UTILIZATION OF DI-AMMONIUM PHOSPHATE BY RUMINANTS AS DETERMINED BY METABOLISM STUDIES

Thesis for the Degree of M. S.

MICHIGAN STATE UNIVERSITY
Donald Keyser

1960

UTILIZATION OF DI-AMMONIUM PHOSPHATE BY RUMINANTS AS DETERMINED BY METABOLISM STUDIES

By

Donald Keyser

A THESIS

Submitted to the College of Agriculture of Michigan
State University of Agriculture and Applied
Science in partial fulfillment of
the requirements for the
degree of

MASTER OF SCIENCE

Department of Dairy

1960

Approved ______________________

ACKNOWLEDGMENTS

The author wishes to express his sincere appreciation to Dr. C. A. Lassiter, Dairy Department, for his guidance and assistance throughout this work and for the critical reading of this manuscript.

I would also like to thank Dr. R. S. Emery, Dairy Department, for his guidance in the laboratory work and for his critical reading of this manuscript.

TABLE OF CONTENTS

LIST OF TABLES

INTRODUCTION

Several years ago, it was demonstrated that ruminants could convert non-protein nitrogen into protein and incorporate it into their bodies. Since that time, many products have been evaluated to establish their value as a substitute for natural proteins. Most of the products evaluated were ammoniated compounds such as urea, biuret, ammonium bicarbonate, and dicyandiamide. It has been demonstrated that these products have a protein sparing effect that varies considerably with the compound tested.

Di-ammonium phosphate is similar to the compounds mentioned above and ruminants should be able to utilize the nitrogen of this product. The nitrogen content of di-ammonium phosphate is about 21 per cent and has approximately one half the nitrogen content of urea. In addition, di-ammonium phosphate contains 30 per cent phosphorus. Since the phosphorus is in the form of a soluble phosphate, it should prove to be a very effective phosphorus supplement.

The manufacturers believe that di-ammonium phosphate may have a place in the commercial feed industry if it can be used both as a source of phosphorus and nitrogen for ruminants.

Preliminary feeding trials by Lassiter (17) showed that di-ammonium phosphate was palatable when fed to dairy cattle at the levels of 1, 2, and 4 per cent of the grain mixture. Similar palatability trials by Shaw (29) indicated that di-ammonium phosphate was palatable up to 3 percent of the grain mixture. Mono-ammonium phosphate was not as palatable in this study and was not recommended for cattle. Both authors indicated that the palatability of the grain mixtures decreased as the level of di-ammonium phosphate increased. There was no sign of toxicity from this product in either trial.

The next phase of this study was to conduct a digestion and nitrogen balance trial to compare the utilization of the nitrogen in di-ammonium phosphate with the nitrogen of urea and soybean oil meal. Phosphorus balance and blood inorganic phosphorus levels were used to indicate the fate of the phosphorus in this product.

REVIEW OF LITERATURE

There appears to have been little previous work with di-ammonium phosphate as a feed for ruminants. Since there is insufficient information on this product, it is the desire of the author to review the literature on the utilization of urea as a non-protein nitrogen source for animals, and the phosphorus metabolism of cattle. It is the author's belief that a review of literature in these two areas will provide the information needed to evaluate the results accumulated in the metabolism trial, where di-ammonium phosphate was compared with urea and soybean oil meal.

Urea Utilization by Ruminants

The research on urea has been quite extensive and is used by many as a standard when comparing other non-protein nitrogen products as a source of nitrogen for animals. Work has been completed with both non-ruminant and ruminant animals. Most of the work has been with ruminants where the utilization of these products shows promise of having some economic value. In addition, "in vitro" studies have been completed to reveal more information on how these products are utilized by the animals.

There is little evidence that urea will ever be of much value to non-ruminants. Work with rats by Rose and co-workers (26) has shown that urea and other ammoniated products can be utilized to some extent when only the essential amino acids were fed to them but they could not utilize enough urea for normal growth.

Liu and co-workers (19) studied the metabolism of urea in pigs with labeled (N^{15}) nitrogen in low protein ration. A small but definite amount of this urea was incorporated into the body.

Hays et al. (12) fed different levels of urea to swine. Slightly higher daily gains were recorded with 0.16 per cent and 0.31 per cent urea in the rations. These gains were small and less efficient. They required more grain per pound of gain, with the feed efficiency decreasing as the urea content increased. When the urea content reached the equivalent of 10 per cent of the protein, it reduced daily gains. These and other studies indicated that this type of feed is of little value to single stomach animals.

Hart et al. (9) compared the utilization of urea and ammonium bicarbonate to casein in growing calves. The calves received milk for the first two weeks and then were gradually changed over to a complete dry ration. The basal ration was approximately a 6 per cent protein ration and was supplemented with the different sources of nitrogen to raise the nitrogen content of the ration to the equivalent of an 18 per cent protein ration. All the calves receiving the extra nitrogen gained more than the calves on the basal ration, indicating that some of the nitrogen from the non-protein sources was being utilized. The calves on the casein gained substantially more than the other groups. The results of the trial are summarized in TABLE I.

Loosli and McCay (21) studied the utilization of urea by two month old calves. The calves were fed milk for a month and gradually changed over to dry feed. The basal ration was a 4.4 per cent protein ration. This was increased to a 16.2 per cent protein ration with the addition of urea or natural proteins. Calves fed on the normal ration gained 80 pounds, while the calves on urea rations gained 61 pounds. This trial indicated that urea was utilized by the calves but not as efficiently as natural protein.

TABLE I

WEIGHT GAINED AND BLOOD UREA NITROGEN LEVEL OF CALVES

Type of Protein	Pounds of Gain		Mg. % of Blood Urea Nitrogen	
	28 weeks	40 weeks	Before exp.	End of exp.
Basal Ration	120	201	16.67	7.2
Ammonium bicarbonate	180	276	14.20	17.2
Urea	220	290	15.58	19.7
Casein	312	427	14.20	17.2

Bartlet and Cotton (2) studied the effect of urea as a substitute for part of the protein for young cattle from 7 to 17 months of age. When 0.127 pounds of urea was added to the rations, it accounted for a 0.24 pound increase in their daily gains indicating that the cattle were utilizing urea. In this experiment, natural proteins produced better gains but they were not significantly greater than those on urea rations. The blood urea nitrogen levels for all animals were fairly close except those on low protein diets. The blood urea nitrogen levels were lower on these animals.

Using young heifers, Parham et al. (24) compared urea, ammoniated molasses, and cotton seed meal as a source of nitrogen. These heifers were from 19 to 33 months of age. Urea and ammoniated molasses made up a third of the protein in an 18 per cent crude protein ration. The average daily gains in pounds were: urea 0.94, ammoniated molasses 1.041, and cotton seed meal 1.23. The results were not significantly different because of the wide variations in gains within groups.

Davis et al. (5) used four groups of milking cows to compare a low protein ration, soybean oil meal, urea, and di-cyandiamide as a protein source in two 56 day trials. The non-protein nitrogen replaced one third of the protein in the urea and dicyandiamide rations. The low protein ration had two-thirds the nitrogen value of the other rations. There was no significant differences in body weight changes, milk production, or blood urea nitrogen levels.

Rust et al. (27) studied the utilization of urea and dicyandiamide as a nitrogen source for low protein rations with 24 cows over a 305 day period. Approximately one-third of the nitrogen was of a non-protein source. Average milk production for these groups were: soybean oil meal 24.4, urea 22.3, and dicyandiamide 22.2 pounds. The average weight gained or lost by these groups were +3, -28, and -86 pounds respectively. The differences in the milk production was not significant but the cows fed urea and soybean oil meal lost significantly less body weight than the cows fed dicyandiamide. The average blood urea nitrogen levels of this group were: soybean oil meal 6.3, urea 6.88, and dicyandiamide 4.07 mg. per cent. This experiment indicated that urea was utilized fairly well but not dicyandiamide. The blood urea nitrogen levels indicated that the dicyandiamide had a low ammonia activity in the rumen.

Archibald et al. (1) studied urea as a partial substitute for natural proteins. Twenty-eight Holstein cows were used in a three year

year trial tc determine the adequacy of urea. The grain ration contained 3 per cent urea. The urea furnished 42 per cent of the nitrogen in the grain and 25 per cent of the total nitrogen in the ration. Milk yields and weight changes were not significantly different but milk yields and weight gains were generally a little better when natural proteins were fed. Blood urea nitrogen levels were slightly higher when the cows were fed urea.

High level urea feeding by Lassiter and co-workers (18) indicated that milk yields and body weight gains would decline slightly as urea supplied 0, 30, 50, and 70 per cent of the total nitrogen in the grain ration. These differences were not statistically significant. Protein digestability values for the rations were: soybean oil meal 53.5, 30 per cent urea nitrogen 50.1, 50 per cent urea nitrogen 50.4, and 70 per cent urea nitrogen 51.8 per cent. The blood urea nitrogen values for the different rations were: 8.8, 10.7, 10.4 and 11.2 mg. per cent respectively.

Ward et al. (30) and Thompson et al. (28) also fed urea and soybean oil meal to cattle with about the same results. The natural proteins seemed to be a little better but the differences were not large enough to be statistically important.

Wegner et al. (31) used a fistulated heifer to study the effects of urea on the ingesta of the rumen. Protein levels averaged 1 to 2 per cent higher in the ingesta when urea was fed in addition to a basal ration of 8.45 per cent protein. The urea was hydrolized in one hour and the ammonia disappeared in 4-6 hours. In another experiment Wegner et al. (32) compared the utilization of urea at different protein levels. When the grain mixture was over 18 per cent, the efficiency of urea utilization decreased. In this study urea was utilized fairly efficiently up to 12 per cent protein equivalent with an 11.5 per cent basal ration composed of natural proteins. The conversion of urea decreased when the protein of the ingesta of the rumen became greater than 12 per cent.

In the digestion of nutrients, Belasco (3) indicated that urea increased cellulose digestion. This work indicated that the microflora that digest cellulose need a readily available supply of ammonia for optimum activity.

"In vitro" studies by Wegner et al. (33) showed that microflora can convert inorganic nitrogen to protein. They found that optimum pH for the microflora to be 5.5 to 7. The carbohydrates used in the medium were corn molasses, cerelose (commercial glucose), starch, and cellulose. All the sources of carbohydrates were readily utilized by the bacteria that converted ammonia to protein except cellulose. The response with cellulose was very poor. In this study, ammonium bicarbonate was just as effectively utilized as urea.

Pearson and Smith (25) indicated that the ability of the ruminant to convert urea to ammonia was very high. One hundred grams of rumen ingesta could convert 100 grams of urea to ammonia in one hour. During the incubation of urea and ingesta with an available starch supply, it was possible for the microorganisms to fix 0.8 mg. of nitrogen per 100 gm. of rumen liquor in 24 hours. At this rate it would be possible for the intact animal to fix 72 grams of nitrogen or 450 grams of protein. Starch proved to be a better source of carbohydrate than glucose, galactose, sucrose, or other sugars.

Studies by Bell et al. (4) indicated that the source of starch did not affect the utilization of urea when it was from corn, dehydrated sweet potatoes, barley, milo, cane molasses, or corn and molasses. The cane molasses and urea combination had the poorest nitrogen retention while corn was the highest. The digestability of barley increased a little when soybean oil meal was fed with barley instead of urea.

Phosphorus Metabolism in Cattle

Phosphorus metabolism is quite difficult to determine in cattle. Most of the phosphorus is excreted in the feces and is mixed with the undigested phosphorus. Urine Phosphorus is usually quite low, amounting to less than 0.1 of a gram per day at times (8). When determining the requirement of phosphorus, it has been the practice to determine the phosphorus balance. The different sources of phosphorus were fed and the requirement determined by how much was needed to keep a constant phosphorus equilibrium. Another factor studied was the inorganic blood phosphorus levels. This has been shown to vary some with the food supply and there is some evidence that it varies with the supply of phosphorus (23). Studies on the effects of normal physiological functions such as eating, exercise, and rest were completed to determine their effect on the blood inorganic phosphorus level.

Many investigators have studied the requirement of phosphorus in cattle. Lamb et al. (16) fed high and low phosphorus rations to cattle. Rations supplemented with bone meal enabled some heavy milk producers to have a positive balance. The cows on low phosphorus rations lost their appetite, and in general were in a negative phosphorus balance. The range of phosphorus retained or lost varied from +0.14 to +10.13 grams per day for the high phosphorus rations and from -11.85 to +8.70 grams for the cows on the low phosphorus rations. The cattle on the high phosphorus rations had a blood inorganic phosphorus level ranging from 5.3 to 7.44 mg. per cent while cows on the low phosphorus diets were below 4 mg. per cent Most of the phosphorus was excreted in the feces. Urine excretion averaged about 0.17 grams per day with a range of 0.07 to 0.54 grams.

Huffman and co-workers (14) studied the phosphorus requirement of cattle when alfalfa hay was the principle source of protein. Alfalfa hay is low in phosphorus and has an adverse ratio between calcium and phosphorus. These studies indicated that the absorption of phosphorus was better if the ratio of calcium to phosphorus was 1.25:1. On low phosphorus rations, blood levels of inorganic phosphorus were 1.85 to 2.3 mg. per cent in calves and as low as 1 mg. per cent in mature cattle. When phosphorus was decreased in the rations, the blood level of phosphorus dropped from around 8 mg. per cent to less than 5 mg. per cent in four days. When the phosphorus supplement was withdrawn from lactating cows to study their phosphorus reserve, the blood level dropped from about 5 mg. per cent to 3.51 mg. per cent in one week, to 1.95 mg. per cent the second week, and to 1.16 mg. per cent the third week. After several months when milk production dropped, the blood level of phosphorus increased to over 5 mg. per cent.

Work reviewed by Forbs (8) indicates that the cattle varied in their absorption and retention of phosphorus. In one study the phosphorus pentoxide (P_2O_5) retention varied from -40.0 to +41.8 grams.

Hart et al. (10) fed high and low amounts of bran ash to milking cows. The high ration contained 190 grams of P_2O_5 and the low ration less than 50 grams of P_2O_5. The balance of phosphorus ranged from -13 grams to +124 grams of P_2O_5.

Eckles et al. (7) studied phosphorus deficiencies in cattle. Blood phosphorus levels on low phosphorus rations were 0.77 to 3.82 mg. per cent for inorganic phosphorus with a balance ranging from +0.05 to -4.03 grams of phosphorus. The high phosphorus rations had a phosphorus balance ranging from +2 to +14.41 grams per day. When cattle on low phosphorus rations were given a phosphorus supplement, the blood levels of inorganic phosphorus raised from 1 to 2.5 mg. per cent to about 6 mg. per cent in five days. Blood phosphorus levels varied from 2.84 to 8.13 mg. per cent during this five day period.

Work by Loosli et al. (20) indicated that phosphorus requirements for cattle varied with age and milk production. Results indicated that heifers needed about 1.8 grams of phosphorus per 100 pounds of body weight for the first year, 1.7 grams per 100 pounds the second year and 1.3 grams per 100 pounds for the third year. Growing calves retained from 3.6 to 5.3 grams of phosphorus per day. Cows require about 1 gram per 100 pounds of body weight for body maintenance and from 0.5 to 0.7 grams per pound of milk.

Kleiber et al. (15) measured endogenous phosphorus in cattle with P^{32}. The work indicated that endogenous phosphorus varies with the supply of phosphorus and other factors. In this trial, the endogenous phosphorus from the feces varied from 43 to 70 per cent of the total phosphorus excreted. The amount excreted depended to some extent on the supply of phosphorus in the feed.

Palmer et al. (23) worked on blood phosphorus levels of cattle as influenced by food intake, exercise, and time of day. Blood inorganic phosphorus levels before feeding ranged from 5.83 to 8.13 mg. per cent. Little change was recorded after eating but the blood inorganic phosphorus level did increase slightly in about one hour and decreased to normal in about three hours. This increase was about a 0.4 mg. per cent increase. Exercise increased the phosphorus level for a short time followed by a decrease for a short period. This work indicated that the time to draw blood for phosphorus determinations was after the cattle had rested for several hours and before they had been fed. Paturition causes a rapid drop in blood phosphorus level, and the calf will have a higher blood phosphorus level than the dam. Young calves have a higher blood phosphorus level than mature cattle and is usually around 8 mg. per cent. Mature cattle usually average about 6 mg. per cent with a range of 4 to 8 mg. per cent for inorganic blood phosphorus.

DESIGN OF EXPERIMENT

This experiment was designed as a 84 day metabolism study to compare the utilization of the nitrogen in di-ammonium phosphate to that of soybean oil meal and urea, and to investigate the value of the phosphorous in this product. Four cows were fed each ration in the same sequence as they appear in TABLE II.

The tentative feeding schedule was a 17 day feeding period for each ration. This was divided into a 10 day adjustment period and a 7 day collection period. Fecal and urine samples were taken daily during the metabolism trial and total weights recorded. Blood samples were taken at the end of the adjustment period and the end of the collection period.

The experiment was started February 7, 1959 and completed June 4, 1959. The tentative date for completion was May 1, but was not possible because the cattle had a tendency to go off feed when di-ammonium phosphate was introduced or increased in their grain mixtures.

Three factors may have been involved in the cows refusal of their grain mixture that contained di-ammonium phosphate. Di-ammonium phosphate did not seem to be as palatable as urea and the cattle never consumed their grain mixture as readily when this product was in their grain. Secondly, the weather turned warm at the time the change was made and this may have caused them to lose their appetite. Third, the

TABLE II

CALCULATED VALUES OF THE RATIONS FED

		CP[2] (lb.)	TDN[3] (lb.)	Ca (gm.)	P (Gm.)
Ration No. I Basal Ration					
Timothy Hay	4540 gm.	.7	5	25	10.5
Starch	1589 gm.		3.5		
Corncobs	681 gm.	.03	.7		.1
Salt (mineralized)	40 gm.				
DiCalcium Phosphate	15 gm.			6	2.7
Total for Ration No. I		.73	9.2	31	13.3
Ration No. II Basal Ration and SBOM					
Basal Ration		.73	8.4[1]	31	13.3
Soybean Oil Meal	454 gm.	.45	.8	.7	2.8
Total For Ration No. II		1.18	9.2	31.7	16.1
Ration No. III Basal Ration Plus Urea					
Basal Ration		.73	9.2	31	13.3
Urea 262	77 gm.	.44			
Total for Ration No. III		1.17	9.2	31	13.3
Ration No. IV Basal plus Urea and Di-ammonium Phosphate					
Basal Ration		.73	9.2	31	13.3
Urea	33.5 gm.	.22			
Di-ammonium Phosphate	77 bm	.22			19
Total for Ration No. IV		1.17	9.2	31	32.3
Ration No. V Basal plus Di-ammonium Phosphate					
Basal Ration		.73	9.2	31	13.3
Di-ammonium Phosphate	154 gm.	.44			38
Total for Ration No. V		1.17	9.2	31	51.3
Requirement for 1100 pound cow by Morrison's Standards (22)		dig. Prot. 0.65-0.71	TDN 7.6-8.6	11	11.0

[1] The corn starch was reduced to allow for the TDN of the SOBM
[2] Crude Protein
[3] Total Digestible Nutrients

grain mixture was never very palatable and does not mask the flavor of the various products in the ration. When corn was sprinkled on top of the grain mixture, the cows would consume their feed.

The adjustment period varied considerably in this experiment. It was 10 days for the basal ration, 11 days for the soybean oil meal, 14 days for urea, 31 days for the urea and di-ammonium phosphate, and 17 days for the di-ammonium phosphate ration. The delay on the urea ration was the result of the digestion stalls being used for another experiment. The 31 day adjustment period for the urea and di-ammonium phosphate ration was the result of the cows going off feed and refusing their grain ration. Corn was used to enhance the grain mixture. When the cows were cleaning up the mixture, the corn was gradually withdrawn. The same method was used with the last ration. All the cows but A-107 cleaned up the last grain mixture. The weigh back of grain for A-107 was very small and did not affect the total consumption.

The cows used in this experiment were dry and supposedly had a long enough dry period to carry out this experiment. Since the trial was extended 34 days, two cows had to be removed from the experiment. They were replaced by two cows with later freshening dates. TABLE III shows the due date and date of calving. All the calves were normal and in good health.

The rations were designed to provide ample carbohydrates and sufficient nitrogen for maintenance of an 1100 pound cow. There was no extra feed allowance for pregnancy as the experiment was to have been completed before the last month of pregnancy. Full bloom timothy hay was used as a roughage. The grain mixture consisted of corn starch, dicalcium phosphate, mineralized salt, corn cobs, and a nitrogen source

TABLE III

RECORD OF WHEN THE COWS WERE DUE AND FRESHENING DATE

Cow No.	Date due	Date calved
A-94	5-9-59	5-12-59
A-98	5-16-59	6-11-59
585	10-2-59	10-2-59
A-110	6-16-59	6-22-59
A-107	6-18-59	6-15-59
K-213	6-19-59	(1)

(1) Not pregnant and sold after the experiment was completed.

of urea, soybean oil meal, or di-ammonium phosphate. Corn cobs were not originally considered for this ration but it was soon evident that the cows would not consume the grain mixture because of its consistency. The rations and their calculated composition are shown in TABLE II.

All the cows remained in about the same condition during this experiment except cow A-107. She refused to consume her grain for long periods of time and lost considerable weight. Her calf was small but normal and there was no apparent harm to either the cow or calf.

Blood samples were taken at the end of the adjustment period and the end of the collection period. The samples were taken at 7 P.M. which was about four hours after they were fed. The blood for the urea nitrogen determination was oxylated to prevent clotting and analyzed by the Van Slyke and Cullen method (11). Another blood sample for the inorganic phosphorus was taken and allowed to clot. It was then refrigerated for about one day and the serum analyzed for inorganic phosphorus. The method used was described in an Eastman Chemical Abstract (6) and is a colorimetric method.

Feces and urine were collected daily and refrigerated. Hydrochloric acid was added to both to reduce bacterial action and loss of ammonia. In addition thymo crystals were added to the feces to decrease mold growth. The analysis of the nitrogen was by the Kjeldahl-Gunning method (13).

The phosphorus analysis was modified from the method described in the Eastman Chemical Abstract (6). One gram of wet feces was weighed into a 50 ml. micro Kjeldahl flask and 2 ml. of 50 per cent H_2SO_4 added. The samples were digested over micro burners. Several drops of 30 per cent peroxide were added to complete the digestion and clear up the solution. Then the samples were diluted with water and boiled for 5 minutes. The samples were then diluted in a 100 ml. volumetric flask. Suitable samples of this solution were quantitatively measured and transferred to a 10 ml. volumetric flask. The reagents were added and the colorimetric readings taken. Standards from a standard solution of KH_2PO_4 were used to determine the concentration of phosphorus.

The urine analysis was similar except 2 ml. of urine replaced the 1 gram of feces. The digestion was more rapid because of the low amount of organic matter in the urine. The solution was diluted in a 50 ml. volumetric flask. The solution proved to be too acid and interfered with reaction of the chemicals. Instead of a blue color, the solution was colorless. After experimenting with the solution, it was evident that a little NaOH solution would decrease the acidity of the solution to the extent that the color was present. Using known standard solutions with and without NaOH present, the readings were similar and no large error was apparent.

RESULTS AND DISCUSSION

The results of this metabolism trial indicated that all the rations except the basal ration furnished sufficient protein for the maintenance of the cows as indicated by the positive nitrogen balance. The basal ration, which was designed to furnish less than the minimal requirement of protein, had a negative nitrogen balance. The nitrogen balance for the different rations were: basal ration -8.4, soybean oil meal +6.4, urea +19.2, urea and di-ammonium phosphate +7.0, and di-ammonium phosphate +5.2 grams per day. The nitrogen balance was probably a little high in this experiment, as there was a little loss of urine due to faulty equipment. The summary of the results of the analysis of the feces and urine are shown on TABLE IV.

The large differences between the retention of nitrogen by the cattle on the urea and soybean oil meal rations cannot be explained. Literature indicates that the retention of nitrogen should be fairly close with these two rations. Lassiter et al. (15) reported slightly higher nitrogen retention with soybean oil meal in their studies. The results were as follows: soybean oil meal +60, 30 per cent urea nitrogen +47.1, 50 per cent urea nitrogen +55.2, and 70 per cent urea nitrogen +52.5 grams per day.

The main difference between the nitrogen retention for these two rations seemed to be the high excretion of nitrogen by the cows numbered A-94 and 585 on the soybean oil meal ration. The reason for the high excretion rate of nitrogen by these two cows is not

TABLE IV

EFFECT OF THE VARIOUS RATIONS ON NITROGEN BALANCE AND DIGESTIBILITY (G/COW DAILY)

	Basal			SBOM			Urea			Urea Di-am Phos			Di-am Phos		
Cow No.	Urine	Feces	Total	Urine	Feces	Total	Urine	Feces	Total	Urine	Feces	Total	Urine	Feces	Total
A-94	25.4	36.2	61.6	47.5	38.7	86.2	25.6	40.8	66.4						
A-98	27.8	35.7	63.5	29.6	38.2	66.1	29.4	38.2	67.6						
A-110	24.6	37.0	61.6	31.7	40.6	72.3	34.7	33.1	67.8	28.5	40.2	68.7	33.4	36.0	69.4
585	25.0	34.4	59.4	42.9	33.8	76.7	27.2	34.4	61.6	25.6	42.6	68.2	30.4	50.6	81.0
A-107										39.5	50.2	89.7	30.1	44.0	74.1
K-213										38.5	47.4	85.9	35.4	53.8	89.2
Average	25.7	35.8	61.5	37.9	37.4	75.3	29.3	36.6	65.9	33.0	45.1	78.1	32.4	47.1	79.9
Total N fed (g)		53.1			85.7			85.1			85.1			85.1	
Nitrogen balance (g)		-8.4			+9.4			+19.2			+7.0			5.2	
Digestibility of N (%)		32.4			55.2			57.0			47.0			44.7	

apparent. It could be from a change in their metabolism, faulty analysis, or poor collection procedure. It is evident that if the two urine determinations were lower, as one would expect from the other urine determinations, the nitrogen retention for the two rations would have been fairly close.

The digestibility of the di-ammonium phosphate rations dropped about 10 per cent below the urea and soybean oil meal rations. This drop is quite large and indicates that di-ammonium phosphate may not be utilized as readily as urea. The digestibility of the protein in the different rations were: basal ration 32.4 per cent, soybean oil meal 55.2 per cent, urea 57.0 per cent, urea and di-ammonium phosphate 47.0 per cent and di-ammonium phosphate 44.7 per cent.

The basal ration had the lowest digestibility of the five rations. The addition of di-ammonium phosphate increased the digestibility coefficient but was not equal to the soybean oil meal and urea rations. The large increase in the fecal nitrogen was not expected. It would be reasonable to expect that a soluble product like this to be absorbed in the digestive tract and excreted in the urine if it was not utilized. Instead, there was a large increase in fecal nitrogen indicating that some of this product may be passing through the digestive tract without being absorbed.

There was no evidence as indicated by the blood urea nitrogen levels, that the ammonia concentration or activity in the rumen was especially low with the exception of the basal ration. The blood urea nitrogen level for the basal ration was very low. There was no evidence in the literature that blood urea nitrogen levels were ever recorded as low as the ones in this experiment. TABLE V summarizes the results of the

blood values for urea nitrogen. There was some doubt that the results of the urea test were accurate as there were very large readings from the blanks. The readings varied from day to day and ranged from about 10 to 50 per cent of the blood urea determinations.

The level of blood urea nitrogen before the experiment was at its highest peak and was never as high again except for about three of the blood tests taken while urea was being fed. In the literature large variations in the blood urea levels have been noted. Archibald (1) recorded a slightly higher levels of blood urea while feeding urea. For urea, it averaged about 11 mg. per cent while regular feeds were about 10.27 mg. per cent. Rust et al. (27) had lower values in his trials with soybean oil meal 6.3, urea 6.8 and dicyandiamide 4.07 mg. per cent. Davis et al. (5) had blood levels as follows: Test I - low protein 12.3, soybean oil meal 13.5, urea 14.0, dicyandiamide 12. Test II - low protein 8.1, soybean oil meal 8.1, urea 10.0, and dicyandiamide 8.1 mg. per cent. Archibald (1) indicated that normal values of urea nitrogen were 14 to 23 mg. per cent. Most of the literature indicated that the blood level of urea nitrogen was 6 to 12 mg. per cent with older cattle and 15 to 20 mg. per cent for calves.

In this trial, there was enough phosphorus in every ration to meet the requirements of the cows. Di-calcium phosphate was used as the phosphorus supplement. The phosphorus supplement was not eliminated in the last two rations even though the di-ammonium phosphate supplied more phosphorus than the cows needed. It was thought that if every ration had the same source of phosphorus with the exception of the di-ammonium phosphate rations any change in blood inorganic phosphorus or phosphorus retention could be attributed to the di-ammonium phosphate. The main fault of this experiment is that it was not designed to compare the availability

TABLE V

EFFECT OF THE RATIONS ON BLOOD UREA NITROGEN VALUES

Ration	Before Trials	Basal		SBOM		Urea		Urea Di-am Phos		Di-am Phos	
Determination[1]	1	2	3	4	5	6	7	8	9	10	11
Cow No.						Mg. %					
A-98	10.98	2.71	3.53	5.24	7.46	5.52	7.46				
A-110	10.17	1.49	2.58	6.10	6.51	9.39	11.60	5.80	4.97	4.70	5.25
585	7.46	1.76	2.89	4.61	5.97	4.97	9.67	7.56	5.25	5.52	5.52
A-94	7.32	2.58	3.39	4.47	6.66	6.63	6.08				
A-107								5.25	7.73	4.42	5.80
K-213								3.87	6.35	5.52	5.25
Average	8.68	2.13	3.10	5.58	6.65	6.63	8.70	5.62	6.07	5.04	5.45

[1]The even numbers represent the blood test at the end of the adjustment period; the odd numbers represent the blood test at the end of the collection period.

of the phosphate is the di-ammonium phosphate with other phosphorus supplements.

The blood serum levels of the inorganic phosphorus increased substantially in this trial when di-ammonium phosphate was fed. The blood phosphorus level was nearly equal for the first three rations and the initial blood test. This was in the 4 to 8 mg. per cent range which is considered normal for cattle. When the di-ammonium phosphate was fed, the blood inorganic phosphorus level increased 80 to 100 per cent. This is fairly conclusive evidence that the phosphorus is readily available to the cattle and was being absorbed. A complete summary of the blood serum inorganic phosphorus determinations are listed in TABLE VI.

Very little can be determined in this trial about how the phosphorus was absorbed or utilized. In all rations, there was slight positive phosphorus retention except the last ration in this test. In the last ration, where the phosphorus level fed was about three times that recommended by Morrison's standards for dry cows, (22), there was a fairly high retnetion of phosphorus. This averaged nearly 10 grams a day for the cows while the other rations had a positive balance ranging from +1 to +4 grams of phosphorus. TABLE VII summarizes the phosphorus balance for this study.

One strange development in this study was the high phosphorus excretion in the urine of A-107 while on the di-ammonium phosphate ration. This cow excreted over 8 grams of phosphorus per day which is abnormal for cattle. In all the determinations, there was a fairly high phosphorus concentration in the urine compared to the levels found by other investigators. This was in part, the result of a fairly high fecal

TABLE VI

BLOOD SERUM INORGANIC PHOSPHORUS VALUES FOR THE VARIOUS RATIONS

Ration	Initial	Basal		SBOM		Urea		Urea Di-am Phos		Di-am Phos	
Determination[1]	1	2	3	4	5	6	7	8	9	10	11
- Cow No.						Mg.%					
A-98	6.2	5.1	5.4	6.8	4.1	5.4	6.0				
A-94	4.5	3.7	5.0	5.9	6.5	5.3	3.6				
A-110	5.2	4.1	3.4	5.2	8.0	5.8	6.0	8.2	8.8	9.6	9.8
585	7.6	4.2	4.0	6.0	7.4	5.8	5.9	7.6	8.8	8.0	5.6
A-107								8.0	13.2	9.6	9.0
K-213								7.6	9.6	9.8	8.2
Average	5.9	4.3	4.5	6.0	6.5	5.6	5.4	7.9	10.1	9.3	8.1

[1]The even numbers represent the blood test taken at the end of the adjustment period; the odd numbers represent the blood test at the end of the collection period.

TABLE VII

THE EFFECT OF THE RATIONS ON PHOSPHORUS RETENTION AND BALANCE

	Basal			SBOM			Urea			Urea Di-am Phos			Di-am Phos		
	Grams per day														
Cow No.	Urine	Feces	Total	Urine	Feces	Total	Urine	Feces	Total	Urine	Feces	Total	Urine	Feces	Total
A-110	0.4	13.5	13.9	1.0	13.2	14.2	1.1	11.7	12.8	0.5	22.4	22.9	1.6	37.1	38.7
585	0.4	13.1	13.5	1.1	10.9	12.0	1.0	13.6	14.6	0.4	29.4	29.8	3.2	39.4	42.6
A-94	0.3	10.7	11.0	0.4	9.7	10.1	0.3	11.4	11.7						
A-98	0.3	10.2	10.5	0.4	11.5	11.9	0.3	9.8	10.1						
A-107										1.3	33.6	34.9	8.7	30.1	38.8
K-213										0.6	37.0	37.6	1.2	41.1	42.3
Average		12.2			12.1			12.3			31.3			40.6	
Phosphorus Fed		13.3			16.1			13.3			32.3			50.3	
Phosphorus Balance		+1.1			+4.0			+1.0			+1.0			+9.7	

contamination in the urine. This still doesn't explain the very high excretion rate in the urine of cow A-107. This may have been the result of some kidney impairment.

SUMMARY

This study was designed to compare the value of the nitrogen in di-ammonium phosphate with the nitrogen in urea and soybean oil meal when fed to ruminants, and to determine the fate of the phosphorus from the di-ammonium phosphate. Metabolism studies were used to determine both nitrogen and phosphorus balance and nitrogen digestability. Blood urea nitrogen levels and blood serum inorganic phosphorus levels were also determined. Four dry Holstein cows were used in this experiment.

Five different grain rations were fed with full bloom timothy hay. The basal grain ration consisted of corn starch, corn cobs, salt, and dicalcium phosphate. The other four rations were supplemented with soybean oil meal, urea, a combination of urea and di-ammonium phosphate, and di-ammonium phosphate. The nitrogen supplements provided the equivalent of 0.44 pounds of protein per day.

There were five feeding trials in this experiment. There was a ten day adjustment period and a seven day collection period for each ration. Blood samples were taken at the end of the adjustment period and at the end of the collection period.

The results of this study indicate that the nitrogen in the di-ammonium phosphate was not utilized as efficiently as the nitrogen in urea. There was a large increase of fecal nitrogen, indicating that this product was not as readily utilized by the bacteria in the rumen. The digestibility of nitrogen for the different rations was: basal 32.4,

soybean oil meal 55.2, urea 57.0, urea and di-ammonium phosphate 47.0, and di-ammonium phosphate 44.7 per cent. Since there was a positive nitrogen balance with di-ammonium phosphate, it is evident that this product was being utilized to some extent by the cows. A growth trial would probably give a more comprehensive comparison between urea and di-ammonium phosphate.

As a phosphorus supplement, there was every indication that the phosphorus in this product was available to the cattle. The blood inorganic phosphorus levels increased nearly 100 per cent when di-ammonium phosphate was added to their rations. These blood levels were above the range considered normal for cattle (4 to 8 mg. per cent). The average phosphorus balance was positive for all the rations, although some of the individual determinations showed a negative balance.

REFERENCES

(1) Archibald, J.G. Feeding Urea to Dairy Cows. Mass. Agr. Exp. Sta., Bull. 406. 1943.

(2) Bartlett, S. and Cotton, A. G. Urea as a Protein Substitute in the Diet of Young Cattle. J. Dairy Res. 9:263. 1938.

(3) Belasco, I.J. Comparison of Urea and Protein Meals as Nitrogen Sources for Rumen Microorganisms: Urea Utilization and Cellulose Digestion. J. Animal Sci. 13:739. 1954.

(4) Bell, M.C., Gallup, W.D., and Whitehair, C.K. Value of Urea Nitrogen in Rations Containing Different Carbohydrate Feeds. J. Animal Sci. 12:787. 1953.

(5) Davis, C.L., Lassiter, C.A., Seath, D.M., and Rust, J.W. An Evaluation of Urea and Dicyandiamide for Milking Cows. J. Animal Sci. 15:515. 1956.

(6) Dryer, R.L., Tammis, A.R., and Touth, J.J. Determination of Phosphorus in Body Fluids. Unpublished. Eastman Chemical Abstract 432. Eastman Organic Chem. Dept., Division of Eastman Kodak Co., Rochester 3, N.Y.

(7) Eckles, C. H., Gullickson, T.W., and Palmer, L.S. Phosphorus Deficiency in the Rations of Cattle. Univ. of Minn. Agr. Exp. Sta., Tech. Bull. 91. 1932.

(8) Forbs, E.B. and Keith, M.H. A Review of the Literature of Phosphorus Compounds in Animal Metabolism. Ohio Agr. Exp. Sta., Tech. Bull. 5. 1914.

(9) Hart, E.B., Bohstedt, G., Deobald, H.J., and Wegner, M.I. The Utilization of Simple Nitrogenous Compounds Such as Urea and Ammonium Bicarbonate by Growing Calves. J. Dairy, Sci., 22:785. 1922.

(10) Hart, E.B., McCollum, E.V., and Humphrey, G.C. The Role of Ash Constituents of Wheat Bran in the Metabolism of Herbivora. Univ. Wis. Agr. Exp. Sta., Res. Bull. 5. 1909.

(11) Hawk, P.B., Oser, B.L., Summerson, W.H. 1949. Practical Physiological Chemistry. 12th ed. Maple Press Co. York, Pa.

(12) Hays, V.W., Ashton, G.C., Lin, C.H., Speer, V.C., and Catron, D.V. Studies of Utilization of Urea by Growing Swine. J. Animal Sci., 16:44. 1957.

(13) Horwitz, William. 1955. Official Methods of Analysis of the Association of Official Agricultural Chemists. 8th ed. Association of Official Agricultural Chemists, Washington, D.C.

(14) Huffman, C.F., Duncan, C.W., Robinson, C.S., and Lamb, L.W. Phosphorus Requirement of Dairy Cattle When Alfalfa Furnishes The principle Source of Protein. Mich. State Agr. Exp. Sta., Tech. Bull. 134. 1937.

(15) Kleiber, M., Smith, A.H., Ralston, N.P., and Black, A.L. Radiophosphorus (P^{32}) as a Tracer for Measuring Endogenous Phosphorus in Cow's Feces. J. Nutr. 45: 253. 1951.

(16) Lamb, L.W., Winter, O.B., Duncan, C.W., Robinson, C.S., and Huffman, C.F. A Study of Phosphorus Requirement of Dairy Cattle. II. Phosphorus, Calcium and Nitrogen Metabolism of the Dairy Cattle When Alfalfa Furnishes the Principle Source of Protein. J. Dairy Sci., 17:233. 1934.

(17) Lassiter, C.A. Palatability Trials with Di-Ammonium Phosphate. Unpublished Data. 1959.

(18) Lassiter, C.A., Grimes, R.M., Duncan, C.W., and Huffman, C.F. High-Level Urea Feeding to Dairy Cattle. III. Effect on Performance and Metabolism of Lactating Dairy Cows. Quarterly Bulletin of the Mich. State Agr. Exp. Sta., 41:326. 1958.

(19) Liu, C.H., Hays, V.W., Svec, H.J , Catron, D.V., Ashton, G.C., and Speer, V.C. The Fate of Urea in Growing Pigs. J. Nutr., 57:241. 1955.

(20) Loosli, J.K., Decker, R.B., Huffman, C.F., Phillips, P.H., and Shaw, J.C. Nutrient Requirements of Domestic Animals. Nat. Ac. Sci. - Nat. Res. Con., Pub. 464. 1956.

(21) Loosli, J.D. and McCay, C.M. Utilization of Urea by Young Calves. J. Nutr., 25:197. 1943.

(22) Morrison, F.B. 1956. Feeds and Feeding. 22nd ed. The Morrison Publishing Co. Ithaca, New York.

(23) Palmer, L.S., Cunningham, W.S., and Eckles, C.H. Normal Variations in the Inorganic Phosphorus Level of the Blood of Dairy Cattle. J. Dairy Sci., 13:174. 1930.

(24) Parham, B.T., Frye, Jr., J.B., Kilpatrick, B.L., and Rusoff, L.L. A Comparison of Ammoniated Molasses, Urea, and Cotton Seed Meal as a Source of Nitrogen in the Ration of Dairy Heifers. J. Dairy Sci., 38:664. 1955.

(25) Pearson, R.M., and Smith, J.A.B. The Utilization of Urea in the Bovine Rumen. 2. Conversion of Urea to Ammonia. Biochem. J., 37:148. 1943.

(26) Rose, W.C., Smith, C.L., Womack, M., and Shane, M. The Utilization of the Nitrogen of Ammonium Salts, Urea, and Certain Other Compounds in the Synthesis of Non-essential Amino Acids in Vivo. J. Biol. Chem., Vol. 181:307. 1949.

(27) Rust, J.W., Lassiter, C.A., Davis, C.L., Brown, L.D., and Seath, D.M. The Utilization of Dicyandiamide and Urea by Lactating Dairy Cows. J. An. Sci., 15:1133. 1956.

(28) Thompson, N.R., Graf, G.C., Eheart, J.F., and Holdaway, C.W. The Utilization of Urea by Dairy Cattle. J. Dairy Sci., 35: 1010. 1952.

(29) Shaw, J.C., Caskey, C.D., and Cairns, G.M. Di-ammonium Phosphate as a Protein Supplement for Ruminants. Feedstuffs, May 25, 1946.

(30) Ward, G.M., Huffman, C.F., Duncan, C.W. Urea as a Protein Extender for Lactating Cows. J. Dairy Sci., 38:299. 1955.

(31) Wegner, M.I., Booth, A.N., Bohstedt, G., and Hart, E.B. The Utilization of Urea by Ruminants as Influenced by the Level of Protein in the Ration. J. Dairy Sci., 24:835. 1941.

(32) Wegner, M.I., Booth, A.N., Bohstedt, G., and Hart, E.B. Preliminary Observation on Chemical Changes of Rumen Ingesta With or Without Urea. J. Dairy Sci., 24:51. 1941.

(33) Wegner, M.I., Booth, A.N., Bohstedt, G., and Hart, E.B. The "In Vitro" Conversion of Inorganic Nitrogen to Protein by Microorganisms from the Cows Rumen. J. Dairy Sci., 23: 1123. 1940.