THE INFLUENCE OF FREQUENCY OF FEEDING
ON THE
UTILIZATION OF UREA NITROGEN

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TABLE OF CONTENTS

INTRODUCTION

During the recent war years and the resulting shortage of protein supplements there has been widespread interest in the possibility of urea utilization by ruminants. Numerous investigators have demonstrated that microorganisms in the rumen can utilize the nitrogen of urea, provided it is fed at a proper level in combination with materials which provide a suitable substrate for growth of the microorganisms. Other questions remain to be answered. Information is needed concerning the exact chemical and biological mechanisms involved in the utilization of urea by the multi-stomached animals. Likewise, there are many problems relating to the practical aspects of urea feeding on the range and in the dry lot that need investigation.

Urea is the chief end product of protein metabolism in mammals. It is a diamide having the formula $CO(NH_2)_2$. In the presence of the enzyme urease, and also upon standing in either acid or basic solutions urea is hydrolyzed to NH_3 and CO_2. It has been demonstrated by Pearson and Smith (1943) that the formation of NH_3 is the first step in protein synthesis from urea; the microorganisms then utilize the NH_3 to form body protein. It is generally accepted that these microorganisms are then digested and the resulting amino acids absorbed by the host. Pearson and Smith (1943) have further shown that when the concentration of NH_3 becomes too high the synthesis of protein is reduced. From these observations it would seem that more urea could be utilized by feeding several small quantities than from one large feeding. Work at the Oklahoma Station by Darlow et al (1946) has supported this hypothesis. Animals receiving one feeding of urea each two days made very poor gains, while Briggs et al (1946) secured good utilization of urea when fed at approximately the same level but twice daily in metabolism studies.

This experiment was designed to study the nitrogen utilization of cottonseed meal and an urea containing pellet when each was fed twice daily as compared to the performance when each was fed on alternate days. The urea pellet had the same composition as those which had given unsatisfactory performance on the range with heavy steers; the pellet had 25 percent of its total nitrogen supplied by urea.

REVIEW OF PREVIOUS INVESTIGATIONS

Armsby (1911) was one of the first to review the literature on the subject of protein synthesis from non-protein sources. He concluded that there was evidence that microorganisms in the rumen of polygastric animals were able to synthesize protein from non-protein sources to a limited extent. Later numerous investigations were conducted in Europe, especially Germany, trying to establish whether or not the nitrogen of urea could be utilized by ruminants. Aselsson (1942) reviewed the literature on the subject of urea utilization and concluded that urea given alone has a toxic effect and that it is best given in such combinations as dried beet pulp, molasses, and urea. According to this summary about 1/3 of the nitrogen of normal rations of ruminants can be replaced by urea provided the energy supply is maintained. Two other German investigators, Schmidt and Kleesch (1943) report poor utilization of urea nitrogen by growing cattle. These results might be attributed to the high level at which they fed the urea.

Hart and co-workers (1939) worked with young dairy stock in a study of urea utilization. They fed a control group a basal ration of corn starch and timothy hay while another lot received this basal ration plus urea and still a third group the basal ration plus casein. They concluded that urea was utilized, that a readily fermentable carbohydrate increased utilization, and that urea was more effective when fed at a level of 43 percent of the nitrogen ingested then at a level of 66 or 70 percent. Wegner, Booth, Bohstedt and Hart (1941) (1943) carried the urea investigation further by using a heifer with a rumen fistula. They determined the total nitrogen, non-protein nitrogen and NH_3 nitrogen of the rumen contents at various intervals following feeding and secured evidence on the conversion of urea nitrogen to protein. They then studied the effect of protein level and urea level on the rate of protein synthesis by using the rate of NH_3 formation and disappearance as an index to the rate of protein synthesis. When the protein level of the rumen ingesta increased above 12 percent the conversion of urea nitrogen to protein began to decrease, and when no protein concentrate was present the urea was utilized up to a level of 4.5 percent of the total ration. Further work at Wisconsin by Mills, Booth, Bohstedt and Hart (1943) using the rumen fistula technique demonstrated that corn starch greatly increased the utilization of urea nitrogen. Later work by Mills, Lardinois, Rupel and Hart (1944) gave similar results. Rupel (1944) reports that the concentration of urea in a ration should not exceed 3 percent, and the protein equivalent of the concentrate should not exceed 18 percent.

Pearson and Smith (1943) used a rumen fistula in a heifer and concluded after determinations of the total nitrogen and non-protein nitrogen of the ingesta that non-protein nitrogen was converted to protein. Similar investigations by Wegner and others (1940) at Wisconsin gave similar results.

Rupel, Bohstedt, and Hart (1943) compared urea and linseed meal as a source of supplemental nitrogen. In one ration urea supplied 46 percent of the nitrogen in the ration of dairy cows while the linseed meal was used as the source of protein in the control ration. They reached the conclusions that there was no significant difference between the two for milk production, and that urea should not be fed above a level of 1 percent of the dry matter of the ration or 3 percent of the concentrate. Owen, Smith and Wright (1943) compared blood meal and urea as sources of dietary nitrogen. In one ration urea supplied 25 percent of the total nitrogen intake, while blood meal furnished the supplement in the other ration. Observations were made concerning nitrogen balance, milk production, and weight gains. There was no significant difference between the two rations for milk productions and weight gains. About 25 percent more nitrogen appeared in the urine of animals fed the urea ration than in that of those fed the blood meal. Bartlett and Cotton (1938) working with young dairy heifers found that additions of .127 lbs. of urea daily to a low protein ration resulted in .24 lbs. more average daily gain.

Harris, Work, and Henke (1943) conducted nitrogen balance experiments on steers in which they compared urea with soybean oil meal when both were added to a basal ration containing 1.44 percent protein. The biological value of the protein of the urea ration was 34 and that of the soybean oil meal 60. They attributed the low biological value for urea to the high level at which it was fed.

Briggs et al. (1946) conducted investigations on the value of urea for nitrogen storage, fattening, and wintering of beef steers. Twenty-five percent and 50 percent of the nitrogen of cottonseed meal was replaced by urea and both cottonseed meal and the urea containing pellets were compared as ration supplements. They concluded from the standpoint of nitrogen retention that the 25 percent pellets were superior to the 50 percent pellets and steers utilized the 25 percent urea pellets as well as cottonseed meal. In the fattening study at the Oklahoma Station both 25 percent and 50 percent urea pellets were satisfactory for calves weighing approximately 425 lbs. The experiment was run for two periods averaging 160 days each and unpublished data give an average daily gain of 1.84 lbs. for cottonseed meal, 1.86 lbs. for 25 percent urea pellets and 1.80 lbs. for 50 percent urea pellets. Darlow and co-workers (1946) compared cottonseed cake, soybean cake, and 25 percent urea pellets in a wintering study with two year old steers. The urea pellets were unsatisfactory giving a daily gain of only .05 lbs. as compared to a daily gain of .26 lbs. for the cottonseed cake and .36 lbs. for soybean cake.

Harris and Mitchell (1941)(1941a) studied the value of urea nitrogen in sheep for maintance and growth. They found that urea nitrogen could replace at least 90 percent of the endogenous nitrogen loss with an efficiency of about 60 percent. In the growth studies an 11 percent protein level, in which urea supplied 50 percent of the nitrogen, produced normal growth. Johnson and co-workers (1944) found that defaunated sheep utilized urea nitrogen as well as normal sheep indicating that bacteria, and not protozoa, are responsible for the protein synthesis.

Hart and associates (1939) reported that kidney damage followed feeding urea at a level of 4.3 percent of the dry matter of the ration and that some damage resulted from feeding it at a 2.8 percent level. Work, Hamre, Henke, and Harris (1943) carried out an investigation on two lots of cattle, one of which received urea at the rate of .88 percent of the dry matter consumed and the other at a 2.29 percent level. Histological studies of the livers and kidneys showed no pathological symptoms. These results indicate that the toxic level of urea lies between 2.29 percent and 2.8 percent. Briggs (1946) working at the Oklahoma Station fed steers that weighed approximately 450 lbs. and intake of .4 lbs. of urea per day for 14 days and observed no apparent ill effects. It was estimated that this was approximately 2.6 percent of the total dry matter intake.

EXPERIMENTAL OBJECTIVES

This study was designed for two purposes: (1) to compare the relative protein supplementing value of cottonseed meal and a feed in which 25 percent of the nitrogen was supplied by urea, and (2) to study the effect of frequency of feeding on urea utilization.

EXPERIMENTAL PROCEDURE

For this study seven 2-year old Hereford steers were placed in false bottom metabolism stalls and fed four experimental rations. The rations are given in table 1.

Table 1. Daily allowance of feeds used in digestion and nitrogen utilization studies with steers.

Feed	Daily allowance in grams			
	Ration A	Ration B	Ration C*	Ration D*
Prairie Hay	4536	4536	4536	4536
Urea Pellets (25% Urea N)	1360	--	1363*	--
Cottonseed Meal	--	1360	--	1363*

* The prairie hay in all rations was fed two times daily at the rate of 2268 grams per feed. In rations A and B the concentrate was fed two times daily, 680 grams per feed; in rations C and D the concentrate was fed on alternate days with 2727 grams being fed per feed.

It will be seen from study of table 1 that ration A is the same as ration C except for frequency of feeding of the concentrate, the same applies to ration B and D. The quantity of nitrogen offered during the experimental periods was approximately the same in all rations.

Originally there were eight steers on the four rations but one refused to eat and was discarded during the first trial. The experiment consisted of four trials, the animals were rotated after each trial so that all the steers were on each ration. Before each collection period the steers were placed on a 10-day preliminary period in order that they might become adjusted to the new ration. This was followed by a 10-day collection period during which daily individual collections of urine and feces were made.

The chemical composition of the constituents of the rations is given in table 2.

Table 2. Chemical analysis of feed fed steers on nitrogen utilization studies.

Feed	Chemical composition of dry matter percent					
	Trial	Protein	Fat	Fiber	NFE	Organic Matter
Prairie Hay	1	4.54	2.50	33.35	52.90	93.29
	2	4.77	2.28	33.38	52.20	92.83
	3	3.87	2.62	32.97	53.67	93.13
	4	4.42	1.90	31.88	53.49	91.69
Urea Pellets	1-4	47.03	5.68	8.76	32.21	93.70
Cottonseed Meal	1-4	43.30	8.90	9.77	32.10	94.10

During the collection periods samples of hay were taken at each feeding and these were analyzed at the conclusion of each 10-day collection period. Since the urea pellets and cottonseed meal fed were all of the same lot only one analysis of these two feeds was made.

The urea pellets used contained the following ingredients by weight:

Urea (Dupont's '262')	4%
Cottonseed Meal	75%
Blackstrap Molasses	10%
Hominy Feed	11%
Total	100%

Approximately 25 percent of the nitrogen was supplied by the urea and the level of urea in the complete ration was approximately 1 percent of the total dry matter offered.

In this study nitrogen storage, apparent coefficient of protein digestability, and biological value were used as criteria of nitrogen utilization. This necessitated quantitative collection of urine and feces and careful weighing of the feed that was fed. No feed was refused during the study. The steers were placed in metabolism stalls and urine was collected by means of funnels strapped to the animals, a hose led from the funnel into a glass collecting bottle beneath the floor of the stall. Enough sulfuric acid was added to the collecting bottle to neutralize the urine and toluene was added to minimize bacterial activity. Comparisons between the nitrogen content of fresh samples and samples after storage gave evidence that no notrogen was being lost through volitalization. Feces were collected by means of a box at the rear of the stalls and an aliquot of each days collection was preserved in the refrigerator with addition of a few drops of a 15 percent solution of thymol in ethyl alcohol. In the first two trials daily determinations were made on the nitrogen excreted in the urine but in the last two trials a 2 percent aliquout was taken from each days collection. This composite sample was stored in the refrigerator and the nitrogen content determined on duplicate 5 ml. samples at the conclusion of each 10-day collecting period. A 2 percent aliquout was taken of each daily collection of feces and the nitrogen determinations were run on triplicate 10 gm. wet samples at the end of each period.

The Kjeldahl method was used for the nitrogen determination and the proximate analysis were made as specified by the A.O.A.C. (1940). The equations of Swanson and Herman (1943) were used to calculate the metabolic and endogenous nitrogen. The data were treated statistically by the analysis of variance method described by Snedecor (1946).

EXPERIMENTAL RESULTS

The nitrogen utilization of each of the rations is given in Table 3. The values are given for each steer when fed each ration and represent the average of ten day periods. There was some variation in nitrogen storage values within each ration, but because of many interacting factors such variations usually occur in this type of study. There was a difference in nitrogen intake of approximately 6 gm. daily between the urea and cottonseed meal supplemented rations but this represents only about 5 percent of the total nitrogen intake. The urinary nitrogen excretions were higher in both urea rations than in the cottonseed meal rations, this is in agreement with work of Owen et al. (1943). In every case the fecal nitrogen was lower in the urea rations but there was no significant difference in fecal nitrogen between rations A and C. The daily nitrogen storage by steers fed the urea pellets was 15.7 gm. when it was fed twice daily as compared to 15.4 gm. for cottonseed meal fed at the same interval. The daily nitrogen storage from the urea containing pellets when the same pellets were fed on alternate days was only 13.1 gm. or 2.6 gm. less per day. This difference in the storage of nitrogen from the same feed fed at two different feeding intervals was not significant at the 5 percent level.

There was an average of 110.5 gm. of dietary nitrogen absorbed from the ration supplemented with urea pellets when the supplement was fed twice daily. This value can be compared to 99.2 gm. of dietary nitrogen absorbed when cottonseed meal was fed two times daily to supplement the basal ration of prairie hay. Corresponding values

Table 3. Average daily nitrogen utilization values for each steer on each ration in metabolism studies.

Ration No.	Steer No.	Dietary N Gm.	Urinary N Gm.	Fecal N Gm.	N Storage Gm.	Dietary N absorbed Gm.	Dietary N retained Gm.	Biological value
A	1	124.0	60.0	41.3	22.7	111.6	59.9	53.7
	2	125.0	65.8	42.0	17.2	112.6	55.6	49.4
	3	119.3	71.2	37.2	10.9	110.9	48.0	43.3
	4	122.9	65.1	42.3	15.5	109.4	53.1	48.5
	5	124.0	67.3	42.9	13.8	110.0	50.6	46.0
	7	119.3	63.5	40.7	15.0	107.5	53.6	49.9
	8	122.9	67.9	40.5	14.6	111.3	51.6	46.3
	Ave.	122.5	65.8	41.0	15.7	110.5	53.2	48.2
B	1	116.5	57.9	47.0	11.6	98.3	49.1	50.0
	2	117.5	55.3	43.7	18.5	102.8	56.1	54.6
	3	118.5	56.3	47.1	15.1	100.1	52.1	52.0
	4	112.9	56.7	40.6	15.6	101.2	53.0	52.3
	5	116.5	61.4	47.8	7.3	97.6	44.5	45.5
	7	118.5	47.6	51.5	19.4	95.8	55.9	58.4
	8	112.9	49.0	43.4	20.5	98.4	57.2	58.1
	Ave.	116.2	54.9	45.9	15.4	99.2	52.5	53.0
C	1	119.5	67.8	45.7	6.0	102.7	43.1	42.0
	2	123.2	76.6	37.0	9.6	115.0	47.3	41.1
	3	124.2	66.3	43.2	14.7	110.0	51.9	47.2
	4	125.2	70.4	44.6	10.2	109.3	47.2	43.2
	5	119.5	69.6	39.0	10.9	109.4	47.7	43.7
	7	124.2	54.4	42.0	27.8	111.1	64.4	58.0
	8	125.2	67.5	45.4	12.3	108.4	48.7	45.0
	Ave.	123.0	67.5	42.4	13.1	109.4	50.1	45.7
D	1	118.8	48.8	49.0	21.0	98.4	58.0	58.9
	2	113.1	52.0	56.6	4.5	83.4	41.9	49.1
	3	116.7	52.4	48.6	15.7	97.0	53.1	54.7
	4	117.8	51.9	50.9	15.0	95.8	52.3	54.6
	5	118.8	54.2	48.5	16.1	99.0	52.9	53.4
	7	116.7	50.5	44.3	21.9	102.3	59.0	57.6
	8	117.8	58.4	45.8	13.6	101.0	50.4	48.9
	Ave.	117.1	52.6	49.1	15.4	96.7	52.5	53.9

of 109.4 gm. and 96.7 gm. respectively were obtained when the same supplements were added to prairie hay on alternate days. These differences in the absorption of nitrogen at the different frequencies of feeding did not prove to be significant. The dietary nitrogen retained was very nearly equal for all rations, being 53.2 gm. when rations contained the urea pellets fed twice daily, 52.5 gm. when cottonseed meal furnished the supplement and was fed twice daily. The values were 50.1 and 52.5 gm. respectively when the same supplements were used but fed on alternate days.

The biological values of the protein in the rations were 48.2 for ration A, 53.0 for ration B, 45.7 for ration C, and 53.9 for ration D. The only significant difference was between ration C in which the urea containing pellets were fed on alternate days and ration D in which cottonseed meal was fed on alternate days.

Table 4 gives the average apparent digestion coefficients of the nutrients in each ration. The apparent digestion of crude protein (Nx6.25) was 67.3 percent for ration A, 60.6 percent for ration B, 64.2 percent for ration C and 58.2 percent for ration D. The crude protein digestability of the urea containing rations was significantly higher than the cottonseed meal supplemented rations when both were fed twice daily and also when both were fed on alternate days. These differences were significant at the 1 percent level. The difference in crude protein digestability between the urea containing pellets when fed twice daily as compared to alternate days was significant at the 5 percent level. There were no significant differences between rations in the digestability of other nutrients, although there

Table 4. The average apparent digestion coefficients of nutrients in each ration fed to steers.

	Apparent digestibility (percent) of:				
Ration Number	Crude Protein	Ether Extract	Crude Fiber	NFE Extract	Organic Matter
A	67.3	67.0	66.1	60.4	63.2
B	60.6	70.9	64.4	58.3	60.6
C	64.2	65.9	68.3	60.7	63.9
D	58.2	72.7	63.5	57.8	60.5

was a general tendency for all digestion coefficients except those of ether extract to be higher in the rations in which urea was used than in those supplemented by straight cottonseed meal.

DISCUSSION

The fact that urea nitrogen can be utilized when properly fed has been demonstrated by numerous investigators. The results of this study further confirm this observation. Steers stored as much nitrogen on the ration in which the urea containing concentrate was fed twice daily as did steers receiving approximately the same nitrogen content in cottonseed meal. In this experiment, urea comprised approximately 1 percent of the total dry matter of the complete ration or 4 percent of the concentrate. At this level approximately 25 percent of the nitrogen was supplied by urea. Work by Rupel et al. (1943) and Briggs and Co-workers (1946) has demonstrated that this is about the optimum level of urea feeding for maximum use of urea nitrogen by cattle.

In this study steers receiving the urea containing concentrate twice daily stored 15.7 gm. of nitrogen, those receiving cottonseed meal stored 15.4 gm. of nitrogen. These values indicate that for nitrogen storage the urea pellets were equal to cottonseed meal at this frequency of feeding. Animals receiving the urea pellets on alternate days stored 13.1 gm. of nitrogen daily as compared to 15.7 gm. for those receiving the same concentrate twice daily. For significance at the 5 percent level a difference of 5.78 would have been required. While the difference obtained was not significant statistically there is a strong indication that the animals utilized the urea nitrogen more effeciently when fed at frequent intervals. When the urea pellets were fed on alternate days the animals received 2727 gm. per feeding, this represents about 99 gm. of pure urea, while animals being fed twice daily received about 27 gm. of pure urea per feeding. Pearson and Smith (1943) and Wegner et al. (1941), (1943) have observed that ingested urea seems to be converted to ammonia in the rumen and that the ammonia is mostly formed within six hours after ingestion. It would seem from these observations that the concentration of ammonia in the rumen of the animal receiving the large feeding would be considerably higher than that in the animal receiving the small feeding. Pearson and Smith (1943) further suggest that the efficiency of protein synthesis seems to vary inversely with the ammonia concentration in the rumen; if this is true the animals receiving the large feedings should not be expected to be as efficient in the utilization of the urea nitrogen as those receiving the smaller amounts at more frequent feeding intervals. The frequency of feeding seemed to have no effect on the utilization of the nitrogen of cottonseed meal because the steers stored 15.4 gm. at both frequencies of feeding.

The dietary nitrogen absorbed was consistently higher for the rations supplemented with urea pellets than for those supplemented with straight cottonseed meal. This increased nitrogen digestability of urea rations was highly significant. Quite possibly the ammonia in the rumen, which is not utilized by bacteria, is absorbed into the blood stream and the nitrogen appears in the urine. The larger urinary nitrogen values obtained in this study for the urea rations support this hypothesis. There was little difference in the dietary nitrogen absorbed between ration A in which urea pellets were fed twice daily and ration C in which they were fed on alternate days, indicating that the frequency of feeding the urea supplemented rations had little effect on the digestability of the dietary nitrogen.

Since the estimated metabolic nitrogen and endogenous nitrogen, as calculated according to the equations of Swanson and Herman (1943), were nearly constant for all the animals the differences in dietary nitrogen retained were about the same as the differences in daily nitrogen storage. These values were 53.2 gm. for ration A, 52.5 gm. for ration B, 50.1 gm. for ration C and 52.5 gm. for ration D.

The estimated biological value of the protein of the urea containing concentrate when fed twice daily was 48.2 as compared to 53.0 for the cottonseed meal. Since biological value bears an inverse relationship to nitrogen digestability this lower value for the ration supplemented with urea pellets is to be expected. The biological value of the protein of ration C was 45.7, this was slightly lower than the value 48.2 obtained for ration A. This would be expected if it is assumed that when urea is fed in large quantities less is converted to protein by the bacteria in the rumen and consequently more is absorbed into the blood stream as ammonia or passed off as gas via the mouth in the process of rumination. Ammonia in the blood stream would not be utilized and the percent of absorbed nitrogen retained would decline. The biological values obtained for the protein of cottonseed meal were affected very little by frequency of feeding; these values were 53.0 when the concentrate was fed twice daily and 53.9 when the cottonseed meal was fed on alternate days.

The nitrogen utilization values were all lower when the urea pellets were fed on alternate days than when the same pellets were fed twice daily; in contrast the values for cottonseed meal were not consistently altered by the frequency of feeding. Since 75 percent of the nitrogen of the urea pellets was supplied by cottonseed meal it would seem that this observed lowering of nitrogen utilization values due to frequency of feeding is to be attributed to the urea in the ration, and quite possibly, as was mentioned earlier to the increased concentration of ammonia produced in the rumen.

SUMMARY

Nitrogen balance trials were conducted on seven 2-year old Hereford steers to compare the nitrogen utilization of the rations when low grade prairie hay was supplemented by cottonseed meal and a pellet in which 25 percent of the nitrogen was supplied by urea. Each steer was fed each supplement at a level to provide approximately the same nitrogen intake; both supplements were fed at two intervals of feeding which consisted of feeding twice daily or one feed on alternate days. Nitrogen storage, apparent coefficient of nitrogen digestability and biological value were used as criteria of nitrogen utilization.

In this study animals receiving the urea pellets twice daily stored slightly more nitrogen than those receiving approximately the same quantity of nitrogen in cottonseed meal, the daily values being 15.7 gm. and 15.4 gm. respectively. Steers receiving the urea containing concentrate at the rate of one feeding each two days stored only 13.1 gm. of nitrogen daily or 2.6 gm. less nitrogen than was stored when the concentrate was fed twice daily. Frequency of feeding made no difference in daily nitrogen storage values when cottonseed meal supplied the supplement; the daily nitrogen storage was 15.4 gm. at both frequencies of feeding.

The apparent coefficients of nitrogen digestability were significantly higher for both rations which were supplemented with the urea pellets than for the cottonseed meal supplemented rations, the values obtained were 67.3 percent for the ration supplemented with the urea pellet fed twice daily, 64.2 percent when the urea pellet was fed on alternate days, 60.6 percent for the ration supplemented with cottonseed meal fed twice daily, and 58.2 percent when the same supplement was offered on alternate days. The difference in the digestibility of the rations supplemented by urea containing pellets, due to the interval of feeding, was significant while

the same comparison for the cottonseed meal supplemented rations was not significant.

The estimated biological values of the protein in the rations ranged from 48.2 to 53.9 but there were no significant differences. The nitrogen utilization of urea was not as efficient when the pellet containing the product was fed on alternate days as when it was fed twice daily. The interval of feeding did not affect the efficiency of cottonseed meal in supplementing the rations.

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