is considerably more finely subdivided than the chromatographic grade, columns containing polyethylene upon the former operate comparatively slowly —e.g., up to 1.5 hours is required, unless pressure is applied on the eluting liquid. For routine use about 1-pound air pressure is applied. In a quantitative test, five extracts from 50-gram samples of canned corn were allowed to run through a regular column of reground polyethylene on chromatographic alumina with 200 ml. of 40% acetonitrile as the eluting agent. The periods required varied from 50 to 91 minutes, the purification ranged from 93 to 97%, and the recovery of parathion added at 7 p.p.m. was from 86 to 91%. A similar set of five extracts put through under 5 pounds of air pressure required 1.5 to 4.9 minutes, giving a purification of 76 to 86% and a recovery of parathion of 86 to 91%.

Successful use of the Heisler polyethylene powder directly as a column material—i.e., without precipitating upon alumina granules—has been reported from the research laboratories of the National Canners Association (*3*). This result has been confirmed in the case of DDT and methoxychlor added at the rate of 1.9 p.p.m. to green beans, carrots, and grapes. With 60% acetonitrile as eluent the purifications with the same order of products were 87, 94, and 78% and the recoveries (DDT, methoxychlor) were 106, 105; 87, 100; 69, 101. The sample of powdered polyethylene which was supplied for the present work varied widely in size and shape of the particles and tended to pack in the column so tightly, that excessively long periods were required to force the eluent through. With heavily contaminated samples there was a tendency to plug completely. Because the coated granules are free from this objection they are preferred, but it may be possible to secure pure polyethylene powder which would perform satisfactorily.

Mode of Operation of Column. At least two processes are concerned in the action of the polyethylene column as it has been used for cleanup of extracts. It is a filter with very fine passages and hence it retains the part of the extractives which is thrown out of solution during the transfer into 40 or 60% acetonitrile. It also is an effective partition column. When small amounts of the three dyes Oil Blue, Oil Orange, and Sudan Yellow, which are somewhat soluble in strong acetonitrile solutions, are put on the column they move in the following decreasing order: Sudan Yellow, Oil Orange, Oil Blue. This is the same order as on a reverse phase paper strip. Thus the more polar the dye the faster it passes through the column, which is in agreement with the interpretation that partition occurs between the highly nonpolar stationary polyethylene phase and the somewhat polar moving acetonitrile phase.

It is unlikely that the alumina plays any part in the action of the column when aqueous acetonitrile solutions are used as eluents, but it might be of importance with nonaqueous systems. Hence a few simple tests were made to detect noncoated alumina granules. In the first place, the untreated alumina powder sinks almost instantly when agitated in water, but the polyethylene-alumina granules are not wetted, since the finer ones persist in floating upon the surface and only the larger ones sink under the influence of gravity. Thus the granules appear to have a polyethylene surface.

Under the microscope the particles may be seen to vary in size and shape in the same manner as the original alumina used. They are of uniform appearance, except a few rather coarse, translucent chunks which probably are pure polyethylene. A typical batch made to contain approximately 20% polyethylene by weight was separated by screening into the following fractions: held on 35 mesh, 10% by weight; held on 60 mesh, 10%; held on 100 mesh, 22%; held on 150 mesh, 33%; fines—i.e., through 150 mesh, 25%. The polyethylene content of each fraction was determined by dissolving in boiling toluene with the following results in the same order: 31, 33, 20, 20, 19.5% by weight. The chunks of polyethylene in the coarse fractions would account for the excess found in them. The very close agreement with the theoretical content in the fine fractions indicates that polyethylene was present as a coating on the alumina particles as their appearance also indicated.

If substantially all the alumina granules are covered by a layer of polyethylene, the addition of a relatively small amount of untreated alumina should change the behavior of the column with nonaqueous eluents, but if noncoated alumina is already present, an extra amount should have relatively little effect. The experiment was made of adding the dye Sudan Yellow and eluting with iso-octane. The dye came through a regular column in the 55- to 95-ml. cut. But when 20% free alumina was mixed in the column, the dye moved very slowly and did not even start to emerge when over 200 ml. of eluent had passed. Thus by these four rather different criteria the column acts as if it consists of granules presenting only a polyethylene surface to the eluting liquids.

Acknowledgment

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RUMINANT NUTRITION

Ammonia Formation in the Bovine Rumen with Various Diets

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During the past decade research has been conducted on the fate of protein and other nitrogenous materials consumed by ruminants. The nitrogen nutrition of the animal is largely controlled by the balance of three processes: the net conversion of dietary protein to microbial protein; breakdown of ammonia from dietary protein and nonprotein nitrogen sources to ammonia and the extent to which the ammonia is absorbed through the rumen wall; and the synthesis of microbial protein from the nonprotein nitrogen of the rumen (*7*). One of the important items in ruminant nutrition is to determine, with certain protein sources and practical diets, which of these processes are dominant (*19*). The interaction of these opposing tendencies is probably a major factor leading to the relative

This work was undertaken to accumulate additional evidence concerning the fate of the nitrogen of soybean oil meal, cottonseed meal, Morea, and cottonseed hulls in the rumen. The results indicate that, although the release of ammonia from Morea is rapid and follows a similar pattern as when urea is fed, the danger of alkalosis may be lessened due to the buffering action of phosphoric acid which keeps the pH near 7. The release of ammonia from cottonseed meal, soybean oil meal, and cottonseed hulls was very low. The high protein level present in the rumen liquor when soybean oil meal is fed may indicate some special advantage for this feed in the diet of ruminants.

constancy of the biological value of food nitrogen for ruminants (*11*). The complexity of the problem is recognized when one realizes that the balance between these processes is largely controlled by the nature and the activity of the microbial flora, which in turn are dependent on the diet of the animal.

The levels of ammonia and crude protein in the rumen contents at intervals after feeding rations with and without urea were determined in a series of investigations at the University of Wisconsin (*15*, *20*, *21*, *22*). These workers reported that ammonia nitrogen was released rapidly from urea in the rumen, reaching its peak at 1 hour in most cases. A peak in ammonia nitrogen in the rumen contents was reached at 3 hours under certain feeding practices (*15*). Very rapid hydrolysis of urea was also observed by Lenkeit and Becker (*12*) and by Wegner *et al.* (*21*). Pearson and Smith (*17*) observed that protein synthesis from ammonia, and protein breakdown with the production of ammonia appeared to proceed at the same time in rumen contents; but that synthesis exceeded breakdown when starch, maltose, or another simple sugar was present. Influence of protein (*5*), sources of energy (*1*), and minerals (*6*) on urea utilization has been reported.

The release of ammonia nitrogen from urea, ammoniated invert molasses, and soybean oil meal in the presence of rumen microorganisms has been compared utilizing both rumen liquor and the artificial rumen technique (*18*). Belasco (*4*) has compared urea with soybean, linseed, cottonseed, and corn gluten meals in an artificial rumen; total volatile fatty acid production and the acetic acid level were unaffected by nitrogen substrate. When urea was present, there were higher levels of propionic acid and lower levels of butyric and valeric acids as compared to protein meals.

The work reported herein was undertaken to accumulate additional evidence concerning the fate of the nitrogen of soybean oil meal, cottonseed meal, Morea, and cottonseed hulls in rumen.

Experimental Procedure

In order to obtain data concerning the release of ammonia nitrogen from Morea (furnished by Feed Service Co., Crete, Neb.), soybean oil meal, and cottonseed meal, it was decided to feed these substances to an animal possessing a rumen fistula and measure the level of free ammonia in the rumen liquor.

Table I. Percentage of Nitrogen of Feedstuffs and Daily Ration Fed in Trials

Ingredient	Nitrogen, %	Ration, Lb. 1	2	3	Control
Cottonseed meal	5.76	3.04			
Soybean meal	6.58		2.66		
Morea	5.22			3.33	
Cottonseed hulls	0.72	36.00	36.00	36.00	36.00

A 7-year-old Holstein steer, fitted with a plastic rumen fistula plug, was used in the investigation. The roughage component of the ration was composed of cottonseed hulls fed ad libitum at 6:00 A.M. and 6:00 P.M. daily. Cottonseed hulls were used, because of their low nitrogen content and their uniformity and ease of mixing with the materials to be studied. The protein supplements were fed at the morning feeding only in amounts necessary to meet the recommendations of the National Research Council for the protein requirements of the animal at a maintenance level of feeding. The protein (or equivalent) of the materials used and the amounts fed daily are presented in Table I. The amounts fed are of equal protein equivalent. Water and a mineral mixture composed of dicalcium phosphate and salt (equal parts) were available at all times except on the day when samples were taken. In the sampling procedure, water was removed 1 hour after feeding, and withheld until the end of the trial, which was 12 hours after feeding. Each ration was repeated four times in a balanced single square design with one extra period for carry-over effects as described by Lucas (*13*) and as outlined below:

1	2	3
2	3	1
3	1	2
3	1	2

1 = cottonseed hulls + cottonseed meal
2 = cottonseed hulls + soybean meal
3 = cottonseed hulls + Morea

Morea is a mixture of cane molasses, urea, ethyl alcohol, phosphoric acid, and trace minerals.

Each ration was fed for 7 days and collections of rumen liquor were made on the eighth day. This has been shown to be sufficient in digestion trials where there are no radical changes in rations (*16*). On the day collections were made, grab samples were taken of rumen contents before feeding and at hourly intervals after feeding until 12 hours had elapsed. The hour before feeding was designated as the zero hour. Care was taken to secure 9 portions of approximately 50 grams each from the top, middle, and lower levels of the rumen ingesta and also at the anterior, medium, and posterior extremities of the rumen. These ingesta were squeezed through four thicknesses of No. 50 cheesecloth. The resultant rumen liquor was then taken immediately to the laboratory.

Determinations of pH were made on a Beckman Model H2 pH meter. Ammonia nitrogen was determined by the method of Conway (*8*, *9*). Total nitrogen was determined by the Kjeldahl method (*2*), and a protein precipitate of rumen liquor was made using the method of Folin and Wu (*10*). This filtrate was used to determine nonprotein nitrogen (*16*). Protein nitrogen was calculated as difference between total and nonprotein nitrogen.

A urine sample was collected whenever possible, and a pH determination made immediately after collection.

Results and Discussion

The data pertaining to the release of ammonia from Morea, cottonseed meal, and soybean oil meal are presented in Table II and Figure 1. There was a very rapid release of ammonia from Morea, following the same order as when urea alone was fed (*18*). A peak was reached at 2 hours. Very high concentrations of ammonia were present up to 4 hours after feeding, following which a rapid decline was noted. The ammonia released in the rumen when soybean meal was fed, reached a small peak at 3 hours following feeding. A

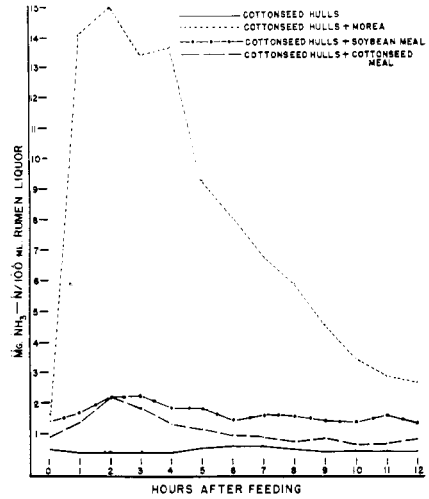

Figure 1. Release of ammonia nitrogen from cottonseed meal, soybean meal, and Morea in the rumen of a fistulated steer

Table II. Level of Ammonia Nitrogen in Rumen Liquor of Fistulated Steer Fed Various Nitrogen Sources

(Mg. NH_3 N/100 ml.)

Time Block	Hour after Feeding	Period	Trial 1	Trial 2	Trial 3	Sum
		1	(1)[a] 1.8	(2)[b] 2.2	(3)[c] 17.1	21.1
	1	2	(2) 3.6	(3) 10.4	(1) 0.6	14.6
		3	(3) 11.6	(1) 1.6	(2) 0.4	13.6
		4	(3) 17.4	(1) 1.2	(2) 0.6	19.2
		Sum	34.4	15.4	18.7	68.5
1						
		1	(1) 4.8	(2) 2.2	(3) 15.4	22.4
		2	(2) 4.7	(3) 15.8	(1) 1.0	21.5
	2	3	(3) 13.5	(1) 2.0	(2) 0.6	16.1
		4	(3) 15.4	(1) 1.1	(2) 1.4	17.9
		Sum	38.4	21.1	18.4	77.9
		1	(1) 2.8	(2) 3.4	(3) 13.4	19.6
	3	2	(2) 4.4	(3) 12.0	(1) 1.2	17.6
		3	(3) 13.2	(1) 2.4	(2) 0.6	16.2
		4	(3) 15.2	(1) 1.0	(2) 0.8	17.0
		Sum	35.6	18.8	16.0	70.4
2						
		1	(1) 1.5	(2) 2.4	(3) 9.8	13.7
		2	(2) 3.1	(3) 9.0	(1) 1.2	13.3
	4	3	(3) 12.1	(1) 1.4	(2) 1.1	14.6
		4	(3) 24.0	(1) 1.0	(2) 0.6	25.6
		Sum	40.7	13.8	12.7	67.2
		Total				
Time block 1		146.4				
Time block 2		137.6				
Over-all		248.0				

[a] Treatment 1 consisted of cottonseed meal and cottonseed hulls. [b] Treatment 2 consisted of soybean meal and cottonseed hulls. [c] Treatment 3 consisted of Morea and cottonseed hulls.

Table III. Summary of Analysis of Variance of Ammonia Release in the Rumen during 0 to 4 Hours

Variance Source	Degrees of Freedom	Sum of Squares	Mean Square
Total	47	1848.03	
Hours	3	5.73	1.91
Periods within hours	12	58.63	4.89[a]
Trials within hours	8	292.33	36.54[a]
Direct effects of treatment	2	1370.82	675.41[a]
Direct effects by time blocks	2	4.71	2.36
Error	20	115.81	5.79

[a] $P < 0.01$.

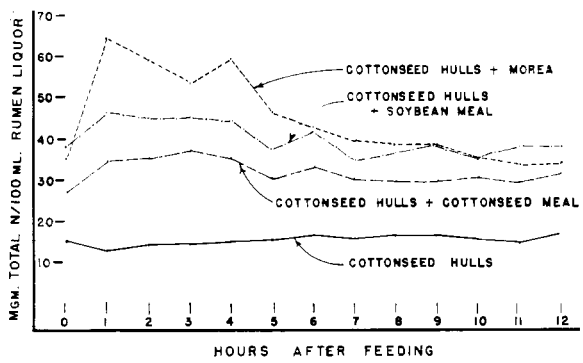

Figure 2. Level of total nitrogen present in the rumen liquor of a fistulated steer fed various nitrogen sources

rather uniform quantity was present from 4 to 11 hours following feeding, indicating that deamination occurred at a rather uniform rate over a longer period of time as compared with a sudden accelerated rate, as was the case with Morea. The release of ammonia from cottonseed meal reached a peak at 2 hours after feeding and declined rather rapidly; from 6 to 12 hours after feeding, it was only slightly above that released from the basal ration of cottonseed hulls. The amount of total nitrogen present in the rumen liquor when Morea, soybean meal, and cottonseed meal were fed, is presented in Figure 2. The concentration of total nitrogen is highest when Morea is fed. This is logical, because a molasses-urea mixture would be very soluble. A relatively high level of nitrogen was present when soybean meal was fed, indicating that relatively large proportions of the nitrogen of that meal are either soluble or suspended as small particles in the rumen liquor. The total nitrogen present in the rumen liquor, when cottonseed meal was fed, paralleled that of soybean meal, though at a slightly lower level. The nitrogen present was much higher when these nitrogen supplements were fed than when a basal ration of cottonseed hulls was fed.

The nonprotein nitrogen present in the rumen liquor following feeding of the nitrogen supplements is presented in Figure 3. The level was highest for Morea. This is logical, since almost all the nitrogen intake on this diet was from urea. Nonprotein nitrogen levels for soybean and cottonseed meals were similar and were above those observed for the basal ration of cottonseed hulls, but considerably below the values observed for Morea, especially during the first 6 hours after feeding.

The levels of protein nitrogen present in the rumen liquor when Morea, cottonseed meal, and soybean meal were fed are presented in Figure 4. The level of protein nitrogen was highest at all times when soybean meal was fed. This may have been due to a higher population of microorganisms in the rumen liquor, or to the fact that more protein is in solution and in suspension than for the other nitrogen sources studied. Further experimental work is needed before valid conclusions can be formulated. The protein values for cottonseed meal and Morea were somewhat variable, though approximately of the same magnitude. The protein levels of all the nitrogen sources were highest during the first 6 hours and were much higher than when the basal ration of cottonseed hulls was fed. It is admitted that these levels of ammonia nitrogen, total nitrogen, nonprotein nitrogen, and protein nitrogen are only semiquantitative in nature. Since the situation in which they exist involves a constantly moving system, heterogeneous in nature, it has opportunities for fluctuation at any time due to movement of nutrients out of the reticulo-rumen to the omasum and by the absorption of ammonia through the wall of the rumen.

The influence of the various rations on the pH of the rumen liquor is presented in Figure 5. The pH was more nearly alkaline when Morea and cottonseed hulls were fed; this was due to the large amount of ammonia released. This trend would have been higher were it not for the phosphoric acid present in the Morea. When the nitrogen supplements were fed, a low point of acid pH was reached 5 to 6 hours after feeding. A rise toward an alkaline pH when the low-nitrogen ration of cottonseed hulls was fed is unexplained. The pH of the urine of samples collected, for the most part, at approximately 2 hours after feeding averaged 5.7, 6.2, and 6.6 when rations containing cottonseed meal, soybean meal, and Morea, respectively, were fed.

The values for ammonia present in the rumen liquor at 1, 2, 3, and 4 hours after feeding are presented in Table II. These data were chosen for statistical analysis, since the 2- and 3-hour collection periods represented a peak in ammonia release for all of the nitrogen sources fed. The data are presented in the sequence outlined in the design for the experiment. The analysis of variance of these data was computed in accordance with procedures outlined by Lucas (*13*). A summary of the analysis of variance is presented in Table III. The direct effects of treatment were significant. Because of differences in direct effects, the mean square for periods within squares and trials within squares is also significant. Direct effects by hour blocks were not significant, indicating that ammonia released tended to follow the same trend during the first and second hours after feeding, as during the third and fourth hours. Although the ammonia levels in the rumen liquor were higher when Morea was fed, the animal suffered no ill effects. The phosphoric acid present in Morea apparently buffers well as the pH of the rumen was near 7.0 at all times. The efficiency of utilization of nitrogen is apparently less, because when there is a high level of ammonia in the rumen liquor, there is passage of nitrogen into the blood stream to be recirculated through the saliva and some is eliminated in the urine (*14*).

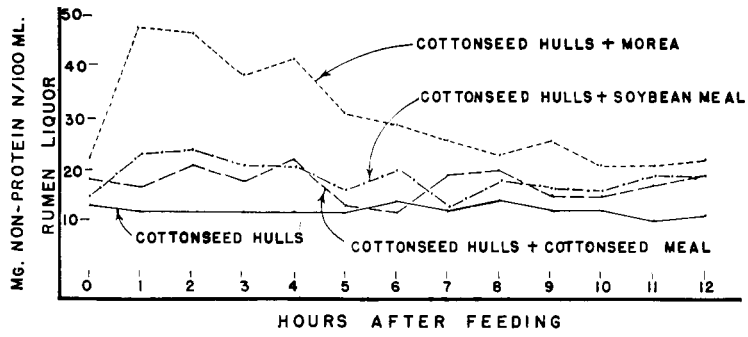

Figure 3. Level of nonprotein nitrogen in the rumen liquor of a fistulated steer fed various nitrogen sources

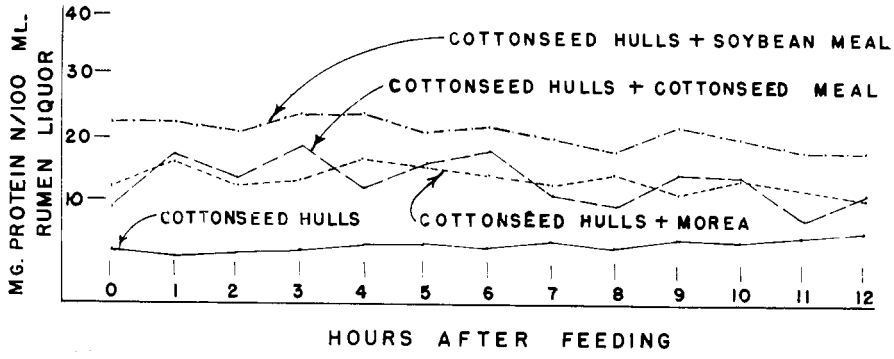

Figure 4. Level of protein nitrogen in the rumen liquor of a fistulated steer fed various nitrogen sources

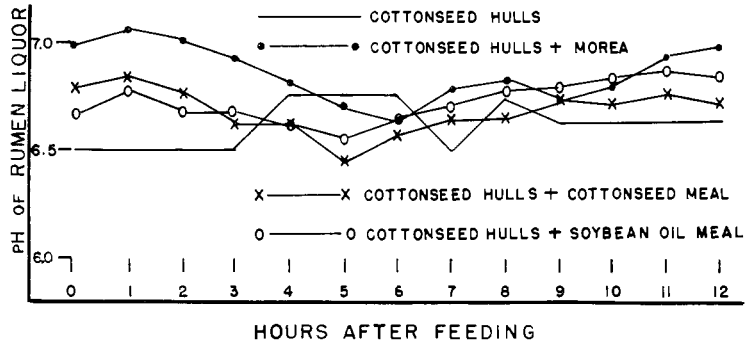

Figure 5. pH of the rumen liquor of a fistulated steer fed various nitrogen sources

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