like energy) were appropriate for the utilization of these additional amino acids. Similarly, this same supplementation may not be beneficial if a deficit does not exist and sufficient quantities of microbial protein are being synthesized; or if a deficit exists but other conditions like energy are not appropriate for the utilization of the additional protein or amino acids.

In summary, therefore, MHA additions to urea supplements have increased production of dairy cows in a limited number of studies, while most trials have shown no response from the addition of MHA to urea supplements fed to cattle on finishing rations. The indications are that further research is needed in this area, because if what has been discussed above is true, then it becomes obvious that there is a need to develop a system that would permit, under practical conditions the prediction of the presence or absence of crude protein and/or

specific amino acid deficits, and the sufficiency of other components of the diet to allow for an efficient utilization of additional amino acids.

C. Rapeseed Meal as a Protein Supplement for Cattle Production

Rapeseed is becoming an increasingly important oilseed crop in Canada, and the utilization of the meal (oil-free residue) as a protein supplement for livestock production has been recommended by the Rapeseed Association of Canada based on work that has been done by many Canadian research workers.

Rapeseed meal (RSM) contains 36 ± 2 per cent crude protein with a maximum of 40 per cent. It has an amino acid composition similar to other valuable oil crops like peanut meal, sunflower meal and soybean meal (Hidlicka et al. 1964, 1965, 1967), and it is characterized by a relatively high methionine, cystine and lysine content (Block et al. 1965). Approximately 72 per cent of the rapeseed meal nitrogen occurs in amino acid compounds with nitrogen and products not precisely identifiable constituting 16 per cent (André and Delaveau 1954). Rapeseed meal is similar to linseed meal in metabolizable energy content and has 92 to 94 per cent of the metabolizable energy of barley (Bell and Delvin 1972). Virtanen (1963) reported that physiological abnormalities associated with thyroid disorders have not been reported when rapeseed meal has been fed to dairy cattle.

Glucosinolates in rapeseed meal are apparently inactivated or altered when the meal is fed to ruminants as those have not been reported in the milk of lactating cows.

In calf-starter and grower rations, Ingalls (1971) and Stone (1971) fed calves up to eight weeks of age and up to eight to ten months of age respectively. Their data suggested that the digestible energy value of rapeseed meal was equal to that of soybean meal. Protein digestibility coefficients were 89 per cent and 93 per cent for rapeseed meal and soybean meal, respectively, which indicated a small difference in protein digestibility between the two meals. With growth studies, the results reported indicate that calf-starter-grower rations containing up to 20 per cent rapeseed meal in place of soybean meal will result in similar feed intake and weight gains. When the rapeseed meal was increased up to 30 per cent, it resulted in decreased daily feed intake and weight gains. In further experiments these two workers reported that a crude protein level of 13 per cent appears adequate in calf-starter rations containing roughage and fed free choice. But with low protein roughage, starter rations may contain up to 20 per cent RSM plus sufficient urea (0.5 to 1 per cent) to balance the protein requirements of the calves. Wood and Stone (1970) have shown that a 10 per cent RSM diet fed to dairy calves achieved an adequate level of performance.

Bell and Delvin (1972) fed calves weighing 185 kg initially with 8 per cent RSM or 7.25 per cent SBM for 56 days, and the average

daily gains were 0.96 and 1.03 kg, respectively. Working with yearling steers weighing about 320 kg initially, the same workers fed some steers with RSM at 10.5 per cent of the diet while some were fed 7.5 per cent of SBM and others a control diet containing barley with no protein supplement. All the diets contained 15 per cent ground straw plus vitamins and minerals. The average daily gains were 1.14 kg for the controls, 1.19 for steers receiving SBM and 1.18 kg for steers fed RSM and corresponding feed conversion ratios were 9.19, 9.22 and 9.42, both results showing no statistically significant differences.

In a 140-day feeding trial with 37 Holstein steer calves using RSM that had received different heat treatments, Donefer (1971) found no significant differences in the average daily gains of the calves. In a 140-day trial with finishing steers, Ingalls and Waldern (1972) compared linseed meal, RSM, sunflower meal and mustard seed meal at 8 to 10 per cent levels in the diet. No statistically significant difference in weight gains for steers fed the different protein supplements was observed. Their further observations were that steers fed the diets with rapeseed meal and mustard seed meal mixtures consumed the diets with rapeseed meal more slowly.

In some breeding studies by Burkitt et al. (1954), pregnant beef cows were fed a low quality hay and wheat straw diet that was supplemented with either 5.6 or 10 per cent RSM or linseed meal. Similar performances were reported but cows fed rapeseed meal required a longer time to consume their diets. Similarly, Bell and Delvin

(1972) fed ewes with low quality non-legume hay that was supplemented with linseed meal or rapeseed meal at 10 per cent of daily intake (i.e., 0.2 kg daily). Daily allowances were always consumed and protein supplementation resulted in improved lambing and lactation performance. However, ewes consumed the rapeseed meal diets less readily than the linseed meal diets.

In general, rapeseed meal has potential as a protein supplement in beef production and the production of uniform high quality varieties that have both low erucic acid and low glucosinolate contents with a protein level that is reasonably high, further strengthens the potentials. More concerted research effort becomes a necessary prerequisite to find better ways of commercialising this by-product since it compares well with other traditional sources of plant proteins for ruminants in North America and elsewhere.

D. Summary of Literature Review

Protein and non-protein nitrogen compounds reaching the reticulo-rumen undergo extensive degradation by rumen microorganisms. The rate and extent of degradation is affected by the solubility of the proteins. Proteolysis and hydrolysis result in the liberation of amino acids from proteins and ammonia from non-protein nitrogen sources, respectively. The amino acids are further deaminated to produce ammonia or may be used in the synthesis of microbial proteins. Amination and transamination are the major pathways of ammonia

assimilation. The major source of nitrogen used by rumen micro-organisms for growth is ammonia, and this ammonia is usually incorporated into microbial protein by bacteria. Both the microbial protein synthesized and the dietary protein that is undegraded in the rumen are enzymatically degraded in the abomasum and small intestines to release their amino acids which are eventually absorbed. The absorbed amino acids are transported mainly to the liver, which releases them to the free amino acid pool in the circulating plasma and other tissues.

Even though ruminants are capable of synthesizing microbial protein, the amount synthesized plus the dietary protein that escapes rumen degradation may or may not meet their protein and/or amino acid requirements for maximum growth performance. This could mean that supplementation with amino acids and/or protein that have been protected against rumen degradation may be necessary under some dietary conditions where these requirements are not met. Usually, the sulphur-containing amino acids are limiting for optimal growth or milk production or both; and evidence available in the literature has shown that quantities of amino acids available to ruminants are usually insufficient to allow maximum productivity.

Several methods to modify dietary protein solubility or amino acid degradation or rumen conditions have been reported, and all of these methods can be broadly divided into physical and chemical methods. Among the chemical methods, formaldehyde treatment of the

dietary proteins has been the most widely researched method. The conditions under which a benefit could be obtained from the application of this method in terms of animal production response still remain to be established. This means a much clearer understanding is necessary of the factors affecting microbial protein synthesis, amount of dietary protein that will escape rumen degradation and the quality and quantity of the net protein or amino acids available for tissue metabolism.

III. OBJECTIVES

1. To compare rapeseed meal and soybean meal as protein supplements for growing cattle.
2. To compare the effect of formaldehyde treatment of rapeseed meal and soybean meal used as protein supplements for growing cattle.
3. To investigate the effect of formaldehyde treatment of rapeseed meal when fed alone or with urea as protein supplements for growing cattle.
4. To compare nutrient digestibility and nitrogen retention of formaldehyde-treated or untreated rapeseed meal when fed to sheep alone or with urea.

IV. EXPERIMENT I: COMPARISON OF RAPESEED AND SOYBEAN MEALS AS PROTEIN SUPPLEMENTS FOR GROWING CATTLE

A. Introduction

Rapeseed meal is becoming increasingly important as a protein supplement for livestock in Canada. Its use in finishing beef cattle has not become widespread in Canada, probably because of the limited research data available.

This experiment was aimed at the direct comparison of rapeseed meal and soybean meal as protein supplements to a corn silage and high moisture corn grain basal ration as measured by the growth performance of steers and heifers.

B. Experimental Procedures

1. Animals

The experiment was initiated in the fall of 1973 utilizing 20 cross-bred steers and 20 cross-bred heifers. The crosses had been produced by Holstein and various beef breed matings and had been purchased as feeder cattle. Their initial weights ranged from 240 to 320 kg, and they were divided into heavy and light groups, with average group weights of 303 and 249 kg, respectively.

2. Experimental design

The animals were randomly distributed in a 2 x 2 x 2 factorial design in which the factors were sex (steers vs. heifers), initial weight (heavy vs. light) and protein supplement fed (rapeseed meal vs. soybean meal). The animals were distributed into eight pens with each pen having five animals.

3. Rations

A corn silage (CS) and high moisture corn grain (HMC) basal ration was fed to all animals with rapeseed meal (RSM) or soybean meal (SEM) supplements. The rations were randomly distributed among the eight pens of animals. Expeller rapeseed meal (Brownowski strain) obtained from CANLIN Industries, Montreal, Quebec, and soybean meal obtained from Supersweet Feeds, Quebec, were used as received as protein supplements throughout the experimental period. The corn silage was initially fed ad libitum but was restricted after the first 28 days of the experiment. The experimental rations were re-calculated every 28 days and adjusted to meet protein requirements according to NRC.¹

4. Management

The cattle were housed in pens measuring 4.8 x 3.9 meters, with each pen having a 3.6 meter long concrete feed manger. Water

¹NRC (1970) Nutrient Requirements for Dairy Cattle. Washington, D.C. Table I. Growing Heifers (Large Breeds) and Growing Bulls (Large Breeds).

and cobalt-iodized salt were made available at all times. The feed offered was weighed and fed once a day and feed not consumed was weighed and recorded the next morning before fresh feed was offered.

Feed samples were collected and analyzed monthly using AOAC methods (1975) for dry matter (DM) and crude protein (CP). These DM and CP values were used in the calculation of the rations for the month to come.

The animals were weighed every 28 days. Before each weigh day, feed but not water was withdrawn from the animals at about five o'clock in the preceding evening. The initial and final weights of the animals were each based on an average of two weighings obtained by weighing the cattle one day, resting them one day and re-weighing them on a third day. This alternative day procedure was used in order to allow the animals a day to "settle down" since the weighing procedure was a stress and affected feed intake.

At the start of the experiment, every animal was injected intramuscularly with 5 cc of vitamin ADE.¹ The experiment lasted for 124 days.

¹Poten A.D.E., Lot 7231, produced by Rogar/STB, London, Ontario.

Vitamin A	500,000 IU
Vitamin D ₃	50,000 IU
Vitamin E	50 IU

5. Statistical analysis

The average daily gains of the animals over the experimental period were analyzed using the analysis of variance technique (Steel and Torrie 1960). The calculations were done using the Statistical Analysis System (SAS) on the McGill IBM 360 computer. The feed consumption data could not be analyzed because the animals were group fed.

C. Results and Discussion

1. Chemical analysis of ration components

Results of analysis conducted on three samples of each feedstuff during Experiment I are summarized in Table 1.

2. Composition of rations

Table 2 indicates the average composition of the rations over the 124 days experimental period.

Ration adjustments made on a monthly basis were designed to meet the protein requirements of each group of cattle based on their average weight.

Table 2 indicates that the corn silage portion of the ration dry matter varied from 74 to 78 per cent, a relatively narrow limit. High moisture corn varied from 18 to 20 per cent of ration DM, thus it also fell into a narrow range.

TABLE 1. Chemical composition of ration components (Experiment I)

Ingredient	NRC Ref. No.	Dry matter (per cent)	Crude protein ¹
Corn, aerial part, ensiled, mature, well-eared mn 30%, mx 50 dry matter	3-08-153	35.5	8.8
Corn, grain, ensiled	-	78.0	9.1
Rapeseed meal [rape, seed mech. extd. grnd]	5-03-870	90.2 ²	35.6
Soybean meal [soybean, seed, solv. extd. grnd, mx 7 fbr]	5-04-504	90.0 ²	46.4

¹Per cent dry matter basis.

²Values are from the United States-Canadian Tables of Feed Composition (1969), second edition. Publ. 1684. National Academy of Sciences, Washington, D.C.

TABLE 2. Average composition of experimental rations (Experiment I)¹

Ingredients	Rapeseed meal				Soybean meal			
	Steers		Heifers		Steers		Heifers	
	Heavy	Light	Heavy	Light	Heavy	Light	Heavy	Light
Corn silage	76.1	74.3	77.9	76.2	77.8	74.5	76.9	77.9
High moisture corn	19.2	20.2	18.1	19.0	18.3	20.6	19.2	18.0
Rapeseed meal	4.5	5.4	3.9	4.6	-	-	-	-
Soybean meal	-	-	-	-	3.7	4.8	3.7	3.9
Dicalcium phosphate	0.2	0.1	0.1	0.2	0.2	0.1	0.2	0.2
	100	100	100	100	100	100	100	100

¹Values are per cent of total dry matter.

The inability of the monthly ration adjustment to supply controlled amounts of protein is indicated in Table 3. As the protein requirements differed for each pen due to cattle weight differences, protein intakes are directly compared to requirements of each pen. The heavy steers had average protein requirements of 0.87 kg and intakes of 1.02 kg compared to the light steers which had average protein requirements of 0.76 kg and intakes of 1.07. The heavy heifers had average protein requirements of 0.73 kg and intakes of 0.93 kg compared to the light heifers that had average protein requirements of 0.67 kg and intakes of 0.98 kg. In general, the steers had higher protein requirements and intakes than the heifers (Table 3). It can thus be seen that in all cases protein was fed from 20 to 60 per cent in excess of stated requirements. This practice could confound the interpretation of the results related to the feeding of the different protein sources, as any differences in protein utilization could be compensated by the apparently large excess of protein supplied.

Table 3 indicates the per cent of daily protein intake supplied by the different ration ingredients. Corn silage was the largest supply of protein, averaging 42 per cent of total protein but ranging from 37 to 46 per cent. High moisture corn supplied an average of 26 per cent of the total protein, ranging from 24 to 27 per cent. The protein supplements supplied an average of 32 per cent of the total protein, ranging from 28 to 37 per cent.

TABLE 3. Summary of average daily protein intake and supply (Experiment I)¹

	Rapeseed meal				Soybean meal			
	Steers		Heifers		Steers		Heifers	
	Heavy	Light	Heavy	Light	Heavy	Light	Heavy	Light
<u>Daily protein</u>								
Intake, kg	1.04	1.06	0.96	1.02	1.00	1.08	0.90	0.94
Requirements, kg	0.87	0.75	0.72	0.64	0.87	0.77	0.74	0.69
Intake as per cent of requirement	120	141	133	159	115	140	122	136
<u>Protein supply per cent of total</u>								
Corn silage	45.9	43.4	42.5	39.7	41.1	42.6	43.0	37.3
High moisture corn	26.1	25.4	26.3	27.1	26.3	23.8	25.4	26.0
Rapeseed meal	28.0	31.2	29.2	33.2	-	-	-	-
Soybean meal	-	-	-	-	32.6	33.6	31.6	36.7
	100	100	100	100	100	100	100	100

¹Each observation is an average for five animals.

The aims of ration adjustment were only partially met, with variation in protein supply and intakes indicating the difficulty in closely controlling this factor with the number and variety of animals being fed under relatively practical conditions.

3. Animal growth performance

A summary of the average daily gains for the 124 days experimental period is shown in Table 4 for each group. The analysis of variance of average daily gains is summarized in Appendix Table 1. Lack of treatment interactions is illustrated in the summary of combinations of treatment effects presented in Table 5. The non-significant interactions (Appendix Table 1) thus allow direct comparisons of main effects as presented in Table 6.

Analysis of variance of the average daily gains showed no significant difference ($P > .1$) between animals of the two different weight categories.

A significant difference ($P < .1$) was observed between sexes with the steers having average daily gains that are significantly higher than the heifers (1.13 kg vs. 1.03 kg). A higher growth rate for steers is expected as indicated in the NRC¹ (1.0 and 0.75 kg for steers and heifers, respectively) with the weight range in Table 4. As compared with NRC¹ expected gains, the heifers in this experiment gained relatively better.

¹NRC (1971) Nutrient Requirements for Dairy Cattle. Washington, D.C. Table I: Growing Heifers (Large Breeds) and Growing Bulls (Large Breeds).

TABLE 4. Summary of average daily gains, average daily feed intake and feed efficiency
(Experiment I) 124 days¹

	Rapeseed meal				Soybean meal			
	Steers		Heifers		Steers		Heifers	
	Heavy	Light	Heavy	Light	Heavy	Light	Heavy	Light
Initial weight, kg	295.9	242.2	316.5	249.5	293.0	250.6	304.8	254.0
Final weight, kg	420.7	358.3	418.9	349.9	418.4	381.5	429.7	382.4
Ave. daily gain, kg	1.05	0.89	0.99	1.02	1.16	1.15	1.12	1.12
<u>Daily feed intake, kg DM</u>								
Corn silage	5.44	4.43	5.90	4.85	5.82	4.37	5.47	5.22
High moisture corn	1.37	1.21	1.37	1.21	1.37	1.21	1.37	1.21
Rapeseed meal	0.32	0.32	0.30	0.30	-	-	-	-
Soybean meal	-	-	-	-	0.28	0.28	0.27	0.26
Total intake, kg/day)	7.13	5.96	7.57	6.36	7.47	5.86	7.11	6.69
<u>Feed efficiency</u>								
Feed DM per kg gain	6.80	6.71	7.62	6.25	6.45	5.10	6.35	5.99

¹Each value is an average for five animals.

TABLE 5. Summary of combinations of treatment effects on average daily gains (Experiment I)

Weight x Proteins

<u>Weight</u>	<u>Protein</u>	<u>ADG (kg)</u>
Heavy	SBM	1.14
Heavy	RSM	1.02
Light	SBM	1.13
Light	RSM	0.95

Sex x Proteins

<u>Sex</u>	<u>Protein</u>	<u>ADG (kg)</u>
Male	SBM	1.15
Male	RSM	0.97
Female	SBM	1.12
Female	RSM	1.01

Sex x Weight

<u>Sex</u>	<u>Weight</u>	<u>ADG (kg)</u>
Male	Heavy	1.10
Male	Light	1.02
Female	Heavy	1.05
Female	Light	1.07

TABLE 6. Summary of average daily gains of main treatment effects
(Experiment I)

	<u>ADG (kg)</u>
<u>Weight</u>	
Heavy	1.09 a
Light	1.07 a
<u>Sex</u>	
Male	1.13 a
Female	1.03 b
<u>Proteins</u>	
Rapeseed meal	1.02 a
Soybean meal	1.13 b

¹Values within Weight, Sex or Protein groups followed by a different letter are significantly different ($P < .1$).

When comparing the effect of the two protein supplements, the steers and heifers fed soybean meal had significantly ($P < .05$) higher average daily gains than those fed rapeseed meal (1.13 vs. 1.02 kg). These data are in contrast to those of Bell and Delvin (1972) who observed average daily gains for yearling steers of 1.14 kg for the control diet containing barley but no protein supplement, 1.19 kg for the diet containing 7.5% SBM and 1.18 kg for the diet containing 10.5% RSM, the differences being non-significant. These same workers have also reported average daily gains of 0.96 and 1.03 kg for beef calves receiving diets containing 8% RSM and 7.25% SBM, respectively, these differences being non-significant.

The difference in animal growth performance between the two protein supplements used in this experiment could be attributed to the fact that the protein of the rapeseed meal had a higher solubility (28 per cent) compared with soybean meal protein (16.1 per cent) as reported by Phillip (1976). This degree of solubility of RSM protein could increase its rumen degradation, thereby resulting in ammonia release from the protein and possible protein wastage. It is of interest that this possible protein wastage was not compensated by the excess level of protein (compared with stated requirements) as fed in this experiment.

4. Feed consumption

Average daily feed intakes for total ration and for individual ingredients are presented in Table 4. Feed intake data could not be statistically analyzed because group (pen) feeding was employed.

A difference exists in total intake due to size, with heavy and light steers having average intakes of 7.30 and 5.91 kg, respectively, while intakes for the heavy and light heifers were 7.34 and 6.53 kg, respectively.

No difference in total intake appears to exist between the RSM and SBM fed to cattle (6.76 and 6.78 kg, respectively).

These differences in feed intake due to cattle size are not reflected in the protein intake as higher amounts of protein supplement were fed to the lighter pens to compensate for their lower intake (Table 3). That there may have been over-compensation is seen by the data of Table 3, indicating the lighter weight pens actually received slightly more protein than their heavier counterparts.

5. Feed efficiency

There was a slight advantage in feed efficiency for the lighter weight animals in all comparisons (Table 4). This is an expected result as feed efficiency generally decreases with age (and weight) due to the increasing proportion of tissue fat being deposited.

On an overall basis, the soybean meal fed cattle had a better feed efficiency (5.97) than those fed rapeseed meal (6.85). This is a

reflection of the larger weight gains with SBM as compared with RSM since total feed intake did not differ. Rapeseed meal fed cattle thus required an average of 0.88 kg more ration DM per kg gain than the SBM fed cattle.

D. Summary of Experiment I

In a 2 x 2 x 2 factorial design, a comparison of RSM and SBM as protein supplements to a corn silage and high moisture corn grain basal ration was made. Cross-bred steers and heifers classified on the basis of weight (heavy vs. light) were used in the 124-day experiment.

The results indicate that there was a significant difference ($P < .05$) in average daily gains for the protein supplements with the cattle on SBM having higher average daily gains than those on RSM. There was also a significant difference ($P < .1$) in average daily gains between the steers and heifers with steers having higher average daily gains than the heifers. But there was no significant difference ($P > .1$) in average daily gains between the heavy and light steers.

Feed consumption was higher for the heavy as compared with the light cattle. However, the lighter cattle had higher protein intakes than the heavier cattle.

The lighter cattle had slightly better feed efficiency than the heavier cattle. Also, the cattle fed soybean meal had a better feed efficiency than those on rapeseed meal.

V. EXPERIMENT II. COMPARISON OF FORMALDEHYDE TREATED
AND UNTREATED RAPESEED AND SOYBEAN MEALS AS PROTEIN
SUPPLEMENTS FOR GROWING CATTLE.

A. Introduction

Reports in the literature have described the use of chemical treatment of protein supplements to decrease their degradation by rumen microorganisms without decreasing their post-ruminal digestibility.

The objective of this experiment was thus to determine the effect of formaldehyde treatment of soybean meal and rapeseed meal used as protein supplements for growing bull calves.

Two replicates (A and B) were conducted in this experiment and the experimental rations and management were basically the same for each replicate. The replicates were based on the supply of two batches of experimental animals available at different times.

B. Experimental Procedures

1. Animals

Replicate A.--This replicate was initiated in August, 1974, using 36 Holstein bull calves averaging about 94.6 kg liveweight and purchased from Mutual Products Inc., Morrisburg, Ontario.

Replicate B.--This replicate was initiated in November, 1974, using 31 Holstein bull calves with an average weight of 112 kg, resulting from a previous calf experiment conducted at Macdonald College Farm.

2. Experimental design

The animals in each replicate were randomly distributed in a 2 x 2 factorial design in which the factors were protein supplement (rapeseed meal vs. soybean meal) and formaldehyde treatment of protein supplements (treated vs. untreated). For Replicate A, the animals were distributed into four pens with each pen having nine animals. One calf died during the course of the experiment, which left one pen with eight animals. For Replicate B, the animals were distributed into four pens with three pens having eight animals and one pen having seven animals.

3. Rations

A timothy hay, barley and molasses ration was supplemented with either rapeseed meal¹ or soybean meal treated or not treated with formaldehyde and fed for an experimental period of 168 days for both replicates. Dicalcium phosphate was fed to all the animals at a rate of 100 g/day.

¹Lower variety (1973 crop).

4. Feed preparation

Timothy hay was chopped to particle sizes varying from 2 to 5 cm. long. Barley hay was coarsely ground using a hammer mill. Blackstrap (final) molasses was obtained in large steel drums from the Canada West Indies Molasses Company, Montreal.

5. Treatment of protein supplements

a. Rapeseed meal

Ten samples from each of the two batches of 1973 Tower rapeseed meal obtained from Saskatchewan (batches no. 940 and 1788) were analyzed and found to contain 29.7 and 34.9 per cent crude protein (DM basis), respectively. A 1:1 blend of the two rapeseed meal batches was made to give an average crude protein analysis of 32.3 per cent. This blended material was used throughout Experiment II. Based on the 32.3 per cent crude protein content of the rapeseed meal blend, 800 ml of 40% histological grade formaldehyde (FA) was used to treat 100 kg. of rapeseed meal. This level of formaldehyde was calculated to give a treatment of 1 g FA/100 g of rapeseed meal protein. To get an even dispersion of the formaldehyde during the treatment of the rapeseed meal, the formaldehyde solution was diluted in a ratio of one part of formaldehyde solution to five parts of water.

The formaldehyde treatment procedure was as follows:

- (1) One hundred kilograms of rapeseed meal were weighed into a horizontal mixer and 4800 ml of the 1:5 formaldehyde-water solution (contained in a five-gallon polyethylene bag fitted

with a spigot) was dripped on the rapeseed meal in the mixer for approximately 30 minutes while the mixer continued to turn.

(ii) After the solution had dripped on the rapeseed meal, the mixer was left in operation for another 30 minutes.

(iii) After mixing was completed, the treated rapeseed meal was then emptied into 47.5 x 105 cm airtight plastic bags, tied, and left for 24 hours to enable the reaction of the formaldehyde with the rapeseed meal protein to go to completion.

(iv) At the end of 24 hours, the rapeseed meal was spread on a concrete floor to air dry and allow for the escape of any formaldehyde gas present.

(v) When the treated rapeseed meal was dry, usually in about five to six hours, it was re-bagged and was ready for use.

This procedure was repeated any time more formaldehyde-treated rapeseed meal was needed.

b. Treatment of soybean meal

Soybean meal obtained from Supersweet Feeds, Quebec, was used in Experiment II. An analysis of ten composite samples gave an average crude protein content of 47.4 per cent (DM basis). Our calculations showed that 1175 ml of 40% histological grade formaldehyde solution were required to treat 100 kg of soybean meal. This gave a treatment of 1 g FA/100 g of soybean meal protein. The formaldehyde solution was diluted in a ratio of one part of formaldehyde solution to five parts of water. The treatment procedure was the same as described above for the rapeseed meal.

c. Control (untreated) protein supplements (H_2O)

The exact mixing and drying procedure was used as previously described, except that only water (no formaldehyde) was added, using the same total volume as employed with the formaldehyde treatments.

6. Sampling feed for chemical analysis

The formaldehyde-treated and untreated rapeseed meal and soybean meal were routinely sampled for crude protein analysis and protein solubility determinations. Other ration ingredients (hay and barley) were also sampled for dry matter and crude protein analysis, conducted according to the AOAC (1975) methods.

7. Management

The animals were dehorned before the start of the experiment and a few of them were given intramuscular injections of streptomycin for colds, while a few were treated for scours.

The rest of the animal management protocol was the same as described for Experiment I.

8. Statistical analysis

The average daily gains of the animals over the experimental period were analyzed using the Regression Procedure (least squares method) for unbalanced data (Steel and Torrie 1960). The calculations were done using the Statistical Analysis System (SAS) on the McGill IBM 360 Computer.

Feed consumption records could not be statistically analyzed because the animals were group fed.

C. Results and Discussion

1. Chemical analysis of ration components

Results of analysis conducted on six samples of each feedstuff during Experiment II are presented in Table 7. Because molasses is fairly constant in its chemical composition, no chemical analysis was conducted and the values presented in Table 7 are from NRC.¹

2. Composition of rations

Table 8 indicates the average composition of the rations over the 168 days experimental period.

Ration adjustments made on a monthly basis were designed to

- (a) meet the protein requirements of each group based on their average weight. An attempt was made not to supply protein in excess of requirements as this could complicate the interpretation of the results;
- (b) supply protein from each supplement at approximately the same level to each pen, taking into consideration the differing protein contents of the RSM and SBM.

Table 8 indicates that the hay, barley, molasses, RSM and SBM portions of the ration dry matter were kept constant at 29, 54, 10, 7 and 7 per cent, respectively. In order for the protein supplements to supply about 20 per cent of the total protein offered (Table 9), it was necessary to use a grass hay (with a relatively low protein content).

¹NRC (1971). Nutrient Requirements for Dairy Cattle. Washington, D.C. Table 4. Composition of feeds commonly used in dairy cattle rations.

TABLE 7. Chemical composition of ration components (Experiment II)¹

Ingredients	NRC Reference number	Dry matter (%)	Crude protein ²
Timothy hay, s-c, mid-bloom	1-04-883	88	7.6
Barley grain	4-00-530	90	11.2
Rapeseed meal [rape, Canada, seeds, cooked, pre-press solv-extd, grnd, Can 1 mx 1% fat]	5-08-135	90	32.3
Soybean meal [soybean, seed, solvent extracted, grnd, mx 7% fbr]	5-04-604	90	47.4
Sugarcane molasses, mn 48% invert sugar mn 79.5 degrees brix ³	4-04-696	75	4.3

¹ Average of six composite samples

² Per cent dry matter basis

³ From NRC

TABLE 8. Average composition of experimental rations (Experiment II)¹

Ingredients	Rapeseed meal		Soybean meal	
	FA	H ₂ O	FA	H ₂ O
Hay	29	29	29	29
Barley	54	54	54	54
Molasses	10	10	10	10
Rapeseed meal	7	7	-	-
Soybean meal	-	-	7	7
Total	100	100	100	100

¹Values are per cent. of total DM

Molasses, also with a low protein content, was fed in an attempt to maintain the available energy level of the ration.

The ability of the monthly ration adjustments to supply controlled amounts of protein is indicated in Table 9. As the protein requirements differed for each replicate due to calf weight differences, then protein intake (supplied) can be directly compared with the requirement.

Table 9 indicates the average daily protein intake for each group of calves. It can be seen that the protein intake for the calves receiving the RSM supplemented diet in Replicate A was lower than those on SBM (0.47 vs. 0.48 kg). These protein intakes of the calves in Replicate A are generally lower than those on Replicate B whose intakes were uniform (i.e., 0.53 kg). The protein intakes for the calves in the two replicates were generally lower than the NRC¹ requirements, with those in Replicate A having comparatively lower intakes.

Table 9 also indicates the per cent of daily protein intake supplied by the different ration ingredients. Barley was the largest supply of protein, averaging 57 per cent of total, but ranging from 56 to 58 per cent. Hay supplied an average of 19 per cent of the total protein, ranging from 18 to 20 per cent. Molasses supplied an average of 3.7 per cent of the total protein, ranging from three to four per

¹NRC (1971): Nutrient Requirements for Dairy Cattle. Washington, D.C. Table I. Growing bulls (large breeds).

TABLE 9. Summary of average daily protein intake and supply (Experiment II)¹

	Replicate A				Replicate B			
	Rapeseed meal		Soybean meal		Rapeseed meal		Soybean meal	
	FA	H ₂ O	FA	H ₂ O	FA	H ₂ O	FA	H ₂ O
Protein intake (kg)	0.47	0.46	0.48	0.48	0.53	0.53	0.53	0.53
Protein requirement (kg)	0.54	0.53	0.53	0.53	0.56	0.56	0.56	0.56
Protein intake as % of requirement	87.0	87.0	90.0	90.0	96.0	96.0	96.0	96.0
<u>Protein supply (% total)</u>								
<u>Ingredients</u>								
Hay	18.9	18.5	18.7	19.5	20.6	20.6	20.0	20.0
Barley	57.5	57.8	56.5	55.9	58.4	58.4	56.4	56.4
Molasses	3.6	3.6	3.5	3.5	4.0	4.0	3.8	3.8
Rapeseed meal	20.0	20.1	-	-	17.0	17.0	-	-
Soybean meal	-	-	21.3	21.1	-	-	19.8	19.8
	100	100	100	100	100	100	100	100

¹Observations are averages per animal.

cent. The protein supplements supplied an average of 20 per cent of the protein, ranging from 19 to 21 per cent.

3. Animal growth performance

A summary of the average daily gains (ADG) for the 168 days experimental period is shown in Table 10 for each group. The analysis of variance of average daily gains is summarized in Appendix Table 2.

A summary of treatment combinations and main treatment effects on average daily gains is presented in Table 11.

A significant difference ($P < .01$) was observed between replicates A and B, with the calves in replicate B having average daily gains that are significantly higher than replicate A (0.88 vs. 0.72 kg). The superior growth performance of the calves in replicate B could be partly attributed to compensatory growth because they were kept on a low protein hay diet for about two months before the experiment was initiated.

Analysis of variance of the average daily gains showed no significant difference ($P > .05$), either between the two protein supplements or among formaldehyde treatments within the protein supplements. However, the calves receiving the RSM supplemented diets had slightly superior average daily gains compared with those receiving SBM supplemented diets (0.82 vs. 0.78 kg). These data partly support the observations of Bell and Delvin (1972) which indicated a non-significant difference in average daily gains between steers fed RSM or SBM protein supplements. The data of these workers

TABLE 10. Summary of average daily gains, average feed intake and feed efficiency
(Experiment II, 168 days trial)¹

	Replicate A				Replicate B			
	Rapeseed meal		Soybean meal		Rapeseed meal		Soybean meal	
	FA	H ₂ O	FA	H ₂ O	FA	H ₂ O	FA	H ₂ O
Number of animals	9	8	9	9	8	8	7	8
Initial weight (kg)	95.9	93.5	94.4	94.6	113.1	110.8	112.9	114.8
Final weight (kg)	224.4	216.4	214.2	212.0	255.9	253.6	250.8	259.9
Average daily gain (kg)	0.75	0.72	0.71	0.69	0.84	0.86	0.81	0.86
<u>Daily feed intake (kg, DM)</u>								
Hay	1.13	1.10	1.17	1.17	1.35	1.35	1.35	1.35
Barley	2.24	1.90	2.24	2.24	2.48	2.48	2.48	2.48
Molasses	0.47	0.45	0.47	0.47	0.47	0.47	0.47	0.47
Rapeseed meal	0.29	0.29	-	-	0.33	0.33	-	-
Soybean meal	-	-	0.21	0.21	-	-	0.22	0.22
Total feed intake	4.13	3.74	4.09	4.09	4.63	4.63	4.52	4.52
<u>Feed efficiency</u>								
Feed DM per kg gain	5.60	5.19	5.76	5.92	5.51	5.38	5.38	5.25

¹Observations are averages per animal.

TABLE 11. Summary of treatment combination and main treatment effects on average daily gains (Experiment II)

Combination effects - Protein x Formaldehyde

<u>Protein</u>	<u>Formaldehyde</u>	<u>ADG (kg)</u>
RSM	FA	0.83 a
RSM	H ₂ O	0.80 a
SBM	FA	0.78 a
SBM	H ₂ O	0.78 a

Main effects

<u>Replicates</u>	<u>ADG (kg)</u>
A	0.72 a
B	0.88 b

Protein supplements

Rapeseed meal	0.82 a
Soybean meal	0.78 a

FA-treatment

FA	0.81 a
H ₂ O	0.79 a

Figures in the protein x formaldehyde, replicates, protein supplement, and FA-treatment groups bearing different letters are significantly different ($P < .01$).

further indicated slightly inferior average daily gains for cattle on the RSM supplement compared with those on the SBM supplement, an observation which is partly in contrast to our data.

Formaldehyde treatment of the protein supplements resulted in slightly superior average daily gains of calves compared with those on the untreated (control) supplements (0.81 vs. 0.79 kg). These data support those of Sharma et al. (1972), who observed non-significant average daily gains of 0.87 kg for all the calves receiving either formaldehyde treated or untreated RSM. Our data are, however, in contrast to those of some workers who have observed a significant improvement in average daily gains of cattle fed formaldehyde-treated protein supplements; whereas Schmidt et al. (1974) fed cattle with rations that had been supplemented with SBM that had received treatments of 0, 0.6 and 1.2 g FA/100 protein and reported average daily gains of 1.31, 1.27 and 1.20 kg, respectively; while Peter et al. (1974) fed sheep with diets that had been supplemented with either untreated or formaldehyde-treated SBM and reported average daily gains of 0.25 and 0.29 kg, respectively, the difference being significant.

It can thus be seen that both our research data and data in the literature indicate inconsistent animal growth performance when fed diets supplemented with formaldehyde treated protein supplements. The inconsistent animal growth performance has been reported to be partly due to the relatively lower biological value of plant protein

supplement when compared with those of animal origin (Colby and Tollert 1973; Faichney and Davies 1972; Nimrick et al. 1972). This is because the biological value of the "protected" protein supplement that escapes rumen proteolysis will determine the final pattern of amino acids that will be available to the animal for productive functions.

4. Feed consumption

Because the animals were group fed, the daily feed intake data could not be analyzed statistically. The average dry matter intakes for replicates A and B are shown in Table 10. In replicate A, total dry matter consumption for the formaldehyde treated and untreated rapeseed meal treatments was 4.1 and 3.7 kg per day, respectively. Calves fed diets supplemented with soybean meal (treated or untreated) apparently had equal total DM intake of 4.1 kg per day.

In replicate B, the dry matter consumption for the calves fed either formaldehyde treated or untreated rapeseed meal supplement was 4.6 kg per day. For the calves fed either formaldehyde treated or untreated soybean meal the dry matter consumption was 4.5 kg per day.

In both replicates the rations were formulated to meet the protein requirements but not the dry matter requirements of this type of calf because the dry matter offered to them was usually slightly lower than that recommended by the NRC.¹ The calves always "cleaned

¹NRC (1971). Nutrient Requirements of Dairy Cattle. Washington, D.C. Table I: Growing bulls (large breeds).

up" the feed offered. Therefore, the calves in replicate B had higher dry matter intakes than the calves in replicate A because the former were started on the experiment with a higher initial weight and finished with a higher final weight than the latter.

The daily protein intakes for replicates A and B are shown in Table 9. The calves in replicate A had daily protein intakes of 0.47 and 0.46 kg for the formaldehyde treated and untreated rapeseed meal treatments, respectively. Those supplemented with soybean meal (treated or untreated) had daily protein intakes of 0.48 kg.

In replicate B, calves fed diets that were supplemented with rapeseed meal (treated or untreated) had daily protein intakes of 0.53 kg. Those fed diets that were supplemented with soybean meal (treated and untreated) had a daily protein intake of 0.53 kg.

It could be seen that the daily protein intake was similar to the dry matter intake pattern. This resulted in the calves in replicate B having higher protein intakes than those in replicate A. This again is reflected by the overall higher daily dry matter intake for the calves in replicate B as a result of their higher initial body weight.

5. Feed efficiency

Table 10 indicates feed efficiencies for the calves in replicates A and B. In replicate A, calves receiving the soybean meal supplement (treated and untreated) had feed efficiencies of 5.76 and 5.92, respectively. These feed efficiencies are slightly inferior

compared with those shown for calves fed formaldehyde treated and untreated rapeseed meal supplement (5.50 and 5.19, respectively).

In replicate B, animals fed the soybean meal supplement (untreated and treated) had feed efficiencies of 5.25 and 5.38, respectively. These feed efficiencies are slightly superior to those for calves on formaldehyde treated and untreated rapeseed meal supplement (5.38 and 5.51, respectively).

On the overall, the data in Table 10 show a slightly better feed efficiency for the calves receiving the rapeseed meal in replicate A compared with those in replicate B. But the calves receiving soybean meal in replicate A had inferior feed efficiencies compared with those fed this protein supplement in replicate B. Comparing calves on soybean meal and rapeseed meal across the two replicates, calves on rapeseed meal had generally inferior feed efficiencies compared with those on soybean meal, although differences were not very large.

D. Summary

In a 168-day replicated 2 x 2 factorial experiment, Holstein calves were fed a hay, barley and molasses ration that was supplemented with either rapeseed meal or soybean meal (treated or untreated with formaldehyde).

The results indicated that there was a significant difference ($P < .05$) between the calves in replicate A and B, with the calves in

replicate B having higher gains than those in replicate A. The results further indicated a non-significant ($P > .05$) difference, either between the rapeseed meal and soybean meal protein supplement or among formaldehyde treatments within protein supplements.

Feed dry matter and protein intakes were higher for the calves in replicate B than those in replicate A. But feed efficiency was better for calves in replicate A than those in replicate B. Feed efficiencies were slightly better for the RSM-fed calves in replicate A, a situation which was reversed in replicate B.

VI. EXPERIMENT III. THE EFFECT OF FORMALDEHYDE TREATMENT
OF RAPESEED MEAL, FED ALONE OR WITH UREA, AS A PROTEIN
SUPPLEMENT FOR GROWING CATTLE: CORN SILAGE BASAL RATION

A. Introduction

The chemical treatment of protein supplements using formaldehyde is usually aimed at decreasing their degradation by rumen microorganisms. Depending on the level of formaldehyde treatment, the rate of degradation of the proteins and consequently ammonia production, can be reduced to such a low level that growth of rumen microorganisms and microbial protein production can be limited. The ideal situation is usually to have ruminant rations containing protected proteins and/or amino acids that would also generate enough rumen ammonia to allow maximum microbial growth. Supplementation of these types of diets with urea, which is highly soluble in the rumen, may be required to achieve this goal.

This experiment was aimed at determining the effect of formaldehyde treatment of rapeseed meal, when fed alone or in combination with various levels of urea, as a protein supplement for growing cattle. Holstein steers, having completed a previous study (Experiment II), were used in this feeding trial, in which corn silage constituted the basal ration.

B. Experimental Procedures

1. Animals

Replicate A.--This replicate was initiated in the spring of 1975 (March 29) and 34 Holstein steers from replicate A of experiment II, with an average initial weight of about 284 kg, were used.

Replicate B.--This replicate was initiated in the spring of 1975 (May 24) and 30 Holstein steers from replicate B of experiment II, with an average initial weight of about 270 kg, were used.

2. Experimental design

The animals in each replicate were randomly distributed in a 2 x 3 factorial design in which the factors were formaldehyde treatment of rapeseed meal (treated vs. untreated) and three levels of urea (zero, medium and high). In replicate A, the animals were distributed into six pens with four pens having six animals each and two pens having five animals each; while in replicate B, the animals were distributed into six pens with each pen having five animals.

3. Rations

A corn silage and molasses ration was supplemented with rapeseed meal¹ (treated or not treated with formaldehyde) in which either 0, 50, or 75 per cent of the protein from rapeseed meal was replaced by urea. Dicalcium phosphate was fed to all the animals at a rate of 200 g/day.

¹Tower variety (1974 crop).

Replicate A lasted for 118 days, while replicate B lasted for 62 days. The difference in length of the experimental period between the replicates was because of a short supply of corn silage at that time of the year, which necessitated the termination of replicate B after only 62 days.

4. Treatment of rapeseed meal

a. Formaldehyde

Ten samples of 1974 Tower rapeseed meal were analyzed and found to contain an average of 37 per cent crude protein (dry matter basis). Based on the 37 per cent crude protein content of the rapeseed meal, 925 ml of 40 per cent histological grade formaldehyde were used to treat 100 kg of rapeseed meal. This level of formaldehyde was calculated to give a treatment of 1 g FA/100 g of rapeseed meal protein. To obtain an even dispersion of the formaldehyde during the treatment of the rapeseed meal, the formaldehyde solution was diluted in a ratio of one part of formaldehyde solution to five parts of water. The formaldehyde treatment procedure was the same as outlined in experiment II, section 5 (a).

b. Control (untreated) rapeseed meal (H_2O)

The exact mixing and drying procedure was used as previously described, except that only water (no formaldehyde) was added, using the same total volume as employed with the formaldehyde treatments.

5. Sampling of feed for chemical analysis

The formaldehyde-treated and untreated rape seed meal was routinely sampled for crude protein analysis and protein solubility determinations. The corn silage was also sampled for dry matter and crude protein analysis, conducted according to the AOAC (1975) methods.

6. Management

The animals were castrated before the start of the experiment. The rest of the management protocol was the same as in experiment I, only that at this stage, the animals were not given any injections of vitamins A, D and E.

7. Statistical analysis

The average daily gains of the animals over the experimental period were analyzed using the Regression Procedure (least squares method) for unbalanced data (Steel and Torrie, 1960). The calculations were made using the Statistical Analysis System (SAS) on the McGill IBM 360 computer.

Feed consumption records were not statistically analyzed because the animals were group fed, so that individual feed intake data were not available.

C. Results and Discussion

1. Chemical analysis of ration components

Results of analysis conducted on six samples of corn silage and rapeseed meal during experiment III are presented in Table 12. Because molasses is fairly constant in its chemical composition, no chemical analysis was conducted and the values presented in Table 12 are from the National Research Council (U.S.).¹

2. Composition of rations

Table 13 indicates the average composition of the rations over the experimental periods (118 days and 62 days for replicates A and B, respectively).

Ration adjustments made on a monthly basis were designed to meet the protein requirements of each group of cattle, based on their average weight.

Table 13 indicates that the corn silage portion of the ration dry matter varied from 59 to 65 per cent among the three levels of urea, a relatively narrow limit. Molasses varied from 28 to 31 per cent of ration dry matter, thus it also fell into a narrow range. The 1.4 to 12 per cent range of ration DM for the RSM was due to the replacement of this ingredient with various levels of urea. Urea was varied from 0 to 1.6 per cent of ration DM, to replace up to 75 per cent

¹United States-Canadian Tables of Feed Composition (1969). Second edition. Publ. 1684, National Academy of Sciences, Washington, D.C.

TABLE 12. Chemical composition of ration components (Experiment III, Replicates A and B)

Ingredient	NRC Ref. No.	Dry matter (%)	Crude protein ¹
Corn - aerial part, ensiled, mature, well-eared, mn 30%, mx 50%, dry matter (3)	3-08-153	30.2	8.3
Sugar cane molasses, mn 48%, invert sugar mn 79.5 degrees brix ²	4-04-696	75.0	4.3
Rapeseed meal (rape, seeds, solvent extracted, grnd)	5-03-871	91.2	37.1
Urea (42% N) ²		98.0	281.0

¹Per cent dry matter basis.

²Values are from the United States-Canadian Tables of Feed Composition (1969), second edition. Publ. 1684, National Academy of Sciences, Washington, D.C.

TABLE 13. Average composition of experimental rations (Experiment III, Replicates A and B)¹

Ingredients	Treatments		
	No urea	Medium urea	High urea
Corn silage	59	64	65
Molasses	28	30	31
Rapeseed meal	12	4	1.4
Urea	0	1	1.6
Dicalcium phosphate	1	1	1
	100	100	100

¹Values are per cent of total dry matter.

of the protein supplied by rapeseed meal. Dicalcium phosphate was fed at a constant level of 1 per cent of ration DM. A relatively high level of molasses, with its low protein content, was fed in an attempt to maintain the available energy level of the ration, without substantially increasing protein supplied.

The ability of the monthly ration adjustments to supply controlled amounts of protein is indicated in Tables 14 and 15 for replicates A and B, respectively. As the protein requirements differed for each replicate due to animal weight differences, protein intakes (supplied) are compared with requirements on a replicate basis. It can be seen that the protein intakes for animals in replicate A were higher than those for the animals in replicate B (0.77 vs. 0.69 kg). The protein intakes for the animals in replicates A and B were slightly lower than the NRC requirements. This was a desirable situation since any excess protein could confound interpretation of the results dealing with utilization of different protein sources. It should also be recognized that the NRC values constitute a guide but may not be an accurate measure of the animal's actual protein requirement.

Tables 14 and 15 also indicate the per cent of daily protein intake supplied by the different ration ingredients. Corn silage was the largest supply of protein, averaging 53 per cent of total. Molasses supplied an average of 13 per cent of the total protein. Rapeseed meal supplied 34, 17 and 9 per cent, and urea supplied 0, 17 and 25 per cent of the total protein supply for the zero, medium and

TABLE 14. Summary of average daily protein intake and supply (Experiment III, Replicate A)¹

	Treatments					
	RSM-FA			RSM-H ₂ O		
	No urea	Medium urea	High urea	No urea	Medium urea	High urea
Protein intake (kg)	0.75	0.75	0.75	0.75	0.75	0.75
Protein requirement (kg) ²	0.77	0.77	0.77	0.77	0.77	0.77
Protein intake as per cent of requirement	97.4	97.4	97.4	97.4	97.4	97.4
<u>Protein supply (per cent of total)</u>						
Corn silage	53	53	53	53	53	53
Molasses	13	13	13	13	13	13
Rapeseed meal	34	17	9	34	17	9
Urea	0	17	25	0	17	25
	100	100	100	100	100	100

¹All treatment groups had 6 animals except the RSM-FA-High urea and RSM-H₂O-No urea groups which had 5 animals; observations are averages per animal.

²Growing bulls (large breeds) Nutrient Requirements of Dairy Cattle, Table I. NRC (1971),⁴ Washington, D.C.

TABLE 15. Summary of average daily protein intake and supply (Experiment III, Replicate B)¹

	Treatments					
	RSM-FA			RSM-H ₂ O		
	No urea	Medium urea	High urea	No urea	Medium urea	High urea
Protein intake (kg)	0.69	0.69	0.69	0.69	0.69	0.69
Protein requirement (kg) ²	0.73	0.73	0.73	0.73	0.73	0.73
Protein intake as per cent of requirement	94.5	94.5	94.5	94.5	94.5	94.5
<u>Protein supply (per cent of total)</u>						
Corn silage	53	53	53	53	53	53
Molasses	13	13	13	13	13	13
Rapeseed meal	34	17	9	34	17	9
Urea	0	17	25	0	17	25
	100	100	100	100	100	100

¹All treatment groups had 5 animals and observations are averages per animal.

²Growing bulls (large breeds) Nutrient Requirements of Dairy Cattle, Table I. NRC (1971), Washington, D.C.

high urea treatments, respectively. Thus, the rapeseed meal-urea combinations contributed about one-third of the total protein supplied, a relatively high level for a protein supplement.

3. Animal growth performance

A summary of the average daily gains for replicates A and B are shown in Tables 16 and 17, respectively. The analysis of variance of average daily gains is summarized in Appendix Table 3. A summary of treatment combinations and main treatment effects on average daily gains is presented in Table 18.

A non-significant difference ($P > .1$) was observed between replicates A and B, with the animals in replicate A having only a slightly higher average daily gain than those in replicate B (0.54 vs. 0.56 kg).

Analysis of variance of the average daily gains showed no significant difference ($P > .1$) between the formaldehyde treated and untreated rapeseed meal. However, the animals receiving the formaldehyde treated rapeseed meal had slightly higher average daily gains compared with those receiving the untreated (control) supplement (0.57 vs. 0.52 kg). These data support those of Sharma *et al.* (1972) who observed non-significant average daily gains of 0.87 kg for all the calves receiving either formaldehyde treated or untreated RSM.

However, the analysis of variance of the average daily gains showed a significant difference ($P < .01$) due to urea levels. The average daily gains decreased as the level of urea increased in the

TABLE 16. Summary of average daily gains, feed intake and feed efficiency
(Experiment III, Replicate A, 118 days)¹

	Treatments					
	RSM-FA			RSM-H ₂		
	No urea	Medium urea	High urea	No urea	Medium urea	High urea
No. of animals	6	6	5	5	6	6
Initial weight (kg)	280.9	280.9	288.4	284.8	288.2	282.9
Final weight (kg)	360.6	344.4	335.2	358.3	351.0	339.6
Average daily gain (kg)	0.68	0.54	0.40	0.62	0.53	0.49
<u>Daily feed intake (kg, DM)</u>						
Corn silage	4.76	4.76	4.76	4.76	4.76	4.76
Molasses	2.26	2.26	2.26	2.26	2.26	2.26
Rapeseed meal	0.78	0.34	0.18	0.78	0.34	0.18
Urea	0.00	0.05	0.07	0.00	0.05	0.07
Total feed intake	7.80	7.41	7.27	7.80	7.41	7.27
<u>Feed efficiency</u>						
Feed DM per kg gain	11.6	13.7	18.2	12.6	14.0	14.8

¹Observations are averages per animal.

TABLE 17. Summary of average daily gains, feed intake and feed efficiency
(Experiment III, Replicate B, 62 days)¹

	Treatments					
	RSM-FA			RSM-H ₂ O		
	No urea	Medium urea	High urea	No urea	Medium urea	High urea
No. of animals	5	5	5	5	5	5
Initial weight (kg)	271.0	270.9	268.3	265.5	277.4	260.5
Final weight (kg)	317.7	305.2	300.5	303.0	303.6	289.8
Average daily gain (kg)	0.75	0.55	0.52	0.60	0.42	0.47
<u>Daily feed intake (kg, DM)</u>						
Corn silage	4.40	4.40	4.40	4.40	4.40	4.40
Molasses	2.09	2.09	2.09	2.09	2.09	2.09
Rapeseed meal	0.68	0.33	0.18	0.68	0.33	0.18
Urea	0.00	0.04	0.06	0.00	0.04	0.06
Total feed intake	7.17	6.86	6.73	7.17	6.86	6.73
<u>Feed efficiency</u>						
Feed DM per kg gain	19.6	12.5	12.9	11.9	16.3	14.3

¹Observations are averages per animal.

TABLE 18. Summary of treatment combinations and main treatment effects on average daily gains (Experiment III, Replicates A and B)

		ADG (kg)
<u>Main effects</u>		
<u>Replicates</u>		
A		0.54 a
B		0.56 a
<u>Treatment</u>		
FA		0.57 a
H ₂ O		0.52 a
<u>Urea levels</u>		
Zero		0.66 a
Medium		0.51 b
High		0.48 b
<u>Combination effects</u>		
<u>Formaldehyde x urea</u>		
<u>Treatment</u>	<u>Urea level</u>	
FA	Zero	0.71 a
FA	Medium	0.54 b
FA	High	0.46 b
H ₂ O	Zero	0.61 c
H ₂ O	Medium	0.47 b
H ₂ O	High	0.49 b

Figures in the formaldehyde x urea, replicates, FA-treatment and urea levels groups bearing different letters are significantly different ($P < .01$).

diet (Table 18). Growth on the zero urea level was significantly greater ($P < .01$) than with any of the added urea levels. Even though there was no significant formaldehyde-urea interaction, the trend was for higher average daily gains with the formaldehyde-treated rapeseed meal when fed alone or with the medium urea level.

4. Feed consumption

Because the animals were group fed, the daily feed intake data represent an average for all animals in a pen and could not be analyzed statistically. The average dry matter intakes for replicates A and B are shown in Tables 16 and 17, respectively.

In replicate A, total dry matter consumption for the animals was 7.8, 7.4 and 7.3 kg per day for the zero, medium and high urea treatments, respectively.

In replicate B, the total dry matter consumption for the animals was 7.2, 6.9 and 6.7 kg per day for the zero, medium and high urea treatments, respectively - the same decrease as noted for replicate A.

The data in Tables 16 and 17 thus indicate a decreasing trend in daily dry matter consumption as the level of urea in the ration increased. This could be attributed to the fact that an increase in the level of urea in the ration resulted in a concomitant decrease in the total dry matter of the ration because of the high nitrogen content of the urea which was used in small amounts, and thus did not contribute significantly to the dry matter content of the ration. The animals

always "cleaned up" the feed offered within a relatively short time. According to the NRC recommendations for growing bulls, daily feed intake (DM) should approximate three per cent of body weight. The dry matter intakes of animals in replicates A and B averaged 2.4 per cent of the average weight throughout the trial. This would indicate that more feed would have been consumed if offered. A greater feed consumption would have resulted in an increased energy intake and probably higher daily gains. Feed intake was restricted in order not to exceed estimated protein requirements and thus confound interpretation of the protein treatments.

The animals in replicate A had higher average dry matter intakes than those in replicate B because the former were started on this experiment with a higher initial weight and finished with a higher final weight than the latter.

5. Feed efficiency

Tables 16 and 17 indicate feed efficiencies for the animals in replicates A and B, respectively. It could be seen that the animals in replicate A had a poorer feed efficiency than those in replicate B. The difference is more outstanding for the animals receiving the formaldehyde-treated RSM. This difference in feed efficiency could be attributed to the fact that the animals in replicate A were larger and had slower rates of gains. This is an expected trend because as animals get heavier the rates of gain tend to decline. Furthermore, even though the feed intakes were similar for animals receiving

formaldehyde-treated and untreated RSM in either replicates A or B, the average daily gains were different. This affected the feed efficiencies, with the animals in replicate A receiving untreated RSM having a slightly better feed efficiency than those receiving the formaldehyde-treated RSM (13.8 vs. 14.5). The situation was, however, reversed in replicate B in which the animals receiving the untreated RSM had poorer feed efficiencies than those receiving the formaldehyde-treated RSM (14.2 vs. 11.7). It could also be seen that feed efficiencies were generally poorer as the level of urea increased in the diets. The only deviation was with the animals in replicate B receiving the untreated RSM and medium urea, that had a better average daily gain than those on the untreated RSM and high urea. This deviation from the pattern is reflected in the average daily gains and feed intakes. Generally, for a slight decrease in feed intakes for the animals in the treatment groups, there was a larger decrease in average daily gains.

D. Summary

In a replicated 2 x 3 factorial experiment that lasted for either 118 days (replicate A) or 62 days (replicate B), Holstein steers were fed a corn silage and molasses ration that was supplemented with rapeseed meal (treated or untreated with formaldehyde) in which 0, 50 or 75 per cent of the RSM protein was replaced by urea.

The results indicated that there was no significant difference ($P > .1$) in average daily gains of the animals, either between replicates A and B or due to formaldehyde treatment. However, there was a significant difference ($P < .01$) in average daily gains of the animals among the three levels of urea, with the average daily gains decreasing as the level of urea increased in the ration.

Feed dry matter and protein intakes were higher for the animals in replicate A than those in replicate B. Feed efficiencies were better for the animals on the untreated RSM than those on the treated RSM in replicate A, a situation which was reversed in replicate B.

VII. EXPERIMENT IV. THE EFFECT OF FORMALDEHYDE TREATMENT
OF RAPESEED MEAL, FED ALONE OR WITH UREA AS A PROTEIN
SUPPLEMENT FOR GROWING CATTLE:
HAYLAGE-MOLASSES BASAL RATION

A. Introduction

This experiment was initiated due to a change in feed supply on the Macdonald College farm where the feeding trials were being conducted. Due to a decreasing supply of corn silage, it became necessary to change the basal ration primarily to alfalfa haylage (harvested 1975). Continuing the same rapeseed meal and urea treatments from the previous experiment, ensiled corn grain (high moisture corn - 1974 crop) and molasses were also used as feeds.

B. Experimental Procedures

1. Animals

Replicate A.--This replicate was initiated in the summer of 1975 (August 7), and 34 Holstein steers (from replicate A of experiment III) with an average weight of 348 kg, were used.

Replicate B.--This replicate was initiated at the same time as replicate A (August 7, 1975), and 30 Holstein steers (from replicate B of experiment III), with an average weight of 303 kg, were used.

2. Experimental design

The animals in each replicate were randomly distributed into a 2 x 3 factorial design in which the factors were formaldehyde treatment of protein supplements (treated vs. untreated) and three levels of urea (zero, medium and high). In replicate A, the animals were distributed into six pens with four pens having six animals each and two pens having five animals each; while in replicate B, the animals were distributed into six pens with each pen having five animals. Treatments and animal distribution were the same as in experiment III, the only change being the switch-over from a corn silage to a haylage-molasses basal ration.

3. Rations

A haylage, corn grain and molasses ration was supplemented with rapeseed meal¹ (treated or not treated with formaldehyde) in which either 0, 50 or 75 per cent of the protein from the rapeseed meal was replaced by urea. Dicalcium phosphate was fed to all the animals at a rate of 200 g/day. The experiment lasted for 84 days.

4. Treatment of rapeseed meal

The same formaldehyde and control treatment procedures as outlined in experiment III, section 4 (a) and (b), were used in this experiment.

¹Tower variety (1974 crop).

5. Sampling of feed for chemical analysis

The formaldehyde-treated and untreated rapeseed meal was routinely sampled for crude protein analysis and solubility measurements. The haylage and corn grain were also sampled for dry matter and crude protein analysis, conducted according to the AOAC (1975) methods.

6. Management

The same procedure as used in Experiment I, only the animals were not given any injections of vitamins A, D and E, was used in this experiment.

7. Statistical analysis

The same procedure was followed as that used in Experiment III.

C. Results and Discussion

1. Chemical analysis of ration components

The results of analysis conducted on six samples of haylage, corn grain and rapeseed meal during experiment IV are presented in Table 19. Because molasses is fairly constant in composition, no chemical analysis was conducted and the values presented in Table 19 are from NRC.¹

¹United States-Canadian Tables of Feed Composition (1969). Second edition. Publ. 1684, National Academy of Sciences, Washington, D.C.

TABLE 19. Chemical composition of ration components (Experiment IV, Replicates A and B)

Ingredient	NRC Ref. No.	Dry matter (%)	Crude protein ¹
Alfalfa - aerial part, ensiled, early bloom, mn 50% dry matter (3)	3-08-151	57.8	13.6
Corn - dent yellow, ensiled, grain gr 2 US mn 54 lb per bushel (4)	4-02-931	81.5	9.8
Sugar cane molasses, mn 48% invert sugar mn 79.5 degrees brix ²	4-04-696	75.0	4.3
Rapeseed meal (rape, seed, solvent extracted, ground)	5-03-871	90.4	37.4
Urea (42% N)		98.0	281.0

¹Per cent dry matter basis.

²Values are from the United States-Canadian Tables of Feed Composition (1969), second edition. Publ. 1684, National Academy of Sciences, Washington, D.C.

2. Composition of rations

Table 20 indicates the average composition of the rations over the 84 days' experimental period.

Ration adjustments made on a monthly basis were designed to meet the protein requirements of each group of cattle based on their average weight.

Table 20 indicates that the haylage portion of the ration dry matter varied from 24 to 27 per cent among the three levels of urea, a relatively narrow limit. Corn grain varied from 32 to 34 per cent, also a relatively narrow range. Molasses varied from 35 to 41 per cent of ration DM, thus it also fell into a narrow limit. The 1 to 4 per cent range of ration DM for rapeseed meal and 0 to 3 per cent range of ration DM for urea were set to supply equivalent amounts of crude protein. A relatively high level of molasses with its low protein content was fed to the animals in an attempt to maintain the available energy level of the ration. Thus, as the level of urea increased, the level of molasses also was increased.

The ability of the monthly ration adjustments to supply controlled amounts of protein is indicated in Tables 21 and 22 for replicates A and B, respectively. As protein requirements differed for each replicate due to animal weight differences, the protein intake (supplied) was directly compared with the specific requirements for the average weight of that replicate.

TABLE 20. Average composition of experimental rations (Experiment IV, Replicates A and B)¹

Ingredients	Treatments		
	No urea	Medium urea	High urea
Alfalfa haylage	27	24	24
Corn grain	34	33	32
Molasses	35	39	41
Rapeseed meal	4	2	1
Urea	0	2	3
	100	100	100

¹Values are per cent of total dry matter.

TABLE 21. Summary of average daily protein intake and supply (Experiment IV, Replicate A)¹

	Treatments					
	RSM-FA			RSM-H ₂ O		
	No urea	Medium urea	High urea	No urea	Medium urea	High urea
Protein intake (kg)	0.85	0.85	0.85	0.85	0.85	0.85
Protein requirement (kg) ²	0.88	0.88	0.88	0.88	0.88	0.88
Protein intake as per cent of requirement	96.6	96.6	96.6	96.6	96.6	96.6
<u>Protein supply (per cent of total)</u>						
Alfalfa haylage	29	27	26	29	27	26
Corn grain	26	26	26	26	26	26
Molasses	11	13	14	11	13	14
Rapeseed meal	34	17	9	34	17	9
Urea	0	17	25	0	17	25
	100	100	100	100	100	100

¹All treatment groups had 6 animals except the RSM-FA-high urea and RSM-H₂O-no urea groups which had 5 animals; observations are averages per animal.

²Daily nutrient Requirements of Dairy Cattle (large breeds). Table I. NRC (1971), National Academy of Sciences, Washington, D.C.

TABLE 22. Summary of average daily protein intake and supply (Experiment IV, Replicate B)¹

	Treatments					
	RSM-FA			RSM-H ₂ O		
	No urea	Medium urea	High urea	No urea	Medium urea	High urea
Protein intake (kg)	0.80	0.80	0.80	0.80	0.80	0.80
Protein requirement (kg) ²	0.81	0.81	0.81	0.81	0.81	0.81
Protein intake as per cent of requirement	98.8	98.8	98.8	98.8	98.8	98.8
<u>Protein supply (per cent of total)</u>						
Alfalfa haylage	29	27	26	29	27	26
Corn grain	26	26	26	26	26	26
Molasses	11	13	14	11	13	14
Rapeseed meal	34	17	9	34	17	9
Urea	0	17	25	0	17	25
	100	100	100	100	100	100

¹All treatment groups had 5 animals and observations are averages per animal.

²Daily nutrient requirements of Dairy Cattle (large breeds), Table I. NRC (1971).
National Academy of Sciences, Washington, D.C.

It can be seen that the protein intakes for the animals in replicate A were higher than those for the animals in replicate B (0.85 vs. 0.80 kg), due to differences in average animal weight. The protein intakes for the animals in both replicates were slightly lower than the NRC¹ requirements. As in the previous experiment, avoidance of feeding excess protein could enhance interpretation of the experimental results.

Tables 21 and 22 also indicate the per cent of daily protein intake supplied by the different ration ingredients. Alfalfa was the largest supply of protein, averaging 27 per cent of total. Corn grain supplied an average of 26 per cent of the total protein; while molasses supplied an average of 13 per cent of the total protein. Rapeseed meal and urea combinations supplied 34 per cent of the total protein supply.

3. Animal growth performance

A summary of the average daily gains for replicates A and B are shown in Tables 23 and 24, respectively. The analysis of variance of average daily gains is summarized in Appendix Table 4. A summary of main treatment effects and treatment combinations of average daily gains is presented in Table 25.

A significant difference ($P < .1$) was observed between replicates A and B, with the larger animals in replicate B having average daily

¹Nutrient Requirements of Beef Cattle. Growing bulls (large breeds). Table I. NRC (1971). National Academy of Sciences, Washington, D. C.

TABLE 23. Summary of average daily gains, feed intake and feed efficiency
(Experiment IV, Replicate A, 84 days)¹

	Treatments					
	RSM-FA			RSM-H ₂ O		
	No urea	Medium urea	High urea	No urea	Medium urea	High urea
No. of animals	6	6	5	5	6	6
Initial weight (kg)	360.6	344.4	335.2	358.3	351.0	339.6
Final weight (kg)	421.6	401.3	388.3	419.5	406.5	394.5
Average daily gain (kg)	0.73	0.68	0.63	0.73	0.66	0.65
<u>Daily feed intake (kg, DM)</u>						
Alfalfa haylage	1.80	2.29	2.74	1.80	2.29	2.74
Corn grain	2.13	2.13	2.13	2.13	2.13	2.13
Molasses	2.97	2.97	2.84	2.97	2.97	2.84
Rapeseed meal	0.30	0.15	0.08	0.30	0.15	0.08
Urea	0.00	0.31	0.22	0.00	0.31	0.22
Total feed intake	7.20	7.85	8.01	7.20	7.85	8.01
<u>Feed efficiency</u>						
Feed DM per kg gain	9.9	11.5	12.7	9.9	11.9	12.3

¹Observations are averages per animal.

TABLE 24. Summary of average daily gains, feed intake and feed efficiency
(Experiment IV, Replicate B, 84 days)¹

	Treatments					
	RSM-FA			RSM-H ₂ O		
	No urea	Medium urea	High urea	No urea	Medium urea	High urea
No. of animals	5	5	5	5	5	5
Initial weight (kg)	317.7	305.2	300.5	303.0	303.6	289.8
Final weight (kg)	388.6	372.3	371.5	372.0	369.2	342.4
Average daily gain (kg)	0.84	0.80	0.85	0.82	0.78	0.63
<u>Daily feed intake (kg, DM)</u>						
Alfalfa haylage	1.99	1.97	1.67	1.99	1.97	1.67
Corn grain	1.98	1.98	1.98	1.98	1.98	1.98
Molasses	2.30	2.40	2.83	2.30	2.40	2.83
Rapeseed meal	0.30	0.29	0.15	0.30	0.29	0.15
Urea	0.00	0.15	0.22	0.00	0.15	0.22
Total feed intake	6.57	6.79	6.85	6.57	6.79	6.85
<u>Feed efficiency</u>						
Feed DM per kg gain	7.9	8.5	8.1	8.0	8.7	10.9

¹Observations are averages per animal and each treatment group had 5 animals.

TABLE 25. Summary of treatment combination and main treatment effects on average daily gains (Experiment IV, Replicates A and B)

		ADG (kg)
<u>Main effects</u>		
<u>Replicates</u>		
A		0.72 a
B		0.79 b
<u>Treatment</u>		
FA		0.73 a
H ₂ O		0.78 a
<u>Urea levels</u>		
Zero		0.78 a
Medium		0.73 ab
High		0.67 b
<u>Combination effects</u>		
<u>Formaldehyde x urea</u>		
<u>Treatment</u>	<u>Urea level</u>	
FA	Zero	0.78 a
FA	Medium	0.73 ab
FA	High	0.69 b
H ₂ O	Zero	0.78 a
H ₂ O	Medium	0.72 b
H ₂ O	High	0.64 c

¹ Figures in the replicates, treatment, urea levels and formaldehyde x urea groups bearing different letters are significantly different ($P < .1$).

gains higher than those of the animals in replicate A (0.79 vs. 0.72 kg). A possible explanation for the difference in performance between the two replicates could be better adaptation to the protein supplements due to a larger feeding period in the previous experiment (III).

Analysis of variance of the average daily gains showed no significant difference ($P > .1$) either due to formaldehyde treatment or urea levels. This was the same trend as in experiment III. This difference in average daily gains might also be partly attributed to the increasing trend in total dry matter consumption as the level of urea increased in the ration (Tables 23 and 24), and partly to the better energy status of the ration because the level of molasses increased as the level of urea in the ration increased.

The analysis of variance also indicated a significant difference ($P < .1$) in average daily gains for the animals receiving the rations containing the various levels of urea with either formaldehyde-treated or untreated RSM.

4. Feed consumption

Because the animals were group fed, the daily feed intake data could not be analyzed statistically. The average dry matter intakes for replicates A and B are shown in Tables 23 and 24, respectively. In replicate A, total dry matter consumption for the animals was 7.20, 7.85 and 8.01 kg per day for the zero, medium and high urea treatments, respectively.

In replicate B, the total dry matter consumption for the animals was 6.57, 6.79 and 6.85 kg per day for the zero, medium and high urea treatments, respectively, within the formaldehyde-treated RSM.

The data in Tables 23 and 24 further indicate an increasing trend in dry matter consumption as the level of urea in the ration increased. This could be attributed to the increase in energy due to molasses addition as the level of urea increased in the ration.

The animals always "cleaned up" the feed offered. Therefore, the animals in replicate A had higher dry matter intakes than those in replicate B, because the former were started on this experiment with higher initial weights and finished with higher final weights than the latter. These dry matter feed intakes were approximately 2.1 and 1.7 per cent of the body weights of animals in replicates A and B, respectively. These dry matter intakes as a percentage of animal body weight are lower than the 2.5 to 3.0 per cent usually recommended by the National Research Council.

5. Feed efficiency

Tables 23 and 24 indicate feed efficiencies for the animals in replicates A and B, respectively. It could be seen that the animals in replicate A had poorer feed efficiencies than those in replicate B (11.4 vs. 8.7). This difference in feed efficiency could be due to the fact that the animals in replicate A were larger than those in replicate B.

The results also indicate that the feed intakes were the same for animals receiving either formaldehyde-treated or untreated RSM in replicates A and B. However, the animals receiving the formaldehyde-treated RSM had better average daily gains than those receiving the untreated material. This trend is reflected in the feed efficiencies of replicates A and B in Tables 23 and 24. It could also be seen from the results that the feed efficiencies in both replicates were poorer as the level of urea increased in the diets. Even though there was a slight increase in the feed intake, there was still a decrease in average daily gains as the level of urea increased in the diet - a trend that was similar to that of experiment III.

D. Summary of Experiment IV

In an 84-day replicated 2 x 3 factorial experiment, Holstein steers were fed a haylage, corn grain and molasses ration that was supplemented with rapeseed meal (treated or untreated with formaldehyde) in which 0, 50 or 75 per cent of the RSM protein was replaced by urea.

The results indicated that there was a significant difference ($P < .1$) in average daily gains of the animals with the animals in replicate B having higher average daily gains than those in replicate A. However, there was no significant difference ($P > .1$) due to formaldehyde treatment of RSM within the formaldehyde treatments.

Feed dry matter and protein intakes were higher for the animals in replicate A than those in replicate B. Feed efficiencies were

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better for the animals on the untreated RSM than those on the treated RSM in replicate A, a situation which was reversed in replicate B. On the overall, the animals in replicate B seemed to have a better feed efficiency than those in replicate A.

VIII. EXPERIMENT V. THE EFFECT OF FORMALDEHYDE TREATMENT
OF RAPESEED MEAL ON RATION DIGESTIBILITY AND
NITROGEN RETENTION OF SHEEP

A. Introduction

The objective of this experiment was to compare nutrient digestibility and nitrogen retention of formaldehyde treated or untreated rapeseed meal when fed alone or with urea. This experiment, utilizing sheep, was designed to complement some of the previous cattle feeding trials, in order to provide additional information on the utilization of rapeseed meal.

B. Experimental Procedures

1. Animals

The experiment was carried out in the winter of 1975-76, and eight mature Columbia wether sheep were used. The animals ranged in weight from 42.2 to 44.9 kg, with the average weight of all sheep over the two periods being 43.6 kg.

2. Experimental design

The animals were randomly distributed in a 2 x 2 factorial design in which the factors were formaldehyde treatment of rapeseed meal (treated vs. untreated) and urea supplementation (urea vs. no urea). Each 2 x 2 factorial was replicated in each experimental period. The experiment constituted two 21-day experimental periods.

3. Rations

The basal ration consisted of corn silage and molasses which was supplemented with rapeseed meal (1974, Tower), treated or not treated with formaldehyde (1 g FA/100 g of protein). This rapeseed meal and treatment were similar to that used in the previous cattle feeding trials. Two of the rations had 25 per cent of the dietary protein provided by urea.

4. Management

The sheep were housed individually in cages which permitted the total collection of urine and feces. Water and cobalt-iodized salt were made available at all times. The sheep were offered daily (at 0700 h) a weighed amount of feed calculated to meet their maintenance requirements for protein. Feed refused was weighed prior to offering fresh feed on the following day.

5. Experimental periods

Each of the four rations was offered to two sheep during each of two 21-day periods. Each experimental period consisted of a 14-day preliminary period followed by a 7-day collection period. The preliminary period enabled the animals to acquaint themselves with the cages, to make the necessary adjustments in order that the feces and urine were collected properly, and to adjust the animals to their intake of feed. The animals were weighed on days 14 and 23 respectively. Daily feed intakes were recorded during the entire period of the experiment. Total fecal and urine collections were

made from day 17 to 23. For calculation of nutrient digestibility coefficients and nitrogen retention, fecal and urine samples collected on day 17 through 23 were apportioned to feed consumed on days 15 through 21, in order to compensate for delay in passage of ingested feed (Donefer et al. 1963).

6. Collection of samples

a. Feed

Corn silage and refused feed samples were collected daily during days 15 through 21. Approximately 200 g of corn silage was randomly collected each day and was dried daily for 72 hours in a forced-air oven at 66°C. Only one composite sample of the rapeseed meal and molasses was collected since these feeds were considered relatively constant in chemical composition during the experimental period.

b. Faeces

Collection of the total faeces was done on days 17 through 23. A 20 per cent aliquot of the faeces was dried daily in a forced-air oven for 24 hours at 66°C and composited for each animal for the total collection period. The composite sample was ground in a hammer mill (1 mm diameter screen) and stored in an air-tight container for chemical analysis.

c. Urine

Urine samples were collected during days 17 through 23. The total volume of the urine excreted by each animal was measured and a two per cent aliquot was taken. Ten ml of concentrated HCl were added to each urine jar prior to each day's collection so as to minimize nitrogen losses. Urine samples for each animal were composited as collected and kept refrigerated under a layer of toluene.

7. Chemical analysis

Analyses were done on corn silage, molasses, RSM and faeces samples for dry matter, crude protein, and ash, by methods of the AOAC (1975); for gross energy using the oxygen bomb calorimeter;¹ and cellulose by the method of Crampton and Maynard (1938) as modified by Donefer et al (1960). Urine was analyzed for nitrogen by the Macro Kjeldahl method (AOAC 1975) and a 5 ml urine sample was used.

8. Calculations

Coefficients of apparent digestibility of different nutrients were calculated using the equation:

$$\begin{array}{l} \text{Coefficient of} \\ \text{digestibility} \\ (\%) \end{array} = \frac{\text{Nutrient intake} - \text{Nutrient fecal excretion}}{\text{Nutrient intake}} \times 100$$

¹Parr Instrument Corp., Inc., Moline, Illinois.

9. Statistical analysis

The data obtained from this experiment were analyzed using the analysis of variance technique (Steel and Torrie 1960). The calculations were done using the Statistical Analysis System (SAS) on the McGill IBM 360 computer.

C. Results and Discussion

1. Chemical analysis of ration components

Results of analysis, conducted on samples of each feedstuff during experiment V are summarized in Table 26. The urea was not analyzed but was assumed to have a crude protein value of 281 per cent and a dry matter content of 98.0, as specified by the manufacturers.

2. Composition of rations

The average composition of the rations over the two 21-day experimental periods is indicated in Table 27.

Ration adjustments made at the start of each 21-day experimental period were designed to meet the protein requirements for maintenance of each sheep, based on its weight.

Table 27 also indicates that the corn silage portion of the ration dry matter varied from 69 to 75 per cent, a relatively narrow limit. Molasses level varied only from 22 to 23 per cent of ration dry matter. The 2 to 8 per cent range of ration dry matter

TABLE 26. Chemical composition of ration components (experiment V)¹

	NRC Ref. No.	Dry matter	Gross energy ²	Crude protein	Cellulose	Ash
Corn, aerial part, ensiled, mature, well-eared mx 50% mn 30% DM (3)	3-08-153	33.5	4.6	7.0	17.4	4.5
Sugar cane molasses, mn 48% invert sugar mn 79.5 degrees brix	4-04-696	76.3	3.9	4.4	0.2	7.8
Rapeseed meal (rapeseed, solvent extracted, ground)	5-03-871	89.7	4.6	35.4	11.2	7.3
Urea (42% N)		98.0	-	281.0	-	-

¹Values are on per cent dry matter basis except for gross energy.

²Values are expressed on Kcal/g.

TABLE 27. Average composition of experimental rations
(Experiment V)¹

Ingredients	Treatments			
	RSM-H ₂ O		RSM-FA	
	No urea	Urea	No urea	Urea
Corn silage	68.4	74.9	69.1	74.5
Molasses	23.3	22.2	23.3	22.6
Rapeseed meal	8.3	2.0	7.6	2.0
Urea	-	0.9	-	0.9
	100.0	100.0	100.0	100.0

¹Per cent of total dry matter

for rapeseed meal and 0 to 0.9 per cent range of ration dry matter for urea were set so that equivalent amounts of crude protein were supplied by rapeseed meal or the rapeseed meal and urea combinations. This was an attempt to parallel the levels used in the previous cattle experiments. A relatively high level of molasses with its low protein content was fed to the animals in an attempt to maintain the available energy level of the ration.

Table 28 indicates the ability of the ration adjustment in the two experimental periods to supply controlled amounts of protein.

It could be seen that the sheep receiving the diets without urea had higher protein intakes than those receiving urea supplemented diets (80.4 vs. 98.8 g/day). The sheep receiving the diets supplemented with RSM-FA had similar protein intakes to those receiving the RSM-H₂O supplemented diets (87.5 vs. 86.7 g/day). These protein intakes were considerably lower than the National Research Council (U.S.) recommendations.

Table 28 further indicates the per cent of daily protein intakes supplied by the different ingredients. Corn silage was the largest supplier of protein, averaging 62 per cent of total. Molasses supplied an average of four per cent of the total protein; while rapeseed meal and urea combinations supplied 34 per cent of the total protein supply.

TABLE 28. Summary of average daily protein intake and supply
(Experiment V)¹

	Treatments			
	RSM-FA		RSM-H ₂ O	
	Urea	No urea	Urea	No urea
Protein intake (g)	81.7	93.3	79.1	94.3
Protein requirement (g) ²	133.0	133.0	133.0	133.0
Protein intake as per cent of requirement	61.0	70.0	59.0	70.0
Protein supply (per cent of total)				
Corn silage	62	62	62	62
Molasses	4	4	4	4
Rapeseed meal	9	34	9	34
Urea	25	-	25	-
	100	100	100	100

¹ Each figure is an average of observations for four sheep.

² Replacement yearlings. Daily Nutrient Requirements of Sheep. Table I, Nutrient Requirements of Sheep, NRC, 1975, Washington, D.C.

3. Nutrient digestibility

Data on the effect of formaldehyde treatment of rapeseed meal, fed alone or with urea, on nutrient digestibility in sheep are summarized in Table 29. The analysis of variance for digestibility of dry matter, gross energy, protein and cellulose are presented in Appendix Tables 5 to 8, respectively.

No significant difference ($P > .05$) in dry matter digestibility was observed either as a result of formaldehyde treatment of rapeseed meal or the addition of urea to the diets. However, there was an observed increase (not significant) in dry matter digestibility due to formaldehyde treatment of RSM (69.9 vs. 72.3 per cent). The trend in this data is rather inconsistent with the observations of other research workers. Sharma and Ingalls (1973) reported a non-significant decrease in dry matter digestibility of formaldehyde treated as compared with untreated RSM. Also, while Ely *et al.* (1971) and Tudor and Morris (1971) have reported an increase in dry matter digestibility due to urea addition, Tillman and Swift (1953) and Bhattacharya and Pervez (1973) reported a non-significant decrease in dry matter digestibility, with the addition of urea to diets.

The inconsistency in the results presented herein could be due to differences in diet ingredients and treatments with the once-a-day feeding protocol, length of the adaptation period to the diet and the age and breed of the sheep used, also being possible factors.

TABLE 29. Effects of formaldehyde treatment of rapeseed meal on nutrient intake and digestibility in sheep (Experiment V)¹

	Treatments			
	RSM-FA		RSM-H ₂ O	
	Urea	No urea	Urea	No urea
<u>Apparent digestibility coefficient (%)</u>				
Dry matter	73.6	71.0	69.9	69.9
Gross energy	73.0	66.7	67.7	68.8
Protein	47.3	52.1	46.6	57.5
Cellulose	51.9	46.1	49.5	45.5
<u>Nutrient intake</u>				
Dry matter (g)	1109.7	1088.8	1037.5	1073.3
Digestible dry matter (g)	779.8	755.8	696.2	734.2
Digestible energy (Kcal/g)	3364.1	3243.5	2989.6	3224.5
Digestible cellulose (g)	66.3	58.0	65.6	56.3

¹Each figure is an average of four observations, and all of the averages for apparent digestibility were not significantly different ($P > .05$).

The data on gross energy digestibility were also non-significant ($P > .05$) for mean effects, as indicated by the analysis of variance in Appendix Table 6. However, there was a slight increase in energy digestibility, a result of formaldehyde treatment of RSM (68.8 vs. 69.9) and the addition of urea to the diets (68.3 vs. 70.3). This trend in gross energy digestibility is similar to that observed for dry matter digestibility. The data are also inconsistent when compared to that of other workers. For example, even though Sharma and Ingalls (1973) reported a non-significant difference in gross energy digestibility between formaldehyde treated and untreated RSM, they observed a decrease in gross energy digestibility due to formaldehyde treatment of RSM.

The data in Table 29 also indicate that the combination of formaldehyde treatment of RSM and urea addition had the greatest effect on increasing gross energy digestibility.

Protein digestibility data in Table 29 also indicate a non-significant difference ($P > .05$) in digestibility as a result of formaldehyde treatment of RSM. The formaldehyde treatment, however, resulted in a decrease in protein digestibility (52.1 vs. 49.7). This observation supports that of several workers. For example, Sharma and Ingalls (1973), Sharma *et al.* (1972), and Stone (1971) reported decreased protein digestibility as a result of formaldehyde treatment of RSM.

The data also indicate a significant decrease ($P < .05$) in protein digestibility as a result of urea addition to the diets

(54.79 vs. 46.93). These data are in contrast to those of Ely et al. (1971) and Tudor and Morris (1971), who indicate an increase in protein digestibility with the addition of urea to diets; but in support of those of Tillman and Swift (1953) and Bhattacharya and Pervez (1973) who observed a non-significant difference in protein digestibility due to urea addition to the diets.

No significant difference ($P > .05$) in cellulose digestibility was observed, either due to the formaldehyde treatment of RSM or the addition of urea to the diets. However, there was a non-significant increase in cellulose digestibility due to formaldehyde treatment of RSM (47.5 vs. 49.0). These data support the reports of Sharma and Ingalls (1973) and Sharma et al. (1972) of increased cellulose digestibility due to formaldehyde treatment of RSM. Also, the addition of urea to the diets increased cellulose digestibility (45.8 vs. 50.7). This increase in digestibility could be due to an increased supply of readily available nitrogen for the cellulolytic microorganisms.

4. Feed consumption

Feed consumption data is summarized in Table 29. The amounts of feed consumed were a result of the calculated and restricted feed offered and thus are not measures of voluntary intake. Feed offered was calculated to meet the maintenance requirements of the sheep, and it was also an attempt not to feed energy and protein in excess of NRC recommendations as this could complicate the interpretation

of the results. This consideration parallels that of the previous cattle experiments. During the last seven days of the experiment in the two experimental periods, the sheep always "cleaned up" the feed offered.

Average dry matter intakes were very similar since fairly uniform sheep were used in the experiment. The differences in intake that did exist were due to the averaging of intakes for four different sheep over the two experimental periods. However, the sheep receiving diets supplemented with formaldehyde treated RSM had higher dry matter intakes than those receiving the untreated meal (1099.3 vs. 1055.4 g/day); while the sheep receiving the urea supplemented diets had slightly lower dry matter intakes than those receiving the unsupplemented diets (1081.1 vs. 1073.6 g/day).

The protein intake pattern (Table 28) was similar to the dry matter intake pattern. The sheep receiving the diets supplemented with formaldehyde treated RSM had slightly higher protein intakes than those receiving the untreated meal (87.5 vs. 86.7 g/day); while the sheep receiving the urea supplemented diets had lower protein intakes than those receiving the unsupplemented diets (80.4 vs. 93.8 g/day).

The protein intakes were generally lower than the NRC recommendations for this category of sheep, as indicated in Table 28.

Intakes are also presented in Table 29 for digestible dry matter, digestible energy (DE), and digestible cellulose. The values are calculated as the product of dry matter intake and the

appropriate digestibility coefficient. They are thus largely influenced by the relatively constant dry matter intake and any differences observed in digestibility due to treatment.

5. Nitrogen metabolism

The data on nitrogen metabolism is summarized in Table 30. The analysis of variance of the nitrogen retention data is presented in Appendix Table 9.

There was a significant difference ($P < .05$) in per cent nitrogen retention in sheep fed diets containing formaldehyde-treated or untreated RSM, with the sheep on the RSM-FA diets having a considerably higher nitrogen retention than those on the untreated RSM (21.1 vs. 3.6 per cent). This observation follows the general observations of most research workers in the area of "protection" of protein supplements (Sharma *et al.* 1972, Sharma and Nicholson 1974, with RSM; Faichney 1974, with peanut meal).

Similarly, there was a significant decrease ($P < .05$) in nitrogen retention due to the addition of urea in the diets (20.4 vs. 4.3 per cent). Nitrogen retention was lowest for those sheep fed the untreated RSM and urea combination, a result which should be expected. These data support those of Sharma and Nicholson (1974) who observed a decrease in nitrogen retention in sheep fed diets supplemented with untreated RSM and urea. Urea nitrogen utilization by ruminants has been found to be inferior to pre-formed protein supplements (Helmer and Bartley 1971), probably due to its rapid

TABLE 30. Effects of formaldehyde treatment of rapeseed meal on nitrogen metabolism in sheep (Experiment V)¹

Parameter	Treatments			
	RSM-FA		RSM-H ₂ O	
	Urea	No urea	Urea	No urea
Nitrogen intake (g)	13.1	14.9	12.7	15.1
Fecal nitrogen (g)	7.9	7.7	7.2	6.8
Absorbed nitrogen (g)	6.6	8.3	6.2	9.4
Urinary nitrogen (g)	5.7	3.6	10.6	7.3
Retained nitrogen (g)	0.95	4.73	-4.42	2.07
Retained nitrogen as per cent of intake ²	12.7	29.5	-4.2	11.4

¹Each figure is an average of four observations, and the averages for nitrogen retention were significantly different ($P < .05$).

²Based on average of individual sheep data (Appendix Table 9).

rate of hydrolysis in the rumen, a situation which could decrease nitrogen retention and utilization.

The decrease in nitrogen retention as a result of feeding untreated RSM and urea is reflected in the high urinary nitrogen excretion as summarized in Table 30. Nitrogen intake was also lowest for this group.

Fecal nitrogen excretion was slightly higher for the sheep fed the RSM-FA diet compared with those fed the RSM-H₂O diet (7.8 vs. 7.0 g/day). These data support that of many research workers. For example, Reis and Tunks (1969) on formaldehyde-treated casein; Nishimuta et al. (1972) and Schmidt et al. (1974) on formaldehyde-treated SEM, Rettray and Joyce (1970) on formaldehyde-treated linseed meal (LSM) and Amos et al. (1974) on formaldehyde-treated sunflower meal (SFM). This observation has usually been attributed to the slight decrease in nutrient digestibility due to formaldehyde treatment of the protein supplements; even though our data showed an increase in digestibility due to formaldehyde treatment of the protein supplements.

The addition of urea to the diets resulted in a slightly increased fecal nitrogen loss from the sheep (7.6 vs. 7.3 g/day). This indicates an inefficient utilization of urea nitrogen for microbial protein synthesis. Fecal nitrogen excretion was highest for the sheep fed RSM-FA plus urea.

Urinary nitrogen excretion was lower for the sheep fed the RSM-FA diet compared with those fed the RSM-H₂O diet (4.2 vs. 9.0

g/day). The sheep fed a combination of untreated RSM and urea had the highest urinary excretion of nitrogen. These data support those reported by Reis and Tunks (1969) on formaldehyde-treated casein, Nishimuta et al. (1972) and Schmidt et al. (1974) on formaldehyde-treated SBM, and Amos et al. (1974) on formaldehyde-treated sunflower meal, that indicate a decreased urinary nitrogen excretion due to formaldehyde treatment.

The addition of urea to the diets resulted in an increase in urinary nitrogen excretion from sheep (8.2 vs. 5.5 g/day). This also indicated an inefficient utilization of urea nitrogen by sheep for microbial protein synthesis. These data support data of Polan et al. (1968, 1970c), Holter et al. (1971) and Leibholz and Naylor (1971), who reported increased urinary nitrogen excretion and decreased nitrogen retention due to urea addition to the diets.

D. Summary of Experiment V

Eight sheep were randomly distributed in a 2 x 2 factorial design in which the factors were formaldehyde treatment of RSM (treated vs. untreated) and urea supplementation (urea vs. no urea). Each 2 x 2 factorial was replicated in each of the two 21-day experimental periods.

In summary, the data from this experiment indicate that there was no significant effect ($P > .05$) of formaldehyde treatment of RSM on the digestibility of dry matter, gross energy, crude protein and cellulose. There was also no significant effect ($P > .05$) of urea

supplementation on the digestibility of dry matter, gross energy and cellulose, but it had an effect in crude protein digestibility ($P < .05$).

Furthermore, formaldehyde treatment of RSM resulted in a significant increase ($P < .05$) in nitrogen retention, an increase in fecal nitrogen excretion but a decrease in urinary nitrogen excretion. Supplementation of the diets with urea resulted in a significant decrease ($P < .05$) in nitrogen retention, an increase in fecal nitrogen excretion and an increase in urinary nitrogen excretion.

IX. GENERAL DISCUSSION

The general assumption till recently was that the quality of proteins was not important in ruminant rations (Snapp and Newmann 1963), and that all proteins of equal digestibility would have the same value. Morrison (1959) found that animal performance was affected by the kind of protein supplement fed in some but not in all feeding trials using practical-type rations. These differences could be attributed to differences in protein utilization which in turn may be due to differences in rates of degradation of proteins in the rumen.

The net protein available to the ruminant animal is the sum of microbial protein synthesized and the amount of dietary protein that escapes rumen degradation. The amount of microbial protein synthesized will depend on the energy available in the rumen, the ammonia available, the source and specific requirement of protein in the rumen, sulphur, other nutrients and the relative rate of release of these different nutrients. The amount of dietary protein escaping rumen degradation will depend on protein solubility, level of protein and feed intake and the physical form of the diet. The above major factors are influenced by an efficiency factor which is actually the efficiency of protein utilization. This depends on the digestibility of the protein, the amino acid composition of the

proteins, the nucleic acid content of the proteins and the amount of energy available to the animal.

In general, the possible reasons for the lack of response in terms of increased weight gains when proteins are treated with formaldehyde could be examined from the point of view that the diets must be adequate in energy yielding ingredients. If the animal is expected to be able to utilize the expected increase in available amino acids in the lower gastro-intestinal tract, then dietary energy should never be limiting. Even though this requirement is somewhat obvious, it may not have been met in our experiments because the diets were not isocaloric. This was primarily due to an attempt to supply protein to the animals at a fixed level that was usually slightly below the National Research Council recommendations. Diet formulation was therefore aimed at an isonitrogenous diet rather than at an isonitrogenous and isocaloric diet, and protein intakes were calculated to meet the requirements of the animals as set by NRC. This practice accounts for the restricted amount of energy fed to the animals, since offering energy feeds ad libitum would have resulted in excess protein intake. This factor may negatively affect microbial protein synthesis, the energy status of the animal and overall animal performance. For example, when Faichney and Davies (1972) restricted dry matter intakes of sheep, there was a significant reduction in volatile fatty acids concentration in rumen fluid, which may be related to a reduced energy availability to the animal and may reflect insufficient microbial activity. This

decrease in VFA production has been associated with formaldehyde treatment of proteins or the diets. If there is a conceivable reduction in rumen digestibility of the energy components of the diet which may be reflected in decreased VFA production, possibly because of a reduced microbial population and/or activity, this could be compensated by digestion in the lower gastrointestinal tract if the diet is high in concentrates. The above situation may tend to parallel that of experiments III and IV which involved the feeding of corn silage and molasses, and haylage, molasses and corn grain, respectively, a situation which resulted in the intake of DM below NRC recommendations. If the expectation is that the starch component of the concentrate would be digested with greater efficiency in the lower gastrointestinal tract than in the rumen, then there should be a decreased energy loss in the form of methane, provided little starch fermentation occurs in the large intestines (Waldo 1973). On the other hand, with the diets high in roughage, as in experiment II, an overall reduction of dietary energy would certainly be expected if ruminal microbial activity is depressed due to formaldehyde treatment because about 90 per cent of the digestion of structural carbohydrates like cellulose and hemicellulose occurs in the rumen (Waldo 1973).

If the energy available in the rumen becomes limiting for microbial growth, then the amino acids coming from microbial protein that will be absorbed from the lower gastrointestinal tract will decrease. Miller (1973), making some assumptions

estimated that a yield of 7.0 g of microbial nitrogen per megacalorie of metabolizable energy fed would be expected. This would be equivalent to 38.15 g protein per megacalorie of metabolizable energy fed. The commonly accepted figures that relate the energy available in the rumen with bacterial growth are 27 g of bacterial nitrogen per kilogram of organic matter truly digested or 36 g bacterial nitrogen per kilogram of organic matter apparently digested in the rumen (Thomas 1973; Miller 1973). At any rate, if energy becomes limiting in the rumen, we would expect a reduction in microbial protein production proportional to the energy deficit in the rumen. Consequently, the more microbial protein production is decreased, the lesser will be the chances of detecting an effect of formaldehyde treatment of proteins because one of the two protein fractions that should be maximized is decreased. The above discussion would seem to indicate that if the treatment of dietary proteins with formaldehyde has any negative effects on rumen microbial activity, then the possibility exists that this would be more serious in limiting the energy available to the animal on a high roughage ration than on a high concentrate ration. The rations in the various experiments reported all contained a relatively high concentrate level. The non-significant effect of formaldehyde treatment on average daily gains of cattle could therefore be attributed to insufficient dry matter intake and improper energy balance to enhance increased net protein synthesis and utilization by the animal.

Another factor which may explain the lack of response due to formaldehyde treatment of the protein supplements is the amino acid composition of the formaldehyde treated rapeseed and soybean meals. A logical theoretical approach would be to protect proteins that are of a high quality from an amino acid composition point of view while the low quality proteins are allowed to be degraded and upgraded to microbial protein (Peter et al. 1971). Even though it is difficult to categorize proteins as either of high or low quality, a useful criteria would be to relate the amino acid composition of the unprotected protein to that of microbial protein, with the understanding that any protein with an inferior amino acid composition would be considered of poor quality. Based on figures given by Purser et al. (1966) for rumen bacteria and protozoa, by Bergen et al. (1968) on rumen bacteria and protozoa and figures given by Wetter (1965) for solvent extracted rapeseed meal and soybean meal, rapeseed meal will be deficient in the amino acids, isoleucine, lysine, phenylalanine, while soybean meal will likely be deficient in isoleucine. Other amino acids may also be slightly below those of microbial amino acids. The question of one or more amino acids being deficient and affecting animal performance could be seen by the positive response on wool growth when sheep are fed formaldehyde-treated casein (Faichney 1971; Barry 1972; Hemsley et al. 1973). This positive response in wool growth has been attributed to the high biological value of casein and the requirements for specific amino acids for wool growth. This contrasts the rather

inconsistent animal response when plant proteins treated with formaldehyde are fed (Colby and Tollert 1973). However, if poor quality proteins are diluted by microbial proteins, then the critical situation of a poor amino acid balance for absorption is gradually eliminated, but if the amount of protein by-passing the rumen is small and rumen microbial protein production is impeded, then total protein reaching the intestines will be reduced.

The more proteins by-pass the rumen compared with microbial protein, the more important would their biological value become in determining the efficiency with which its proteins or amino acids are utilized because of the reasons already discussed above. As already stated in the earlier part of the discussion, the main factors which will determine the extent of this by-pass are the solubility of the proteins and the level of feed intake. As indicated earlier, proteins like casein will be degraded to a degree of about 90 per cent or more in the rumen (Hume 1974). Plant proteins like peanut meal or rapeseed meal whose solubility is similar and is about 40 per cent (Wholt et al. 1973) and soybean meal and zein (Hume 1974) may be considered highly insoluble and a substantial proportion will normally escape rumen proteolysis. This relationship between rumen degradation and solubility of dietary proteins is not necessarily in a 1:1 ratio. Since the proportion of insoluble protein which reaches the lower gastrointestinal tract undegraded will also depend on the level of feed intake and rate of passage (Miller 1973; Ørskow et al. 1971, 1973), it could be

expected that at the high level of crude protein intake required for growth, which is about 2.63 times the maintenance level for a 300 kg steer growing at 1.3 kg per day (NRC 1970), a large amount of untreated dietary proteins would by-pass the rumen and be digested in the lower gastro-intestinal tract in a similar way as the formaldehyde-treated proteins. It is obvious that under these circumstances, the chances of finding an effect that is due to formaldehyde treatment of the protein would be minimal. In the same way, if the total protein coming from formaldehyde-treated protein is small compared with the total crude protein intake, then chances of detecting an effect due to formaldehyde treatment are small.

Under feeding conditions where urea is added to diets in an attempt to ensure that ammonia nitrogen would not be limiting for maximum microbial growth, the feeding of protein supplements that have been treated with formaldehyde has not resulted in any positive effect on animal growth (Wachira et al. 1974). This does not necessarily mean that there are no situations where addition of urea would not be advisable. Theoretically, when a considerable proportion of dietary protein has been treated with formaldehyde, a situation which would be compared with the feeding of very insoluble proteins like zein may occur in the rumen. When zein was the only source of nitrogen, the rate of VFA production in the rumen was lowest compared with diets where casein, soybean and urea were added (Hembry et al. 1975). Under these circumstances, ammonia nitrogen may become a limiting factor for maximum microbial growth

(Hembry et al. 1975) and certainly a positive effect of urea could be expected. This may not have been the case in experiments III and IV where there was a decrease in animal performance as the level of urea increased in the diets. But this was attributed to low dry matter intake and hence low energy consumption due to restricted feeding protocol employed during the experiments.

Finally, Sharma et al. (1974) and Faichney (1972) have used the argument that the dietary crude protein fed in trials whose formaldehyde protection has been studied is usually too high and the implication being that these diets would provide sufficient protein without treatment. The beneficial effects of formaldehyde treatment will usually be masked if the protein is not very soluble; if it is very soluble, then the higher its level in the diet the easier it should be to detect a positive effect of protection. This argument would not be true in our own experiments because our diets usually contained less protein compared with the NRC recommended requirements. This was an attempt in our own case not to complicate the interpretation of the results. But while we ensured lowered protein intakes, energy was also restricted and this could be expected to complicate the interpretation of the results.

This energy effect was clearly demonstrated in the nitrogen metabolism studies conducted with sheep. Even though there was a loss of nitrogen in the faeces, a situation that is usually attributed to a decreased digestibility of the diets (even though this was not the case with our sheep studies), it was clearly demonstrated that

the addition of urea to the diets resulted in an increased loss of both fecal and urinary nitrogen. In a situation where energy sufficiency was ensured, one would expect that the loss of nitrogen due to the addition of urea would not be significant. This would therefore mean an increase in microbial protein production, and an increase in by-pass of dietary protein in the case of the formaldehyde treated protein supplements. However, the sheep used in our experiment were almost mature and the situation of energy insufficiency may not be the same as in the previous cattle experiments.

If positive effects of formaldehyde treatment of protein are expected, then the above discussed situations and factors should be introduced simultaneously into future designed research. Also, a carcass study should be conducted to see whether there are any differences in carcass profile in terms of lean to bone ratio because most research workers have reported a better nitrogen retention with formaldehyde treatment of the dietary proteins, without reporting either improved animal performance or improved carcass characteristics.

X. SUMMARY AND CONCLUSIONS

Five experiments were conducted to

1. compare rapeseed meal and soybean meal as protein supplements to a corn silage and high moisture corn grain basal ration on the growth of Holstein cattle (experiment I);
2. determine the effects of feeding formaldehyde-treated or untreated RSM and SBM as protein supplements to a hay and barley basal ration on the growth of young Holstein cattle (experiment II);
3. determine the effects of feeding formaldehyde-treated or untreated RSM with or without urea as a protein supplement to a corn silage and molasses basal ration on the growth of Holstein steers (experiment III);
4. determine the effects of feeding formaldehyde-treated or untreated RSM with or without urea as a protein supplement to a haylage, molasses and corn grain ration on the growth of Holstein steers (experiment IV);
5. determine the effects of feeding formaldehyde-treated or untreated RSM, with or without urea on nutrient digestibility and nitrogen retention of sheep (experiment V).

In experiment I, twenty cross-bred steers and twenty cross-bred heifers were randomly distributed to a 2 x 2 x 2 factorial

design in which the factors were, sex (steers vs. heifers), weight (heavy vs. light), and protein supplement (SBM vs. RSM). The animals were fed a corn silage and high moisture corn basal ration that was supplemented with either SBM or RSM. The animals were distributed into eight pens with each pen having five animals. The experiment lasted for 124 days. The results indicated that there was a significant difference ($P < .05$) in average daily gains between cattle on SBM and RSM, with the former having higher ADG than the latter. There was also a significant effect ($P < .1$) of sex on ADG with the steers having higher ADG than the heifers. The difference in ADG due to initial weight differences was non-significant ($P > .1$).

Experiment II involved two replicates, A and B, with 36 and 31 Holstein cattle, respectively. The cattle were randomly distributed to a 2 x 2 factorial design in which the factors were protein supplements (SBM vs. RSM) and formaldehyde treatment (treated vs. untreated). The proteins were supplemented to a hay and barley basal ration. For replicate A, the animals were distributed into four pens with each pen having nine animals. One calf died during the course of the experiment, which left one pen with eight animals. For replicate B, the animals were distributed into four pens with three pens having eight animals and one pen having seven animals. The experiment lasted for 168 days.

The results indicated that there was a significant difference ($P < .05$) in average daily gains between the cattle in replicates A and B, with the cattle in replicate B having higher ADG. However,

there was no significant difference ($P > .1$) in ADG between the cattle on SBM and RSM, but the cattle on RSM had slightly higher ADG. There was also no significant difference ($P > .1$) in ADG between cattle fed the formaldehyde-treated or untreated SBM or RSM but the cattle on the formaldehyde-treated meals had higher ADG.

Experiment III involved two replicates, A and B, with 34 and 30 Holstein cattle, respectively. The cattle were randomly distributed into a 2 x 3 factorial design in which the factors were formaldehyde treatment (treated vs. untreated), and urea supplementation (zero, medium and high). This protein was used as a supplement to a corn silage and molasses basal ration. In replicate A, the animals were distributed into six pens, with four pens having six animals each and two pens with five animals each. In replicate B, each pen had five animals. Replicate A lasted for 118 days, while replicate B lasted for 62 days. The results indicated that there was no significant difference ($P > .1$) in ADG between cattle in replicates A and B, but the cattle in replicate B had slightly higher ADG. There was also no significant ($P > .1$) difference in ADG of cattle due to formaldehyde treatment of RSM, but the cattle fed the formaldehyde-treated RSM had slightly higher ADG. However, there was a significant effect ($P < .01$) of urea supplementation on ADG, with ADG increasing as the level of urea in the diet decreased.

Experiment IV was set up the same as experiment III, only that the protein was used to supplement a haylage, molasses and corn grain basal ration, and it lasted for 84 days. The result indicated that

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there was a significant difference ($P < .1$) in ADG between cattle in replicates A and B, with the cattle in replicate B having higher ADG. However, there was no significant difference ($P > .1$) in ADG between cattle fed formaldehyde-treated or untreated RSM, but the cattle on formaldehyde-treated RSM had higher ADG. There was also no significant effect ($P > .1$) of urea supplementation on ADG of the cattle but ADG increased as the level of urea decreased in the diets. A significant ($P < .1$) formaldehyde x urea interaction was also observed.

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In experiment V, eight mature Columbia wether sheep were randomly distributed to a replicated 2 x 2 factorial design in which the factors were formaldehyde treatment of RSM (treated vs. untreated) and urea supplementation (no urea vs. urea). The protein was used to supplement a corn silage and molasses basal ration. The experiment was conducted for two 21-day periods. The results indicated that there was no significant effect ($P > .05$) of formaldehyde treatment of RSM on nutrient digestibility, but nutrient digestibility increased with formaldehyde treatment. Urea supplementation did not significantly affect ($P > .05$) nutrient digestibility. However, there was a slight increase in nutrient digestibility due to urea supplementation. Nitrogen retention was significantly increased ($P < .05$) by formaldehyde treatment of RSM, while urea supplementation significantly decreased ($P < .05$) nitrogen retention, especially on the diets with untreated RSM.

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In conclusion, it would seem evident that the chances of detecting an improvement in the efficiency of utilization of dietary protein supplements for animal growth by protecting them with formaldehyde are not high.

The chances of detecting an effect of formaldehyde treatment can be increased if it is ensured that the energy of the animals' ration is not limiting in relation to the potential increase in amino acids availability resulting from formaldehyde treatment. Therefore, basal diets high in concentrates (energy feeds) would seem to have a better chance of giving a positive response when supplemented with the formaldehyde-treated protein supplements than roughage-basal diets. The indication is that under any dietary conditions, microbial protein synthesis should not be impaired or the chances of detecting a positive significant effect are minimized. The proteins treated should be of high solubility such that when treated with formaldehyde, their rate of ammonia release is decreased considerably. However, protein and ammonia supply in the rumen should not be limiting for maximum microbial growth. If this situation is predicted, then it would be advisable to add urea to the diet. Furthermore, the treatment with formaldehyde should be carefully controlled so that post-ruminal digestibility is not decreased. Also, the biological value of the protected protein should be high compared with microbial protein and this factor would become more important the higher the proportion of protected protein compared with microbial protein. Therefore, the

formaldehyde-treated proteins should provide a high proportion of the animal's crude protein intake (a situation which is more likely to occur in practice in rations high in certain concentrates). As energy feeds vary in their protein content, treated protein supplements would appear to be more effective when used with the lower protein energy feeds such as those based on corn or sugarcane (i.e., molasses). Since, under many feeding conditions, it may not be practical to feed high levels of treated protein supplements, the use of this practice may be restricted to certain of the most promising situations.

Since the factors in the rumen that seem to be important in maximising microbial protein production are adequate fermentable energy, presence of some untreated dietary protein, adequate sulphur concentration, as well as optimum ammonia nitrogen concentration, then these factors must be monitored and controlled. The protected protein should serve as an additional source of protein for the animal, in addition to microbial protein.

Finally, since high crude protein intake or high feed intake are required for maximum growth and this is associated with high rates of passage and ruminal by-pass, then only those proteins whose solubility is high should be expected to give beneficial growth responses when treated with formaldehyde. Among the highly soluble proteins, those with a high biological value would probably benefit most from formaldehyde treatment. This would indicate that research based on consideration of the above factors should be designed and carried out.

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APPENDIX TABLE 1. Analysis of variance on average daily gains
(Experiment I)

Source	df	SS	MS	Fcal
Sex	1	0.4603	0.4603	3.5508 *
Weight	1	0.0294	0.0294	0.2270 ns
Protein	1	0.6253	0.6253	4.8231 **
Sex x weight	1	0.0424	0.0424	0.3274 ns
Sex x protein	1	0.1876	0.1876	1.4468 ns
Weight x protein	1	0.0205	0.0205	0.1585 ns
Sex x protein x weight	1	0.0509	0.0509	0.3927 ns
Error	32	4.1484	0.1296	
Total corrected	39	5.5648	0.1427	

* Significant ($P < .1$)

** Significant ($P < .05$)

ns Non-significant ($P > .1$)

APPENDIX TABLE 2. Analysis of variance on average daily gains
(Experiment II)

Source	df	SS	MS	Fcal
Regression	4	0.4389	0.1097	
Block (replicates)	1	0.4086	0.4086	30.8699***
Protein	1	0.0186	0.0186	1.4062 ns
Formaldehyde	1	0.0112	0.0112	0.8464 ns
Formaldehyde x protein	1	0.0013	0.0014	0.0963 ns
Error	60	0.7941	0.0132	
Total corrected	64	1.2330		

***Significant ($P < .01$)

ns Non-significant ($P > .1$)

APPENDIX TABLE 3. Analysis of variance on average daily gains
Experiment III (replicates A and B)

Source	df	SS	MS	Fcal
Regression	6	0.5149	0.0858	
Replicates	1	0.0046	0.0046	0.3218 ns
Formaldehyde	1	0.0339	0.0339	2.3946 ns
Urea	2	0.4168	0.4168	14.7089***
Formaldehyde x urea	2	0.0514	0.0514	1.8146 ns
Error	58	0.8218	0.0142	
Total corrected	64	1.3367		

***Significant (P < .01)

ns Non-significant (P > .1)

APPENDIX TABLE 4. Analysis of variance on average daily gains
Experiment IV (replicates A and B)

Source	df	SS	MS	Fcal
Regression	6	0.3619	0.0603	3.9792
Replicates	1	0.1759	0.1759	3.9792*
Formaldehyde	1	0.1013	0.1013	2.2916 ns
Urea	2	0.0643	0.0643	0.7278 ns
Formaldehyde x urea	2	0.2138	0.1069	2.4182*
Error	56	2.4756	0.0442	
Total corrected	62	2.8375		

* Significant ($P < .1$)

ns Non-significant ($P > .1$)

APPENDIX TABLE 5. Digestibility of dry matter ($\bar{X}$) (Experiment V)

	Rapeseed meal treatment		Urea means
	FA	H ₂ O	
<u>No urea</u>	(2) 70.54	(9) 73.31	
	(6) 73.13	(10) 62.42	
	(7) 70.51	(3) 70.92	
	(8) 69.83	(5) 72.95	
Means	71.01	69.90	70.45
<u>Urea</u>	(9) 80.02	(2) 73.74	
	(10) 69.42	(6) 66.61	
	(3) 70.48	(7) 66.29	
	(5) 74.55	(8) 73.00	
Means	73.61	69.91	71.76
RSM treatment means	72.31	69.90	

Analysis of variance				
<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>Fcal</u>
Total	15	238.50	15.90	
Treatments	3	36.80	12.26	
RSM	1	23.26	23.26	1.38 ns
Urea	1	6.83	6.83	0.40 ns
RSM x Urea	1	6.71	6.71	0.39 ns
Error	12	201.70	16.80	

Figures in parentheses () are sheep numbers
 ns, non-significant ($P > .05$)

APPENDIX TABLE 6. Digestibility of energy (%) (Experiment V)

	Rapeseed meal treatment		Urea means
	FA	H ₂ O	
<u>No urea</u>	(2) 66.19	(9) 72.31	
	(6) 69.21	(10) 61.02	
	(7) 66.21	(3) 69.92	
	(8) 65.38	(5) 71.99	
Means	66.74	68.81	67.77
<u>Urea</u>	(9) 79.44	(2) 71.79	
	(10) 68.54	(6) 64.24	
	(3) 69.83	(7) 63.79	
	(5) 74.00	(8) 70.81	
Means	72.95	67.65	70.30
RSM treatment means	69.85	68.23	

<u>Analysis of variance</u>				
<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>Fcal</u>
Total	15	308.95	20.59	
Treatments	3	90.11	30.03	
RSM	1	10.45	10.45	0.57 ns
Urea	1	25.53	25.53	1.39 ns
RSM x Urea	1	54.13	54.13	2.96 ns
Error	12	218.84	18.23	

Figures in parentheses () are sheep numbers
 ns non-significant ($P > .05$).

APPENDIX TABLE 7. Digestibility of protein (%) (Experiment V)

	Rapeseed meal treatment		Urea means
	FA	H ₂ O	
<u>No urea</u>	(2) 50.87	(9) 61.32	
	(6) 55.98	(10) 45.51	
	(7) 51.69	(3) 61.80	
	(8) 49.67	(5) 61.51	
Means	52.05	57.53	54.79
<u>Urea</u>	(9) 60.80	(2) 53.59	
	(10) 40.22	(6) 40.49	
	(3) 39.93	(7) 40.06	
	(5) 48.19	(8) 52.22	
Means	47.28	46.59	46.93
RSM treatment means	49.66	52.06	

Analysis of variance				
Source	df	SS	MS	Fcal
Total	15	921.55	64.77	
Treatments	3	307.97	102.65	
RSM	1	22.92	22.92	0.44 ns
Urea	1	246.89	246.86	4.75 **
RSM x Urea	1	38.15	38.15	0.73 ns
Error	12	623.58	51.96	

** Significant (P < .05)

ns Non-significant (P > .05)

Figures in parentheses () are sheep numbers

APPENDIX TABLE 8. Digestibility of cellulose (%) (Experiment V)

	Rapeseed meal treatment				Urea means
	FA		H ₂ O		
<u>No urea</u>	(2)	45.04	(9)	51.55	
	(6)	50.27	(10)	32.00	
	(7)	45.45	(3)	46.84	
	(8)	43.70	(5)	51.54	
Means		46.11		45.48	45.79
<u>Urea</u>	(9)	62.47	(2)	54.97	
	(10)	43.00	(6)	45.55	
	(3)	47.38	(7)	44.26	
	(5)	54.71	(8)	53.29	
Means		51.89		49.51	50.70
RSM treatment means		49.00		47.50	

<u>Analysis of variance</u>				
<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>Fcal</u>
Total	15	696.72	46.44	
Treatments	3	108.29	36.09	
RSM	1	9.03	9.03	0.18 ns
Urea	1	96.22	96.22	1.96 ns
RSM x urea	1	3.04	3.03	0.06 ns
Error	12	588.43	49.05	

ns Non-significant ($P > .05$)

Figures in parentheses () are sheep numbers

APPENDIX TABLE 9. Retention of nitrogen (%) (Experiment V)

	Rapeseed meal treatment				Urea means
	FA		H ₂ O		
<u>No urea</u>	(2)	24.87	(9)	27.18	
	(6)	33.09	(10)	0.02	
	(7)	34.31	(3)	18.40	
	(8)	25.69	(5)	0.01	
Means		29.49		11.40	20.44
<u>Urea</u>	(9)	36.44	(2)	-1.06	
	(10)	-0.12	(6)	0.03	
	(3)	-0.13	(7)	-0.08	
	(5)	14.74	(8)	-15.65	
Means		12.73		-4.19	4.22
RSM treatment means		21.11		3.60	

Analysis of variance

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>Fcal</u>
Total	15	3975.57	265.03	
Treatments	3	2273.57	757.85	
RSM	1	1225.52	1225.52	8.64 **
Urea	1	1046.68	1046.68	7.37 **
RSM x urea	1	1.37	1.37	0.01 ns
Error	12	1702.00	141.83	

** Significant ($P < .05$)ns Non-significant ($P > .05$)

Figures in parentheses () are sheep numbers