The effect of partially replacing urea nitrogen with protein N on N capture in the rumen of sheep fed a purified diet

BY D. BEN-GHEDALIA*, N. P. MCMENIMAN† AND D. G. ARMSTRONG

Department of Agricultural Biochemistry, University of Newcastle upon Tyne, Newcastle upon Tyne, NE1 7RU

(*Received* 23 *July* 1976 – *Accepted* 26 *January* 1977)

1. The influence of replacing 10% of the urea nitrogen in a purified diet with casein, maize gluten or white fish meal on the efficiency of conversion of dietary-N into microbial N was examined using sheep equipped with rumen fistulas and duodenal re-entrant cannulas.

2. Total nitrogen (TN), non-ammonia nitrogen (NAN) and amino acid nitrogen (AAN) flowing to the proximal duodenum were significantly higher ($P < 0.05$) when maize gluten was added to the diet, and this appeared to be due to an increased efficiency of microbial protein production.

3. Pepsin secretion was not significantly different between treatments and the daily amount of pepsin N flowing to the proximal duodenum was very small (40–53 mg). The peak of pepsin activity in duodenal digesta was reached 6–8 h after feeding.

4. The possible practical implications of the results are discussed.

Oltjen (1969), from a survey of published experiments, concluded that the complete substitution of non-protein nitrogen (NPN) for natural proteins in purified diets given to ruminants resulted in a 35% reduction in growth rate, feed efficiency and N retention. If the assumption is made that the efficiency of microbial protein production is not influenced by the source of dietary-N, an explanation of these findings is that when proteins are included in the diet a certain proportion escapes degradation in the rumen and so increases the quality of amino acids reaching the small intestine.

Alternatively, the supply of protein to the rumen may stimulate microbial production, in which case the explanation may lie in part with dietary protein escaping rumen fermentation and in part be due to increased microbial protein production, or be entirely due to the latter. It has been shown in in vitro studies that, in addition to ammonia-N, some species of rumen bacteria can utilize amino-N and peptides; in these species, peptides usually stimulated more growth than mixtures of individual amino acids (Bryant & Robinson, 1962; Pittman & Bryant, 1964; Wright, 1967; Allison, 1969). The addition of various amino acids either singly or in combination to urea-based, purified diets have, in general, not improved animal performance (Harbers, Oltjen & Tillman, 1961; Oltjen, Sirney & Tillman, 1962; Clifford, Bourdette & Tillman, 1967).

In experiments with sheep, Hume (1970) observed that when casein or zein replaced 50% of the N in a purified diet containing urea as the only source of N, efficiency of microbial protein production was increased by 12·2% and 15·6% respectively. The following experiment was designed to determine whether the substitution of 10% of the N in a urea-based purified diet by different proteins would enhance ruminal microbial protein production when these diets were given to sheep.

* Present address: Department of Animal Nutrition, Faculty of Agriculture, The Hebrew University of Jerusalem, P.O. Box 12, Rehovot, Israel.

† Present address: Pastoral Laboratory, Department of Primary Industries, Box 282, Charleville, Queensland, 4470, Australia.

Table 1. *The composition of the purified diet containing urea* (g/kg, air-dried basis)*

Solka flock (cellulose)	300
Starch	340
Cerelose (glucose)	222
Urea†	45
Mineral mixture‡	61
Maize oil	30
Choline chloride	1
Spices	1

Vitamins A, D and E were added at the rate of 4000, 800 and 20 i.u./kg respectively.

* The above relates to the control diet (U). In the other three diets 10% of the urea-N was replaced by N from casein (C), maize gluten (MG) and fish meal (F).

† The nitrogen content of all diets was 24·5 g/kg dry matter.

‡ The composition of the mineral mixture was (g/kg): $CaHPO_4$, 461·0; K_2CO_3, 323·5; $MgSO_4$, 103·1; NaCl, 931; $FeSO_4$, 7·1; $MnSO_4.4H_2O$, 5·2; $Na_2B_4O_7.10H_2O$, 3·1; $ZnSO_4.7H_2O$, 3·8. To this was added (mg/kg of mix): $CuSO_4.5H_2O$, 27·5; KI, 1·6; $CoCl_2.6H_2O$, 0·5; $Na_2MoO_4.2H_2O$, 0·24.

EXPERIMENTAL

Animals

Four wethers, 1–2 years old and weighing 45–50 kg, were each equipped with a rumen fistula and duodenal re-entrant cannulas (Brown, Armstrong & MacRae, 1968). The sheep were housed in metabolism crates and had free access to water.

Experimental design

A 4×4 Latin square design was used. Each period consisted of at least a 14 d adaption period. Subsequently, duodenal digesta and rumen contents were sampled during two 24 h periods each period being separated by at least 2 d.

Diets

Four purified diets were fed. In the control diet (U) urea was the only source of N whereas in the other three diets (C, MG and F) 10% of the urea-N was replaced by casein, maize gluten and white fish meal respectively. The composition of the purified diet containing urea is presented in Table 1. Each sheep was fed 600 g dry matter/d in equal portions at 09.00 and 21.00 hours.

Collections and sampling

The 24 h duodenal collections were performed according to the method described by Beever, Thomson, Pfeffer & Armstrong (1971). Flows of duodenal digesta were corrected for 100% recovery of Cr EDTA which was infused continuously into the rumen; infusions were begun at least 5 d before duodenal digesta collections. Representative samples of duodenal digesta were kept at −20° until required for analysis. In order to determine the pepsin activity in digesta flowing into the proximal duodenum, digesta samples were obtained every 2 h during the four 24 h duodenal digesta collections performed during each of experimental periods 1 and 2; the pepsin analyses were carried out immediately the samples were obtained. This procedure was adopted to prevent loss of pepsin activity which may occur during storage (Ben-Ghedalia, unpublished results; Hunner, Hudson & Fletcher, 1969). During experimental periods 3 and 4, pepsin was determined on the pooled 24 h duodenal digesta samples, the analyses being performed immediately after the completion of the collections. Rumen liquor was sampled every 2 h during the 24 h period of sampling; pH in rumen liquor was measured immediately after sampling, but ammonia and volatile

fatty acid (VFA) analyses were carried out later on the samples of liquor that were stored at −20°. At the end of each experimental period a large sample (1 l) of rumen digesta was obtained from each sheep for the isolation of rumen bacteria.

Analytical procedures

Cr EDTA was prepared and analysed according to the procedure of Binnerts, van't Klooster & Frens (1968). Organic matter (OM) was determined as the loss in weight of dry matter resulting from ashing in a muffle furnace at 550° for 24 h. The concentration and molar proportions of the VFA in the rumen liquor were measured on a Pye 104 gas liquid chromatograph (Pye Unicam Ltd, Cambridge) equipped with a flame ionization detector.

The total N contents of feed, duodenal digesta and rumen bacteria, were determined by the Kjeldahl procedure. For the determination of ammonia in rumen liquor and in duodenal digesta the distillation and titration part of the Kjeldahl procedure was adopted. Individual amino acids in the protein supplements, duodenal digesta and rumen bacteria were determined on freeze-dried samples of these materials; 50 mg of the respective sample were hydrolysed in 10 ml 6 M-HCl in a tube stoppered by a screw cap. The hydrolysis was carried out under N gas for 24 h at 110°. Norleucine was used as the internal standard. The succeeding steps in the amino acid analyses were done as described by Coelho da Silva, Seeley, Thomson, Beever & Armstrong (1972) as modified by G. P. Savage (personal communication).

Pepsin activity in the digesta flowing into the proximal duodenum was determined using the technique of Anson (1938); immediately after sampling, the digesta was diluted ten times with 0·01 M-HCl and 1 ml of the diluted digesta was then incubated with 2 ml substrate (haemoglobin solution, 2% pH 2) for 15 min at 37°. The reaction was stopped by the addition of 5 ml of a 0·3 M solution of trichloroacetic acid (TCA). In the reaction blanks the 5 ml of 0·3 M-TCA were added to the diluted digesta before the addition of the substrate. After the cessation of the enzymatic reaction the suspension was allowed to settle for 0·5 h and then filtered. The absorbance of the filtrate was determined at 280 nm using a Unicam SP.800 spectrophotometer (Pye Unicam Ltd, Cambridge). Purified crystalline pepsin was used as a standard.

The zein contents of maize gluten and of the digesta samples obtained when maize gluten was present in the diet were determined, using the technique of McDonald (1954).

RESULTS

The amino acid composition, total amino acid N (TAA-N) and total N content of the protein sources added to the purified diets are presented in Table 2. In comparison to casein, maize gluten had higher proportions of leucine and alanine and lower proportions of lysine, while fish meal was higher in arginine, alanine, glycine and aspartic acid and lower in glutamic acid, proline and tyrosine. It is obvious from Table 3, however, which shows the amino acid compositions of the duodenal digesta from the four treatments, that the differences in the amino acid compositions of the proteins added to the purified diets were not reflected in the respective digesta samples. The amino acid profiles of these digesta samples (Table 3) were very similar and closely resembled those of the rumen bacteria samples isolated from the rumens of the sheep given the respective diets (see Table 4).

Quantitative results of the nitrogenous components of the digesta flowing through the proximal duodenum are presented in Table 5; flows of total N (TN), non-ammonia-N (NAN) and amino acid N (AAN) were significantly higher ($P < 0.05$) when maize gluten was included in the diet. Although the amino acid compositions of the digesta samples were similar and did not reflect the compositions of the dietary protein sources, there was a

Table 2. *The amino acid composition* (g amino acid/100 g total amino acid) and the contents of total amino acid nitrogen (TAA-N) and total N (g/100 g dry matter) of the casein, maize gluten and white fish meal used in purified diets*

	Dietary component		
Amino acid	Casein	Maize gluten	Fish meal
Arginine	3·4	2·6	7·0
Histidine	3·3	2·4	3·5
Isoleucine	6·1	4·3	5·1
Leucine	9·2	14·9	7·4
Lysine	8·2	1·7	7·9
Methionine	3·0	2·5	2·7
Phenylalanine	4·8	5·6	3·8
Threonine	4·3	3·9	4·1
Valine	6·5	4·6	5·6
Alanine	2·5	8·1	6·7
Aspartic acid	6·9	5·9	9·2
Glutamic acid	19·2	21·0	13·5
Glycine	1·9	2·9	9·5
Proline	10·1	9·5	5·3
Serine	4·7	4·8	5·2
Tyrosine	5·5	5·1	3·6
TAA-N	13·0	8·3	9·3
Total N	14·6	10·7	10·9

* Tryptophan and cystine are not included.

Table 3. *The amino acid composition (g amino acid/100 g total amino acids) of the duodenal digesta from sheep fed purified diets in which urea was the sole source of nitrogen (U) or in which 10% of the urea-N had been replaced by casein (C), maize gluten (MG) or fish meal (F)*

	Diet				
Amino acid	U	C	MG	F	SEM
Arginine	5·97	6·36	5·15	5·88	0·76
Histidine	2·96	2·74	2·65	2·61	0·24
Isoleucine	6·06	5·92	6·09	6·20	0·24
Leucine	7·57	7·72	8·02	8·71	0·61
Lysine	9·30	8·93	8·64	8·96	0·44
Methionine	2·05	2·09	2·16	2·14	0·10
Phenylalanine	5·09	4·69	4·94	4·66	0·32
Threonine	5·97	5·66	5·49	5·62	0·37
Valine	6·20	6·74	6·23	6·46	0·35
Alanine	7·36	7·77	7·83	7·34	0·29
Aspartic acid	11·42	11·25	10·57	10·93	0·61
Glutamic acid	12·20	12·40	12·98	12·73	0·30
Glycine	5·56	5·57	5·63	5·37	0·27
Proline	4·06	3·97	4·91	4·19	0·39
Serine	4·40	4·28	4·53	4·34	0·20
Tyrosine	3·88	3·91	4·18	3·86	0·27

possibility that the increased amounts of TN, NAN and AAN reaching the proximal duodenum when maize gluten was added to the diet were due, at least in part, to maize gluten escaping digestion in the rumen. As maize gluten contains zein, the quantity of zein-N in the TCA-precipitable fraction of duodenal digesta from sheep fed the maize gluten-supplemented diet was determined. No zein-N was detected. However, as only 14 g of maize gluten were given daily it was possible that the method used for the detection of

Table 4. *The amino acid composition (g amino acid/100 g total amino acid) of the mixed rumen bacteria isolated from sheep fed purified diets in which urea was the sole source of N (U) or in which 10% of the urea N was replaced by casein, (C) maize gluten (MG) or fishmeal (F)*

	Diet			
Amino acid	U	C	MG	F
Arginine	7·35	6·44	7·45	5·63
Histidine	1·78	1·73	1·76	2·21
Isoleucine	6·31	6·17	6·57	6·54
Leucine	7·07	7·55	8·02	7·91
Lysine	9·46	10·46	11·38	9·38
Methionine	1·61	1·79	1·78	1·63
Phenylalanine	4·46	4·89	4·79	4·67
Threonine	5·31	5·65	4·70	5·32
Valine	5·85	5·90	5·76	5·91
Alanine	8·40	8·48	9·51	7·89
Aspartic acid	11·22	11·45	7·88	10·46
Glutamic acid	11·18	10·98	11·95	12·91
Glycine	6·66	5·58	5·64	6·21
Proline	4·42	3·75	3·73	4·27
Serine	3·87	3·98	3·82	4·29
Tyrosine	5·05	5·20	5·26	4·86
Amino N (g/100 g dry matter)	6·92	7·24	6·75	6·31
Total N (g/100 g dry matter)	7·96	8·33	7·76	7·25

Table 5. *The flow of nitrogenous components (g/d) of digesta through the proximal duodenum of sheep fed purified diets in which urea was the sole source of N (U) or in which casein (C), maize gluten (MG) or fish meal (F) replaced 10% of the urea N*

	Diet				
	U	C	MG	F	SEM
Total nitrogen (TN)	$12·12^{b}$	$11·77^{b}$	$14·25^{a}$	$12·30^{b}$	0·43
Non ammonia nitrogen (NAN)	$11·33^{b}$	$10·83^{b}$	$13·26^{a}$	$11·40^{b}$	0·35
Essential amino acid nitrogen	4·87	4·64	5·55	5·16	0·24
Non-essential amino acid nitrogen	3·42	3·32	3·99	3·61	0·17
Total amino acid nitrogen (AAN)	$8·29^{b}$	$7·96^{b}$	$9·54^{a}$	$8·77^{b}$	0·32
Pepsin	0·715	0·635	0·843	0·811	0·135
Pepsin nitrogen	0·045	0·040	0·053	0·051	0·008

Values with different superscripts are significantly different ($P < 0·05$).

zein-N in duodenal digesta was not sensitive enough to detect the small quantities that would have been present even if all the maize gluten had passed into the small intestine. To examine this possibility, the zein N content of the maize gluten was determined and was found to be 32·8 g N/kg maize gluten. A sample of duodenal digesta from a sheep given a purified diet was then taken and an amount of maize gluten added to it in the proportions that would have been present had all of the maize gluten used to replace 10% of the urea-N in the purified diet escaped fermentation in the rumen. Subsequent analysis of this sample for zein-N showed that all of it could be accounted for. Similarly, when one-half of the above amount of maize gluten was added to the digesta sample the zein-N could be quantitatively accounted for. It was therefore concluded that no dietary maize gluten was present in the duodenal digesta of sheep given the diet containing a small supplement of maize gluten.

Endogenous abomasal secretions are another source of N in duodenal digesta. Pepsin,

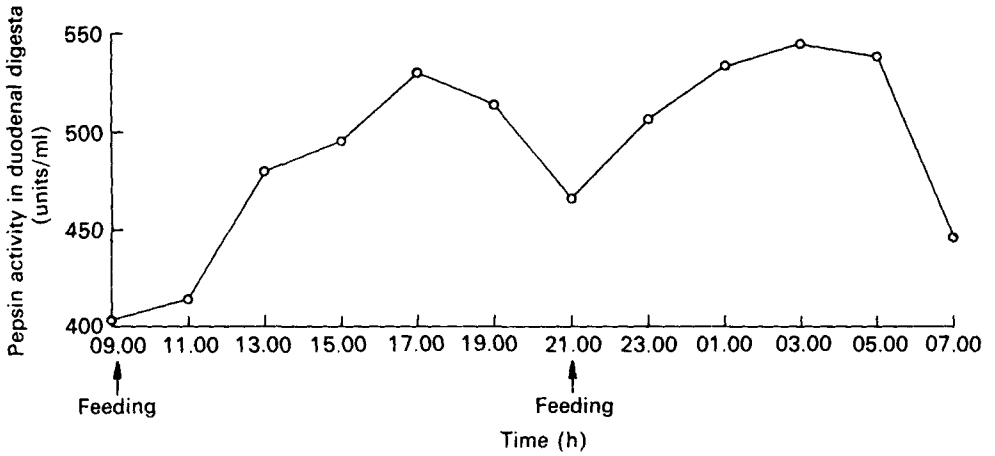

Fig. 1. The distribution of pepsin activity during the day, in digesta flowing to the duodenum of sheep fed a purified diet containing urea. (Each point represents sixteen analyses done during four collections on four sheep.) A unit of pepsin activity is defined as an increase in absorbance of 0·001/min at 280 nm under the conditions of the assay.

Table 6. *The efficiency of energy utilization for microbial growth*

	Diet				
	U	C	MG	F	SEM
Organic matter (OM) intake (g/d)	551	558	557	556	—
OM apparently digested in the rumen (g/d)*	306	297	276	306	29·8
Flow of microbial OM to the duodenum (g/d)	122[b]	112[b]	147[a]	135[b]	4·2
OM actually digested in the rumen (g/d)†	428	409	423	441	28·9
Microbial nitrogen produced (g/kg OM actually digested in the rumen)	26·5	26·5	31·3	25·8	2·7

Values with different superscripts are significantly different ($P < 0.05$).
* OM intake − OM entering duodenum.
† OM intake − OM entering duodenum + microbial OM entering duodenum.

a major nitrogenous component of gastric secretion, was measured in the digesta flowing to the duodenum and the values obtained are presented in Table 5. The contribution that pepsin N made to the total N reaching the proximal duodenum in each of the treatments was neglible. As there were no differences between treatments in pepsin activity, the results from all treatments were bulked in order to examine the pattern of pepsin activity over a 24 h period; these results are presented in Fig. 1. Examination of the figure shows that pepsin secretion appears to be affected by feeding; the peak of activity was reached between 6 and 8 h after feeding.

Making the assumption from the foregoing that all of the NAN and AAN in the duodenal digesta was of microbial origin and knowing the total N content of rumen bacteria and the flow of NAN through the proximal duodenum, it was possible to calculate the efficiency of microbial N production and these values are presented in Table 6. When maize gluten was added to the diet, significantly more bacterial OM ($P < 0.05$) flowed to the proximal duodenum; efficiency of production of microbial N was also increased but the differences between the treatments was not significant.

The mean concentrations and molar proportions of the VFA ammonia-N concentrations

Table 7. *Mean pH, ammonia nitrogen concentrations and the mean concentrations and molar proportions of the volatile fatty acids in the rumen liquor of sheep fed purified diets in which urea was the sole source of N (U) or in which casein (C), maize gluten (MG) or fish meal (F) replaced 10% of the urea N*

	Diet				
	U	C	MG	F	SEM
pH	6·1	6·1	6·4	6·3	0·1
NH_3-N, mmol/l	23·4	22·7	14·9	15·7	3·35
Total VFA, mmol/l	117·2	108·0	102·1	108·9	8·7
Proportions of individual VFA:					
Acetate	0·523[a]	0·562[b]	0·560[b]	0·570[b]	0·009
Propionate	0·332	0·269	0·271	0·267	0·033
Butyrate	0·105	0·123	0·129	0·123	0·023
Isobutyrate	0·006	0·006	0·006	0·005	0·001
Valerate	0·020	0·033	0·022	0·026	0·005
Isovalerate	0·012	0·008	0·012	0·013	0·002

Values with different superscripts are significantly different ($P < 0·05$).

and pH in the rumen liquor of the sheep given the various diets are shown in Table 7. Apart from the lower proportion of acetic acid in the liquor of sheep receiving the control diet, no other statistically significant results were present. The ammonia-N concentrations in the rumen liquor of sheep receiving maize gluten and fish meal were appreciably, but not significantly, lower than in the liquor of sheep receiving the casein-supplemented or unsupplemented diets.

DISCUSSION

The results presented show that adding maize gluten to a purified diet in which urea was the only source of N increased the flow of TN, NAN and AAN at the proximal duodenum and the indication was that this was due to an improved efficiency of microbial protein production. This result is in agreement with that of Hume (1970), who fed zein. The cause of this improved efficiency of microbial growth is not known although it could be postulated that it was due to the release from the maize gluten of particular peptides which stimulated microbial growth. The lack of response found when casein was added to the purified diet is in contradiction to the results determined by Hume (1970). However, as casein is very soluble in the rumen, it is possible that the preformed peptide(s) or amino acid(s) or both necessary for the stimulation of microbial growth were not present for a long enough period or in sufficient concentrations to be effective in the present experiment; Hume (1970) replaced 50% of the urea-N with casein-N and gave the diet at two-hourly intervals, whereas in the present study only 10% of the urea-N was replaced by casein and the diet was given twice daily. Adding fish meal to the diet did not result in any increased flow of AAN at the proximal duodenum or influence the efficiency of microbial protein production. Ramirez (1972) also found that fish meal supplementation failed to increase microbial protein flow at the proximal duodenum of bulls given a urea-based diet containing molasses.

The results of this experiment suggest that for certain ruminant diets, that is those containing little or no preformed protein, a small supplement of maize gluten or possibly some other slowly degradable protein may be beneficial. However, before any recommendations of a practical nature can be made, production experiments using natural diets of low-protein content would have to be carried out to confirm these findings.

The amino acid compositions of the bacteria isolated from the rumen (see Table 4) were similar to those reported by other workers (e.g. Weller, 1957; Purser & Beuchler,

1966; Bergen, Purser & Cline, 1968; Williams & Dinusson, 1973) and further support the conclusion of Purser (1970) that the amino acid composition of rumen bacteria shows little variation.

The small quantity of N added to the digesta in the abomasum as pepsin is surprising. Clarke, Ellinger & Phillipson (1966) estimated that the abomasal secretions of the sheep could contribute approximately 2·0 g of N daily to the material flowing through this organ. Harrop (1974) determined the main nitrogenous components of the gastric juice of sheep using innervated pouches in the fundic and pyloric regions of the abomasum and estimated that from 0·51 to 2·86 g N were secreted daily. In the light of the results of the present experiment a large proportion of this N would have to be in forms other than pepsin, possibly muco-proteins.

The senior author gratefully acknowledges financial assistance from Beecham Limited and from the Friends of the Hebrew University, UK.

REFERENCES

Allison, M. J. (1969). *J. Anim. Sci.* **29**, 797.
Anson, M. L. (1938). *J. Gen. Physiol.* **22**, 79.
Beever, D. E., Thomson, D. J., Pfeffer, E. & Armstrong, D. G. (1971). *Br. J. Nutr.* **26**, 123.
Bergen, W. G., Purser, D. B. & Cline, J. H. (1968). *J. Dairy Sci.* **51**, 1698.
Binnerts, W. T., van't Klooster, A. Th. & Frens, A. M. (1968). *Vet. Rec.* **82**, 470.
Brown, G. F., Armstrong, D. G. & MacRae, J. C. (1968). *Br. Vet. J.* **124**, 78.
Bryant, M. P. & Robinson, I. M. (1962). *J. Bacteriol.* **84**, 605.
Clarke, E. H. W., Ellinger, B. M. & Phillipson, A. T. (1966). *Proc. R. Soc.* B **166**, 63.
Clifford, A. J., Bourdette, J. R. & Tillman, A. D. (1967). *J. Anim. Sci.* **26**, 917.
Coelho da Silva, J. F., Seeley, R. C., Thomson, D. J., Beever, D. E. & Armstrong, D. G. (1972). *Br. J. Nutr.* **28**, 63.
Harbers, L. H., Oltjen, R. R. & Tillman, A. D. (1961). *J. Anim. Sci.* **20**, 880.
Harrop, C. J. F. (1974). *J. agric. Sci., Camb.* **83**, 249.
Hume, I. D. (1970). *Aust. J. agric. Res.* **21**, 305.
Hunner, M. C., Hudson, F. B. & Fletcher, T. L. (1969). *Proc. Soc. exp. Biol. Med.* **130**, 1246.
McDonald, I. W. (1954). *Biochem. J.* **56**, 120.
Oltjen, R. R. (1969). *J. Anim. Sci.* **28**, 673.
Oltjen, R. R., Sirney, R. J. & Tillman, A. D. (1962). *J. Anim. Sci.* **21**, 277.
Pittman, K. A. & Bryant, M. P. (1964). *J. Bacteriol.* **88**, 401.
Purser, D. B. (1970). *J. Anim. Sci.* **30**, 988.
Purser, D. B. & Beuchler, S. M. (1966). *J. Dairy Sci.* **49**, 81.
Ramirez, A. (1972). *Rev. Cubana Ciene. Agric.* **6**, 195.
Weller, R. A. (1957). *Aust. J. biol. Sci.* **10**, 384.
Williams, P. O. & Dinusson, W. E. (1973). *J. Anim. Sci.* **36**, 151.
Wright, D. E. (1967). *Appl. Microbiol.* **15**, 547.

Printed in Great Britain