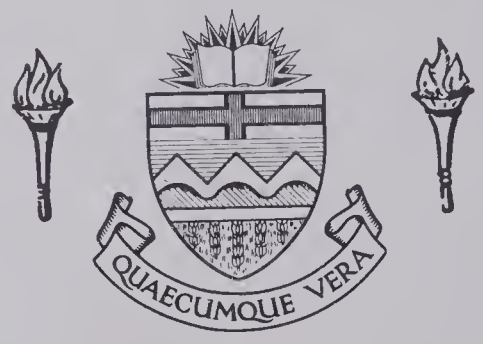

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THE UNIVERSITY OF ALBERTA

UREA VERSUS FURFURAL UREA POLYMER AS A SOURCE OF NON-PROTEIN NITROGEN FOR FATTENING CALVES

by

© GORDON ARTHUR WELLS

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

DEPARTMENT OF ANIMAL SCIENCE

EDMONTON, ALBERTA

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THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Urea versus furfural urea polymer as a source of non-protein nitrogen for fattening calves" submitted by Gordon Arthur Wells, B.S.A., in partial fulfilment of the requirements for the degree of Master of Science.

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Date.....April 3rd, 1969.....

ABSTRACT

In the first experiment a feeding trial was carried out with two lots of Holstein-Friesian calves (4 calves per lot) to compare two non-protein nitrogen sources in ruminant rations. Calves in one lot were fed a ration consisting of barley plus urea, and calves in the other lot were fed a ration of barley plus furfural urea polymer. Prior to and during the first week of the experiment calves in the first group were fed a calf meal containing urea, while calves in the second group were fed a concentrate mixture formulated for dairy cows and which did not contain urea.

During the feeding trial, calves fed the ration of barley and urea consumed 58% more feed daily, gained 121% more weight and used 28% less feed per unit of gain than those fed the ration of barley and furfural urea polymer. These differences appeared to be caused by low palatability of the ration with furfural urea polymer. Feed intake had to be restricted with these calves to reduce sorting of the ration and ensure consumption of the pelleted supplement containing the polymer.

Three metabolism studies were conducted with two calves from each lot at intervals of three weeks, commencing with the first week of the experiment. Apparent coefficients of digestion of dry matter, crude protein and gross energy were high for all of the rations, and there were no significant differences between the rations or between the three trials.

In the first metabolism trial, percentage nitrogen retention was low in calves fed the urea calf meal or dairy concentrate. This appeared to be caused primarily by high urinary excretion of nitrogen, particularly by calves fed the urea calf meal. In the second metabolism trial, calves fed the barley ration with urea retained very low levels of nitrogen as a result of low feed consumption and low intake of nitrogen. In the following trial, these calves had the highest retention of nitrogen, and their urinary excretion of nitrogen was low in both trials. Calves fed the furfural urea ration had low rates of urinary excretion of nitrogen and high retention of nitrogen in both metabolism trials.

The metabolizable energy values of the urea calf meal and dairy concentrate were higher than expected, and were higher than those of the two experimental rations. The values for the barley rations, when supplemented with urea or furfural urea polymer, were similar to metabolizable energy values quoted for barley, and there was very little difference in the values determined in the two trials or for the two rations.

There were small differences between groups of calves and between metabolism trials in the concentrations and proportions of volatile fatty acids and in the pH of rumen contents. The proportions of acetate were low and of propionate were high, which would be expected when ruminants are fed all-concentrate rations.

In a second experiment, samples of rumen contents and jugular blood were obtained at three intervals from the calves used in the metabolism trials and from 2 calves from each of 4 lots made available from another research project.

High levels of rumen ammonia and blood urea were found in calves fed the urea calf meal. When the ration was changed to barley and urea, the levels of rumen ammonia and blood urea were significantly lower. Low levels of rumen ammonia and blood urea were obtained in calves fed a barley ration without nitrogen supplementation. Supplementation of barley with soybean meal, urea or furfural urea polymer resulted in slightly higher levels of rumen ammonia and blood urea than when barley was fed alone.

Nitrogen appeared to be utilized with approximately the same efficiency in all-concentrate rations when it was supplied by soybean meal, urea or furfural urea polymer.

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I wish to thank Dr. L. W. McElroy, Chairman of the Department of Animal Science, for placing the facilities of the Department at my disposal. The enthusiasm, encouragement and guidance of Dr. C. M. Grieve, Assistant Professor of Animal Nutrition, during the course of the study and in the preparation of the manuscript, are gratefully acknowledged. I am thankful to Dr. L. P. Milligan, Assistant Professor of Animal Biochemistry for his assistance with analytical procedures. I am indebted to Dr. R. T. Hardin, Associate Professor of Poultry Genetics, for assistance with the statistical treatment of the data.

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INTRODUCTION

The problem of replacing protein by the addition of urea as the main source of supplemental nitrogen in the diet of ruminant animals is of interest from the scientific as well as the economic point of view. Nitrogen supplied in the form of urea is more economical than that supplied by protein, since proteins are among the more expensive constituents of natural feedstuffs. Incorporation of urea nitrogen into high-quality animal protein could increase the supply of protein required by the rapidly increasing human population. In addition, the use of urea in ruminant rations would permit the diversion of available vegetable protein to meet the requirements of monogastric species of animals that are not able to make appreciable use of non-protein nitrogen in their diets.

Recent research has shown the benefits of urea as a feedstuff, but has also pointed out some of the problems in its use. One of the principal problems has been the rapid hydrolysis of urea in the rumen, resulting in excess ammonia for the first hour or two after feeding and then followed by deficiency. The temporary surplus of available ammonia is believed to be one of the reasons for inefficient utilization of urea in many rations.

The search for a nitrogen source that would be made available gradually and over a longer period of time in the rumen began in the nineteen fifties, and is still in progress. Ammoniated feedstuffs, biuret and other non-protein nitrogen compounds, and products of

chemical reaction with urea have shown little promise.

Consequently, an experiment was undertaken to compare a ration containing urea with a ration containing a urea derivative, a polymer of furfural urea. Their effects were measured on feed intake, growth rate, digestibility, energetic efficiency, rumen fermentation, rumen ammonia levels and blood urea concentrations in calves fed high-concentrate rations during early growth.

REVIEW OF LITERATURE

Nitrogen metabolism in the ruminant

The concepts of nitrogen metabolism are based on what is called the "Nitrogen Cycle" in the ruminant animal (Fig. 1). Protein entering the rumen is fermented by the micro-organisms present giving rise to peptides, amino acids, and ammonia. The non-protein nitrogen (NPN) in the diet can also be converted to ammonia. Simultaneously with these breakdown reactions there is a synthesis of microbial protein using the NPN compounds in the rumen (Annison and Lewis, 1959). Amino acids and peptides can be used in these synthetic reactions, and ammonia nitrogen also can be incorporated readily into microbial protein (McLaren, 1964; Phillipson et al., 1959). The relative rapidity of the breakdown and synthetic reactions exerts a controlling effect on the nutritive value of the protein fed (Lewis, 1961). Some unchanged protein, microbial protein and NPN are continually passed along the alimentary tract. After leaving the rumen, the digestive processes are essentially the same as in non-ruminants.

The significance of the rumen action lies partly in the extent to which food protein is converted into microbial protein. This effect is dependent upon the relative nutritive values of the ingested protein and that synthesized in the rumen or, alternatively, it may be stated that it depends upon the degree of synthesis of essential amino acids (Lewis, 1961). Nitrogen metabolism, therefore, is dependent upon the complex metabolism of nitrogen compounds by the

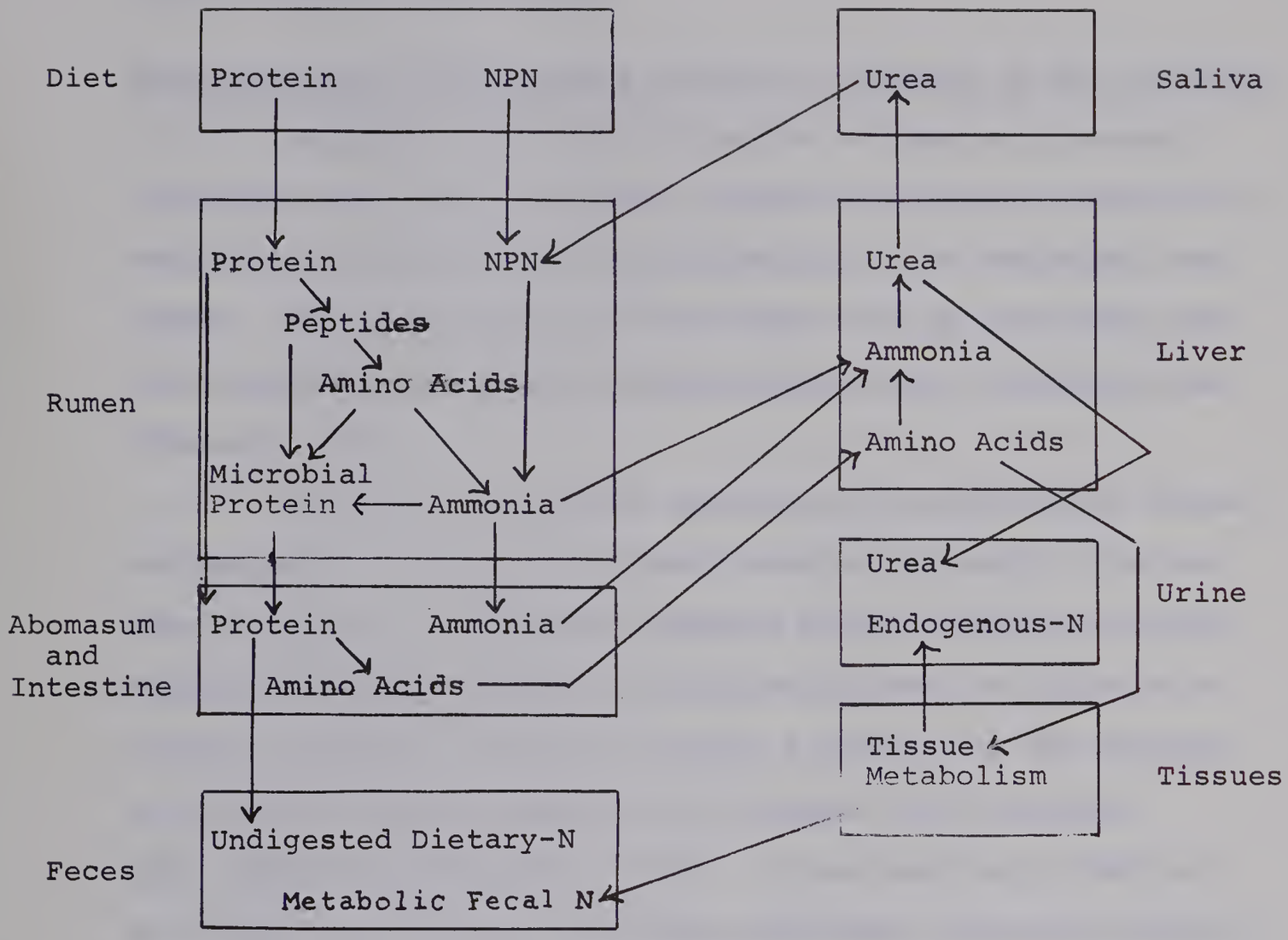

Fig. 1. Nitrogen metabolism in the ruminant. (Annison and Lewis, 1959).

rumen microbial population.

The utilization of urea as a source of nitrogen by the ruminant

Research into the utilization of urea as a protein supplement was begun by several German researchers towards the end of the nineteenth and the beginning of the twentieth centuries. The recognition of the unique role of the rumen and its microflora was brought forward from their investigations (Stangel, 1967).

Isolated experiments carried out in the United States and England in the late nineteen twenties and early thirties were a result of conflicting reports from the German studies. These experiments studied the ability of urea and other non-protein nitrogen sources to replace a portion of the protein in ruminant rations (Bartlett and Cotton, 1938; Krauss, 1927: Watson and Ferguson, 1936). It was not until Hart et al. (1939) at Wisconsin published the first intensive research in the United States on the use of urea and ammonium bicarbonate in ruminant rations that the possible value of urea as a protein supplement was realized.

Research into urea metabolism by the ruminant was intensified. Two approaches were taken to determine the value of urea as a protein supplement. The first approach was based on the study of growth in ruminants which were fed rations supplemented with various levels of urea. Growth studies involved feed efficiency and average daily gain (Bartlett and Cotton, 1938; Harris and Mitchell, 1941b; Hart et al., 1939; Mills et al., 1942, 1944; Work and Henke, 1939).

Hart et al. (1939) found that urea could be used for at least a partial source of protein nitrogen. It was shown from these studies that with urea nitrogen constituting 43% of the nitrogen of the ration, the growth rate of calves was only slightly less than that secured with a ration containing 66% of its nitrogen as casein nitrogen. Work and Henke (1939) found similar results but also concluded that growth in dairy heifers was superior when urea supplemented a low protein ration (8.40 percent) as compared to a high protein ration (20.69 percent). Harris and Mitchell (1941b) found this to be the case for lambs that were unable to support appreciable growth when a low protein ration was fed.

The second approach to urea utilization by ruminants was based on nitrogen balance studies since this method provided an exact measure of the actual protein requirements in terms of a specific ration by determining the minimum intake which would provide maximum retention. Harris and Mitchell (1941a) found that sheep could be maintained in body and nitrogen equilibrium for over 100 days on rations containing urea, when protein provided only one-tenth the amount of nitrogen needed for equilibrium. These workers also found that at nitrogen equilibrium, the biological value of urea nitrogen was 62, and that of casein nitrogen was 79. Loosli and McCay (1943) showed that calves, two months of age, were in negative nitrogen balance when receiving a basal ration containing only 4.4 percent protein, while those receiving a urea supplemented ration containing 16.2 percent

protein equivalent were in a positive nitrogen balance. Harris et al. (1943) found that the biological value of urea nitrogen when fed to 6-8 month old steers was 34 while that of soybean meal nitrogen was 60 when fed at the 12 and 14 percent protein equivalent levels. Loosli et al. (1949) found biological values for urea to be 56 and for casein to be 82 when each supplement was fed in separate rations to lambs.

Wegner et al. (1941) noted that the presence of high concentrations of protein in a ration decreased the ruminal protein concentration on a diet containing urea. Belasco (1954) found, using in vitro techniques, that 35 percent replacement of protein by urea gave maximum urea utilization by the microorganisms. Johnson et al. (1944) observed that starch increased the ruminal protein concentration on a diet containing urea. Smith et al. (1960) noted an improvement in nitrogen utilization as a function of length of time of urea feeding and called the improvement an "adaptation response". Virtanen (1966) found higher digestion coefficients and higher levels of nitrogen retention in lactating cows that were adapted to urea as compared to cows that were unadapted. Increasing the urea content of the diet increased the protein content of the milk but maintained the fat content. Increased milk yields also resulted from increased urea levels in the diet. Other investigators have observed that a considerable period must elapse after the initial feeding of diets rich

in non-protein nitrogen before maximum utilization of the diet occurred (Caffrey et al., 1967; Campbell et al., 1963).

Guidelines, based on the efficiency with which urea could be utilized by the microorganisms, were developed concerning urea supplementation to ruminant diets. Reid (1953) summarized the earlier findings on urea utilization and concluded that the amount of protein which could be replaced by urea depended on the following factors:

i) the amount and nature of true protein contributed by the ingredients of the ration

ii) the amount and kinds of carbohydrates in the ration

iii) level of urea causing toxicity.

The utilization of other non-protein nitrogen sources by the ruminant

Pearson and Smith (1943), using *in vitro* techniques, incubated liquid rumen contents under different conditions and with different substances. Using different non-protein nitrogen sources they found that protein synthesis occurring during the incubation of rumen liquor was accompanied by protein breakdown. Either process might predominate according to the general conditions or to the substances present. Burroughs et al. (1950 a,b,c) improved this *in vitro* technique and introduced the artificial rumen. Belasco (1954), using the artificial rumen, was the first to evaluate different

non-protein compounds as substrates for the rumen microorganisms. Since evidence presented by McDonald (1948, 1952) showed that ammonia was the major end-product in the metabolism of urea, and its rapid release limited urea utilization, Belasco (1954) looked for other non-protein nitrogen compounds which would not have this limitation. Urea derivatives, amides, amidines and ammonium salts of organic and inorganic acids were all examined using the artificial rumen. Cellulose digestion, bacterial growth and ammonia utilization were measured and compared to urea. Ammonium salts of organic and inorganic acids were shown to be similar to urea, but other non-protein nitrogen compounds proved to be less efficient. No generalization could be given to these results under in vivo conditions. Feeding trials, in which non-protein nitrogen sources were examined, had to be carried out before any conclusions could be reached (Berry et al., 1956; Hatfield et al., 1959; Kay et al., 1967; McElroy, 1968).

The effect of energy level and volatile fatty acid production in nitrogen metabolism

The balance between the amounts and availabilities of nitrogen and energy to the microbial population has an important effect on the form of nitrogen in the rumen and the utilization of both nitrogen and energy. Inadequate nitrogen results in low utilization of nitrogen and carbohydrate (Waldo, 1968).

In the ruminant, nitrogen metabolism cannot be considered separately from carbohydrate digestion. The balance

of nitrogen and carbohydrate can influence nitrogen utilization, carbohydrate utilization and feed intake. Harris and Mitchell (1941a) found that when urea was added to a low nitrogen ration the digestibility of cellulose was improved as well as the digestibility of the urea. Mills et al. (1942) noticed that when starch was fed in conjunction with urea, the urea was hydrolyzed in less than one hour, and the ammonia thus formed had practically all disappeared in six hours. As the ammonia nitrogen level fell there was a concurrent rise in the protein content of the rumen indicating ammonia was being utilized by the microorganisms to produce microbial protein. Loosli and McCay (1943) showed that the apparent digestibility of the dry matter and carbohydrate of a basal diet was improved when urea was added to the ration of calves two months of age.

Pearson and Smith (1943) concluded from in vitro experiments that starch had the most stimulating effect on protein synthesis in the rumen. Other sources of carbohydrates tested failed to perform as well as starch. Mills et al. (1944), experimenting with molasses which contains a more soluble carbohydrate, and urea, found that normal growth rate could not be attained in young calves. Addition of a small quantity of starch resulted in a higher growth rate than was attained with molasses. Similar observations were noted by Bell et al. (1951). Warner (1956), using the artificial rumen, showed that when digestion was complete about half the nitrogen and carbon of added casein could be recovered as ammonia and volatile fatty acids (VFA),

respectively. Most of the remainder could not be accounted for analytically, and was presumed to be used for the microbial growth which had occurred. When starch was added with casein to the artificial rumen, the production of ammonia was lowered. This was shown not to be due to any effect on proteolysis or deamination, and was presumed to be due to the increased utilization for microbial growth of some breakdown product of casein. It appeared there was a more efficient combination of VFA and ammonia to produce microbial protein. Lewis and McDonald (1958) indicated from experiments that the best utilization of protein supplements was probably obtained when a carbohydrate was also present that could be fermented at a comparable rate resulting in conditions which stimulated microbial synthesis in the rumen.

Van de Horst (1961) carried out an investigation of the occurrence of keto-acids in the rumen liquid of cattle, examining amino acids and amines at the same time. Adding glucose to rumen liquid increased the pyruvic acid concentration. On further examination it was found that the concentration of amino acids was also increased. Bryant and Robinson (1962) showed that a large number of ruminal bacteria require one or more of the volatile fatty acids, n-valeric, isovaleric, isobutyric and 2-methylbutyric for growth. There were indications that some of these acids were precursors of certain amino acids and longer-chained fatty acids and aldehydes in some of the species.

Acetate is considered important in the nutrition of

many species of ruminal bacteria. Hoover et al. (1963), using in vitro tracer studies, conducted experiments to determine the rate at which several volatile fatty acids and glucose were utilized by rumen bacteria, and the amount of carbon from these sources incorporated into bacterial proteins, nucleic acids, and polysaccharides. These studies showed that nucleic acid contained activity from acetate and glucose, but little or no activity from propionate, butyrate, or valerate. Activity from all labeled metabolites was found in the proteins. Of the activity that disappeared from the labeled acetate, propionate, butyrate, valerate, and glucose during incubation, the average amounts incorporated into proteins were 2.5, 2.1, 1.6, 2.6, and 2.9%, respectively. These results were in agreement with experiments of Bryant and Robinson (1962).

Studies of nitrogen metabolism in steers fed purified diets which contained either isolated soybean protein or urea, and either starch or starch and glucose monohydrate, were carried out by Oltjen and Putnam (1966). They found that the replacement of glucose monohydrate by additional starch altered fecal and urinary losses of nitrogen and also the ratio of the ruminal volatile fatty acids. Nitrogen retention was significantly greater when the isolated soy diets were fed. Serine and glycine were detected in significantly greater quantities, whereas valine, isoleucine, leucine, and phenylalanine were detected in significantly smaller quantities in the blood plasma when steers were fed the urea diets. It was also suggested that the lowered nitrogen retention might have been

caused by a deficiency of these amino acids or an imbalance of certain amino acids synthesized by ruminal microorganisms.

McLaren (1964) concluded that improvement in nitrogen utilization as a function of the level of readily available carbohydrates in the ration might result from increased microbial protein synthesis. This could be related to the availability to the microorganisms of an excess of carbohydrates or intermediates of carbohydrate metabolism at the time of release of ammonia nitrogen from urea or protein. It could also be related to increased concentrations of carbohydrates or intermediates of carbohydrate metabolism in the animal's tissue. These substances might enhance the utilization of absorbed ammonia by providing energy for synthetic needs and by providing the carbon skeletons of non-essential amino acids.

Hydrolysis of NPN compounds and absorption of ammonia

McDonald (1952) established the fact that the breakdown of protein in the rumen was of quantitative importance to the ruminant. Ammonia was shown to be a major end-product of the degradation of several different proteins and was the main component of the NPN fraction. Pearson and Smith (1943) demonstrated the action of bacterial urease in the hydrolysis of urea but were unable to isolate it. Bloomfield et al. (1960) indicated that urea hydrolysis in the rumen occurred four times faster than synthetic reactions using the liberated ammonia, and resulted in an eventual loss of nitrogen

available for microbial synthesis.

Chalmers (1961) stressed that the concentration of ammonia determined in rumen liquor at any one time was the result of many factors acting simultaneously. It did not give any direct indication of the total quantity of ammonia evolved from the protein, nor any measure of the rate of evolution or absorption. Therefore, high ammonia concentrations indicated that the deamination of the protein had exceeded the synthetic capabilities of microorganisms to use the degraded products for synthesizing their own protein. When the ammonia concentration in rumen liquor was determined at intervals throughout a whole day it became obvious that the form of the curve closely followed the intake of nitrogen (Lewis, 1957).

Recent studies have done much to clarify the use of ammonia in the biosynthesis of amino acids in ruminant animals. Van de Horst (1961) suggested the involvement of amination and transamination reactions as important mechanisms in the synthesis of amino acids by rumen bacteria, since many keto acids, including α-ketoglutaric and pyruvic acids, were found in rumen liquor. Bryant (1961) demonstrated the relatively simple nitrogen requirements of many bacteria from the examination of 44 strains of rumen bacteria in which he found 80% could be grown with ammonia as the sole nitrogen source, 26% would not grow unless ammonia was present and 55% could use either ammonia or amino nitrogen. Hungate (1966) also showed that ammonia was an essential nutrient for the growth of many rumen bacteria.

Ammonia is absorbed from the rumen; some may return to the rumen after passage through the liver, by secretion as urea in the saliva, while some is excreted in the urine as urea (McDonald, 1948, 1952). Hogan (1961) showed ammonia absorption to be dependent on the concentration gradient at pH 6.5. Thus an additional measure of the significance of rumen action in relation to protein nutrition is achieved by an assessment of the proportion of NPN absorbed from the rumen that is either excreted or recycled in the saliva. The total nitrogen of rumen contents is usually between 0.3 and 0.5 percent and is a relatively constant amount. The NPN value in strained rumen liquor, however, can fall within a very wide range. The ammonia-nitrogen level under relatively normal conditions may be 10-60 mg/100 ml, amino-nitrogen may be present at 0.5-10 mg/100 ml and peptide nitrogen at 0.2-5 mg/100 ml (Annison, 1956). Protein leaving the rumen by passage in the ingesta to the more distal parts of the gastrointestinal tract consists of a mixture of undigested food protein and the protein of the microorganisms.

Blood ammonia in relation to urea toxicity

McDonald (1948, 1952) demonstrated that ammonia may be directly absorbed through the rumen epithelium into the venous blood draining the organ. Lewis et al. (1957) found that there was a direct relationship between portal-blood ammonia levels and the concentration of ammonia produced in the rumens of sheep fed different diets. Further investigations showed no significant difference between urea concen-

trations in portal and peripheral blood. The ammonia absorbed is carried to the liver, where, unless the concentration is unusually high, it is converted to urea. The urea so formed may be excreted in the urine, or re-cycled into the rumen via the saliva.

Inability of the liver to convert all absorbed ammonia to urea is responsible for the presence of ammonia in peripheral blood which may result in toxicity. Dinning et al. (1948) found that urea, in amounts exceeding 100 grams administered as a drench to steers, produced a rapid rise in the levels of both urea and ammonia in the systemic blood. When urea nitrogen levels reached 16-18 mg percent, and ammonia nitrogen levels reached approximately 2.5 mg percent in the systemic blood of steers, ataxia appeared. Symptoms of alkalosis followed by death occurred at a blood ammonia level of about 4 mg percent. However, urea produced no ill effects in steers when amounts as large as 400 grams daily were mixed with other concentrates in the feed. Lewis (1961) concluded that ammonia poisoning in the ruminant is a complex process probably affected by the following factors:

i) a direct toxic effect of the ammonium ion

ii) a disturbance of the acid-base status, though probably not great enough to give rise to clinical signs of toxicity

iii) a change in electrolyte balance that might be sufficiently great to modify the signs of toxicity.

EXPERIMENTS AT THE UNIVERSITY OF ALBERTA

The experiments listed below were conducted during the summer and fall of 1968.

Experiment I — Measurements of feed intake, growth rate, digestibility and nitrogen retention by calves, and concentrations of volatile fatty acids in rumen fluid, when two groups of calves were fed rations supplemented with urea or furfural urea polymer.[1]

Experiment II — Effects of different nitrogen supplements in rations on concentrations of rumen ammonia and blood urea in young calves.

[1] Produced by the Alberta Research Council in co-operation with Dr. L. P. Milligan, Department of Animal Science, University of Alberta, Edmonton.

Experiment I

Growth and metabolism studies

Preliminary period

Two lots of Holstein-Friesian calves, each with 4 calves averaging approximately 3 months of age, were made available from the University herd. The calves in Lot 1 (Table 1) had been fed a calf meal containing 1.5% urea (Table 2) from birth to commencement of this experiment. The calves in Lot 2 had been fed a low protein calf meal without urea (Table 2) from birth to 2 months of age, followed by dairy concentrate (Table 2) to commencement of this experiment.

During the first week of the experiment calves in Lot 1 were fed the calf meal with urea and calves in Lot 2 were fed the dairy concentrate, to enable metabolism studies with the pre-experimental rations. Thereafter, the two lots of calves were fed the experimental rations (Table 3).

Experimental rations

Two rations (Table 3) were formulated with comparable levels of protein equivalent (N x 6.25), one with urea and one with furfural urea polymer.

Each ration was formulated to contain a calculated level of 14% crude protein, with the same amount of nitrogen in each ration supplied by urea or furfural urea polymer. However, the barley used in the experiment had an average crude protein content of 9.0%, which was higher than the

Table 1: Average weight and average age of the calves

Lot number	Average weight (kilograms)	Average age (days)
1	61.8[a]	82.0[a]
2	69.4	92.7

[a] Only three calves were in this group initially but a fourth was added when it had completed a prior experiment at 60 days of age.

expected level of 8.4%, and the furfural urea polymer was found to contain 23.2% nitrogen instead of the expected level of 21% nitrogen. Consequently, both rations were higher in crude protein than had been calculated (Table 3).

All supplements to each ration were mixed and pelleted, and the pellets mixed with the appropriate level of rolled barley to form the complete ration. Since the pellets containing furfural urea polymer were rejected initially by the calves, more barley was incorporated into these pellets until the pellets composed 20% of the ration in Lot 2.

Management of experimental animals

The two groups of calves were housed in the dairy barn, each group in a separate pen bedded with shavings. Rations were full-fed at 0530 and 1530 hours, and records maintained of the amounts fed and any unconsumed feed that was periodically removed. The calves had access to water, cobaltized-iodized salt and dicalcium phosphate free-choice.

Table 2. Formulation of pre-experimental rations

Ration Ingredients (%)	Urea calf meal	Low protein calf meal	Dairy concentrate
Wheat	57.0	58.5	
Oats	25.0	25.0	25.0
Barley			56.0
Dehydrated alfalfa meal	5.0	5.0	
Dried molasses	5.0	5.0	2.0
Fishmeal	5.0	5.0	
Soybean meal			10.5
Urea	1.5		
Bran			3.0
Limestone	0.2	0.2	
Trisodium phosphate			2.0
Cobaltized-iodized salt	0.5	0.5	1.0
Vitamin A & D mix[1]	0.6	0.6	
Vitamin B mix[2]	0.2	0.2	
Vitamin premix[3]			0.5
Total	100.0	100.0	100.0
Dry matter, %	95.0	88.4	95.3
Crude Protein, %	18.5	16.5	15.8

[1] Vitamin A and D mixture: vitamin A (10,000 I.U./g), 60 g; vitamin D (35,000 I.U./g), 3 g; aureomycin (Aurofac 25), 60 g; shorts, 477 g.

[2] Vitamin B mix: riboflavin, 4.4 g; calcium pantothenate, 8.8 g; niacin, 19.8 g; choline chloride, 21.4 g; folic acid, 132.0 mg.

[3] Vitamin premix: soybean meal, 3.6 kg; vitamin A (10,000 I.U./g), 360 g; vitamin D_2 (35,000 I.U./g), 22 g.

Table 3: Composition of supplements and experimental rations

Lot no. Ration Ingredients, (kg)	1 Urea	2 Furfural urea
A. Supplements		
Urea	1.9	
Furfural urea		4.107
Limestone	.7	.7
Vitamin D_2, (35,000 I.U/g)	.005	.005
Vitamin A (10,000 I.U/g)	.08	.08
Cobaltized-iodized salt	.5	.5
Sodium metabisulfite	.024	.024
Alfalfa	2.79	.584
Barley		14.0
Total supplement	6.0	20.0
B. Rations		
Barley	94.0	80.0
Supplement	6.0	20.0
Total	100.0	100.0
C. Analyses		
Urea in ration, %	1.9	1.9
NPN in ration from urea or furfural urea polymer, %	0.9	0.9
Protein equivalent in ration, %	14.6	14.8
Dry matter, %	91.8	90.6
Protein equivalent in pellet, %	101.2	37.8

The calves were weighed every two weeks until the end of the 150-day experimental period. Final weights were obtained after 15 hours without feed or water. At slaughter, the carcasses were inspected for abnormalities of the rumen, heart, lungs, liver, and kidney, and carcass grades and weights were obtained.

Digestion studies

a) Collection of samples

Three digestibility trials were conducted; the same two animals from each lot were used in each trial. Each digestibility trial lasted 5 days with a 16-day interval between each trial. The first digestibility trial was begun 5 days prior to the initial feeding of the experimental rations, to enable a comparison of digestibility prior to feeding the experimental rations with that obtained early in the experimental period.

Samples of the rations fed and of unconsumed feed were obtained, ground in a laboratory mill and stored for further chemical analysis.

Total feces was collected and weighed twice daily at 0900 and 1700 hours. Five percent by weight of each collection was retained and frozen. At the end of each trial the fecal samples were dried in a forced-draft oven for 48 hours at 70°C, ground in a laboratory mill, and retained for chemical analysis.

Total urine with 15 ml of 50% (v/v) H_2SO_4 was collected twice daily from each animal at 0900 and 1700 hours and the

volume was recorded. Ten percent by volume of each collection was transferred to polyethylene bottles and stored at 4°C for further chemical analyses.

b) Urine and fecal collection apparatus

The apparatus for collection of feces and urine was similar to that described by Balch et al. (1951) with some modifications developed in conjunction with Hoogendoorn (1968) and Kehoe (1969). Since young steer calves were being used in this experiment, a different urine collection device and a much smaller harness had to be constructed. The urine collection device is illustrated in Fig. 2. The urine was drained into large glass bottles located in the gutter of the dairy barn, by a 7/16" (o.d.) nylon-reinforced rubber hose. The bottles were emptied at each collection after the volume accumulated had been recorded.

The design of the harness is shown in Fig. 3. The straps for the harness were made of 1/16" neoprene-on-nylon. All joins between the straps and between straps and metal buckles were glued and then sewn to provide maximum strength.

The fecal deflection pad is illustrated in Fig. 4. This device was attached to the innermost buckles at the back of the harness and the two straps (A) at the bottom of the fecal deflection pad went underneath the back legs of the animal and attached to buckles lettered A in Fig. 3.

The fecal collection bag (Fig. 5) was constructed from synthetic canvas (silver-on-silver), and had a moulded hood with a hole for the calf's tail. The straps designated B and C attach to the harness buckles designated B and C in

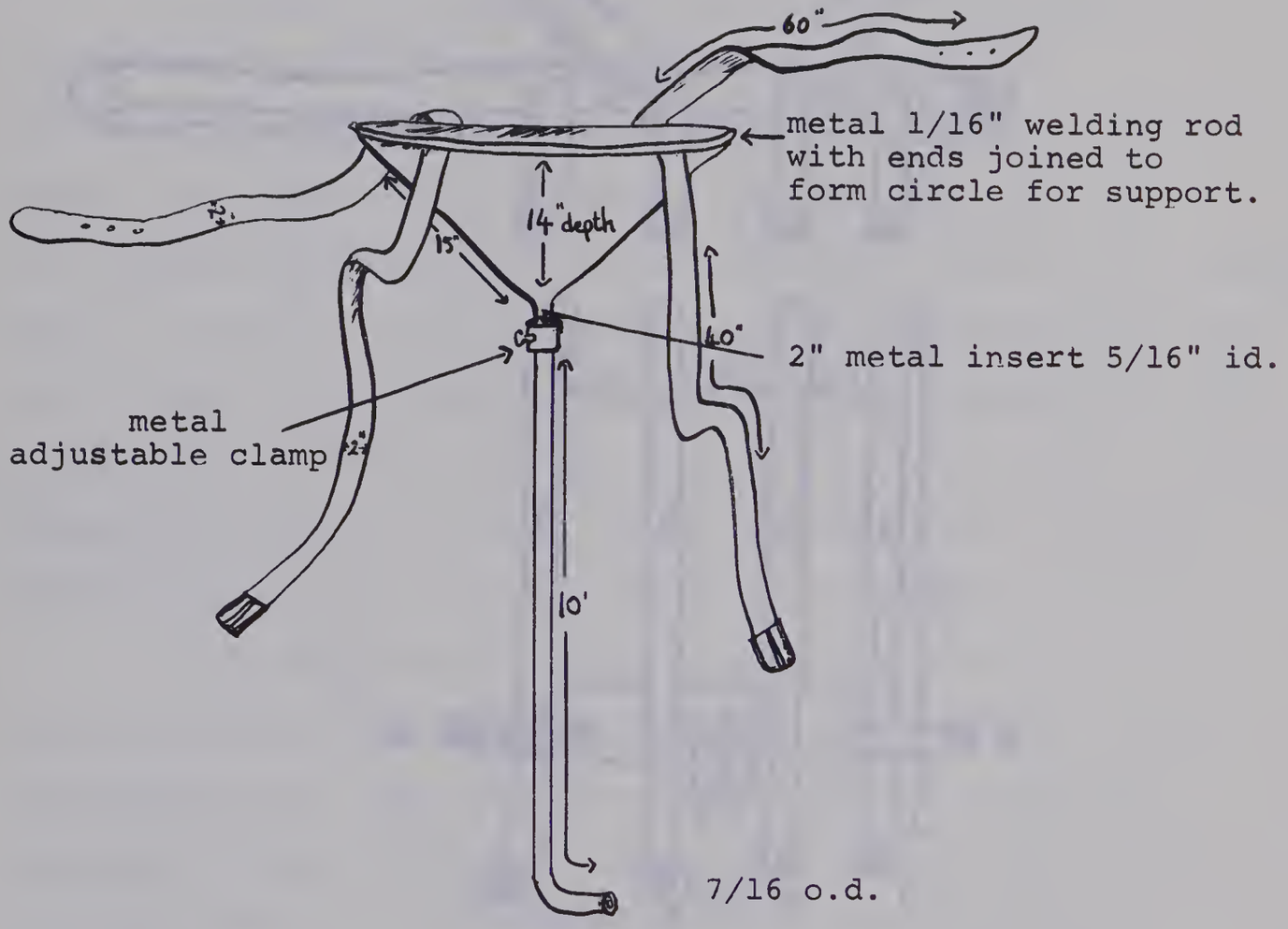

Fig. 2. Urine collection apparatus for steers.

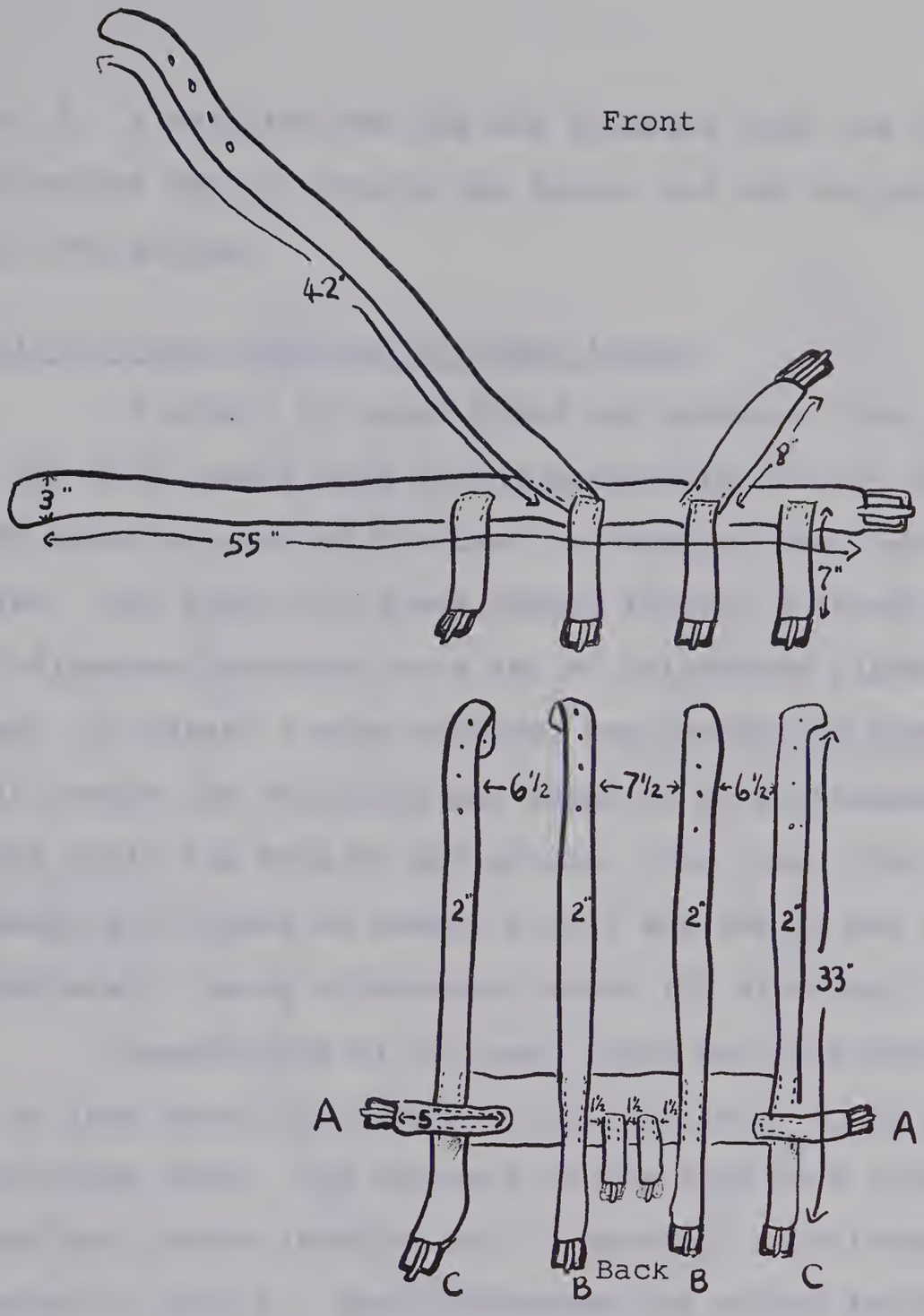

Fig. 3. Harness measurements for calves.

Fig. 3. A polyethylene bag was inserted into the fecal collection bag to receive the feces, and was replaced at each collection.

Collection and sampling of rumen liquor

A sample of rumen fluid was obtained from each of the four steers used in the metabolism studies at 0900 hours on each of the last two days of each metabolism trial. The rumen fluid was pumped through a stomach tube (1" diameter) attached to a set of Erlenmeyer flasks and a vacuum pump. To obtain a more uniform, representative sample from each calf, water and feed were not supplied to the animals from 0500 hours until the time of collection. The rumen fluid was filtered through six layers of cheese cloth, and the pH was determined immediately, using a Photovolt model 125 electronic pH meter.

Twenty-five ml of rumen fluid was acidified to a pH of less than 2 by adding 0.5 ml 50% (v/v) H_2SO_4 in a 90 ml centrifuge tube. The contents of the tube were thoroughly mixed and, after standing for 30 minutes, centrifuged for 20 minutes at 3020 g. The supernatant was poured into glass vials, sealed, and stored at -18°C until analyzed for volatile fatty acids.

Chemical analyses

Dry matter and nitrogen were determined in feed, unconsumed feed, and fecal samples, and nitrogen was determined in urine samples by AOAC (1960) methods. Gross energy in feed, unconsumed feed, urine, and fecal samples was

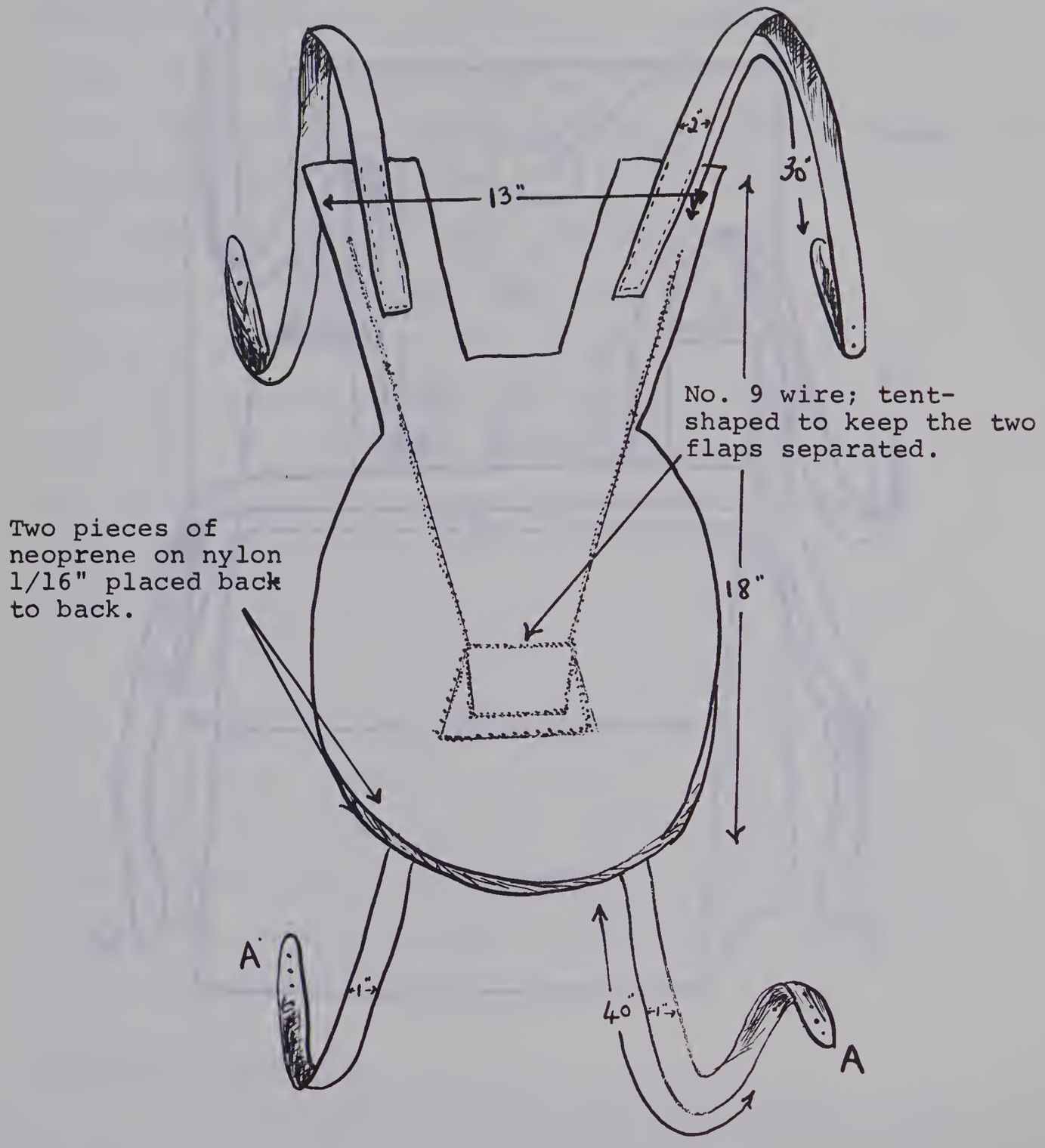

Fig. 4. Fecal deflection pad.

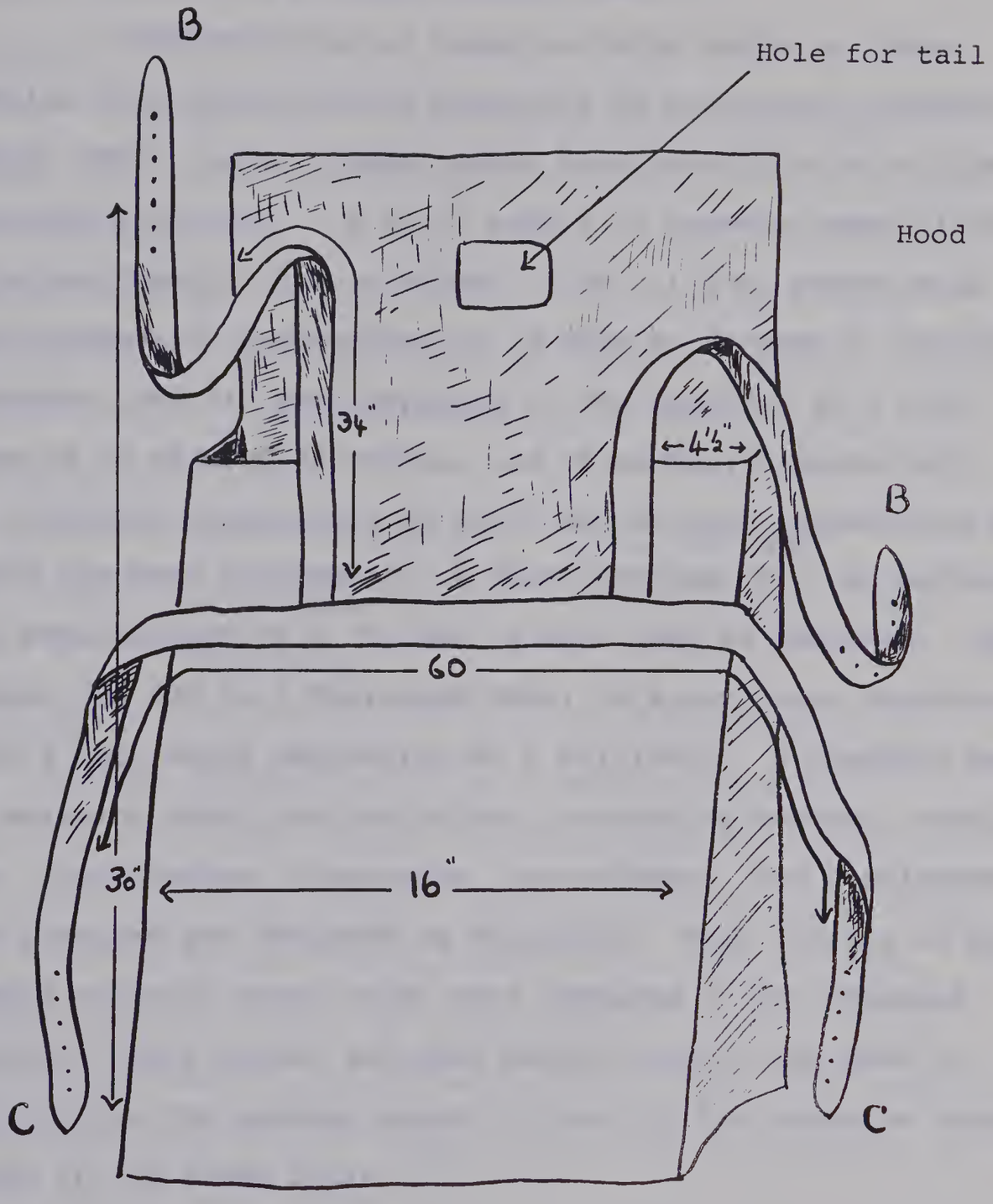

Fig. 5. Fecal collection bag.

measured by combustion in a Parr oxygen bomb calorimeter. Dry matter in urine was determined by freeze-drying a 5 ml sample of urine in a freeze-drying bulb.

Concentrations of volatile fatty acids in rumen samples were determined in duplicate by gas-liquid chromatography (GLC), using a model 600-D Aerograph GLC with a flame ionization detector. A 20 ul sample of aqueous rumen fluid was injected directly into a column (3 mm x 1.8 m) packed with a semi-commercial preparation of 3% FFAP on Porapak Q. Helium, hydrogen, and air were supplied to the detector at a flow rate of 75 ml/min, 40 ml/min, and 40 ml/min, respectively. An injection temperature of 215°C and an oven temperature of 205°C was used throughout. A flame setting of 1 was maintained and attenuations of 4, 8, and 16 were used as required. The output was fed to a Microcord Model 44 strip chart recorder with a full scale deflection of 1 millivolt. A standard set of volatile fatty acid solutions, containing acetate, propionate, iso-butyrate, n-butyrate, iso-valerate, and n-valerate was prepared and analyzed as described. Peak heights of the sample volatile fatty acids were compared to the standard volatile fatty acids, and peak height ratios were used in determining the unknown amount of each of the volatile fatty acids in the rumen fluid.

Statistical analysis

An IBM 360 computer was used to calculate digestibility coefficients and analysis of variance. The computations followed procedures outlined by Steel and Torrie (1960). Mean squares obtained by analysis of variance are recorded in the appendix.

Experiment II

Studies of rumen ammonia levels and blood urea concentrations

General

The same two calves from each lot described in Experiment I were used to provide rumen and blood samples in this experiment. In addition, two calves from each of 4 lots were made available from another project being carried out at the same time and described elsewhere (Kehoe, 1969). This resulted in 6 treatments, with 2 calves per treatment as follows:

Lot 1 - as in Experiment I - urea supplement

Lot 2 - as in Experiment I - furfural urea polymer supplement

Lot 3 - all barley ration - no protein supplement

Lot 4 - barley ration - soybean meal supplement

Lot 5 - barley ration - urea supplement as in Lot 1

Lot 6 - barley ration - urea plus mineral supplement.

The calves in Lot 1 were fed a urea calf meal during the first week of the experiment, and all other calves were fed the dairy concentrate mixture described in Experiment I during the first week of the experiment.

Rumen samples

Rumen samples were obtained at 0930 hours on the fourth and fifth days of each of the three metabolism studies as previously described in Experiment I. The filtered rumen

fluid was prepared for subsequent ammonia analysis by the Somogyi reaction (Somogyi, 1945) as follows:

A 2 ml sample of strained rumen fluid was transferred to a 90 ml polyethylene centrifuge tube. Then 18 ml of ammonia-free demineralized water, 10 ml of 1.8% (w/v) $Ba(OH)_2 \cdot 8H_2O$, and 10 ml of 2.0% (w/v) $ZnSO_4 \cdot 7H_2O$ were added to the centrifuge tube successively, with the contents of the tube being mixed after each addition. The tube and its contents were centrifuged for 20 minutes at 3020 g. The supernatant was poured into a 4 oz polyethylene container, and the pH was determined and adjusted to pH 7.0 by adding either $Ba(OH)_2$ or $ZnSO_4$. The sample was stored at $-18^{o}C$ until required for the analysis of rumen ammonia.

Blood samples

Blood samples were obtained from the 12 animals described above at 0930 hours on the fourth and fifth days of each of the three metabolism studies. Approximately 10 ml of whole blood was taken from the jugular vein, using a vacuum tube containing two drops of heparin to prevent coagulation. The sample was centrifuged for 15 minutes at 1100 g, and the plasma was stored at $-18^{o}C$ in glass vials sealed with polyethylene film until required for analysis of blood urea.

Chemical analyses

Blood urea and rumen ammonia were determined by the colour reaction method developed by Fawcett and Scott

(1960). The analysis of blood urea consisted of releasing the urea ammonia from the blood plasma with the aid of a buffered urease[1] solution. The colour reaction outlined by Fawcett and Scott (1960) was used to determine the amount of ammonia. The determination of rumen ammonia employed the same colour reaction but no buffered urease solution was used. A neutral sample of rumen fluid was used as prepared by the method previously outlined (Somogyi, 1945). In each case, a set of standards for blood urea and a set of standards for rumen ammonia was prepared. The colour reaction (Fawcett and Scott, 1960) was carried out on these standards each time samples were analyzed. Optical density of the coloured samples and standards was read on a "Spectronic 20" spectrophotometer set at 630 mu. Graphs were drawn in which optical density was compared to the known standard urea and ammonia concentrations. Concentrations in the unknown samples were determined from standard curves prepared each day that the analysis was conducted. Since optical density decreased with time, a continuous injection syringe was used to decrease the time needed to add the colour producing reagents. A 10 ul sample of blood plasma was analyzed using a dilution factor of 1:100. A 1 ml sample of diluted rumen fluid (dilution 1:20), which had been neutralized in the Somogyi reaction (1945) was analyzed.

[1] Worthington Biochemical Corporation, Freehold, New Jersey, U. S. A.

RESULTS AND DISCUSSION

Experiment I

Feed intake, growth rate and feed utilization

The calves in Lot 1, fed the basal ration plus urea and alfalfa, consumed 58% more feed daily than those in Lot 2 fed the basal ration plus furfural urea polymer (Table 4). They gained 121% more weight per day and required 28% less feed per unit of gain.

The furfural urea ration was not consumed readily by the calves. Since low-protein barley was used in this experiment to ensure a requirement for nitrogen in excess of that supplied by the grain, a level of 1.9% urea was required in each ration to raise the protein-equivalent level to 14 percent. This required the addition of a large amount of the furfural urea polymer.

The pelleted supplement containing furfural urea polymer had a pronounced odour, particularly when it became moistened in the feed bunk. The calves found it unpalatable and sorted the grain out of the ration, leaving most of the pellets.

The proportion of barley in the supplement was increased until the supplement constituted 20% of the ration, but palatability problems were still encountered; total feed had to be restricted to reduce sorting and obtain reasonable consumption of the pelleted supplement. It seems likely that lower levels of furfural urea polymer, which would be used with barley containing high levels of crude protein, would be

less affected by palatability problems.

The ration supplemented with urea and alfalfa (Lot 1) did not appear to be affected by reduced palatability, and sorting of the grain and pellets was not a problem.

Table 4: Average feed consumption, daily gain and feed utilization

Lot no.	1	2
Ration	Urea alfalfa	Furfural urea
Initial weight (kg)	83.3	95.3
Final weight (kg)	236.0	166.8
Days on experiment	147	156
Average daily feed (kg)	4.36	2.75
Average daily gain (kg)	1.04**	.47
Average feed/kilogram gain (kg)	4.19	5.85

** Significant ($P < .01$)

The metabolism trials caused considerable stress on the two calves from each lot that were used in these studies. These calves grew at a slower rate throughout the experiment than the calves that were not used in the metabolism studies. In each of Lots 1 and 2, the two calves used in the metabolism trials gained an average of 17 and 22% less weight, respectively, than the two calves that were not subjected to this treatment.

Carcass characteristics

Carcass data of the calves are shown in Table 5. Only three calves were slaughtered in the group fed furfural urea polymer, since the fourth animal was a heifer and was retained in the University herd. Dressing percentages were not calculated since the animals were marketed as calves and dressed carcass weights were taken with the hides on the carcasses.

Grades were slightly higher in Lot 1, since half of these calves graded Commercial, whereas all of the calves in Lot 2 graded Utility. However, all of the calves were lacking in finish, which may be indicated by the low weights of kidney fat as well as the low grades.

There was no evidence of rumenitis in the rumens of any of these calves, and none of the livers was condemned because of abscesses.

One calf in Lot 2 was found to have one lung adhering to the wall of the body cavity. This could have resulted from an injury during sampling of rumen contents early in the experiment, and could have influenced feed intake and growth rate.

Table 5: Carcass characteristics

Lot no.	Ration	Calf no.	Plant live weight (kg)	Warm carcass weight (kg)	Kidney fat weight (kg)	Grade
1	Urea alfalfa	812	277.2	181.8	2.9	Commercial
		813	250.0	157.2	1.1	Commercial
		814	218.1	187.2	1.7	Utility
		815	195.4	123.6	2.2	Utility
2	Furfural urea	808	181.8	118.1	1.3	Utility
		810	150.0	96.3	1.1	Utility
		811	136.6[1]	85.4	0.7	Utility

[1] Calf which was found to have one lung adhered to the body wall.

Apparent digestion coefficients

There were no significant differences between digestion coefficients for dry matter, gross energy or crude protein (Table 6) in the two rations fed during the first metabolism trial. Digestibility of crude protein tended to be slightly higher in the calf meal containing urea (Lot 1) than in the dairy concentrate (Lot 2), and digestibilities of dry matter and gross energy were almost equal in the two rations. The coefficients of approximately 80% digestibility agree with values of 77 to 82% for young growing calves reported by Gonzalez-Jimenez and Blaxter (1962).

In the second metabolism trial, when the experimental rations were being fed, all digestion coefficients were lower than in the first metabolism trial and they increased slightly in the third metabolism trial. None of these differences were found to be significant, which could be attributed to variation between animals within each treatment and to the small number of animals involved.

With the exception of the digestibility of crude protein in the second metabolism trial there was little difference between the two experimental rations in their digestion coefficients. One steer fed the ration supplemented with urea and alfalfa had a very low apparent digestibility of crude protein in the second metabolism trial, resulting in a low average digestion coefficient for that treatment.

It appeared that some adaptation in the calves may have occurred when the experimental rations were fed. The decrease in apparent digestibility during the second metabolism trial, followed by an increase in the third metabolism trial suggests that a period of at least three weeks was required for the adaptation to take place. However, this is difficult to assess because of the large variation between animals, particularly in the second trial. The calves in Lot 1 had been fed a calf meal containing urea for two months prior to being fed the ration of barley plus urea and alfalfa. The high apparent digestion coefficients obtained for the urea calf meal indicate the calves were well-adapted to the calf meal and able to digest it as well as the dairy concentrate. Consequently,

it seems unlikely that the change from the calf meal to the ration of barley plus urea and alfalfa should have caused any marked change in rumen fermentation or digestibility of the ration.

Table 6: Apparent digestion coefficients

Lot no.	Metabolism trial	Ration	Calf no.	Dry matter %	Gross energy %	Crude protein %
1	1	Urea calf meal	813	81.5	79.8	84.9
			814	84.3	82.6	86.6
		Av		82.9	81.2	85.8
2	1	Dairy conc.	810	84.3	82.9	78.0
			811	82.7	82.0	78.5
		Av		83.5	82.4	78.2
1	2	Urea alfalfa	813	80.9	80.3	70.7
			814	70.3	69.6	49.2
		Av		75.6	75.0	60.0
2	2	Furfural urea	810	71.7	73.7	63.7
			811	74.8	79.0	76.5
		Av		73.2	76.4	70.1
1	3	Urea alfalfa	813	77.1	76.0	76.9
			814	78.8	78.0	79.0
		Av		78.0	77.0	78.0
2	3	Furfural urea	810	78.5	78.4	70.8
			811	82.3	81.8	81.5
		Av		80.4	80.1	76.2

Nitrogen retention

In the first metabolism trial at the beginning of the experiment, calves fed the urea calf meal consumed almost as much nitrogen daily as those fed dairy concentrate (Table 7). They retained 46% of the nitrogen consumed as compared with 54% by

those fed the dairy concentrate. The slightly lower retention was the result of higher rates of excretion in the urine, and indicates that the urea nitrogen was not utilized in the formation of body protein quite as well as the nitrogen from soybean meal in the dairy concentrate.

In the second metabolism trial, calves fed the urea alfalfa ration (Lot 1) consumed less nitrogen than those fed the furfural urea polymer (Lot 2), excreted almost as much nitrogen in the feces and urine, and retained only 18% as compared with 44% by calves in Lot 2. This suggests that nitrogen from the furfural urea polymer was used more efficiently than that from urea alone during the third week that the experimental rations were fed. However, the low nitrogen intake by calves fed the urea alfalfa ration during this metabolism trial resulted in the low apparent percentage retention. Both calves excreted the same amount of nitrogen, but calf no. 813 consumed 38% more nitrogen than calf no. 814, resulting in a retention of 32% as compared with only 5%, respectively. This difference can be attributed almost entirely to the fact that losses of endogenous and metabolic nitrogen made up a very high proportion of the total nitrogen excretion by these calves, particularly calf no. 814, resulting in low apparent retention relative to the low level of nitrogen consumed. If these losses could be assessed accurately, it is unlikely there was any real difference in true percentage nitrogen retention between calves fed the supplements of urea with alfalfa or furfural urea polymer.

In the third metabolism trial, calves fed the urea alfalfa ration consumed more nitrogen, had a lower level of excretion and retained more nitrogen than those fed the furfural urea polymer (58% vs 37%).

None of the differences was found to be significant; this could be due to animal variation and the small numbers involved.

In general, there did not appear to be marked differences in the ability of the calves to utilize nitrogen from urea or the furfural urea polymer, as compared with nitrogen from plant protein. Although nitrogen retention by calves in Lot 1 was very low in the second metabolism trial, this could have been the result of low nitrogen intake, and nitrogen retention by these calves in the following trial was slightly higher than when the dairy concentrate was fed to calves in Lot 2. Nitrogen retention by calves in Lot 2, fed the furfural urea polymer, was relatively high in each metabolism trial, but did not reach as high a level as that obtained with the dairy concentrate or the urea alfalfa ration. Reduced nitrogen retention associated with increased urinary nitrogen losses has been noted elsewhere (Oltjen and Putnam, 1966) when urea was added to the ration.

Although there was no definite response to adaptation to urea rations in this experiment, in other reports it has been observed that a period of adaptation is required (McLaren, 1964; Smith et al., 1960). It is possible that the use of all-concentrate rations, with the high levels of starch provided by

these rations, results in more rapid and efficient utilization of nitrogen from non-protein sources.

The values for nitrogen retention found in this experiment are comparable with values reported in the literature (Loosli and McCay, 1943; Stobo et al., 1967; Whitelaw and Preston, 1963). Stobo et al. (1966) have reported nitrogen retention values of about 32% for 17-week old calves fed all-concentrate rations. The values reported in this experiment are slightly higher which would point to a more efficient utilization of the nitrogen consumed by the calves. Lower urinary nitrogen losses would tend to substantiate this view. Since growth is an efficient process, the values found in this experiment would not be unreasonable.

Table 7: Average daily nitrogen balance and percentage retention

Lot no.	Metabolism trial	Ration	Calf no.	N consumed (g)	N in feces (g)	N in urine (g)	N retained (g)	N retained (%)
1	1	Urea calf meal	813	44.8	6.3	15.3	23.2	51.8
			814	59.2	7.5	27.5	24.2	40.9
			Av				23.7	46.4
2	1	Dairy conc.	810	46.4	9.6	9.0	27.8	59.9
			811	59.6	9.9	11.6	28.1	47.2
			Av				27.9	53.5
1	2	Urea alfalfa	813	28.8	8.1	11.6	9.1	31.6
			814	20.8	10.2	9.5	1.1	5.3
			Av				5.1	18.4
2	2	Furfural urea	810	41.6	14.4	8.9	18.3	44.0
			811	49.6	11.4	16.8	21.4	43.2
			Av				19.8	43.6
1	3	Urea alfalfa	813	54.4	12.1	11.1	31.2	57.4
			814	70.4	14.1	15.3	41.0	58.2
			Av				36.1	57.8
2	3	Furfural urea	810	36.8	10.2	19.8	6.8	18.5
			811	57.6	10.1	15.6	31.9	55.4
			Av				19.3	36.9

Metabolizable energy

Values for metabolizable energy in the rations are shown in Table 8. The metabolizable energy of the ration 'as fed' was determined by deducting energy losses in the feces, urine and methane from the gross energy consumed. Losses due to the production of methane were calculated by using a value of 8 kcal per 100 kcal of gross energy consumed (Agricultural Research Council, 1965). Since metabolizable energy declines with increasing feed intake above the level of maintenance (Blaxter, 1962), the values for metabolizable energy in the ration 'as fed' were adjusted for the level of feed above maintenance as outlined by the Agricultural Research Council (1965).

There was considerable variation in the feeding level above maintenance requirements during the metabolism studies. Since the calves appeared to be under considerable stress as a result of frequent handling and the restrictions imposed by harnesses for collection of excreta, the differences in feeding levels between rations do not appear to correlate closely with acceptability of the rations fed during the experiment.

There was little difference between the urea calf meal and dairy concentrate in metabolizable energy at maintenance, but their values were higher than those for the rations supplemented with urea and furfural urea polymer (Table 8). There was almost no difference in the metabolizable energy values at maintenance between the rations supplemented with urea or with furfural urea polymer in the second and third metabolism trials. On the average, the metabolizable energy was 2.98 Mcal/kg dry

matter when the urea supplement was fed, and 3.02 Mcal/kg dry matter when the furfural urea polymer was fed.

The energy value of the experimental rations agrees fairly closely with expected values for these rations, since barley is quoted elsewhere at 3.09 Mcal/kg of dry matter (Agricultural Research Council, 1965), but the values for the urea calf meal and dairy concentrate appear higher than would be expected.

Table 8: Metabolizable energy

Lot no.	Metabolism trial no.	Ration	Calf no.	Feeding level[1]	Metabolizable energy Mcal/kg dry matter as fed	Metabolizable energy Mcal/kg dry matter at maintenance
1	1	Urea calf meal	813	-0.11	3.17	3.17
			814	0.40	3.27	3.30
			Av		3.22	3.24
2	1	Dairy conc.	810	0.30	3.28	3.30
			811	0.48	3.25	3.28
			Av		3.26	3.29
1	2	Urea alfalfa	813	-0.03	3.16	3.16
			814	-0.26	2.77	2.77
			Av		2.96	2.96
2	2	Furfural urea	810	0.08	2.88	2.89
			811	0.31	3.04	3.06
			Av		2.96	2.98
1	3	Urea alfalfa	813	0.07	2.93	2.94
			814	0.47	3.02	3.06
			Av		2.98	3.00
2	3	Furfural urea	810	-0.12	2.95	2.95
			811	0.18	3.15	3.16
			Av		3.05	3.06

[1] Feeding levels expressed as increments above maintenance.

Volatile fatty acids in rumen fluid

In the first metabolism trial there was no appreciable difference in the concentrations of total VFA (Table 9) in the rumen fluid from calves fed the urea calf meal (Lot 1) or the dairy concentrate (Lot 2). Rumen fluid from calves fed the urea calf meal had lower proportions of acetate and butyrate and higher proportions of propionate than that from calves fed the dairy concentrate.

In the second metabolism trial after the experimental rations had been fed for three weeks, there were no marked changes in the concentrations of total VFA, but there was an increase in the proportion of acetate and a decrease in propionate in the rumen fluid from calves fed the furfural urea ration.

In the third metabolism trial there was a marked increase in the concentration of total VFA, (significant; $P < .05$) in the rumen fluid from calves fed the furfural urea ration, but no further change in the molar proportions of acetate and propionate. The concentration of VFA in rumen fluid from calves fed the urea ration did not change appreciably, but the proportion of acetate increased and of propionate decreased to levels similar to that obtained with the furfural urea ration.

The pH of rumen contents varied from 5.7 to 6.9 and was slightly higher in calves fed the urea alfalfa ration than in calves fed the furfural urea polymer.

In general, the concentrations and proportions of VFA, and the pH of rumen contents were within the range expected when ruminants are fed all-concentrate rations. These

rations are associated with relatively lower levels of acetate, higher levels of propionate and lower pH than would be obtained with rations containing roughage.

There were no definite indications from VFA concentrations or proportions, or from rumen pH, of any adaptation in the rumen to the experimental rations. Some of the variation found could be attributed to sampling and analytical errors; sampling errors can result in considerable variation when the samples are taken by stomach tube.

Table 9: Mean VFA levels in rumen fluid

Metabolism trial	1		2		3	
Ration	Urea calf meal	Dairy conc.	Urea alfalfa	Furfural urea	Urea alfalfa	Furfural urea
Lot no.	1	2	1	2	1	2
Total rumen VFA (mmoles/100 ml)	8.75[b]	8.98[b]	8.53[b]	9.60[b]	7.92[b]	16.47[a]
Molar proportions, %						
Acetate	43.4	47.8	41.5	57.5	49.7	55.7
Propionate	44.0	33.5	43.9	26.1	27.8	28.9
iso-Butyrate	0.6	0.5	0.8	1.0	1.3	0.9
Butyrate	7.4	15.2	9.0	12.3	11.1	15.3
iso-Valerate	0.5	0.6	0.6	0.7	3.3	0.7
Valerate	4.0	2.6	4.1	2.4	6.8	0.7
	99.9	100.2	99.9	100.0	100.0	102.2
Rumen pH	6.3	5.7	6.8	6.5	6.9	5.7

[ab] Values with the same superscript are not significantly different ($P < .05$).

Experiment II

Rumen ammonia

During the first metabolism trial calves in Lot 1, fed the urea calf meal, had significantly ($P < .05$) higher levels of ammonia in rumen fluid than did calves in the other five lots fed dairy concentrate (Table 10). This indicated that the urea in the calf meal was being converted to ammonia at a rapid rate in the rumen, even though this ration had been fed for at least two months. Among the calves fed dairy concentrate, those in Lot 2 had slightly higher (non-significant) levels of rumen ammonia than those in Lots 3, 4, 5 and 6. This might be attributed to the younger age of the calves in Lot 2, since they were only 70 days of age as compared to 150 days of age for the calves in the other four groups. The levels of rumen ammonia are in agreement with those reported by Annison et al. (1954) for sheep fed a diet of flaked maize.

In the second metabolism trial, calves in Lot 1, fed the basal ration plus urea and alfalfa, had significantly ($P < .05$) lower levels of rumen ammonia than in the first trial, although there was a higher level of urea in this ration. There was a further decrease (significant; $P < .05$) in rumen ammonia levels in these calves by the third metabolism trial.

Calves in Lot 2, fed the furfural urea polymer in the second and third metabolism trials, had comparatively low levels of rumen ammonia which were comparable to those obtained with calves fed the dairy concentrate in the first trial, and with calves in Lots 3 and 4 fed the barley and barley plus

soybean meal rations, respectively, in the second and third metabolism trials. This suggests that the furfural urea polymer was broken down to ammonia at a rate comparable to that of plant protein in barley and soybean meal, and that no lengthy adaptation period was required.

In Lots 5 and 6, fed the barley plus urea and alfalfa and the barley plus urea and minerals, respectively, rumen ammonia levels were comparatively high during the second trial. However, they were significantly lower ($P < .05$) than the average value obtained for Lot 1 in the first metabolism trial, which indicates that any effect of adaptation must have been of minor importance. In the third metabolism trial, rumen ammonia decreased to levels comparable to those obtained in the other four groups.

It seems apparent that nitrogen from urea or from furfural urea polymer was used in the rumen as efficiently as that from barley or barley plus soybean meal.

Table 10: Rumen ammonia concentrations (mmoles/liter)[1]

Lot no.	1	2	3	4	5	6
	Pre-experimental rations					
Metabolism trial 1	Urea calf meal	Dairy conc.	Dairy conc.	Dairy conc.	Dairy conc.	Dairy conc.
	22.10	3.65	0.38	0.28	0.35	0.40
	7.65	1.40	1.35	1.60	1.45	1.30
Av	14.87[a]	2.52[cd]	0.86[de]	0.94[de]	0.90[de]	0.85[de]
	Experimental rations					
Metabolism trial 2	Barley & urea & alfalfa	Barley & furfural urea	Barley	Barley & soybean	Barley & urea & alfalfa	Barley & urea & minerals
	3.2	0.72	0.95	1.40	4.30	4.90
	2.2	1.28	0.49	0.53	7.30	3.93
Av	2.7[bcd]	1.00[de]	0.72[de]	0.93[de]	5.80[b]	4.41[bc]
Metabolism trial 3	0.30	0.48	0.32	0.53	0.40	1.68
	0.34	0.50	0.27	1.70	0.47	0.25
Av	0.32[e]	0.49[e]	0.29[e]	1.12[de]	0.43[de]	0.96[de]

[1] Data are shown for each of the two calves in each Lot, and the average for the two calves.

[abcde] Values with one or more common superscripts are not significantly different ($P < .05$).

Blood urea concentrations

During the first metabolism trial calves in Lot 1, fed the urea calf meal, had average blood urea concentrations approximately twice as high as those of calves in Lots 3, 4, 5 and 6, which were fed dairy concentrate (Table 11). This difference was statistically significant ($P < .01$). It was noted previously that the calves in Lot 1 had higher rumen ammonia levels than the other calves in the experiment. This suggests that the urea was being broken down rapidly in the rumen, the microorganisms were not able to utilize all of the ammonia, and a considerable proportion was being absorbed into the blood stream and synthesized into urea by the liver. It appears that these calves were not able to utilize urea as efficiently as plant protein, although they had been fed this ration for two months.

Calves in Lot 2 had higher blood urea levels than those in Lots 3, 4, 5 and 6, although all of these calves were fed the same ration. The younger age of the calves in Lot 2 may have had some effect on nitrogen utilization.

The calves in Lot 1 appeared to adjust rapidly to the barley plus urea and alfalfa ration. At the end of three weeks on this ration, their blood urea concentrations were comparable to those of calves fed dairy concentrate in the first metabolism trial, and there was very little change at the end of the third metabolism trial.

When calves in Lot 2 were fed the furfural urea ration, blood urea concentrations were low at the end of the second metabolism trial. They increased at the third metabolism trial

Table 11: Blood urea concentrations[1] (mg urea/100 ml plasma)

Lot no.		1	2	3	4	5	6
		Pre-experimental rations					
Metabolism trial 1		Urea calf meal	Dairy conc.	Dairy conc.	Dairy conc.	Dairy conc.	Dairy conc.
		31.8	16.1	17.1	7.5	11.6	12.4
		25.2	18.4	11.4	18.7	19.8	19.8
	Av	28.5^{a}	17.2^{bc}	14.2^{cd}	13.1^{de}	15.7^{cd}	16.1^{bcd}
		Experimental rations					
		Barley & urea & alfalfa	Barley & furfural urea	Barley	Barley & soybean	Barley & urea & alfalfa	Barley & urea & minerals
Metabolism trial 2		15.1	6.7	7.0	18.7	25.7	14.6
		14.3	13.5	7.7	16.7	27.3	18.4
	Av	14.7^{cd}	10.1^{ef}	7.4^{f}	17.7^{bc}	26.5^{a}	16.5^{bcd}
Metabolism trial 3		11.8	13.4	6.3	17.0	18.6	14.8
		15.8	22.5	6.2	19.6	20.8	20.7
	Av	13.8^{d}	18.0^{bc}	6.2^{f}	18.3^{bc}	19.7^{b}	17.8^{bc}

[1] Values are shown for each of the two calves in each Lot, as well as the average for the two calves.

abcdef Values with one or more common superscripts are not significantly different ($P < .01$).

to levels comparable to those of calves fed urea-supplemented rations (Lots 5 and 6) or the soybean supplemented ration (Lot 4). This suggests that the furfural urea polymer was broken down to ammonia at a slow rate throughout both trials. Most of the ammonia was utilized by the rumen microorganisms, and blood urea concentrations were never particularly high.

Calves fed the unsupplemented barley ration (Lot 3) had low concentrations of blood urea in both the second and third metabolism trials. This suggests that nitrogen may have been a limiting factor with this ration, resulting in utilization of a higher proportion in the rumen.

Supplementation of the barley ration with soybean meal (Lot 4) resulted in blood urea concentrations over twice as great as those with the barley ration alone. There was no change in the concentrations between the second and third metabolism trials, suggesting that no period of adaptation was required.

When the barley ration was supplemented with urea and alfalfa (Lot 5), very high levels of blood urea were obtained at the end of the second metabolism trial, but these had declined to a level comparable to that of calves in Lot 4 at the end of the third metabolism trial. This suggests that an adaptation period of at least three weeks is required to obtain efficient utilization of urea nitrogen. However, calves in Lot 6, fed urea plus minerals, did not appear to require three weeks for adaptation to a urea ration. Blood urea concentrations in these calves showed very little change at any of the three sampling periods.

GENERAL DISCUSSION AND SUMMARY

Two experimental rations with comparable levels of crude protein equivalent, one with urea (Lot 1) and one with furfural urea polymer (Lot 2), were fed to two lots of calves for an average period of 152 days. Prior to and during the first week of the experiment, calves in Lot 1 were fed a calf meal containing urea, and calves in Lot 2 were fed a concentrate mixture for dairy cows. Feed intake, growth rate, feed utilization and carcass characteristics were measured for each lot at the end of the experiment. Metabolism trials were conducted with two calves from each lot during the first week of the experiment when calves were fed the pre-experimental rations, and during the fourth and seventh weeks when calves were fed the experimental rations. The metabolism trials were conducted to enable determinations of apparent digestion coefficients, nitrogen retention and metabolizable energy concentrations. At the end of each metabolism trial measurements were made of VFA and pH in rumen contents.

In a second experiment with the two lots of calves noted above, and four lots of calves made available from another project, rumen and blood samples were obtained at the end of each of the three metabolism trials for determination of rumen ammonia and blood urea concentrations.

Over the whole experimental period, calves fed the barley ration supplemented with urea and alfalfa (Lot 1) consumed 58% more feed daily, gained 121% more weight and used 28% less feed per unit of gain than did the

calves fed the ration supplemented with furfural urea polymer. These differences appeared to be caused by low palatability of the ration supplemented with furfural urea polymer. This supplement had a distinct odour, particularly when it became moistened in the feeder, and the calves tended to eat the barley and leave the pelleted supplement. Even when the level of barley was increased in the supplement, until 20% of the ration was composed of the pelleted supplement, palatability was still a problem. Calves fed this ration never had a high level of feed consumption, as compared with those fed the urea ration. No palatability problems were encountered with the pelleted supplement containing urea, since it was consumed readily by the calves in Lot 1.

Since these were young calves and were presumed to have fairly high requirements for crude protein, and since low-protein barley was used as the principal ingredient in the rations, the levels of urea and furfural urea polymer were higher than would be required in most feedlot rations. It is probable that lower levels of furfural urea polymer, particularly if it were mixed with the complete ration to give maximum dilution, would be a more acceptable nitrogen source than it appeared to be in this experiment.

The average daily gain of 1.04 kg by calves fed the urea ration was low by comparison with expected gains of 1.4 kg or more by steers fed high-concentrate rations. However, it must be considered that the stress caused by the metabolism trials during the first third of the experimental period would

reduce feed consumption and growth rate and have a marked effect on average values for the whole experiment.

Apparent coefficients of digestion were high with all rations and in all three metabolism trials. There did not appear to be any appreciable differences in digestibility of the urea calf meal, dairy concentrate or the barley rations supplemented with urea and alfalfa or furfural urea polymer. Although lower coefficients were obtained for the barley rations than for the calf meal and dairy concentrate, no significant differences were detected. The values of approximately 80% digestibility were similar to those reported for comparable rations in other experiments (Gardner, 1968; Gonzalez-Jimenez and Blaxter, 1962).

In the first metabolism trial, calves fed the urea calf meal had a high rate of excretion of urinary nitrogen, resulting in low percentage retention of dietary nitrogen. High levels of rumen ammonia and blood urea were also found in these calves. This suggests that the urea in the ration was degraded to ammonia faster than the rumen bacteria could use the ammonia to synthesize microbial protein. Consequently, ammonia was absorbed into the blood stream and converted into urea in the liver, resulting in a high rate of urinary excretion. Since the calves had been fed this ration for a period of two months, they should have been well-adapted to urea and able to use the nitrogen efficiently. The most probable explanation for the low percentage retention of nitrogen from the calf meal is that it contained a higher level of nitrogen

than was required by calves of this age. Consequently, the excess nitrogen could not be utilized, resulting in the high rate of urinary excretion.

In this experiment nitrogen from urea appeared to be utilized as well as nitrogen from soybean meal. Although percentage retention of nitrogen was low in the second metabolism trial, this was attributed to very low feed and nitrogen intake during that week by calves fed the urea ration (Lot 1). Consequently, endogenous and metabolic nitrogen losses were high in relation to the amount of dietary nitrogen consumed, resulting in a low apparent retention. If these losses were accounted for, it seems unlikely that percentage nitrogen retention in that trial would have been markedly different to that obtained in the third trial, or in trials with the other rations. Rumen ammonia levels and blood urea concentrations were low in calves in Lot 1 during the second metabolism trial, indicating that nitrogen was being used as efficiently as in the third trial.

In experiment II, rumen ammonia and blood urea concentrations in calves fed barley supplemented with urea (Lots 1, 5 and 6), were comparable to those in calves fed barley supplemented with soybean meal (Lot 4). These results, along with the data for nitrogen retention, support the conclusion that urea can be used as a source of supplemental nitrogen resulting in utilization as efficient as that obtained with soybean meal.

Percentage nitrogen retention was relatively high in both metabolism trials by calves fed the barley ration supplemented with furfural urea polymer. At the same time rumen ammonia and blood urea concentrations were low. This suggests that the furfural urea polymer was broken down slowly in the rumen, resulting in efficient utilization of the urea nitrogen contained in the polymer. Although this compound does not appear to have any advantages over urea in all-concentrate rations, the fact that it is broken down slowly in the rumen would be a definite advantage when feeding rations with high proportions of roughage. Under those conditions, where digestion occurs at a slower rate and less starch is available, a slower and more prolonged release of ammonia is required for efficient use of the ammonia in protein synthesis.

There was little or no indication in this experiment of any adaptation in the rumen to rations containing urea, although other reports have noted an adaptation response (McLaren, 1964; Virtanen, 1966). Differences between the second and third metabolism trials were generally small, so that if adaptation did take place, it was virtually completed within two weeks.

There was no apparent difference in the metabolizable energy values of the barley rations when supplemented with urea or with furfural urea polymer. These values were similar to those expected for barley, and this was the main energy source in these rations. The metabolizable energy

values of the calf meal and dairy concentrate were slightly higher than expected, but there was no apparent reason for this difference other than the effect of stress on the calves.

The concentrations and proportions of VFA, and the pH of rumen contents were within the ranges expected when ruminants are fed all-concentrate rations. Such rations are associated with low proportions of acetate, high proportions of propionate and relatively low pH. The high concentrations of total VFA from calves fed the furfural urea polymer in the third metabolism trial might be attributed to sampling and analytical errors, since relative proportions of acetate and propionate did not change.

It seems evident from the data in this experiment that urea can be used as a source of supplemental nitrogen in rations that are low in crude protein content and high in available carbohydrate. Dinning et al. (1948) obtained quantitative evidence to show that rumen microorganisms utilize urea nitrogen to synthesize amino acids. The ingested NPN is of primary nutritional significance since the microbial protein synthesized from NPN is degraded in the abomasum and intestinal tract. The amino acids are absorbed into the blood stream and carried to the tissues where they can be utilized for protein synthesis.

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Appendix Table 1. Experiment I - mean squares obtained by analysis of variance.

Variable	Source of variation	Degrees of freedom	Mean squares
Rate of gain	Ration	1	0.6441**
	Error	6	0.0078
Digestibility coefficients			
Dry matter	Between all means	5	33.2900
	Error	6	12.3400
Crude protein	Between all means	5	154.2313
	Error	6	62.2507
Gross energy	Between all means	5	17.6358
	Error	6	13.9301
Nitrogen balance	Between all means	5	213.7428
	Error	6	66.7291
Nitrogen retention	Between all means	5	392.3793
	Error	6	194.5566
Total VFA	Between all means	5	20.4324*
	Error	6	2.6218

* Significant at $P < 0.05$.

** Significant at $P \leq 0.01$.

Appendix Table 2. Experiment II - mean squares obtained by analysis of variance

Variable	Source of variation	Degrees of freedom	Mean squares
Rumen ammonia	Between all means	17	24.3274*
	Error	18	6.5059
Blood urea	Between all means	17	61.0419**
	Error	18	15.1689

* Significant at $P < 0.05$.

** Significant at $P \leq 0.01$.