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Urea Supplementation of High Roughage Rations for Wintering Calves

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UREA SUPPLEMENTATION OF HIGH ROUGHAGE RATIONS

FOR WINTERING CALVES

BY

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FOR WINTERING CALVES

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable as meeting the thesis requirements for this degree, but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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INTRODUCTION

Ruminant animals have the unique ability to convert large quantities of nonprotein nitrogen (NPN) to protein. This process is mediated by the microorganisms present in the rumen. By taking advantage of this fact, it is possible to reduce the cost of protein supplementation by replacing a certain amount of true protein by cheaper NPN compounds such as urea in the protein supplement.

Several factors affect the efficient utilization of urea. Among these are level and source of energy, level and source of protein, and percent urea in the total ration and in the supplement. The ration should also be properly balanced as to other essential nutrients. It is generally agreed that a source of readily available carbohydrate is necessary for efficient utilization of urea and that the total protein in the ration should not exceed about 12%.

There appears to be no optimum level of urea to feed under all conditions. Generally accepted guidelines are to feed not more than 3% urea in the concentrate mix, less than 1% urea in the ration dry matter and not more than one-third of the protein equivalent in the total ration should be from urea (Chalupa, 1968).

Performance of fattening cattle being fed urea at high levels in the supplement is generally quite comparable to those fed conventional protein supplements. The high grain rations used furnish a readily available source of energy and the amount of protein to be supplemented in most fattening rations will be less than for a growing ration.

Response of wintering cattle to a supplement in which urea furnishes a major portion of supplemental protein often has been reported to be inferior to that of cattle being fed plant protein supplements. Wintering rations for cattle and sheep are generally composed largely of roughages. Such roughages are generally high in crude fiber content and often low in crude protein. Thus energy in such rations may not be readily available to the microorganisms in the rumen and a greater portion of the total protein from supplements will be needed. Although the low protein content of such rations offers an advantage for the use of larger amounts of urea, safe and efficient levels may be exceeded. In addition, the high fiber content of the rations will restrict the amount of urea that can be utilized efficiently unless properly supplemented.

The objective of this research was to study methods of increasing the efficiency of utilization of urea in supplements fed with prairie hay to wintering beef calves. Four wintering experiments were conducted in which weight gains, feed consumption and feed efficiency were used as measures of performance. In addition, a series of <u>in vitro</u> trials was conducted with ammonia (NH_3) and total volatile fatty acid (VFA) production being measured.

REVIEW OF LITERATURE

Much work has been reported on the utilization of nonprotein nitrogen (NPN) by ruminant animals. Much of the earlier work in the 1940's was concerned with establishing the fact that urea could be used as a substitute for true protein in the diet of ruminants. Subsequent investigations were concerned with dietary factors affecting safe and efficient use of nonprotein nitrogen. This review of literature will not be an intensive survey of all of the reports concerned with the feeding of nonprotein nitrogen but rather will attempt to review more thoroughly that research concerned with factors affecting the utilization of nonprotein nitrogen.

Value and Problems in the Use of Urea

Several review articles have been published in recent years which discuss factors affecting nonprotein nitrogen, primarily urea, utilization (Reid, 1953; Lewis, 1961; Chalmers, 1961; McLaren, 1964; Blackburn, 1965; Hungate, 1966; Chalupa, 1968). These papers discuss results and conclusions of several research workers and further discuss many problems involved in the feeding of urea to livestock.

Research has shown that urea is rapidly converted to ammonia and carbon dioxide in the rumen. Efficient utilization of the nitrogen from urea depends on the ability of microorganisms in the rumen to convert this large amount of ammonia to bacterial protein. Ammonia is readily absorbed from the rumen and is therefore available to the microbes for only a limited period of time. Factors which affect either the rate of release of ammonia from urea or the rate of assimilation of microbial protein from ammonia will therefore affect efficiency of utilization of urea.

Experiments dealing with nitrogen balance, ruminal ingesta composition and isotopic tracers have been employed to demonstrate that urea nitrogen is converted to and stored as protein nitrogen. The conversion of urea to protein is mediated by the microorganisms of the rumen and reticulum. Subsequent digestion of bacteria avails the host animal of the protein contained therein (Reid, 1953).

The diet to which urea is added influences the growth and multiplication of ruminal bacteria and, therefore, the extent to which ruminants are able to utilize nitrogen. Urea is soluble in water and rapidly hydrolyzed, thus indicating a need for available carbohydrates at time of ingestion. Sugars are found to disappear too quickly from the rumen while cellulose becomes available too slowly to satisfy energy needs of microorganisms for maximum utilization of urea. A mixture of readily available and more complex slowly available carbohydrates appears to be satisfactory. It is generally agreed that a low level of true protein and a high level of starch in the ration favor urea utilization (Stangel, 1967).

Frequency of feeding of urea has been shown to influence its utilization. Campbell _et al._ (1963) reported that by feeding a grain ration containing urea (3.3%) to dairy heifers six times daily rather than twice daily growth and feed efficiency were comparable to animals fed a soybean oil meal supplemented diet. There was a significant decrease (11.8%) in estimated TDN requirement per pound of body weight gain resulting from six times daily feeding as compared to feeding twice daily. Raleigh and Wallace (1965) found that feeding calves a high urea ration three times daily as compared to once daily had no effect on performance at a high-energy level in the diet. However, animals on a low-energy, high-protein diet where urea supplied one-half of the protein were benefited significantly by three times a day feeding.

Age of the ruminant appears to influence the degree to which urea is utilized. Calves as young as 2 months of age have been shown to use some urea nitrogen. In experiments with calves in which urea nitrogen has been compared with the nitrogen of conventional high-protein feeds, urea nitrogen has generally been at least slightly inferior. Less difference between the two forms of nitrogen has been found, however, in the response of milking cows than in growing cattle. Also, less difference has been observed in the weight gains of older steers than in those of fattening calves (Reid, 1953).

Problems with urea (ammonia) toxicity were reported frequently in the 1940's and 1950's. Lewis (1961) indicated that the toxicity of ammonia in ruminants is a complex problem involving (1) a direct toxic effect of the ammonium ion, (2) a disturbance of the acid-base status which would probably not give rise to clinical symptoms of toxicity, however, and (3) a change in electrolyte balance which might modify the signs of toxicity.

Several researchers have outlined the symptoms of urea toxicity which appears when peripheral blood ammonia levels reach a critical value of approximately 1 to 4 mg. per 100 ml. of blood. These include respiratory difficulties, excessive salivation, muscular tremors, incoordination, bloat and death within 1.5 to 2.5 hours after the initial appearance of symptoms (Dinning et al., 1948; Repp et al., 1955; Lewis et al., 1957; Oltjen et al., 1963). Feeding urea according to established guidelines (i.e., less than 1% of the total ration or not more than 3% of the concentrate mix) will not normally cause toxicity (Davis and Roberts, 1959). Urea toxicity does not appear to be a significant problem under practical feeding conditions, but more research is needed to confirm this observation.

Large amounts of urea produced by the liver are returned to the rumen via the saliva and by direct diffusion across the rumen wall (Houpt, 1959; Decker et al., 1960; Somers, 1961; Packett and Groves, 1965; Cocimano and Leng, 1967). Re-entry of urea into the rumen is undoubtedly important in the efficient utilization of dietary urea nitrogen. An additional supply of nitrogen to the rumen when dietary sources are low should benefit cellulolytic and other bacteria (Chalupa, 1968).

Cocimano and Leng (1967) used radioactive tracer methods to study urea metabolism in sheep. These workers found that the percentage of urea nitrogen recycled in ruminants was higher in animals fed low-nitrogen diets, while the quantity of urea nitrogen recycled was higher for those animals on high nitrogen intakes.

These workers also showed that amount of nitrogen diffusing from the blood to the rumen is quantitatively more important than that entering by way of the saliva.

Feedlot Adaptation to Urea

Several workers have noted an improvement in nonprotein nitrogen utilization as the period during which animals were fed was extended. Smith *et al.* (1960) fed 63 lambs in 19 digestion trials a semipurified ration containing 1.7% nitrogen of which 66% was contributed by urea. By using multiple regression analysis, these workers showed that retention of absorbed nitrogen was significantly improved by approximately 0.2 percentage units with each consecutive 10-day feeding period up to 50 days with no accompanying change in organic matter or crude fiber digestibility. Retention of absorbed nitrogen was decreased 12 percentage units by increasing the percentage of total nitrogen supplied as urea from 54 to 68%. This improvement in nitrogen utilization as a function of length of time of urea feeding has been called an adaptation response.

Digestion and nitrogen metabolism data obtained with 83 lambs fed semipurified rations containing urea as the sole source of nitrogen were used by McLaren *et al.* (1965c) to estimate the influence of length of time of urea feeding and level of readily fermentable carbohydrates on the retention of absorbed nitrogen. Regression analysis indicated that retention of absorbed nitrogen was significantly improved by 3 percentage units with each 10-day period of urea feeding. Improvement in retention of absorbed nitrogen due to level of readily fermentable carbohydrates was observed in lambs regardless of the degree of their adaptation to urea feeding.

Caffrey et al. (1967a) conducted experiments to investigate intraruminal and nitrogen balance changes occurring in lambs during adjustment to a urea-rich diet. *In vitro* rumen fermentation studies showed that rate of ammonia assimilation by ruminal microorganisms was significantly greater using inocula from lambs that had been adapted to a urea-rich (3.5% urea) diet than with inocula from lambs fed a nonurea diet. Changes in ruminal ammonia concentrations relative to polyethylene glycol were taken as an index of the rate of ammonia assimilation by ruminal microorganisms. The basic assumption was that ammonia absorption from the rumen and endogenous ammonia production within the rumen remain constant during the different determinations. There was a significant ($P < .05$) difference between regression coefficients for days 3 and 19 but not between days 19 and 40. These data suggest that the maximum ability of ruminal microorganisms to assimilate ammonia was reached before the lambs had been 19 days on a urea-rich diet.

McLaren et al. (1959) showed a reduction in the urea adaptation period by feeding diethylstilbestrol (DES). Two digestion and nitrogen metabolism trials were conducted with wether lambs fed a ration of wheat straw, molasses, soybean protein, starch, dextrose, corn oil, minerals and vitamins with urea and crude biuret in various proportions. The rations were 9.6% protein, one-third of which was nonprotein nitrogen. Part of the animals received 2 mg. DES daily in the feed. Utilization of absorbed nitrogen was increased as a function of length of the preliminary period with a maximum reached between 30 and 40 days. DES reduced this to about 10 days. Karr et al. (1964) showed a similar reduction in the adaptation of lambs to urea by feeding DES.

The mechanism involved in urea adaptation does not appear to be agreed upon by all workers. Reports in the literature show variations in length of the adaptation period. There is much disagreement among researchers on the site of urea adaptation in the animal body. Dowdy et al. (1964) suggested that the adaptation response is specific to the ruminant, possibly occurring as a result of the urea recycling mechanism which the ruminant possesses. McLaren et al. (1960) observed that the pattern of blood ammonia and urea nitrogen levels, urinary excretion of biuret, plasma protein-bound iodine values and associated digestion and nitrogen metabolism data make it appear that DES and time influence retention of absorbed nitrogen through direct action of tissues to promote better utilization of nonprotein nitrogen. These workers found that blood ammonia concentration in lambs was significantly increased as the number of days on the nonprotein nitrogen supplemented rations was increased. Although blood ammonia nitrogen was increased with time, when DES was fed this increase was less. Blood urea nitrogen data indicate that the number of days on the ration caused a significant depression in concentration of blood urea nitrogen. When DES was present in the ration, depression with time was quite marked. This lack of increase in blood urea nitrogen despite elevated blood ammonia levels might suggest more efficient utilization of ammonia by tissues for synthesis of nonessential amino acids.

Caffrey et al. (1967b) used balance studies, conducted after two lambs had consumed a 3.5% urea diet for 4, 20 and 41 days, and showed there was no significant difference in nitrogen balance between these times. Also, length of time on the high-urea diet did not significantly alter the regression of blood urea nitrogen on time after injection of urea or saline solution or the corresponding 12-hour urinary nitrogen excretion pattern. Therefore, it appears that rate of urea recycling was not affected by length of time lambs had been fed a urea-rich diet.

Virtanen (1966) has shown that synthesis of bacterial protein in the rumen of lactating cows fed on purified diets with urea and ammonia salts as the sole source of nitrogen can be increased through feed adaptation to levels adequate not only for maintenance of the cow but also for relatively high milk production. This researcher stated that observations on the rapid utilization of ammonia in the rumen of cows adapted to high-urea diets could be explained only by supposing that microbial flora of the rumen content had been effectively adapted to utilization of ammonia nitrogen.

Data which may indicate that adaptation of ruminants to urea may take place in the body rather than in the contents of the digestive system were presented by Clifford and Tillman (1968). In a nitrogen balance trial using 20 lambs fed various combinations of urea and isolated soybean protein, these workers showed an apparent improvement in nitrogen retention with time on all rations. Fecal nitrogen was not affected by time; however, urinary nitrogen excretion was higher ($P<.01$) during the first 10-day period and there was no further change with time. Nitrogen retention improved ($P<.01$) during the first two 10-day periods and there was no further improvement.

It appears that there is much need for additional research on the mechanisms involved in urea adaptation. Conditions under which urea adaptation has been studied have been quite variable. Reported ranges in the length of the adaptation period have been from 10 days for sheep and up to 40 days for cattle. Such factors as age of animal, species of animal, ration components and pre-experimental conditions may all affect the length of this adaptation period and should be taken into account when studying this.

Effects of Energy and Protein Level on Urea Utilization

Efficient utilization of urea nitrogen by ruminal microorganisms is dependent upon several factors, including level and source of energy and nitrogen. Urea is readily hydrolyzed to ammonia and carbon dioxide in the rumen. Optimum use of the resulting ammonia nitrogen requires a readily available source of carbohydrates. Therefore, urea utilization is a function of both level and source of carbohydrate (energy) in the diet.

Fixation of urea nitrogen by rumen microorganisms has been shown by in vitro incubations (Bloomfield et al., 1964) to be a quantitative function of energy level. Bacterial assimilation required 55 gm. carbohydrate for each gram of nitrogen fixed. An *in vivo* experiment was conducted by these same workers to study the effects of two different energy levels on urea and soybean oil meal utilization by 28 wintering steers divided into four equal groups. Groups I and II received corn silage (28.7% estimated TDN) and groups III and IV received forage sorghum silage (21.7% estimated TDN) *ad libitum.* Groups I and III received 2.5 lb. per head daily of a urea supplement (70% NPN) and groups II and IV received 2.5 lb. per head daily of a soybean oil meal supplement. Average daily gains, blood urea nitrogen (mg. N/100 ml.) and feed efficiency for 56 days were as follows: (I) 1.67, 8.5, 1790; (II) 1.63, 8.4, 1715; (III) 0.34, 13.9, 6330; and (IV) 0.81, 13.4 and 2784. These data may indicate that level of urea in the ration is not restricted by a fixed percent but can be fed as a function of energy level.

Improvement in retention of absorbed nitrogen due to level of readily fermentable carbohydrates was observed in lambs regardless of the degree of their adaptation to urea feeding (McLaren *et al.*, 1965c). Digestion and nitrogen metabolism data obtained with 83 lambs fed semipurified rations containing urea as the sole source of nitrogen showed an improvement in retention of absorbed nitrogen by approximately 2 percentage units for each kcal. of readily fermentable carbohydrates.

Results *in vitro* (Belasco, 1955) showed that urea utilization and the production and distribution of volatile fatty acids were dependent on the amount and type of carbohydrate used as the energy substrate. In these trials, the extent of urea utilization and fatty acid production were generally greater with starch than with comparable amounts of cellulose. In combination with cellulose, dextrose was as effective as comparable amounts of starch in urea nitrogen fixation. Xylan and pectin also promoted urea utilization but not to the same extent as starch. At high input levels, starch and dextrose inhibited cellulose digestion.

While it has been shown that starch provides the most readily available source of carbohydrate for optimum utilization of urea, several workers have shown that the addition of starch to the ration of ruminant animals will depress the digestion of cellulose in these animals (Burroughs *et al.*, 1949; Arias *et al.*, 1951; Hunt *et al.*, 1954; El-Shazley *et al.*, 1961). Cellulose digestion *in vitro* is inhibited by the presence of large quantities of starch (El-Shazley *et al.*, 1961). This inhibition was reported by these workers to be due to competition between cellulolytic and amylolytic groups of bacteria for nutrients. The main limiting nutrient was nitrogen. This could be partly overcome by higher levels of urea nitrogen *in vitro*. Using digestibility of filter paper in nylon bags in the rumen of fistulated sheep as a criteria of bacterial activity, the same inhibition was demonstrated *in vivo*. This inhibition could also be alleviated by the addition of urea nitrogen to the ration. The degree of alleviation depended on the proportion of corn to hay, or energy content, in the ration. Although other nitrogen supplements were effective, urea appeared to be the most efficient source tested.

Burroughs *et al.* (1949) noted a substantial decrease in roughage dry matter digestion when starch was included in a ration in which corncobs or corncobs and limited alfalfa hay made up the roughage portion of the ration. This decrease in roughage digestion occurred even though percent protein in the rations varied widely. Arias *et al.* (1951) observed that small amounts of a readily available carbohydrate aided cellulose digestion which in turn increased urea utilization, whereas large amounts of starch markedly inhibited cellulose digestion *in vitro.*

In addition to the effects of level and source of energy *per se* in the ration, the efficient utilization of nonprotein nitrogen by ruminants is also dependent on level of protein in the ration and ratio of protein to energy. Waldo (1968) stated that the relationship of dietary carbohydrate level and form to dietary nitrogen level and form is the primary factor affecting ammonia concentration in the rumen.

Data were presented by Preston *et al.* (1964) which indicated that gain and feed efficiency are affected by protein to energy ratio (P:E). In an experiment involving eight lambs, various ratios of crude protein (mg.) to energy (kcal. of estimated net energy) were fed in an extra period Latin Square changeover design to determine the effects of these ratios upon blood urea nitrogen, gain and feed consumption. Each period was 21 days during which the lambs were full-fed a ration of 50% cottonseed hulls, 8% cane molasses, 0.9% minerals and vitamins and a variable percent of corn and soybean meal to give P:E ratios of 54, 70, 102 and 118 mg. protein per kcal. ENE. Daily gains increased ($P<.005$) with increasing P:E ratio (61, 168, 251 and 251 gm.). Daily feed consumption also increased ($P<.05$) with increasing P:E ratio (1700, 1800, 1920 and 1890 gm.). Blood urea nitrogen increased ($P<.005$) with increasing P:E ratio (2.5, 3.5, 10.0 and 14.5 mg./100 ml.). These data indicate that gain would be maximal and feed/gain minimal at a P:E ratio of 83 and 73, respectively.

Fontenot et al. (1955) conducted a series of three experiments with steers to determine the effect of adding different levels of cerelose to wintering rations containing 8, 10 and 12% protein. The basal wintering rations were composed of prairie hay, cottonseed meal and minerals. Additions of cerelose to the extent of 350, 700 and 1050 gm. to the 8% ration and 700 and 1050 gm. to the 10 and 12% rations resulted in a significant depression in nitrogen retention when the basal ration contained 8% protein, a significant increase in nitrogen retention when the basal ration contained 10% protein and a small but nonsignificant increase when the basal ration contained 12% protein. Cerelose additions generally resulted in significant decreases in apparent digestibilities of crude protein and crude fiber and in a significant increase in apparent digestibility of nitrogen-free extract.

A feeding trial with sheep was conducted by Williams et al. (1953) to study the influence of starch and protein levels on the digestibility and utilization of protein. Nine diets were used in this trial and were composed of oaten chaff to which wheaten starch and wheat gluten were added to provide 3 levels of starch and 3 levels of protein. The concentration of free microorganisms in the rumen was found to be markedly affected by the levels of dietary protein and by levels of starch, but the extent to which either of these constituents affected this concentration was greatly influenced by the proportion of the other constituent present in the diet. At all levels of starch, the addition of protein was found to increase significantly the digestibility of dry matter and true protein as well as number of rumen microorganisms. On the other hand, the addition of starch to low-protein diets markedly reduced these counts without significantly influencing digestibility of dry matter.

The nature and level of protein in the ration will influence the utilization of urea. Burroughs _et al._ (1951) reported that urea utilization _in vitro_ was greatest in the absence of other nitrogenous materials. These workers postulated at that time that microorganisms of the rumen have a requirement primarily for simple nitrogen in the form of ammonia and that remaining needs can be met by energy and minerals. Belasco (1954) suggested from his data that availability of nitrogen from all sources is important for good cellulose digestion, presumably through promotion of growth of cellulose digesting bacteria. When urea was fed in a 1:1 mixture with feed proteins, utilization _in vitro_ was very efficient and the activity of the mixture was better than with feed protein alone.

An increase in the level of urea used as a sole source of nitrogen resulted in an increase in cellulose digestion as well as in the amount of urea utilized.

Adding urea to a basal ration in amounts to produce the equivalent of 12% crude protein on the dry basis induced a retention of nitrogen in growing lambs that could not be bettered by further urea additions but could be bettered by raising the true protein content of the ration (Johnson et al., 1942).

Hamilton et al. (1948) compared the utilization of nitrogen of urea with that of some feed proteins (dried skimmilk, dried skimmilk plus cystine, gluten feed, casein or casein plus cystine and linseed oil meal). Urea was shown to be as satisfactory a source of nitrogen for growing lambs as that from most ordinary feeds provided at least 25% of the feed nitrogen is in the form of preformed protein and provided further that the total protein equivalent of the ration does not exceed about 12%.

Wegner et al. (1941) fed a fistulated heifer twice daily a ration consisting of corn silage (15 lb.), timothy hay (4 lb.) and grain mixture (4 lb. equal parts ground corn and ground oats). Linseed oil meal and urea were varied to increase the protein level of the concentrate from 11.3 to 31.1%. Samples of the rumen contents were collected and analyzed for nonprotein nitrogen, total nitrogen and dry matter. It was found that urea was utilized up to a level of 4.5% (protein equivalent of 12%) in the grain mixture. Rate of conversion of urea nitrogen to protein in the rumen decreased as the protein level of the rumen contents became greater than 12%.

Urea in amounts to provide one-half and all the nitrogen supplement in 6, 9 and 12% protein rations was compared with cottonseed meal in a wintering ration for steer calves fed a low-quality native meadow hay (Raleigh and Wallace, 1963). Level of nitrogen significantly affected digestibility of cellulose, dry matter and organic matter with the 9 and 12% crude protein levels having higher values than the 6% level and hay alone (5.5% crude protein), but they were not different from each other. Nitrogen digestibility significantly increased with each increase in nitrogen level of the diet regardless of the source of supplement. There was, however, an interaction of source and level of nitrogen in the 6 and 9% diets on nitrogen digestibility. Nitrogen digestibility was lowest at the 6% level but highest at the 9% level when urea was the sole source of supplemental nitrogen.

Chalupa (1968) concluded, after reviewing the literature, that some preformed protein may be necessary as a source of carbon skeletons for the utilization of the ammonia formed from urea. The main function of amino acids (from protein degradation) may not be as a supply of amino nitrogen but as a source of carbon skeletons following their deamination. It is possible that the availability of carbon structures may be a limiting factor in growth of certain rumen bacteria.

Level and source of energy and protein markedly affect urea utilization. A readily available source of energy has been shown to be necessary for efficient utilization of urea. On the other hand, such high energy diets have been shown to depress crude fiber digestibility. Some true protein in the ration may be necessary for urea utilization. Urea has been shown to be utilized rather efficiently in rations with up to 12% protein and less efficiently in rations containing higher levels of protein. It should be added, however, that rations for cattle and sheep seldom need to exceed 12% protein.

Additions to Urea Containing Supplements

Many substances have been investigated as additives to urea-based supplements. These additions are used to try to increase production either by specifically improving utilization or to increase production by their effects on the total ration.

Variable results have been reported on the effects of added dehydrated alfalfa meal, alfalfa ash or alfalfa hay on digestibility of low-protein roughages. Burroughs et al. (1950) conducted a series of three digestion trials with four steers to measure the influence of alfalfa hay upon corncob digestion. Corncob digestion was improved progressively with four respective additions of alfalfa hay. A water extract of dehydrated alfalfa meal or the ash of the alfalfa meal fed at a rate equivalent to 4 lb. meal daily improved corncob digestion materially.

Tillman *et al*. (1954) conducted two digestibility trials involving 52 sheep to study the effect of supplementing a ration containing low-quality prairie hay with a complete mineral mixture or alfalfa ash. In the first trial, the basal ration contained natural feedstuffs in addition to the hay. In the second, a semi-purified diet was used in which prairie hay was the only natural feedstuff. In both trials neither alfalfa ash nor a complete mineral mixture was found to improve apparent digestibility of the ration or any of its proximate components.

The results of Embry *et al*. (1955) indicated that levels of 0, 10 and 20% dehydrated alfalfa meal have little if any effect on ration digestibility by calves and lambs. These levels were added to both high- and low-protein prairie hay and fed to steer calves and wether lambs in a series of digestion trials. Quality of the hay did not influence the value of dehydrated alfalfa meal.

Gossett and Riggs (1956) added dehydrated alfalfa meal and trace minerals to a basal growing ration composed of poor-quality prairie hay, 2 lb. of cottonseed meal and 4 lb. of ground milo grain. Levels of 1, 2 and 3 lb. of dehydrated alfalfa leaf meal substituted for hay produced higher and more efficient gains. Steers which received the supplemental alfalfa meal gained 15.4% faster and required 15.2% less feed per unit of gain. Trace minerals did not alter the performance of the steers in this trial and were without effect when added to a poor-quality prairie hay ration.

A 2 x 2 x 2 x 3 factorially designed experiment involving 216 northwestern lambs was used by Karr et al. (1963a) to study factors affecting utilization of nitrogen from soybean meal, urea or biuret. Rations involved consisted of 60% concentrate and 40% roughage with a crude protein equivalent of 13.5%. Half of the rations contained 40% corncobs as their roughage source while the roughage of the remaining rations was made up of 20% corncobs and 20% dehydrated alfalfa meal. Lambs receiving dehydrated meal gained significantly faster (0.42 lb. per head daily compared to 0.24 lb.) and required 53% less feed per pound of gain for the 78-day feeding trial. Average daily gains for lambs fed dehydrated alfalfa meal with soybean meal, urea or biuret were 0.45, 0.39 and 0.43 lb., respectively. Respective gains for these supplements with corncobs were 0.31, 0.19 and 0.24 lb. In a digestion trial by these workers, 36 lambs receiving diets containing dehydrated alfalfa meal retained 17% more dietary nitrogen and digested 14% more dry matter than similar lambs fed diets containing corncobs as their sole roughage source. Dehydrated alfalfa meal appeared to have a very significant effect on the utilization of urea and biuret nitrogen.

The beneficial effects of dehydrated alfalfa meal on utilization of nonprotein nitrogen is due at least partially to the minerals contained therein (Karr et al., 1965). However, as pointed out by Chalupa (1968), the fact that alfalfa meal also supplies peptide nitrogen and readily available carbohydrates should not be overlooked.

Karr et al. (1963b, 1965) studied the effects of diethylstilbestrol implants on the utilization of nitrogen from different sources by lambs. Two hundred sixteen lambs were used in a 2 x 2 x 2 x 3 factorially designed experiment. When lambs being fed a 60% concentrate and 40% roughage diet were implanted with 3 mg. DES implant at the beginning of the 78-day feeding trial, they gained 44% more than nonimplanted lambs. The differences favoring implanted lambs represented 45, 62 and 32% faster gains for lambs receiving soybean meal, urea and biuret, respectively. In a digestion trial, 36 DES implanted lambs retained 17% more nitrogen than a similar group of nonimplanted lambs. DES increased nitrogen retention 34% in lambs receiving diets containing urea compared to 8% for soybean meal and 12% for biuret.

In an experiment designed to study the combined effects of dehydrated alfalfa meal and DES, Karr et al. (1965) showed improved gains from DES of 9% with soybean meal as the supplemental nitrogen source, 30% with dehy-urea and 18% with dehy-biuret. The authors suggested that dehydrated alfalfa meal increases rumen microbial activity and thus improves utilization of soluble dietary nitrogen while DES improves the utilization of absorbed nitrogen at the tissue level.

Supplementation of low-protein diets with urea and amino acids has been reported by several workers. Methionine supplementation at the rate of 3 or 6 gm. per day failed to improve a low-protein ration. However, when added to a urea ration at the rate of 1.6, 2.0 and 3.0

gm. per day, methionine increased the average digestibility of nutrients and nitrogen utilization. These differences were not statistically significant, however (Gallup _et al.,_ 1952).

Barth _et al_. (1959) found that supplementation of a high-urea diet (87% of total dietary N) with either methionine or tryptophan or both amino acids increased ($P < .05$) the percentage retention of absorbed nitrogen. Digestibility of nutrients was not influenced by either amino acid supplementation or time.

McLaren _et al_. (1965a) reported results of experiments to study the influence of methionine and tryptophan on nitrogen utilization by lambs fed high levels of nonprotein nitrogen. Two digestion and nitrogen balance trials were conducted with grade wether lambs fed semipurified rations composed of wheat straw, cane molasses and a concentrate mixture. Urea contained in the concentrate mixture was the sole source of supplemental nitrogen and accounted for 85% of the total nitrogen which amounted to 1.6% of the ration. Substitution of either 1.5 gm. of DL-methionine or 0.8 gm. of L-tryptophan for isonitrogenous amounts of urea nitrogen increased ($P < .05$) retention of absorbed nitrogen above that of lambs fed the urea basal ration. However, lambs fed rations containing both amino acids did not retain more absorbed nitrogen than lambs fed rations containing either one. Average increase in retention of absorbed nitrogen by lambs fed rations containing either or both amino acids was 15% above that of lambs fed the urea basal ration. This increase in nitrogen retention was not related to the adaptation of lambs to nonprotein nitrogen utilization associated with time.

Some of the beneficial response reported from feeding of amino acids to ruminants on high-urea diets may be due to the addition of sulfur via methionine. Many studies have been reported on the additions of sulfur to rations high in nonprotein nitrogen. Starks et al. (1953) used paired feeding techniques to show that elemental sulfur can be used by sheep to supply partially the dietary needs of sulfur when added to a low-protein diet where the major source of nitrogen is urea. Lambs receiving elemental sulfur retained more nitrogen ($P = .015$) and more sulfur ($P < .01$), their wool growth was increased ($P < .01$) and they came closer to maintaining their weight than those on the basal ration ($P = .033$).

Starks et al. (1954) studied the utilization of inorganic sulfur and urea in the nutrition of lambs in an experiment in which 40 growing-fattening lambs were fed a purified ration (consisting primarily of wood cellulose, wheat straw, starch, cerelose and urea). Weight gains and wool growth were increased by the addition of three graded levels of sulfur of different sources. This increase was highly significant. Lofgreen et al. (1953), however, reported that the addition of 0.2% sodium sulfate to a basal ration made up of approximately 87% natural feeds with urea furnishing 40% of the total nitrogen was without effect on gains in body weight, efficiency of feed utilization, nitrogen retention, serum sulfate levels or wool growth.

Corn distillers dried solubles (DDS) is recognized as a potent source of unidentified factors which stimulate cellulose digestion. Baker *et al.* (1957) reported results of *in vivo* studies on the cellulolytic factors present in distillers dried solubles. DDS *per se* and all fractions except the $CHCl_3$ extract significantly improved digestibility of cellulose of the basal ration. These results demonstrate that the cellulolytic factors contained in DDS are organic in nature and are associated with the protein and carbohydrate fractions.

Little *et al.* (1964) has reviewed work which reveals many of the chemical and physical properties of these unidentified cellulose digesting factors in DDS. It was established that the rumen stimulatory activity of DDS was completely extracted in water and not extracted in several concentrated organic solvents. It was not steam distillable, thus not directly associated with volatile fatty acids. The activity was not lost when dialyzed in aqueous solution and, therefore, probably not present in DDS as a simple carbohydrate, free amino acid or similar short-chained molecule. It was not removed by acid hydrolysis suggesting that if associated with proteins the gross structure of the protein was not critical.

Results of research reported by Beeson and Horn (1967) showed that the addition of corn distillers dried solubles to a high-urea diet produced a significant increase in the retention of dietary nitrogen. DDS increased the utilization of dietary nitrogen 79%. These data suggest that DDS furnishes a factor, or factors, which improves the bacterial synthesis of protein from urea. The diets used in these studies contained 71% corncobs with urea supplying 56% and 46% of the total dietary crude protein in the basal and basal plus DDS diets, respectively.

The role of vitamin B_{12} in one-carbon metabolism is widely recognized, and it is possible that the contribution of methyl groups of creatine to the one-carbon pool could result in a transitory vitamin B_{12} deficiency (McLaren et al., 1965b). Investigations by these workers have shown that the addition of vitamin B_{12} or folic acid or both vitamins did not influence the digestibility of nutrients or the utilization of nitrogen in rations where approximately 75% of the total ration nitrogen was furnished by urea. Retention of absorbed nitrogen by lambs was significantly ($P<.05$) increased when one-third of the urea nitrogen of the urea basal ration was replaced by an isonitrogenous amount of creatine. These data suggest that changes in rumen microbial population resulting from prolonged feeding of nonprotein nitrogen bring about increased synthesis of vitamin B_{12} required in the one-carbon metabolism associated with creatine degradation.

Bradley et al. (1966) produced a consistent depression in rate of gain in steers by simultaneous additions of fat and urea. Adding fat to the control ration reduced ($P<.01$) digestibility of dry matter, energy and NFE. When urea was added along with fat, digestibility of crude protein was also reduced ($P<.01$). Addition of calcium, lysine or corn distillers dried grain solubles to rations containing both animal fat and urea did not improve feedlot performance.

After reviewing research reported on additions to urea containing rations, Briggs (1967) concluded that of the many feed additives reported fed there is little evidence that most are in any way beneficial under all feeding circumstances. Sulfur supplementation is undoubtedly of value where deficiencies of this element occur in feedstuffs. Steroid hormones act just as well with urea containing feeds as with conventional rations. Most other additives do not seem to be of any general value.

Certain additives have been shown to be of some value in increasing utilization of urea. Diethylstilbestrol has been shown to increase performance of urea-fed animals to a greater extent than for animals not being fed urea. In addition, DES has been shown to decrease the urea adaptation period. Dehydrated alfalfa has been reported by several researchers to increase efficiency of urea utilization. However, other workers have not noted such a response. Additions of amino acids, sulfur and distillers dried solubles have increased performance in some cases but were without benefit in others.

METHOD OF PROCEDURE

The objective of this research was to investigate methods of improving utilization of urea in supplements with low-quality prairie hay fed to calves. The research consisted principally of four feeding experiments with wintering steer calves. In addition, a series of four *in vitro* experiments was conducted to study ammonia and volatile fatty acid production by ruminal microorganisms using supplement and prairie hay samples from the wintering experiments as substrates.

Feeding Experiments

Experiment 1. This experiment was conducted at the Range Field Substation, Cottonwood, during the winter of 1966-67. The research was initiated to determine the effects of adding various feed ingredients on utilization of a corn-urea supplement by calves wintered on low-protein prairie hay. Ingredients tested were soybean meal (SBOM), dehydrated alfalfa (dehy) and corn fermentation extractives (CFS) substituted on an equal nitrogen basis for urea and corn in a corn-urea supplement.

For this experiment 96 Angus x Hereford steer calves were purchased from an auction salebarn in west central South Dakota and had an average weight of about 410 lb. For the first 4 weeks after arrival at the substation, the calves were given a full feed of prairie hay plus 2 lb. per head daily of a 40% protein supplement (containing no urea) fortified with an antibiotic, vitamin A and minerals.

Two steers died during this 4-week feedlot adjustment period, leaving 94 steers to be used in the experiment. The steers were randomly allotted on basis of stratified filled weights to 6 pens of 12 steers each and 2 pens of 11 steers each. Four supplement treatments were used with a full feed of prairie hay and each treatment was replicated. Composition of supplements is shown in table 1. Length of this feeding experiment was 147 days.

The supplements fed were formulated to contain 32% protein and were fed at a rate of 2 lb. per head daily. Samples of the prairie hay fed were taken at various intervals throughout the experiment and were analyzed to contain an average of 4.9% protein. Ground limestone, dicalcium phosphate and trace mineral salt were offered separately on a free-choice basis.

Calves had access to sheds with outside exercise pens. The hay was fed once daily inside the sheds and the protein supplement in feed bunks in the outside pens.

Filled weights were taken at 28-day intervals to determine progress of performance. Initial and final shrunk weights were taken after an overnight stand without feed and water. Shrunk weights were used to calculate performance for the experiment.

During the course of the experiment, two steers accidentally died. In each case the steer was assigned a feed intake of the average for the pen up to the time of death and that amount was subtracted from the total consumption for the pen. This was done in

TABLE 1. PERCENTAGE COMPOSITION OF SUPPLEMENTS FOR EXPERIMENTS 1 AND 2

	Type of supplement[a]			
Ingredient	Corn + urea	Corn + urea + CFS	Corn + urea + dehy	Corn + urea + SBOM
Ground corn	91.15	67.65	55.25	78.65
Urea (281%)	8.50	7.00	7.40	7.00
Soybean meal	--	--	--	14.00
Corn fermentation extractives	--	25.00	--	--
Dehydrated alfalfa	--	--	37.00	--
Antibiotic premix[b]	0.35	0.35	0.35	0.35
Vitamin A premix[c]	0.07	0.07	0.07	0.07

[a] CFS = corn fermentation extractives, SBOM = soybean meal, dehy = dehydrated alfalfa.

[b] Chlortetracycline at 35 mg. per pound of supplement.

[c] 10,000 I.U. vitamin A per pound of supplement.

order that feed consumption and feed efficiency could be calculated for the remaining steers.

The data for this experiment were analyzed by a general analysis of variance using procedures as outlined by Steel and Torrie (1960). Since losses occurred in two of the pens during the experiment and two other pens had only 11 steers to begin the experiment, one steer was randomly eliminated from each of the remaining pens with 12 steers each. An average feed consumption was assigned to these steers and was subtracted from the total for that pen. This gave a total of 11 steers per pen to be used for the analysis of data.

<u>Experiment 2.</u> This experiment was conducted at the Central Substation, Highmore, during the winter of 1967-68. Design of the experiment was the same as for experiment 1, except treatments were not replicated. The objective was to test the same additive treatments with rations higher in protein than in the first experiment.

For this experiment 48 steer calves weighing an average of 365 lb. were purchased from a single herd in south central South Dakota. During a 24-day feedlot adjustment period, the steers were full-fed prairie hay plus 2 lb. per head daily of a 40% protein supplement (containing no urea) fortified with an antibiotic, vitamin A and minerals. The steers were randomly allotted on basis of stratified filled weight to 4 pens of 12 steers each for the experiment. Experimental treatments and ingredient composition of the supplements were the same as for experiment 1 (table 1).

Samples of the prairie hay fed were taken at weekly intervals and were composited at monthly intervals. These samples were analyzed to contain an average of 7.0% protein, which was considerably higher than that fed in experiment 1. Ground limestone, dicalcium phosphate and trace mineral salt were offered separately on a free-choice basis. Facilities used were similar to those for experiment 1.

A filled weight was taken at 28-day intervals to calculate periodic gains and feed efficiency. The initial and final shrunk weights were taken after an overnight stand without feed and water.

The data from this experiment were analyzed by a general analysis of variance using procedures as outlined by Steel and Torrie (1960).

Experiment 3. Experiment 3 was conducted at the Range Field Substation, Cottonwood, during the winter of 1967-68. The purpose of the experiment was to compare performance of cattle on a full feed of prairie hay plus a limited grain ration with soybean meal or urea as the source of supplemental protein. The trial was of 169 days duration.

Sixty-four steer calves weighing about 420 lb. were purchased from an auction salebarn in western South Dakota. The calves were offered a full feed of prairie hay for the first week following their arrival at the substation. In addition, they were fed 1 lb. of ground corn per head on the sixth and seventh days after arrival. After this 1-week adjustment period, the cattle were weighed and allotted by a gate cut into 2 pens with 32 head each. These pens were located away from any buildings and the steers did not have access to shelter.

During the experiment the cattle were full-fed prairie hay plus 4 lb. per head daily of a grain-protein supplement mix consisting of corn with soybean meal or urea on a protein equivalent basis as the source of supplemental protein. The grain-supplement mixture was formulated to contain 20% protein. At the rate of feeding, the urea furnished approximately 57% of protein in the mix with urea. The prairie hay fed was sampled at weekly intervals and composited at monthly intervals. These samples were analyzed to contain an average of 6.5% protein.

The grain-supplement mix contained 17.5 mg. of chlortetracycline and 2,500 I.U. of vitamin A per pound. For the first 4 weeks of the experiment, the calves were fed 300 mg. of chlortetracycline per head daily in addition to that present in the grain and supplement mixture. Trace mineral salt, dicalcium phosphate and ground limestone were offered separately on a free-choice basis.

Filled weights were taken at 28-day intervals to calculate periodic gains and feed efficiency. Initial and final shrunk weights were taken after an overnight stand without feed and water.

Data from this experiment were analyzed by a "Student's" _t_ test using procedures as outlined by Steel and Torrie (1960).

Experiment 4 - Adaptation Phase. Experiment 4 consisted of two phases--an adaptation phase (37 days) and a wintering phase (126 days). The experiment was conducted at the Range Field Substation, Cottonwood, during the winter of 1967-68 using the same facilities as for experiment 1. The adaptation phase was conducted to compare performance of calves fed protein supplements with and without urea (4%) during feedlot adaptation following weaning and shipping.

For this experiment 64 steer calves were purchased from a rancher in western South Dakota in November. The calves were offered prairie hay free choice for the first 3 days after arriving at the substation. After 3 days, a filled weight was taken and the cattle were randomly allotted on basis of stratified filled weights to 8 pens of 8 steers each. Two supplement treatments were used with a full feed of prairie hay and each treatment was replicated 3 times. Composition of supplements is shown in table 2.

Supplements used in the adaptation phase were formulated to contain 40% protein and were fed at the rate of 2 lb. per head daily. Prairie hay fed was sampled at weekly intervals and was composited at the end of the adaptation phase. This composite sample was analyzed to contain 6.9% protein. Trace mineral salt, dicalcium phosphate and ground limestone were offered separately on a free-choice basis. At the end of 37 days, the cattle were weighed and reallotted to begin the wintering phase.

The data from the adaptation experiment were analyzed by a general analysis of variance using procedures as outlined by Steel and Torrie (1960).

Experiment 4 - Wintering Phase. The wintering phase was designed to determine effects of an adaptation period of about 5 weeks with low level of urea on later performance of calves at higher levels of urea with low-protein prairie hay. To begin the wintering phase the cattle were reallotted, within adaptation treatment group, on basis of stratified filled weights to 8 pens of 8 steers each. Supplements were formulated with 4% or 8% urea and contained about 31% protein. Composition of supplements is shown in table 2. Rations used in this experiment consisted of a full feed of prairie hay plus 2 lb. per head daily of the appropriate wintering supplement. Prairie

TABLE 2. PERCENTAGE COMPOSITION OF SUPPLEMENTS FOR EXPERIMENT 4

Ingredient	Adaptation supplement (40% protein)		Wintering supplement (31% protein)	
	4% urea	SBOM control	4% urea	8% urea
Ground corn	27.00	--	64.55	91.58
Urea (281%)	4.00	--	4.00	8.00
Soybean meal	59.18	90.18	31.03	--
Trace mineral salt	4.00	4.00	--	--
Dicalcium phosphate	4.00	4.00	--	--
Antibiotic premix[a]	1.75	1.75	0.35	0.35
Vitamin A premix[b]	0.07	0.07	0.07	0.07

[a] Chlortetracycline at 175 and 35 mg. per pound of adaptation and wintering supplements.

[b] 10,000 I.U. vitamin A per pound of supplement.

hay was sampled weekly and samples were composited at monthly intervals. These samples were analyzed to contain an average of 6.5% protein. Trace mineral salt, dicalcium phosphate and ground limestone were offered separately on a free-choice basis.

Filled weights were taken at 28-day intervals to calculate periodic gains and feed efficiency. Initial and final shrunk weights were taken after an overnight stand without feed and water.

The data from this phase of experiment 4 were analyzed by a general analysis of variance using procedures as outlined by Steel and Torrie (1960).

In Vitro Experiments

A series of *in vitro* experiments was conducted to study ammonia (NH_3) and volatile fatty acid (VFA) production from the rations fed in wintering experiment 1. The *in vitro* fermentation procedure of Cheng *et al.* (1955) was modified by omitting the washing step prior to suspending the microorganisms in the medium. The medium used was as described by Yoder *et al.* (1966) except that cellulose and urea were omitted. The bacterial inoculum was obtained from a rumen fistulated steer fed a ration composed of alfalfa hay (60%) and a grain mixture (40%).

One liter of whole rumen fluid was collected from the top layer of rumen ingesta. This fluid was strained through four layers of cheesecloth and the pulp was saved. Four lb. of pulp were then suspended in 2 l. of phosphate buffer (1.05 gm. of $Na_2HPO_4 \cdot 7H_2O$ and 0.436 gm. of KH_2PO_4/l.) and this suspension was strained through four layers of cheesecloth and the filtrate was added to the 1 l. of whole rumen fluid.

The 3 l. of fluid were centrifuged for 2 minutes at about 1500 rpm in an International Centrifuge, strained through eight layers of cheesecloth and centrifuged in a Sharples centrifuge. The sediment was collected and suspended in 1 l. of bacterial medium and the pH was then adjusted to 6.9. Twenty ml. aliquots of the bacterial suspension were pipetted into a series of 75 ml. centrifuge tubes containing preweighed finely ground substrate. The tubes were incubated anaerobically in a 39° C. water bath for 24 hours with CO_2 bubbling through the suspension.

The substrates were prepared for study by grinding in a Wiley mill using a 1 mm. screen. Prairie hay and protein supplements in the proportion as consumed by steers in the wintering trial were used to make up 500 mg. of substrate.

Total VFA production was determined by the steam distillation procedure and was used as an indication of total ration digestibility. Rumen ammonia production was measured using the microdiffusion analysis technique as described by Conway (1950). Ammonia production was used as an indication of protein digestion.

Experiments 1 and 2. A 3 x 4 factorial design was used in experiments 1 and 2 to study VFA and NH_3 production from the 4 protein supplements (table 1) and 3 prairie hay samples fed in feeding experiment 1. The prairie hay samples used had a protein content of 4.3, 4.7 and 5.9% for samples 1, 2 and 3, respectively. Each hay x supplement treatment was replicated twice. The substrate consisted of 75 mg. supplement and 425 mg. of prairie hay.

Data from in vitro experiment 1 was analyzed using a general analysis of variance as outlined by Steel and Torrie (1960). The loss of a number of tubes from experiment 2 resulting in unequal subclass numbers necessitated the use of least squares method to compute the analysis of variance (Harvey, 1960).

Experiments 3 and 4. For these two experiments a 2 x 4 factorial design was used to study the effects of increasing energy levels and of adding sulfur plus energy to a urea-based supplement. Supplements used were the corn-urea and the corn-urea-soybean meal supplements as for in vitro experiments 1 and 2. Seventy-five milligrams of supplement plus 425 mg. prairie hay made up the basal substrate. Soluble starch was substituted for prairie hay at levels of 0, 45 or 90 mg. and a fourth treatment of 45 mg. starch plus 12.5 mg. Glauber's salt. These amounts of starch were calculated to be equivalent to 0, 2 or 4 lb. additions of corn per day in the ration fed in wintering experiment 1, and the Glauber's salt was calculated to furnish an equivalent of 0.33 lb. of added sulfur per day. Each treatment was replicated 3 times.

Data from experiment 3 were analyzed by a general analysis of variance as outlined by Steel and Torrie (1960). A least squares analysis as outlined by Harvey (1960) was used to analyze data from experiment 4 since some tubes were missing.

RESULTS AND DISCUSSION

Feeding Experiments

Experiment 1. Weight gain and feed data for experiment 1 are presented in table 3. Data were analyzed statistically and no differences were found to be significant.

During this experiment calves receiving the corn-urea soybean meal (SBOM) supplement gained only 0.07 lb. more daily but amounted to 10.3% more than for steers fed the corn-urea supplement. Calves fed the corn-urea-corn fermentation extractives (CFS) supplement gained only slightly more (4.7%) than did the corn-urea fed cattle. The addition of dehydrated alfalfa (dehy) resulted in the same rate of gain as for cattle fed the corn-urea supplement.

Feed consumption varied only slightly among treatments resulting in similar differences in feed efficiency as for weight gains. Calves fed protein supplements with SBOM or CFS required 10.3 and 5.7%, respectively, less feed per 100 lb. of gain than those fed the corn-urea supplement. Dehydrated alfalfa resulted in essentially no change in feed efficiency in comparison to the corn-urea supplement.

The hay fed in this experiment was low in protein and protein content of the rations calculated from feed intakes shown amounted to only 8.1%. Feed intakes shown are for the amounts fed and include an unknown amount of wastage. Therefore, percent of protein in the rations consumed would be some higher than for the rations fed. It is quite likely that performance of the cattle could have been improved

TABLE 3. WEIGHT GAINS AND FEED DATA
(EXPERIMENT 1 - COTTONWOOD, 1966-67, 147 DAYS)

	Type of supplement			
Item	Corn + urea	Corn + urea + CFS	Corn + urea + SBOM	Corn + urea + dehy
Number of steers	22	22	22	22
Av. init. wt., lb.	410	408	412	418
Av. final wt., lb.	517	520	530	525
Av. daily gain, lb.	0.73	0.77	0.80	0.73
Av. daily ration, lb.				
Prairie hay	14.8	14.6	15.0	15.0
Supplement	2.0	2.0	2.0	2.0
Feed/cwt. gain, lb.				
Prairie hay	2043	1922	1836	2039
Supplement	274	262	242	271

some by feeding a higher level of protein. On the other hand, rations borderline to slightly deficient in protein might be expected to offer a more sensitive measure of the value of the various additions to the corn-urea supplement.

An apparent urea adaptation response was noted in this experiment. Weight gains for the first 28-day period were lower than for any subsequent 28-day period (figure 1). Low weight gains were noted in all pens regardless of the experimental ration treatment. This "adaptation response" has been noted by several researchers (Smith et al., 1960; McLaren et al., 1965; Virtanen, 1966). In these rations urea supplied 75% of the supplemental nitrogen in the corn-urea supplement, 61% in both the corn-urea-CFS and the corn-urea-SBOM supplements and 65% in the corn-urea-dehy supplement. From the hay

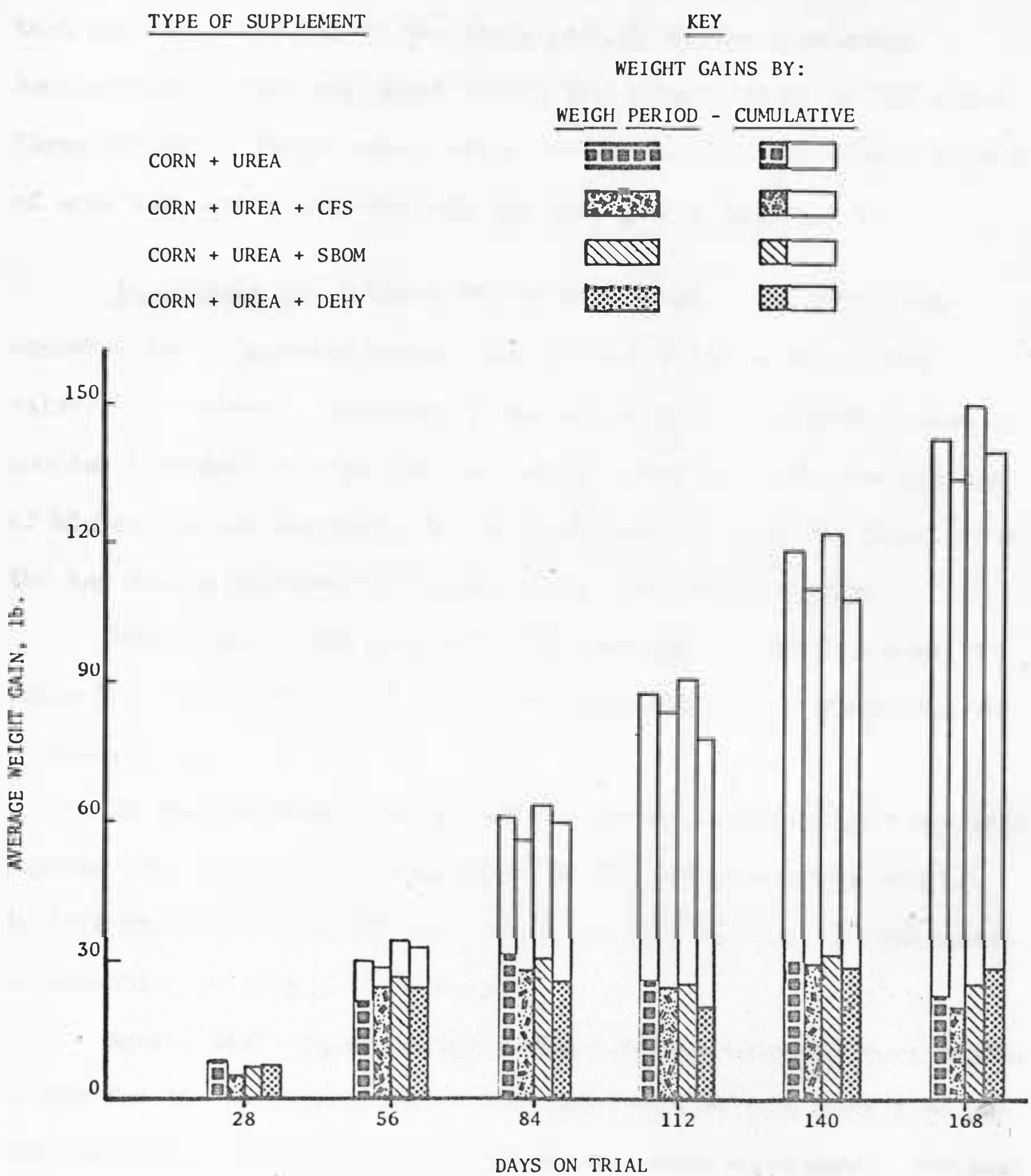

Figure 1. Cumulative and periodic weight gains (Experiments 1 and 2).

consumed and the protein content of the hay, it can be calculated that urea supplied 39% of the total protein in the corn-urea supplemented ration and about 35% of the total protein in the other three rations. Weight gains after the first month with these levels of urea were quite satisfactory for the type of rations fed.

Experiment 2. Rations fed in experiment 1 may have been somewhat low in protein content for optimum performance of the calves. Therefore, experiment 2 was conducted the following year at another location to study the same supplements fed with prairie hay of higher protein content. The rate of protein supplementation with the hay fed in experiment 2 gave rations with 10.8% protein.

Weight gains and feed data for experiment 2 are presented in table 4. Statistical analysis of the data showed no differences to be significant.

In this experiment only calves fed the protein supplement with soybean meal gained more than those fed the corn-urea supplement. In this instance the difference was somewhat less than in experiment 1, amounting to only 3.9% more gain.

Again, differences in feed intake among treatments were small. Calves fed the corn-urea-SBOM supplement required 7.1% less feed per 100 lb. of gain than did those fed the corn-urea supplement. Neither corn fermentation extractives nor dehydrated alfalfa meal improved feed efficiency.

TABLE 4. WEIGHT GAINS AND FEED DATA
(EXPERIMENT 2 - HIGHMORE, 1967-68, 168 DAYS)

	Type of supplement			
Item	Corn + urea	Corn + urea + CFS	Corn + urea + SBOM	Corn + urea + dehy
Number of steers	12	12	12	12
Av. init. wt., lb.	364	364	365	366
Av. final wt., lb.	545	526	553	536
Av. daily gain, lb.	1.08	0.96	1.12	1.01
Av. daily ration, lb.				
Prairie hay	11.3	10.9	10.8	11.1
Supplement	2.0	2.0	2.0	2.0
Feed/cwt. gain, lb.				
Prairie hay	1047	1130	966	1096
Supplement	186	208	179	198

Combined weight gain data from experiments 1 and 2 are presented graphically in figure 1 by weigh periods and as cumulative gains. For the first 28-day period, daily gains for all cattle in both experiments averaged only 0.2 lb. per head. Gains after this initial period did not vary greatly among ration treatments and averaged about 1.0 lb. per head daily.

Urea furnished a smaller percentage of the total protein in those rations supplemented with dehydrated alfalfa, corn fermentation extractives or soybean meal. Additions of these ingredients to the corn-urea supplement fed with prairie hay to wintering calves did not significantly improve performance in the two experiments. However, soybean meal did appear to have a slight advantage over the other ingredients in both experiments. Even though protein content of the rations varied considerably between the two experiments, the results were quite similar.

Experiment 3. The results of experiment 3 are shown in table 5. Calves fed the grain-supplement mix with soybean meal as the source of supplemental protein gained 0.19 lb. per head daily more (33 lb. total) than did calves which received urea. This difference was highly significant ($P < .01$).

Gains by weigh periods and cumulative gains are shown graphically in figure 2. Even though a readily available source of energy was fed in this experiment, performance of urea-supplemented calves was inferior to that of cattle receiving soybean meal. Urea-fed calves performed at a somewhat lower rate during most weigh periods throughout the experiment. There was no obvious adaptation period evident in this experiment as in experiments 1 and 2. These results are in agreement with those of Thomas et al. (1953) and Bloomfield et al. (1964).

Feed consumption was the same for both treatment groups. Therefore, feed required per 100 lb. gain was less for cattle receiving the soybean meal. Average consumption of the grain-supplement mixture was less than 4 lb. per steer daily because only 1 lb. per head daily was fed at the beginning of the experiment and this amount was progressively raised to 4 lb. per head per day by the end of 1 week.

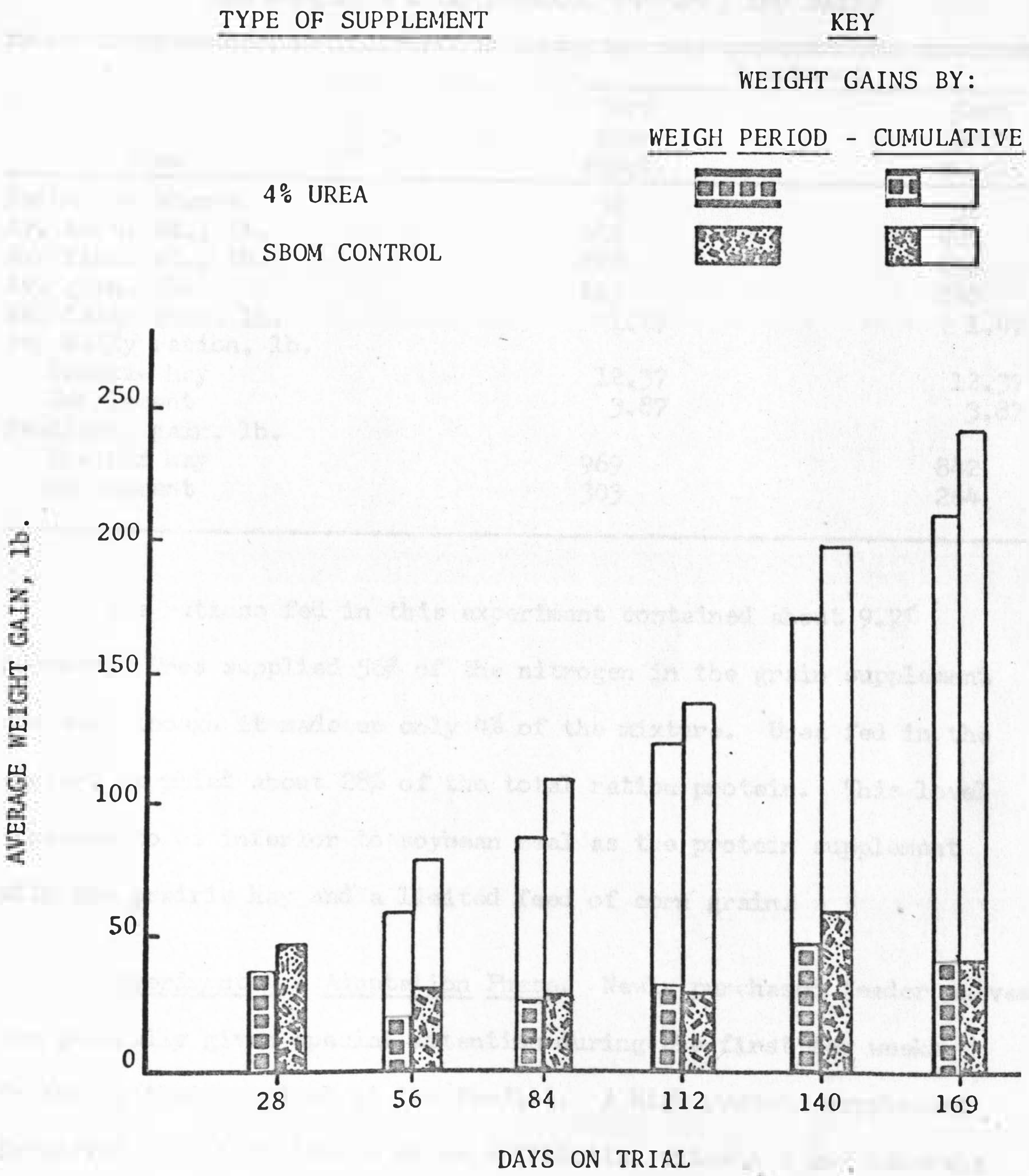

Figure 2. Cumulative and periodic weight gains (Experiment 3).

TABLE 5. WEIGHT GAINS AND FEED DATA
(EXPERIMENT 3 - COTTONWOOD, 1967-68, 169 DAYS)

Item	Treatment: Corn urea suppl.	Treatment: Corn SBOM suppl.
Number of steers	32	32
Av. init. wt., lb.	411	428
Av. final wt., lb.	626	676
Av. gain, lb.	215	248
Av. daily gain, lb.	1.27	1.47
Av. daily ration, lb.		
Prairie hay	12.37	12.37
Supplement	3.87	3.87
Feed/cwt. gain, lb.		
Prairie hay	969	842
Supplement	303	264

The rations fed in this experiment contained about 9.7% protein. Urea supplied 56% of the nitrogen in the grain supplement mix even though it made up only 4% of the mixture. Urea fed in the mixture supplied about 28% of the total ration protein. This level appeared to be inferior to soybean meal as the protein supplement with the prairie hay and a limited feed of corn grain.

Experiment 4 - Adaptation Phase. Newly purchased feeder calves are generally given special attention during the first few weeks following their arrival at the feedlot. A high protein supplement fortified with high levels of an antibiotic, vitamin A and minerals is often fed during this time. This experiment was conducted to determine the effects of also feeding a low level of urea during a period of feedlot adaptation and the effects on later performance when fed higher levels of urea.

Table 6 shows the results of the adaptation phase of experiment 4. Calves fed the supplement with 4% urea gained significantly ($P<.01$) less than did the cattle fed the soybean meal control supplement. This difference in gain averaged 0.27 lb. per day (10 lb. total) over the 37-day period. Rates of gain from both treatments, however, would generally be considered as quite satisfactory for calves on this type of ration for the first few weeks following weaning and shipping.

The calves were trucked about 120 miles from the buying point to the substation and were started on experiment only 3 days after arriving. Part of the gain during this adaptation period would be represented by recovery of shrinkage from weaning and shipping.

Calves receiving the 4% urea supplement consumed only slightly less prairie hay than did the control cattle. They required 134 lb. more hay per 100 lb. gain than did the controls. Consumption of protein supplement averaged less than 2 lb. per head daily for the urea-supplemented calves since some difficulty was encountered in getting the calves in two of the pens to accept the supplement with urea for the first 10 days of the experiment. The supplement was readily consumed thereafter, however.

TABLE 6. WEIGHT GAINS AND FEED DATA (EXPERIMENT 4 - ADAPTATION PHASE, COTTONWOOD, 1967, 37 DAYS)

Item	Type of supplement: 4% urea	Type of supplement: SBOM control
Number of steers	32	32
Av. init. wt., lb.	422	422
Av. final wt., lb.	472	482
Av. daily gain, lb.	1.34	1.61
Av. daily ration, lb.		
Prairie hay	11.07	11.23
Supplement	1.87	2.00
Feed/cwt. gain, lb.		
Prairie hay	833	699
Supplement	147	124

Experiment 4 - Wintering Phase. Results of the wintering phase of experiment 4 are shown in table 7. Calves which received the control supplement during the adaptation phase and an 8% urea supplement during the wintering phase gained significantly ($P < .01$) less than did cattle on any of the other three treatments. Only very small differences in gain were noted between the other three treatments.

Feed data show that cattle going from a supplement with no urea to an 8% urea supplement consumed slightly more hay and required considerably more feed per 100 lb. gain than did cattle on any one of the other three treatments. Again, only small differences were noted between the other treatments.

Calves not fed urea during the feedlot adaptation phase of the experiment showed an apparent adaptation response to urea when first offered during the wintering phase. Daily rate of gain during the first 28-day period was 0.76 and 0.18 lb. for calves with and without

TABLE 7. WEIGHT GAINS AND FEED DATA (EXPERIMENT 4 - WINTERING PHASE, COTTONWOOD, 1967-68, 126 DAYS)

		Type of supplement			
	Adaptation	4% urea	4% urea	SBOM control	SBOM control
Item	Wintering	4% urea	8% urea	4% urea	8% urea
Number of steers		16	16	16	16
Av. init. wt., lb.		471	465	476	475
Av. final wt., lb.		601	594	602	583
Av. daily gain, lb.		1.04	1.03	1.00	0.86
Av. daily ration, lb.					
Prairie hay		12.82	13.00	12.88	13.12
Supplement		2.00	2.00	2.00	2.00
Feed/cwt. gain, lb.					
Prairie hay		1240	1269	1288	1534
Supplement		194	195	200	235

urea previously. Differences between those fed the supplements with 4 and 8% urea were small during this time.

Figure 3 shows weight gains by weigh periods and cumulative gains for both phases of experiment 4 combined. Calves fed the 4% supplement during the adaptation phase and the 8% urea supplement during the wintering phase gained 15 lb. more per calf over the 163-day total than did calves going from the no urea control supplement to the one with 8% urea.

Calves gained at a lower rate during the first month when first offered the supplement with 4% urea whether this was shortly after arrival or about 5 weeks later. However, depression in gain in comparison to calves without urea was less when administered upon arrival at the feedlot than for 5 weeks later. Total gain for both

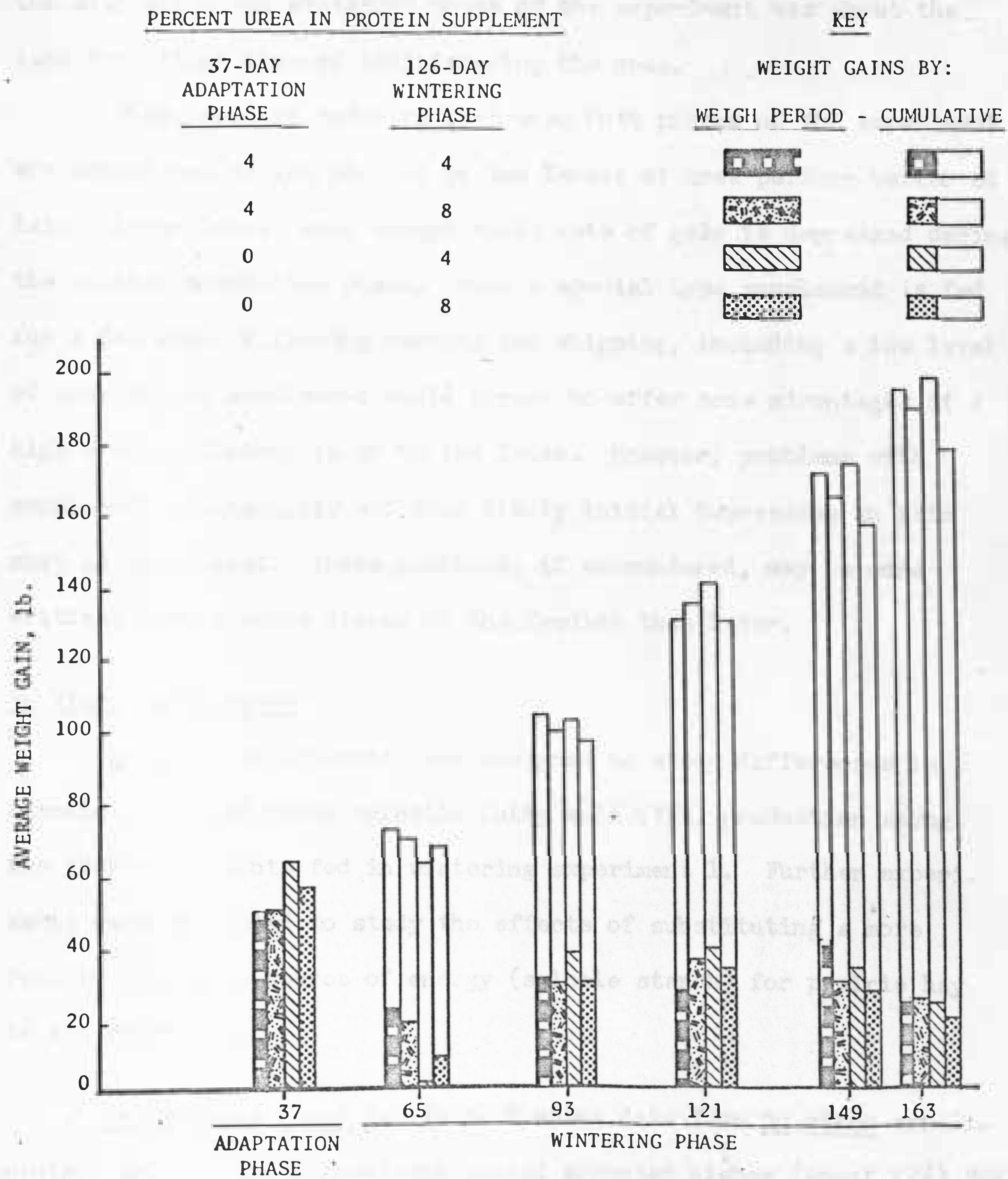

Figure 3. Cumulative and periodic weight gains (Experiment 4).

the adaptation and wintering phase of the experiment was about the same for either time of administering the urea.

These results indicate that when both phases of the experiment are considered calves adapted to low levels of urea perform better at later higher levels even though their rate of gain is depressed during the initial adaptation phase. When a special type supplement is fed for a few weeks following weaning and shipping, including a low level of urea in the supplement would appear to offer some advantages if a high urea supplement is to be fed later. However, problems with supplement palatability and some likely initial depression in gain must be considered. These problems, if encountered, may be more critical during early stages in the feedlot than later.

In Vitro Experiments

In vitro experiments were designed to study differences in ammonia (NH_3) and total volatile fatty acid (VFA) production among the four supplements fed in wintering experiment 1. Further experiments were initiated to study the effects of substituting a more readily available source of energy (soluble starch) for prairie hay in the substrate.

Experiments 1 and 2. Table 8 shows data from *in vitro* experiments 1 and 2. The ingredients tested promoted higher (about 12%) VFA production than the corn-urea supplement. However, the differences were not statistically significant and only small differences existed

TABLE 8. AMMONIA AND VOLATILE FATTY ACID PRODUCTION (*IN VITRO* EXPERIMENTS 1 AND 2)

	Prairie hay sample number[a]							
	1		2		3		Average	
	um/ml. VFA	mg. % NH_3	um/ml. VFA	mg. % NH_3	um/ml. VFA	mg. % NH_3	um/ml. VFA	mg. % NH_3
Corn-urea	41.0	44.5	46.6	44.6	45.3	47.5	44.3	45.5
Corn-urea-CFS	46.5	42.4	54.7[b]	46.2[c]	53.1	47.5	50.0	46.0
Corn-urea-SBOM	46.2	45.9	46.4	45.0	55.6	52.1	49.4	47.7
Corn-urea-dehy	47.3	47.7	50.9[d]	40.8[e]	46.9	47.2	46.0	46.9
Average	45.2	45.1	49.6	44.2	50.2	48.6		

[a] Prairie hay samples contained 4.34, 4.71 and 5.94% protein for samples 1, 2 and 3, respectively.
[b,c,d,e] Results of experiment 1 only.

among the three ingredients. Only very small differences in NH_3 production occurred among the treatments.

Significant differences were obtained among the three prairie hay samples used in these experiments ($P < .01$ in experiment 1 and $P < .05$ in experiment 2) in both VFA and NH_3 production. Prairie hay samples 2 and 3 (4.71 and 5.94% protein) promoted higher VFA production than did sample 1 (4.34% protein). Sample 3 promoted higher NH_3 production than did samples 1 and 2.

The higher VFA production from soybean meal, CFS or dehydrated alfalfa meal did not appear to be associated with a higher rate of gain from these ingredients in the feeding trials, with the possible exception of soybean meal. By using hay of higher protein content, VFA and NH_3 production was increased but relative differences among the supplements did not change in general. Larger daily gains were obtained in feeding experiment 2 where the hay contained more protein than in experiment 1, but results from the various supplements were similar for the two experiments.

Experiments 3 and 4. Results of *in vitro* experiments 1 and 2 indicate that only a small, if any, benefit in performance was derived from adding soybean meal, dehydrated alfalfa or corn fermentation extractives to a corn-urea supplement. Experiments 3 and 4 were designed to study the effects of adding an energy supplement to a ration of prairie hay supplemented with a corn-urea or a corn-urea-SBOM supplement. Also, the effects of added energy plus sulfur were studied.

Results of *in vitro* experiments 3 and 4 are presented in table 9. The addition of starch plus sulfur, but not starch alone, resulted in increased VFA production. Results differed for the two experiments, being statistically ($P < .01$) significant in experiment 3 but non-significant in experiment 4. There were only small differences between the corn-urea and the corn-urea-soybean meal supplements.

Additions of 45 or 90 mg. of starch or 45 mg. starch plus 12.5 mg. of Glauber's salt gave higher values (nonsignificant) for NH_3 production as compared to the basal substrate. The values were slightly higher for the supplements with soybean meal. These additions would be equivalent to feeding 2 or 4 lb. of additional corn or 2 lb. corn plus 0.33 lb. of sulfur in wintering experiment 1.

TABLE 9. AMMONIA AND VOLATILE FATTY ACID PRODUCTION (*IN VITRO* EXPERIMENTS 3 AND 4)

	Type of supplement					
	Corn-urea		Corn-urea-SBOM		Average	
Additions	um/ml. VFA	mg. % NH_3	um/ml. VFA	mg. % NH_3	um/ml. VFA	mg. % NH_3
0 mg. starch	50.9	52.3	48.0	55.5	49.4	53.9
45 mg. starch	51.9	55.5	51.8	58.6	51.8	57.0
90 mg. starch	51.6	54.9	49.2	56.9	50.4	55.9
45 mg. starch plus 12.5 mg. Glauber's salt	55.0	55.6	55.8	56.7	55.4	56.2
Average	52.4	54.6	51.2	56.9		

These results on VFA production without supplemental sulfur are not in agreement with those reported by Bloomfield *et al.* (1964) and Belasco (1955). These workers reported significant increases in VFA and NH_3 production by adding starch to a urea-based substrate. However, it has been reported by other workers (Burroughs *et al.*, 1949; Arias *et al.*, 1951; Hunt *et al.*, 1954; El-Shazley *et al.*, 1961) that starch markedly depressed cellulose digestion both *in vitro* and *in vivo*. This may have been a factor in the results obtained by offsetting increased VFA production which may have been promoted by adding starch. Individual VFA production was not measured in these trials. It is possible that some changes in proportion of individual fatty acids may have differed among treatments, while total VFA production did not change.

SUMMARY

This research was designed to study methods of improving utilization of urea-containing supplements fed with low-protein prairie hay to beef calves. Four feeding experiments involving 272 steer calves were conducted. In addition a series of four *in vitro* experiments was conducted.

Additions of soybean meal, dehydrated alfalfa or corn fermentation extractives to a corn-urea supplement fed with prairie hay to wintering calves did not significantly improve performance in either of two experiments. Soybean meal did appear to have a slight advantage over the other ingredients. Urea supplied 35 to 40% of the protein in the rations and furnished a smaller percentage of the total protein in those rations supplemented with dehydrated alfalfa, corn fermentation extractives or soybean meal. Even though protein content of the total ration consumed varied considerably between the two trials, the results were relatively similar. Slower gains were noted during the first 28-day period than for the remainder of the trials. This urea-adaptation response was noted in all pens in both trials. The feed ingredients tested in these experiments did not improve performance during this adaptation period.

In a third feeding experiment, calves fed a full feed of prairie hay plus 4 lb. of grain and supplement per day performed less favorably ($P < .01$) with 4% urea in the mixture than with soybean meal on a protein equivalent basis.

A fourth feeding experiment was conducted to study effects of adapting calves to a low level of urea during feedlot adaptation following weaning and shipping and subsequent performance of these calves at higher levels of urea. During a 37-day adaptation phase, calves fed 2 lb. of 4% urea supplement gained at a slower rate (1.34 vs. 1.61 lb. daily) than did cattle receiving a similar supplement without urea. During a subsequent wintering phase, however, those cattle fed a 4% urea supplement during adaptation gained significantly more (21 lb.) when fed an 8% urea supplement than did calves fed the control supplement during the adaptation phase. These results indicate that when both phases of the experiment are considered calves adapted to low levels of urea perform better later at higher levels even though their rate of gain is depressed during the initial adaptation phase.

In vitro experiments were conducted to study VFA and ammonia production from rations used in feeding experiment 1. The feed ingredients tested resulted in some improvement in VFA production *in vitro.* Prairie hay of higher protein content also resulted in higher VFA and NH_3 values. However, rations resulting in higher VFA values did not always result in higher rates of gain.

Substitution of a more readily available carbohydrate (soluble starch) for prairie hay in the substrate (*in vitro* experiments 3 and 4) did not significantly improve either VFA or NH_3 production. Significant ($P<.05$) increases in VFA production were noted in *in vitro* experiment 3 when sulfur plus energy was added to the substrate. However, this increase was not noted in *in vitro* experiment 4.

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