Nitrogen metabolism in the reticulo-rumen of steers on diets containing extracted soya-bean meal and infrared-toasted soya beans*

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1. Diets containing cottonseed hulls and flaked infrared-toasted soya beans, cottonseed hulls and soya-bean meal or cottonseed hulls alone were fed to three fistulated steers. 2. The diet containing toasted soya-bean flakes was associated with the highest concentrations of total nitrogen, protein N, and ammonia N in the rumen liquor; the diet of cottonseed hulls was associated with the lowest concentrations; and soya-bean meal was associated with intermediate concentrations. 3. Concentrations of residual N were highest in rumen liquor during the first 3 h after feeding when soya-bean meal was given and highest with the other diets between 10 and 12 h after feeding; concentrations of non-protein N were highest with the toasted soya-bean flakes at all times except approximately 2 h after feeding when they were highest with soya-bean meal. 4. The rumen liquor was more acid with soya-bean flakes than with the other diets. 5. The total concentration of volatile fatty acids (m-moles/100 ml) was highest with the diet containing toasted soya-bean flakes, followed by that with soya-bean meal and cottonseed hulls and lowest with the cottonseed-hull diet. The molar percentage of acetic acid was highest with soya-bean meal, whereas its concentration (m-moles/100 ml) was highest with soya-bean flakes. Propionic acid concentrations were highest with the toasted soya-bean flakes. 6. These results indicate that the metabolism in the reticulo-rumen of infrared-toasted whole soya beans given as flakes differs from that of soya-bean meal.

The results of previous work (Stallcup, 1954; Stallcup & Looper, 1958) have shown that a more significant amount of ammonia nitrogen was produced in rumen liquor from a diet of extracted soya-bean meal and cottonseed hulls than when cottonseed hulls alone were given to fistulated steers. The amount produced, however, was small compared to the amount produced when urea was given. In further experiments (Davis & Stallcup, 1964) soya-bean meal has been compared to casein and urea when these materials were added to a basal diet of cottonseed hulls. The concentration of ammonia in the rumen liquor over a 12 h period with the urea or casein ration was significantly higher than that observed with the basal ration. Feeding with urea resulted in a significantly higher concentration of ammonia than did feeding with casein. The casein ration was associated with greater quantities of propionic, butyric and valeric acids in rumen liquor than the other rations. The urea ration was associated with the greatest quantity of acetic acid. Annison (1956) examined the rates of breakdown of several proteins in vitro by washed cell suspensions obtained from the rumen of sheep. Casein, soya protein and arachin were more readily attacked than were zein, bovine albumin and wheat-gluten proteins. Belasco (1954) used the artificial rumen to study the rates of degradation of urea, soya-bean, cottonseed, linseed and maize-gluten meals.

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Urea was associated with the formation of higher levels of propionic acid and lower levels of butyric and valeric acids than were the other N sources. The acetic acid level was unaffected by the type of N substrate. The study reported here was undertaken to compare the fate of N of soya-bean meal with that from infrared-toasted whole soya-beans in the rumen of fistulated steers.

EXPERIMENTAL

Three dairy-type steers, fitted with plastic rumen cannulas were used in these studies. Animals were housed in concrete stalls with automatic waterers. A 3×3 Latin-square experimental design was used with the three steers and three diets as follows: cottonseed hulls (basal); basal + soya-bean meal; and basal + infrared-toasted soya beans. Cottonseed hulls were used because of their low N content, high cellulose content, and their uniformity and ease of mixing with other materials. The soya-bean meal used was solvent-extracted meal from a local supplier. The infrared-toasted soya beans were processed in a Soytron Infra Toaster (Harry Truax and Sons Co., Inc., Mooresville, Indiana, USA), in which the whole beans were processed dry. The beans were then flaked in a mill at the Arkansas Agricultural Experiment Station Farm.

Table 1. *Daily diets (lb) given to fistulated steers*

Ingredient	Nitrogen content (%)	Diet 1	Diet 2	Diet 3
Soya-bean meal	7·36	3·40	—	—
Toasted soya-bean flakes	5·52	—	4·53	—
Cottonseed hulls	0·52	15·00	14·33	14·00

The cottonseed hulls were given twice daily at 6 am and 5 pm for all diets. The soya-bean meal and flaked toasted soya beans were given at the morning feeding in amounts only sufficient to meet maintenance requirements for protein as outlined by Morrison (1956). The quantities of protein supplements given to each steer were such as to provide equal amounts of N (Table 1). The majority of the cottonseed hulls, at least 12 lb/animal, were consumed at the morning feeding. Water and a mineral mixture of equal parts of dicalcium phosphate and trace-mineralized salt were available at all times except on the day when rumen liquor samples were taken; on those days water was removed from the animals 1 h after feeding and withheld for the remainder of the 12 h sampling period.

Each diet was given for 2 weeks. A collection of rumen liquor was made during the 3rd week. On the days when collections were made, a rumen liquor sample taken before feeding was designated as the 0 h sample. Further samples were taken at hourly intervals for 6 h after feeding and every 2 h thereafter until 12 h after feeding. Samples of rumen contents were withdrawn through the fistula with an aluminum dipper. Care was taken to secure samples from the top, middle and lower levels and also the anterior, median, and posterior extremities of the rumen. The digesta were squeezed through three layers of cheese cloth and the resultant liquid was designated as rumen liquor, which was taken immediately to the laboratory for analysis. Portions

of samples of rumen liquor taken 3 h after feeding were centrifuged and preserved with $HgCl_2$; these were placed in glass bottles, frozen, and stored for volatile fatty acid (VFA) analysis at a later date.

Determinations of pH were made on rumen liquor immediately upon collection after due precautions were taken to avoid the escape of CO_2. This was accomplished by placing the sample in an atmosphere of N_2 immediately following collection, whereupon pH readings were taken.

Ammonia N was determined by the method of Conway (1933) and Conway & Byrne (1933). Total N was determined by the Kjeldahl method (Association of Official Agricultural Chemists, 1960). Non-protein N was calculated as the difference between total N and the protein N determined on a precipitate of the rumen liquor (Folin & Wu, 1919). The essential steps of the procedure involved the addition of 25 ml of rumen liquor to 175 ml of distilled water. To the resultant mixture was added 25 ml of 10 % (w/v) sodium tungstate solution. To this was added slowly, and with shaking, 25 ml of two-thirds normal sulphuric acid. After shaking, the mixture was allowed to stand for 10 min. The mixture was then filtered and the precipitate together with the filter paper upon which it was collected, was placed in a Kjeldahl flask and the N determined by the Kjeldahl method (Association of Official Agricultural Chemists, 1960). The trace of N in the filter paper was determined for each batch of papers and subtracted from each determination of protein N. Residual N was calculated as the difference between non-protein and ammonia N. VFA were determined by the method of Baumgardt (1964) using a gas chromatograph. Statistical procedures used were those outlined by Steele & Torrie (1960).

RESULTS

Values pertaining to ammonia concentration in the rumen liquor from the diets are presented in Fig. 1. Both of the diets containing N supplements were associated with increased concentrations of ammonia N in the rumen liquor as compared to those with the control diet of cottonseed hulls. Mean values for ammonia N with their standard errors at 3 h after feeding were $19{\cdot}4 \pm 5{\cdot}6$, $9{\cdot}8 \pm 4{\cdot}5$, and $0{\cdot}6 \pm 0{\cdot}1$ for diets 2, 1 and 3, respectively. The rumen concentration of ammonia N with soya-bean meal was approximately that observed in previous investigations (Stallcup & Looper, 1958; Davis & Stallcup, 1964). With soya-bean meal, peak ammonia-N concentration occurred 2 h after feeding and 3 h after feeding with infrared-toasted soya-bean flakes.

Concentrations of total N in the rumen liquor when the diets were given are presented in Fig. 2. The level was highest throughout the 12 h period with the diet containing infrared-toasted soya-bean flakes. The concentration was extremely low with the diet containing only cottonseed hulls and intermediate with the diet containing soya-bean meal and cottonseed hulls. The concentration with soya-bean meal was approximately that observed in a previous study (Davis & Stallcup, 1964). With all the diets studied a peak was reached at 1 h after feeding.

The concentration of protein N is presented in Fig. 3. It was highest when the toasted soya-bean flakes were given. A peak was reached at 3 h after feeding. The level

remained high, resulting in a rather uniform quantity present in the rumen liquor from 0 to 12 h after feeding. By comparison, the level of protein N was of a lower magnitude when soya-bean meal was given. The protein N level was extremely low, in comparison to the other diets, when only cottonseed hulls were given.

The concentration of residual N was rather erratic with all diets (Fig. 4). The level was highest in the early hours after intake of soya-bean meal. There was a dramatic increase in the concentration of residual N in the rumen liquor of steers 10–12 h after intake of cottonseed hulls or toasted soya-bean flakes.

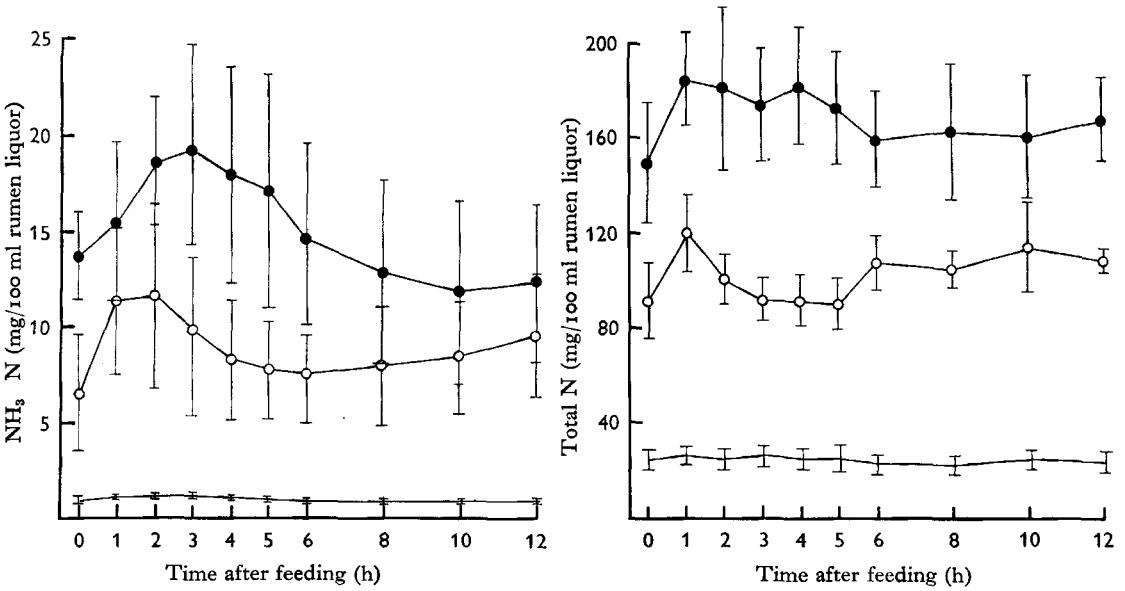

Fig. 1. Concentration of ammonia N in the rumen liquor of steers receiving cottonseed hulls with infrared-toasted soya-bean flakes, ●—●, or with soya-bean meal, ○—○, or cottonseed hulls alone, ——; vertical lines, SES. Three steers received each diet.

Fig. 2. Concentration of total N in the rumen liquor of steers receiving cottonseed hulls with infrared-toasted soya-bean flakes, ●—●, or with soya-bean meal, ○—○, or cottonseed hulls alone, ——; vertical lines, SES. Three steers received each diet.

Trends in the concentration of non-protein N in rumen liquor are presented in Fig. 5. In steers given soya-bean meal it was highest at 2 h after feeding. Concentrations were highest for the remainder of the 12 h period with toasted soya-bean flakes. Non-protein N concentrations were extremely low when only cottonseed hulls were given.

Rumen pH was observed in these sudies and results are presented in Fig. 6. When only cottonseed hulls were given the pH of the rumen liquor was consistently slightly above 7·0. This is in accordance with previous investigations (Davis & Stallcup, 1964). The pH was more acid with toasted soya-bean flakes, particularly during the first 6 h after feeding. The rumen pH observed with soya-bean meal was consistent with previous results obtained in this laboratory.

The concentrations of acetic, propionic, butyric and valeric acids are presented in Table 2. The diet containing soya-bean flakes was associated with a significantly

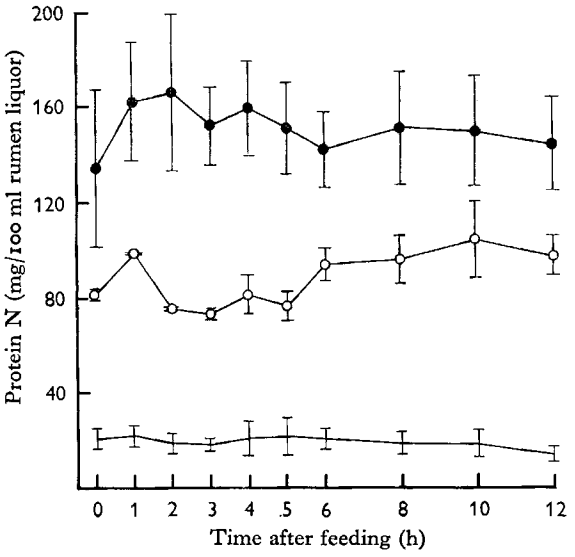

Fig. 3

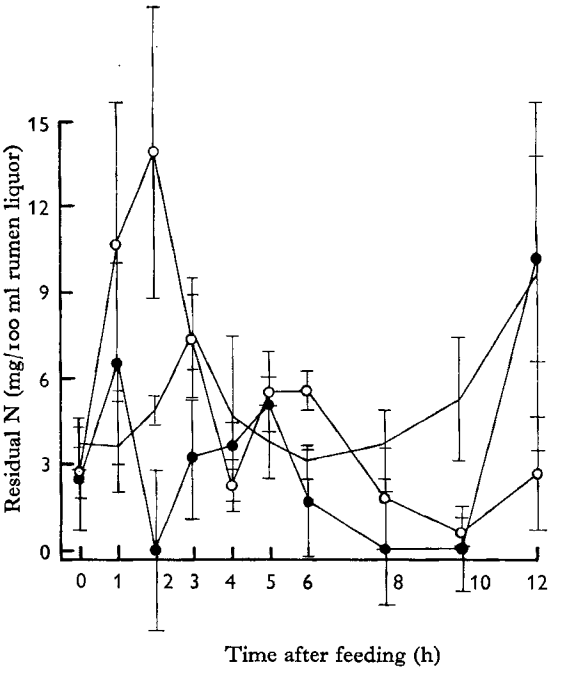

Fig. 4

Fig. 3. Concentration of protein N in the rumen liquor of steers receiving cottonseed hulls with infrared-toasted soya-bean flakes, ●—●, or with soya-bean meal, ○—○, or cottonseed hulls alone, ——; vertical lines, SEs. Three steers received each diet.

Fig. 4. Concentration of residual N in the rumen liquor of steers receiving cottonseed hulls with infrared-toasted soya-bean flakes, ●—●, or with soya-bean meal, ○—○, or cottonseed hulls alone, ——; vertical lines, SEs. Three steers received each diet.

Table 2. *Concentration of volatile fatty acids in the rumen liquor of steers given varied diets*

(Samples taken 3 h after feeding. Mean values with their standard errors for three steers)

Diet containing	Valeric acid m-moles/100 ml	Valeric acid Molar %	Isovaleric acid m-moles/100 ml	Isovaleric acid Molar %	Butyric acid m-moles/100 ml	Butyric acid Molar %
Soya-bean meal	0·48 ± 0·20	7·66 ± 0·32	0·48 ± 0·05	7·66 ± 0·83	0·69 ± 0·09	11·00 ± 1·46
Soya-bean flakes	0·53 ± 0·03	6·39 ± 0·30	0·55 ± 0·03	6·63 ± 0·32	1·02 ± 0·08	12·30 ± 0·91
Cottonseed hulls alone	0·40 ± 0·01	9·15 ± 0·23	0·34 ± 0·03	7·78 ± 0·61	0·44 ± 0·10	10·07 ± 2·10

	Isobutyric acid m-moles/100 ml	Isobutyric acid Molar %	Propionic acid m-moles/100 ml	Propionic acid Molar %	Acetic acid m-moles/100 ml	Acetic acid Molar %
Soya-bean meal	0·26 ± 0·04	4·14 ± 0·69	0·55 ± 0·09	8·77 ± 1·38	3·81 ± 0·24	60·77 ± 1·26
Soya-bean flakes	0·27 ± 0·02	3·27 ± 0·21	1·16 ± 0·03	14·12 ± 0·37	4·76 ± 0·43	57·42 ± 5·14
Cottonseed hulls alone	0·22 ± 0·05	5·03 ± 1·05	0·50 ± 0·09	11·44 ± 2·00	2·47 ± 0·44	56·52 ± 10·13

	Total volatile acids (m-moles/100 ml)
Soya-bean meal	6·27 ± 0·22
Soya-bean flakes	8·29 ± 0·32
Cottonseed hulls alone	4·37 ± 0·59

higher concentration (m-moles/100 ml) of VFAs ($P < 0.05$). The lowest values were associated with the basal diet. The concentration of acetic acid in rumen fluid was similar on all diets but was highest on the diet containing soya-bean meal. The concentration of propionic acid was significantly higher ($P < 0.05$) when the ration contained toasted soya-bean flakes. The molar percentage of isobutyric acid was highest in animals given the basal diet, whereas concentrations (m-moles/100 ml) were not significantly different among the animals given the various diets. The concentrations of butyric acid were highest in animals given the diet containing the toasted soya-bean

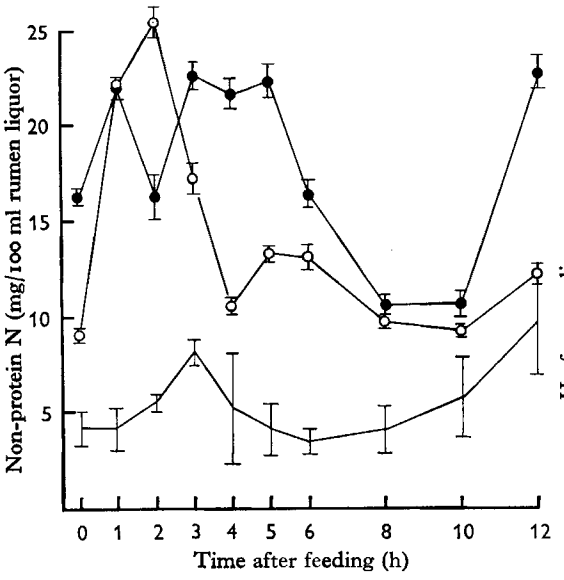

Fig. 5

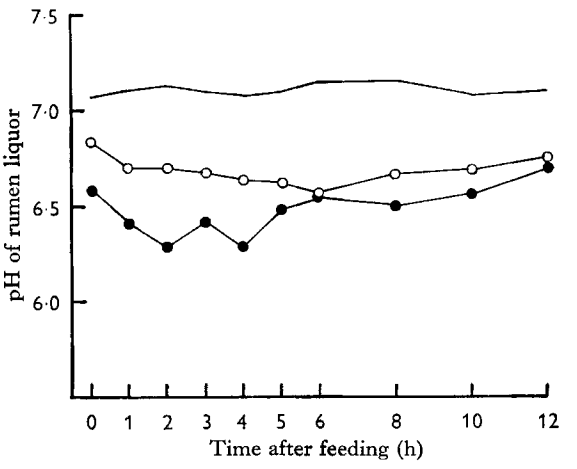

Fig. 6

Fig. 5. Concentration of non-protein N in the rumen liquor of steers receiving cottonseed hulls with infrared-toasted soya-bean flakes, ●—●, or with soya-bean meal, ○—○, or cottonseed hulls alone, ——; vertical lines, SES. Three steers received each diet.

Fig. 6. The pH of the rumen liquor of steers receiving cottonseed hulls with infrared-toasted soya-bean flakes, ●—●, or with soya-bean meal, ○—○, or cottonseed hulls alone ——. Three steers received each diet.

flakes. The highest molar percentages of valeric and isovaleric acids were associated with the basal diet, whereas their concentrations (m-moles/100 ml) were significantly higher ($P < 0.05$) in animals when the diet contained the soya-bean flakes.

DISCUSSION

These results suggest that the N in infrared-toasted soya-bean flakes metabolized quite differently from that of soya-bean meal. This is evident since the concentrations of total N, ammonia N and protein N in the rumen liquor were considerably higher with the toasted soya-bean flakes. Rumen non-protein N concentrations were also higher when the diet contained the flakes, except for a brief time 2 h after feeding. Residual N levels were highest during the first 3 h following feeding when soya-bean

meal was given, but were highest near the end of the 12 h period when the flakes were given. Another interesting observation was the pH of the rumen with soya-bean meal and toasted soya-bean flakes. Volatile fatty acid concentrations (m-moles/100 ml) were highest and the pH more acid with the toasted soya-bean flakes. This observation together with the higher levels of ammonia N maintained might suggest that the amino acids in the flakes were being deaminated more readily. Ammonia-N content was only 0·095 and 0·097 % for the infrared-toasted soya beans and the soya-bean meal respectively. Thus, it appears that the higher levels of ammonia N observed with the toasted soya beans were associated with changes in the activity of the rumen micro-organisms. The higher concentration of protein N in the rumen may indicate that the extra ammonia N present in the rumen when flakes were given was being used effectively in the synthesis of protein by micro-organisms. The net advantage, for ruminants, of using the infrared-toasted soya-bean flakes awaits further investigation. Observations by Perry (1963) indicated that, in pigs, infrared-treated whole soya beans were associated with more rapid gains and greater feed efficiency than were solvent-extracted soya-bean meals.

REFERENCES

Annison, E. F. (1956). *Biochem. J.* **64**, 705.
Association of Official Agricultural Chemists (1960). *Methods of Analysis*, 9th ed., p. 12, para. 2036. Washington, D.C.: Association of Official Agricultural Chemists.
Baumgardt, B. R. (1964). *Bull. Dep. Dairy Sci. Univ. Wis.* no. 1.
Belasco, I. J. (1954). *J. Anim. Sci.* **13**, 748.
Conway, E. J. (1933). *Biochem. J.* **27**, 430.
Conway, E. J. & Byrne, A. (1933). *Biochem. J.* **27**, 419.
Davis, G. V. & Stallcup, O. T. (1964). *J. Dairy Sci.* **47**, 1237.
Folin, O. & Wu, H. (1919). *J. biol. Chem.* **38**, 81.
Morrison, F. B. (1956). *Feeds and Feeding*, 22nd ed. Ithaca, New York: The Morrison Publishing Co.
Perry, T. W. (1963). *Feedstuffs*, **35**, no. 43, p. 14.
Stallcup, O. T. (1954). *J. Dairy Sci.* **37**, 1449.
Stallcup, O. T. & Looper, C. G. (1958). *J. agric. Fd Chem.* **6**, 916.
Steele, G. D. & Torrie, J. H. (1960). *Principles and Procedure of Statistics*. New York: McGraw-Hill Book Co.

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