The effect of dietary content of plant protein on the utilization of urea in the bovine rumen

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1. Four young Friesian bulls with rumen fistulas were given four isocaloric all-concentrate diets containing different amounts and sources of nitrogen in a Latin square arrangement. Diet HP (high-protein) contained 2·31% plant nitrogen; diet MPU (medium-protein with urea) 1·67% plant nitrogen and 0·69% urea nitrogen (total 2·36%); diet LPU (low-protein with urea) 0·95% plant nitrogen and 0·69% urea nitrogen (total 1·65%); diet HPU (high-protein with urea) 2·28% plant nitrogen and 0·69% urea nitrogen (total 2·97%), calculated on an air-dry basis.

2. The rumen pH varied between 5·8 and 6·1 with diets HP, MPU and HPU, but was significantly lower with diet LPU with values between 5·4 and 5·8.

3. The results showed no differences between the isonitrogenous diets HP and MPU except that replacement of plant nitrogen with urea was followed by an increase in the concentration of ammonia in the rumen. With the diets containing urea, the concentrations of rumen ammonia varied inversely with the amount of dietary plant nitrogen supplied, indicating a negative effect of plant nitrogen on urea utilization.

4. Concentrations of alkali-labile nitrogen (amide) were not increased with diets containing urea except with diet HPU, which produced the highest concentrations of ammonia in the rumen.

5. The concentration of true protein in the rumen and the amino acid distribution were similar with all four diets, indicating the ability of the microflora to adapt to qualitative and quantitative differences in dietary nitrogen intake.

6. Ration acceptability was lower with diets LPU and HPU than with diets HP and MPU.

7. Large differences between individual animals in rumen pH, percentage of dry matter and total nitrogen concentration in the rumen were noted.

Nearly 30 years ago Pearson & Smith (1943) showed how protein synthesis or catabolism may predominate in the rumen digesta according to the conditions present. More recently Chalupa (1968) and Tillman & Sidhu (1969) have emphasized that a major factor limiting the efficient use of urea nitrogen is the difficulty in obtaining comparable rates of urea hydrolysis and of fixation of the liberated ammonia into microbial protein. Since one mode of ammonia storage in the rumen has been postulated to be as glutamine (Hoshino, Sarumaru & Morimoto, 1966; Chalupa, Clark, Opliger & Lavker, 1970), it was of interest to discover if amide concentrations in the rumen are raised as a result of feeding with urea and if there is any relationship between the concentrations of amide and ammonia in the rumen. The utilization of non-protein nitrogen for microbial protein synthesis is probably affected by the dietary content of plant protein, digestible carbohydrate and minerals, of which the first is considered in the present paper. An investigation, in which four all-concentrate diets for young growing bulls were used, has been carried out in three parts: (1) an examination of the rumen digesta; (2) measurement of microbial protein synthesis in

vitro and (3) a feeding experiment under production conditions. The results of the first part are presented here. Observations have been made on rumen pH, ammonia concentration and amino acid content. A brief account of some of these results has been given elsewhere (Nikolić, Jovanović, Stošić & Pavličević, 1970).

EXPERIMENTAL

Animals

Four 10-month-old Friesian bulls with rumen fistulas received 1·5 kg portions of the experimental diets four times daily and water *ad lib.* The animals were kept on concrete floors with no bedding.

Diets

The four diets were designed to contain about 3100 kcal digestible energy/kg and were supplemented with equal quantities of minerals and vitamins (Table 1). The control diet (HP) contained no urea whereas the other diets all contained 1·5% crystalline urea in the air-dry feed. Analyses showed that the amounts of total

Table 1. *Composition and analysis of the experimental diets*

	Diet HP (high-protein diet)	Diet MPU (medium-protein diet with urea)	Diet LPU (low-protein diet with urea)	Diet HPU (high-protein diet with urea)
Composition (as percentage of air-dry feed)				
Yellow maize meal	51	56·5	38·5	49·5
Starch (feed grade)	—	—	20	—
Sunflower-oil meal	15	3	—	15
Sugar-beet pulp (dried)	25	30	30	25
Maize bran	—	—	6	—
Dried lucerne meal	5	5	—	5
Urea (feed grade)	—	1·5	1·5	1·5
Mineral and vitamin mixture*	4	4	4	4
Analysis (per 100 g air-dry feed)				
Nitrogen (g)	2·31 ± 0·42	2·36 ± 0·12	1·64 ± 0·25	2·97 ± 0·32
Ammonia (mg)	24·9	20·6	25·7	28·6
Amide ($mgNH_3$)	73·1	43·4	48·4	73·9
Dry matter (g)	85·9	85·8	86·4	85·9

* Mineral mixture supplying limestone (1·5%), NaCl (0·5%), bone meal (Kostan, 1·0%) which contained (g/kg): Ca, 320·00; P, 100·00; Fe, 2·45; Cu, 0·25; Mn, 3·00; Co, 0·01; I, 0·02; and vitamin mixture (Premiks; 1·0%) which contained (per kg): vitamin A, 500 000 i.u.; cholecalciferol, 80 000 i.u.; vitamin E, 200 mg.

ammonia and alkali labile nitrogen (amide) in the diets were low. The protein equivalents, calculated on an air-dry basis from the total nitrogen content were: diet HP, 14·4%; diet MPU, 14·7%; diet LPU, 10·3% and diet HPU, 18·6%, with urea supplying 29%, 42% and 23% of the total nitrogen in diets MPU, LPU and HPU respectively. At the levels given, diets HP and MPU supplied the daily crude-protein requirement for finishing yearling cattle, diet HPU supplied an excess of crude

protein, whereas diet LPU contained enough nitrogen only to fulfill the protein requirement for normal growth (National Research Council, 1963). The dietary components were ground through a 5 mm screen before being mixed thoroughly.

Procedure

Each diet was given to each young bull for a 5-week period in a Latin square arrangement. Rumen samples (300 ml) were taken for analysis during the last week of each feeding period. Samples were collected through polyethylene tubing of internal diameter 1 cm into plastic bottles containing 1·5 g mercuric chloride as a preservative, in the morning before the first feed and then at 1, 2, 3 and 5 h after placing a portion of diet before the animal. The time taken for each animal to consume its portion was noted. The pH of the rumen contents was measured immediately with a pH meter (Model 27; Radiometer, Copenhagen). The samples were stored for further analysis at $-13°$. All measurements were made on a weight basis and the samples were used unfiltered.

Analyses

Total nitrogen was determined by the Kjeldahl method on 5 g samples of the rumen contents and 0·5 g samples of the diets.

Dry matter was measured after heating 10 g samples of rumen digesta in an oven at 105° to constant weight.

Ammonia and amide concentrations were determined in 25 g diluted rumen contents (1:3) according to the technique of Varner, Bulen, Vanecko & Burrell (1953), modified as follows. Distillation was carried out for 15 min periods under reduced pressure at a water-bath temperature of 80° for ammonia and 98–100° for amides. The distillate was collected into 100 ml 1% (w/v) boric acid. The recoveries and standard errors of single determinations based on forty-one measurements of a mixture of 10 ml 0·005 M-ammonium sulphate solution and 10 ml 0·01 M-asparagine solution were found to be $97·1 \pm 4·0\%$ and $91·2 \pm 6·8\%$ respectively. Under the conditions used for amide determination the recovery from 10 ml 0·005 M-urea solution was 5·4%.

For amino acid determinations, 10 g rumen samples were hydrolysed in 100 ml 6 M-hydrochloric acid for 22 h at 110° in an open reflux system, filtered and evaporated nearly to dryness under reduced pressure at 40°. The samples were dissolved in citrate buffer (pH 2·2), filtered and adjusted to a predetermined volume before analysis on the amino acid analyser (Model 120B; Beckman Instruments Inc., Palo Alto, California). Careful adjustment of the buffer eluants permitted separation of methionine from α, ϵ-diaminopimelic acid.

Statistical analyses were made according to Snedecor (1956) on an IBM Computer.

RESULTS

Conditions in the rumen

Rumen pH. The changes in the pH of the rumen contents up to 5 h after the animals began to eat are shown in Fig. 1; the pH fell to a minimum at 2–3 h. No significant differences were observed between diets HP, MPU and HPU, but the

pH after a feed of diet LPU, which contained the low level of nitrogen, was significantly lower ($P < 0.05$) except at 5 h.

Nitrogen concentration. The concentration of total nitrogen in rumen contents was significantly greater ($P < 0.05$) with diet HPU than with each of the other diets (Fig. 2), whereas no significant differences were detected among the other diets. A similar picture occurred when the results were expressed in relation to the dry

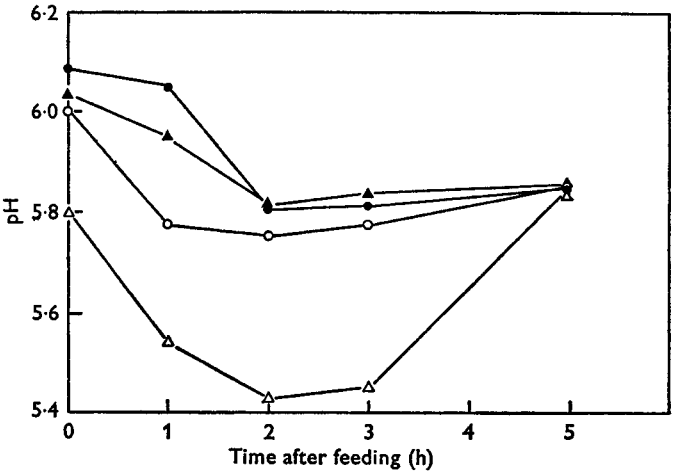

Fig. 1. Mean values for rumen pH of four young bulls in relation to time after beginning to feed on different diets. O—O, high-protein diet (HP); ●—●, medium-protein diet with urea (MPU); △—△, low-protein diet with urea (LPU); ▲—▲, high-protein diet with urea (HPU).

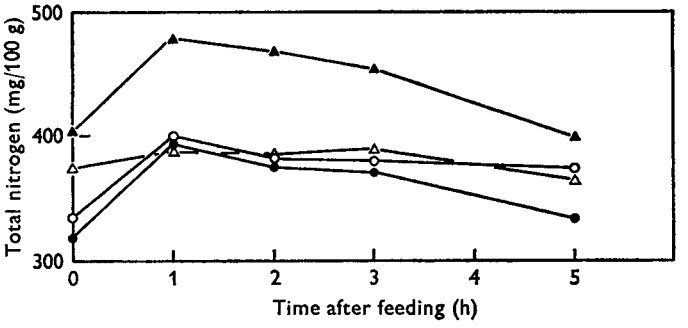

Fig. 2. Mean values for total nitrogen concentrations in rumen contents of four young bulls in relation to time after beginning to feed on different diets. O—O, high-protein diet (HP); ●—●, medium-protein diet with urea (MPU); △—△, low-protein diet with urea (LPU); ▲—▲, high-protein diet with urea (HPU).

rumen contents. With diet HPU the concentration of nitrogen was mainly between 5% and 6% of the dry matter, which is significantly larger than for diet LPU (4–5%). The nitrogen concentrations with the isonitrogenous diets HP and MPU were very similar and between the extremes found with diets LPU and HPU. Thus the total nitrogen content of the dry matter in the rumen appeared to depend on the dietary intake to a certain extent (Fig. 3).

Ammonia concentration. Large differences in the levels of free volatile bases, calculated as ammonia, in the rumen contents were found with the four diets (Fig. 4). When diets containing urea (MPU, LPU and HPU) were given, sharp peaks in ammonia concentration occurred 1 h after feeding. The height of these peaks increased

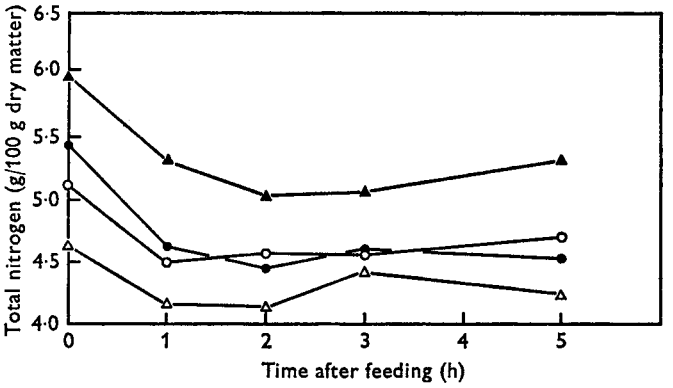

Fig. 3. Mean values for total nitrogen concentrations in dry rumen digesta of four young bulls in relation to time after beginning to feed on different diets. ○—○, high-protein diet (HP); ●—●, medium-protein diet with urea (MPU); △—△, low-protein diet with urea (LPU); ▲—▲, high-protein diet with urea (HPU).

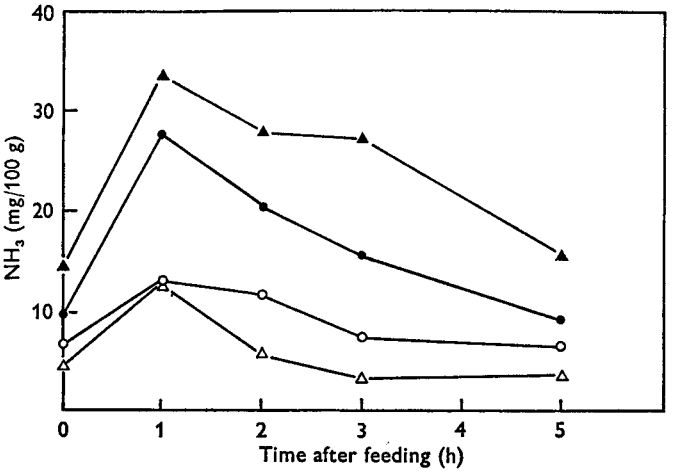

Fig. 4. Mean values for ammonia concentrations in rumen contents of four young bulls in relation to time after beginning to feed on different diets. ○—○, high-protein diet (HP); ●—●, medium-protein diet with urea (MPU); △—△, low-protein diet with urea (LPU); ▲—▲, high-protein diet with urea (HPU).

as the dietary content of plant protein nitrogen was raised. The rounded curve that occurred with diet HP was much lower than that with the isonitrogenous diet MPU. The mean values for ammonia concentration in rumen digesta were 8·9 mg, 16·4 mg, 5·9 mg and 23·8 mg NH_3 per 100 g digesta with diets HP, MPU, LPU and HPU respectively. Only the difference between diets HP and LPU was not statistically significant.

Amide concentrations. There were no significant differences between diets HP, MPU and LPU (Fig. 5), but the amide concentration in rumen contents was greater with diet HPU ($P < 0.05$). Since this difference was still apparent when the amide nitrogen was calculated as a percentage of total nitrogen, a greater proportion of amide groups occurred only when dietary nitrogen was supplied at a high level.

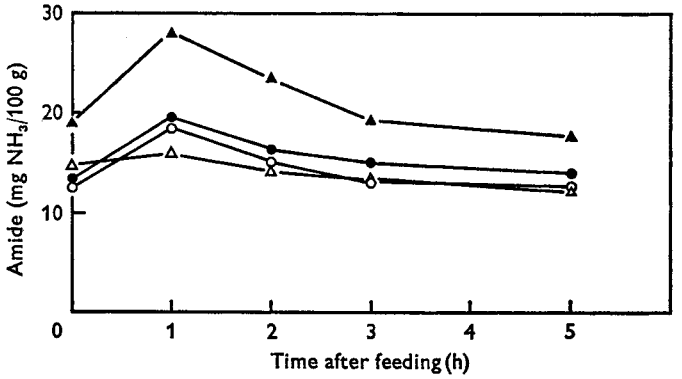

Fig. 5. Mean values for labile amide groups in the rumen digesta of four young bulls in relation to time after beginning to feed on different diets. O—O, high-protein diet (HP); ●—●, medium-protein diet with urea (MPU); △—△, low-protein diet with urea (LPU); ▲—▲, high-protein diet with urea (HPU).

Table 2. *Amino acid composition of hydrolysates of rumen contents from young bulls 3 h after feeding*

(Mean values and standard deviations expressed as g residue/100 g amino acid residues recovered)

Amino acid	Diet HP (high-protein)	Diet MPU (medium-protein +urea)	Diet LPU (low protein +urea)	Diet HPU (high-protein +urea)
Lysine	7·89±0·53	8·22±0·97	7·80±1·51	7·69±1·38
Histidine	2·47±0·28	2·46±0·29	2·42±0·22	2·25±0·24
Arginine	5·54±1·02	5·16±0·26	5·54±0·34	5·53±0·60
Aspartic acid	11·20±0·32	11·67±0·20	11·09±1·15	11·74±1·18
Threonine	5·20±0·10	5·41±0·23	5·89±0·73	5·38±0·27
Serine	4·76±0·34	4·78±0·20	5·48±0·76	4·80±0·21
Glutamic acid	15·98±0·78	15·54±0·73	16·10±1·69	16·38±0·69
Proline	4·20±0·40	4·19±0·29	4·10±0·62	4·05±0·53
Glycine	4·88±0·10	4·76±0·20	4·78±0·16	4·79±0·16
Alanine	6·28±0·29	6·30±0·56	6·74±0·11	6·28±0·38
Cystine	1·34±0·45	1·14±0·20	0·68±0·23	1·11±0·77
Valine	5·78±0·21	5·62±0·61	5·71±0·21	5·79±0·32
Methionine	1·38±0·69	1·41±0·48	1·15±0·46	0·99±0·72
Isoleucine	4·89±0·91	5·08±0·38	4·78±0·34	4·98±0·36
Leucine	8·89±0·42	8·99±0·69	8·46±1·14	8·88±0·27
Tyrosine	3·80±1·03	3·71±0·94	4·25±0·92	3·93±0·62
Phenylalanine	5·54±0·18	5·58±0·88	5·00±0·97	5·40±0·53

Amino acid distribution. The distribution of amino acids in the rumen contents 3 h after feeding is shown in Table 2. The similarity of the results with the various diets was striking. The proportions of some essential amino acids, such as phenylalanine, leucine, isoleucine and the sulphur acids, tended to be very slightly lower and those

of non-essential amino acids, such as tyrosine, serine and alanine, to be slightly higher with diet LPU, but these differences were not significant. Tryptophan was not determined but the content of α, ϵ-diaminopimelic acid, a constituent of the cell wall of many bacteria (Work, 1951; Rhuland, 1960), was estimated in the last three feeding

Table 3. *Distribution of nitrogen in the rumen contents of young bulls 3 h after feeding*

(Mean and standard deviations for four animals, expressed as mg N/100 g dry matter)

	Diet HP (high-protein)	Diet MPU (medium-protein + urea)	Diet LPU (low-protein + urea)	Diet HPU (high-protein + urea)	*F*
Ammonia N	81 ± 56	147 ± 62	29 ± 14	291 ± 138	7·57*
Amide N	130 ± 10	146 ± 19	127 ± 23	172 ± 50	1·53
Amino acid N	2903 ± 263	2888 ± 227	2830 ± 568	2862 ± 240	0·75
Total N	4530 ± 405	4560 ± 483	4410 ± 994	5060 ± 888	0·45

* Significant at the 0·05 level of probability.

Table 4. *Statistical analysis of the components of variance (mean square) for the analytical results*

Measurement	Source of variation	Time after feeding (h) 0	1	2	3	5
pH	Sampling period	0·123	0·198	0·154	0·132	0·118
	Animals	0·963	0·749	0·849	0·719	0·581
	Treatments	0·064	0·202	0·133	0·131	0·000
	Error	0·180	0·087	0·065	0·095	0·149
Dry matter (g/100 g)	Sampling period	4·45	0·34	0·75	0·21	0·42
	Animals	15·35	18·32	21·13	19·20	16·49
	Treatments	3·39	0·95	1·29	0·75	1·07
	Error	4·62	7·09	6·25	6·66	4·04
Total nitrogen (mg/100 g rumen contents)	Sampling period	9600	3040	4870	4650	9090
	Animals	37020	57500	56320	55070	32160
	Treatments	6100	7530	7590	5890	2980
	Error	3730	5120	5270	5660	4480
Ammonia (mg NH_3/ 100 g rumen contents)	Sampling period	17·1	62·6	60·3	17·8	19·7
	Animals	9·4	6·4	28·9	11·4	7·7
	Treatments	73·3	566·4	390·9	454·8	105·9
	Error	31·8	166·9	119·7	57·7	36·4
Amide (mg NH_3/ 100 g rumen contents)	Sampling period	5·6	6·2	4·3	7·8	5·9
	Animals	84·2	146·0	28·9	86·7	38·9
	Treatments	107·9	74·3	34·4	34·8	28·7
	Error	13·7	30·0	20·2	50·3	14·4

periods. This acid occurred at concentrations of 0·20 ± 0·03 g, 0·23 ± 0·12 g, 0·40 ± 0·13 g and 0·26 ± 0·14 g per 100 g dry rumen contents with diets HP, MPU, LPU and HPU respectively. The total amount of amino acid nitrogen per 100 g rumen dry matter was closely similar with all diets (Table 3). The factors for converting amino acid nitrogen (excluding tryptophan) into protein were calculated to be 6·39, 6·36, 6·31 and 6·36 respectively for diets HP, MPU, LPU and HPU.

Animal variation

Analysis of variance of the pH values showed that the greatest component was provided by individual differences between the animals (Table 4). Thus animals nos. 3 and 4 had rumen pH values significantly higher than animals nos. 1 and 2 ($P < 0.05$). The same animals had lower concentrations of dry matter and total nitrogen in the rumen contents ($P < 0.05$) and consumed their rations more quickly. However, there were no significant differences between the cattle in total nitrogen content, when calculated as a percentage of dry rumen contents (Fig. 6), or in the concentrations of rumen ammonia. This indicates that the individual variations arose from differences among the animals in factors, such as rate of salivary secretion, water consumption and rate of outflow from the rumen, that were not studied in these investigations.

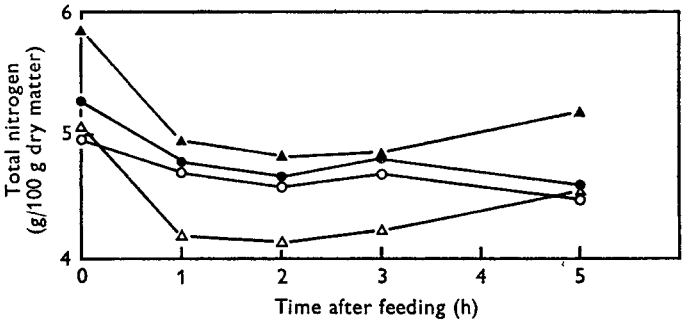

Fig. 6. Mean values for total nitrogen concentrations in dry rumen digesta of four young bulls in relation to time after beginning to feed on the same diets. ○—○, animal no. 1; ●—●, animal no. 2; △—△, animal no. 3; ▲—▲, animal no. 4.

Health of the animals

The cattle were healthy throughout the experiment except for the occasional occurrence of frothy bloat, particularly with diet LPU; this did not happen in the feeding experiment under production conditions (Part 3) when the animals had access to maize stalks. Nevertheless, the cattle generally consumed their rations readily; thus portions of diets HP and MPU were eaten in a mean time of 11 min after being placed before the animal, which indicates that replacement of most of the dietary sunflower meal by maize and urea in the latter diet had no effect on the palatability of the ration. The rates of consumption of diets LPU and HPU, in which the nitrogen contents were different, were slower (26 min and 17 min respectively). All the animals chewed the wood of their pens.

DISCUSSION

Many factors control the rumen pH, but the values are generally low when all-concentrate diets are given. This appears to be partly due to the higher concentrations of volatile fatty acids (Storry & Rook, 1966; Emmanuel, Lawlor & McAleese, 1969; Whitelaw, Hyldgaard-Jensen, Reid & Kay, 1970) and the lower rates of salivary flow

(Oltjen, Putnam & Davis, 1965; Lawlor, Giesecke & Walser-Kärst, 1966; Putnam, Yarns & Davis, 1966) that occur with such diets. Generally a low pH is associated with a reduction or complete absence of rumen protozoa which, by rapid ingestion of starch, provide a further buffer against changes in the environment (Purser & Moir, 1959; Eadie, Hyldgaard-Jensen, Mann, Reid & Whitelaw, 1970). Although no conclusions as to the dominant factor operating in this investigation can be made because salivary flow rate, volatile fatty acid concentrations and protozoal numbers were not determined, it can be suggested that feeding with diet LPU led to lowered numbers of protozoa and, from the increase found in the concentrations of diaminopimelic acid, raised numbers of bacteria. However, since diaminopimelic acid was not determined during the first feeding period, the statistical benefit of a Latin square arrangement was not applicable here and the difference was not statistically significant. Moreover the use of the concentration of diaminopimelic acid as an indicator of bacterial numbers has been criticized, but nevertheless it does indicate a tendency in a particular direction (Virtanen, 1967).

The amino acid distribution in the whole rumen contents in this investigation approximates more closely to the results obtained for rumen bacteria by Purser & Buechler (1966) and Bergen, Purser & Cline (1968) than for those for protozoa, indicating that bacteria form a predominant part of the rumen digesta 3 h after feeding. The proportion of methionine was lower than that found by these authors and was nearer to the value for rumen total proteins reported by Virtanen (1967), who also successfully separated diaminopimelic acid from the methionine peak. Nevertheless the presence of undegraded dietary proteins is suggested by the high proportion of glutamic acid, which constitutes a considerable fraction of the total amino acids in both sunflower-oil meal and maize meal (Stošić & Čuperlović, 1967). Zein, in particular, has been reported to be slowly degraded in the rumen (Lewis, 1961).

A striking fact to emerge from these studies is the closely similar amount of amino acid nitrogen that occurred with each of the four diets (Table 3). Thus, although the increased intake of dietary nitrogen with diet HPU, led to a greater concentration of nitrogen in the rumen, the concentration of true protein was not affected by either the qualitative or quantitative changes in food nitrogen sources. The proportion of amino acid nitrogen was less than that reported for separated rumen bacteria by Purser & Buechler (1966) but greater than the values given by Richardson & Tsien (1963) for strained rumen fluid. This similarity in concentration and quality of rumen protein is probably responsible for the failure to observe any differences in growth rate and carcass quality when these four diets were used under production conditions (Ševković, Stošić, Rajić, Zotović & Bezbradica, 1970). It can thus be concluded that 10·3% crude protein, of which 42% is supplied as urea, is sufficient for beef cattle when given in a high-energy ration. The successful utilization of such a diet by ruminants is partly due to the metabolic recycling of urea which has been found with diets of low nitrogen content (Gray, Pilgrim & Weller, 1958; Packett & Groves, 1965). The rate of absorption of ammonia through the rumen wall depends largely on its concentration and the pH in the rumen (Chalupa, 1968). A balance between this loss and the return of nitrogen to the rumen, as salivary urea for example, has been postulated to occur at about 7 mg

of ammonia nitrogen per 100 ml rumen contents (Waldo, 1968). In this investigation values were generally near or below this figure for diets HP and LPU but above it with diets MPU and HPU. A net loss of food nitrogen as ammonia from the rumen and eventually as urea in the urine may thus be assumed to occur with the latter two diets. Loss of nitrogen in the urine is an important factor in urea feeding (Lewis, 1961; Hembry, Pfander & Preston, 1969).

Since urease activity in the rumen is invariably very high (Caffrey, Hatfield, Norton & Garrigus, 1967; Pearson & Smith, 1943; Jones, MacLeod & Blackwood, 1964), rumen ammonia concentration can be taken as a reflection of the protein synthesis activity of the rumen microflora when the level of urea feeding and the rumen pH are the same. Thus the addition of urea to diet HP (diet HPU) leads to an almost triple increase in the mean value for rumen ammonia concentration. When the level of plant nitrogen is reduced (diet MPU) the value falls to a level which is still nearly double that of the isonitrogenous diet HP. With diet LPU, however, the low value indicates that the synthetic activity of the bacteria is commensurate with the rate of release of ammonia from urea with this diet. Djordjević, Jovanović, Stošić, Nikolić & Jovančević (1970), using $Na_2{}^{35}SO_4$ have shown a greater incorporation of radioactivity into rumen protein in vitro with diet LPU than with diets HPU and MPU, which supports the above postulate. Owen (1967) proposed that diets must contain less than 12% protein nitrogen for efficient use of urea supplements.

The similarities in the concentrations of primary amide nitrogen with the various diets show that urea feeding *per se* does not lead to changes. Only when rumen concentrations of ammonia are high is nitrogen present as amide in an increased amount. The 30% of rumen nitrogen uncharacterized (Table 3) may occur largely as nucleic acids, and compounds such as purines and nitrates. This fraction is being further investigated.

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