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In vitro caecal fermentation of nitrogenous substrates in rabbits

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SUMMARY

Caecal contents of 3-month-old rabbits were analysed and used as inoculi of cultures supplied with plant proteins (gluten, gliadin, zein), mucin, casein, ribonucleic acid (RNA) and glucose. The caecal digesta contained dry matter, total N, protein, trichloroacetic acid (TCA)-soluble N and NH_3-N at 234, 10·2, 42·6, 3·2 and 0·27 mg/g (on average), respectively. One g of the caecal digesta contained 180 μmol of glycine equivalents of amino acids in TCA-soluble fraction. Because ninhydrin reaction was almost three times greater after HCl hydrolysis, peptides rather than amino acids were the primary component in this fraction. Urea concentration was only 2·3 μg N/g.

Net production of volatile fatty acids (VFA) in cultures with casein and mucin was higher than in cultures with gluten, gliadin, zein and RNA. Zein was the least fermentable substrate tested. Acetate was the principal fermentation end-product, followed mostly by butyrate and propionate. Branched-chain VFA proportions in VFA produced in cultures with casein, gluten, gliadin and zein were higher than in those supplied with glucose, mucin and RNA. In the former cultures, branched-chain VFA accounted for 10·2–17·7 molar % in the total VFA. In cultures supplied with proteins, there was a significant correlation between methane and VFA production, and between VFA and ammonia. The highest concentration of ammonia was found in cultures with casein. Production of methane was increased on glucose and nitrogenous substrates containing a carbohydrate moiety (mucin, RNA). It can be concluded that the caecal contents of rabbits are rich in nitrogenous compounds. The caecal fermentation of nitrogenous substrates supplies nutrients both for caecal microorganisms (branched-chain VFA, NH_3) and for the host (VFA).

INTRODUCTION

In rabbits, the caecum and proximal colon are important sites of organic matter digestion. Approximately 40% of the digested organic matter of the feed is digested in the caeco-colic segment (Gidenne 1992). Caecal microorganisms convert available nutrients (polysaccharides and nitrogenous substances) into mixtures of metabolites, and compounds of bacterial cells. No detailed information is available on fractions of nitrogen and fermentation of proteins and nucleic acids, in spite of the fact that the concentration of N compounds in rabbit caecal contents is high. Hoover & Heitmann (1975) reported a mean value of 47 mg non-ammonia N per g dry matter of the caecal contents of rabbits fed on a commercial concentrate feed. This value (equivalent to 294 g crude protein/kg) is similar to the findings of Piattoni *et al*. (1995), and also to values reported by several authors for rabbit soft faeces (reviewed by Emaldi *et al*. 1979). Bacterial cells account for most of the caecal crude protein (Spreadbury 1978). It can be calculated from the data of Gidenne (1992) on ileal and overall digestibility of protein (0·45–0·65 and 0·61–0·73 respectively) that, in a rabbit consuming 25 g of crude protein daily, *c*. 9–14 g of crude protein passes into the lower intestinal tract, where *c*. 2 g is apparently digested. As overall digestibility measurements include caecal microbial protein synthesized *de novo* from ammonia, the amount of dietary protein digested in the caecum is probably underestimated in this calculation. The quantity of protein supplied into the caecum by mucin and sloughed epithelial cells is not known, but cannot be ignored. In rats fed on a protein-free diet, the amount of nitrogen secreted or sloughed into the digestive tract was equivalent to the amount consumed by rats fed on a diet containing 100 g dietary protein/kg (Twombly & Meyer 1961).

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The presence of these substances and ammonia levels in the range 4–18 mmol/l (Gidenne 1997) suggests substantial decomposition of nitrogen-containing compounds in the rabbit caecum. Undoubtedly, the caecal fermentation of proteins and nucleic acids contributes significantly to the total VFA production in the rabbit digestive tract. The VFA are absorbed into the blood stream and satisfy a part of the energy requirement of the rabbit. The current study was carried out (i) to determine the amount of the total and TCA-soluble nitrogen, protein, ammonia, amino acids and urea in the caecal digesta, and (ii) to examine fermentation patterns in cultures of the caecal contents supplied with plant proteins (gluten, gliadin, zein), mucin and nucleic acids. For comparison, the fermentation pattern in cultures with casein and glucose was also investigated.

MATERIALS AND METHODS

Rabbits

Three-month-old Hyla 2000 rabbits were fed on granulated concentrate feeds (Table 1). Eleven rabbits were slaughtered, the caeca emptied and their contents weighed. Samples of digesta were taken for analyses. These rabbits had been fed on diet 1. Similarly, 12 rabbits were slaughtered, their caeca were emptied and pooled caecal contents were mixed and used for inoculation of *in vitro* cultures. These rabbits had been fed on diet 2.

Analyses of digesta and feeds

The pH of the digesta was measured immediately after sampling. Dry matter (DM) of the caecal contents was determined by drying at 105 °C overnight. Total nitrogen in the caecal contents was determined using the Kjeltec Auto 1030 Analyser (Tecator AB, Sweden). To determine TCA-soluble N, ammonia, urea and amino acids in TCA-supernatant, caecal contents (5 g) were mixed with 7% (w/v) trichloroacetic acid (15 ml). The mixture was refrigerated overnight, then centrifuged at 8000 ***g*** for 20 min. TCA-soluble N was determined in the supernatant by the Kjeldahl procedure, ammonia colorimetrically with Nessler reagent (after prior separation from interfering compounds by microdiffusion in Conway units) and urea by the diacetyl monoxime reagent using a commercial kit (Lachema Brno, Czech Republic). TCA-precipitate was washed with 7% trichloroacetic acid and protein extracted with 1 M NaOH at 100 °C for 1 h. Extracted protein was determined by the Lowry method (Herbert *et al.* 1971). Free amino acids in the TCA-supernatant were detected by the ninhydrin reagent (Spies 1957); the method was calibrated with glycine. A portion of the TCA-supernatant was mixed 1:1 with 12 M HCL and heated at 110 °C for 24 h. The hydrolysate was neutralized and free amino acids determined using the same procedure. Feeds were analysed by standard AOAC (1980) procedures.

Table 1. *Ingredients* (%) *and determined chemical composition* (*g*/*kg*) *of rabbit diets*

	Diet 1	Diet 2
Alfalfa meal	35	30
Wheat bran	22	30
Extracted sunflower meal	20	—
Extracted soya-bean meal	—	8
Barley	10	12
Oats	9	—
Wheat meal	—	8
Distillers dried grains	—	8
Rapeseed oil	1	1
Mineral supplement	2	2
Vitamin supplement	1	1
Dry matter	881	897
Crude protein	169	165
Crude fibre	146	137
Fat	37	35
Ash	89	81

Incubation

Incubations were carried out in quadruplicate on a shaking water bath at 39 °C, in 100 ml bottles. The caecal contents (3 g) were added to 30 ml of the Burroughs buffer (Burroughs *et al.* 1950) with 10% (v/v) of the caecal extract. CO_2 was bubbled through the buffer before use. The caecal extract was prepared by autoclaving (at 110 °C for 45 min) equal quantities of the caecal contents of rabbits and distilled water. The extract was clarified by centrifugation (8000 ***g*** for 1 h), autoclaved and re-centrifuged before use. Substrates were added at 0·2 g per incubation. Casein, gliadin from wheat and mucin from pig stomach were purchased from Fluka. Gluten from wheat and zein from maize were purchased from Sigma. Sodium ribonucleate (RNA) from yeast was supplied by ICN. Lipids from gluten were removed by diethylether extraction. Sodium sulphide was added to the incubation fluid at 0·5 g/l as a reducing agent. Bottles were thoroughly flushed with CO_2, hermetically closed with rubber stoppers and incubated for 20 h. Blank incubations were carried out by incubating caecal contents without substrate.

Analyses of incubation fluid and gas

The VFA concentrations were determined by titration, after steam distillation. Molar percentages of acetate (C_2), propionate (C_3), isobutyrate (i-C_4), butyrate

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(C_4), isovalerate (i-C_5) and valerate (C_5) were determined on a gas chromatograph with a FI detector, equipped with a column of the Chromosorb WAW with 15% SP 1200 and 1% H_3PO_4 (Supelco). The separation was carried out at 140 °C. Lactate was estimated by the microdiffusion method (Conway 1957). Ammonia was determined in samples clarified by centrifugation (8000 ***g*** for 20 min), as described above. Samples of the headspace gas were taken at the end of the incubation and analysed with the gas chromatograph, using the same column operated at room temperature. Production of metabolites from substrates tested was calculated by correcting findings for amounts present in incubated blanks. Data were analysed statistically by one-way analysis of variance (GraphPAD software, version 1.14, Birthe Avery, MD). The Bonferroni test was used for means comparison, where appropriate.

Concentration of substrates before and after incubation was measured against substrate-free blanks. Protein was determined by the Lowry method and RNA by orcinol reagent (Herbert *et al*. 1971). Glucose was assayed enzymatically using a commercial kit (Lachema Brno, Czech Republic).

RESULTS

The average caecal content weight of 3-month-old Hyla 2000 rabbits was 121·9 g. The quantity of caecal content ranged between 82 and 167 g, and represented 3·4–6·3% of the live weight. Both caecal pH and VFA concentration varied significantly: pH from 5·82 to 6·56 and VFA from 46·1 to 151·0 μmol/g. One g of the caecal digesta contained on average 234 mg of DM (Table 2). Crude protein (calculated using the factor 6·25) and true protein (determined by the Lowry method) represented 27·2 and 18·2% of digesta DM, respectively. Nitrogenous compounds soluble in 5·25% trichloroacetic acid accounted for 31·4% of total N in the caecal digesta. Caecal contents contained 0·27 mg of ammonia N per g and 2·3 μg of urea N per g, on average. One g of the caecal contents contained 65 μmol of glycine equivalents of TCA-soluble amino acids. Acid hydrolysis increased this amount to 180 μmol/g.

Production of metabolites in rabbit caecal cultures is shown in Table 3. The values were corrected for metabolites produced in corresponding incubated blanks. Incubation of proteins and RNA resulted in the net production of VFA, methane and ammonia, but not lactate. Proteins differed in their fermentability. Fermentation of casein and mucin yielded significantly more VFA than fermentation of other proteins (gluten, gliadin, zein) and RNA ($P < 0·001$). Zein was the poorest substrate out of six nitrogen compounds tested. In VFA produced from RNA a significantly higher proportion of acetate and a lower proportion of propionate was found ($P < 0·01$). Branched-chain VFA accounted for 0·102–0·177 molar proportion of the total VFA produced from casein, gluten, gliadin and zein. The concentration of isovalerate was greater than that of isobutyrate in all cultures, except those grown on glucose. Average production of methane and ammonia from proteins correlated significantly with that of VFA ($r = 0·96$ and 0·94, respectively; $P < 0·02$). Fermentation of casein produced significantly more ammonia than fermentation of other nitrogen-containing substrates ($P < 0·05$). Fermentation of nitrogenous substrates and glucose differed. Cultures of caecal microbes grown on glucose produced relatively more propionate and butyrate, and less branched-chain VFA than cultures supplied with proteins. Glucose and RNA fermentation yielded the same amount of methane, significantly more than fermentation of other substrates ($P < 0·01$). Ammonia was utilized instead of produced in glucose-supplied cultures.

Table 2. *Content of dry matter, total N, protein, TCA-soluble N, ammonia N, urea N and amino acids in the caecal digesta of rabbits. Standard errors are given in parentheses* (*D.F. = 10*)

	Per g of digesta	Per g of digesta DM	Per organ
Dry matter (g)	0·234 (0·0018)	1·00	28·4 (1·96)
Total N (mg)	10·2 (0·19)	43·8 (0·76)	1242 (86·1)
Protein (mg)	42·6 (2·91)	182·3 (12·18)	5203 (489·2)
TCA-soluble N			
Total (mg)	3·2 (0·10)	13·8 (0·39)	390 (27·4)
NH_3-N (mg)	0·27 (0·062)	1·15 (0·271)	33 (7·2)
Urea (μg)	2·3 (0·12)	9·8 (0·48)	280 (22·4)
Amino acids in TCA-supernatant*			
Before hydrolysis (mmol)	0·065 (0·0029)	0·28 (0·012)	7·9 (0·35)
After hydrolysis (mmol)	0·180 (0·0074)	0·77 (0·032)	21·9 (0·90)

DM, dry matter.
* Ninhydrin-reactive compounds expressed as glycine equivalents.

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Table 3. *Effect of substrate on production* (μmol/flask) and molar proportions of volatile fatty acids (VFA), production* of methane (μmol/flask) and ammonia (mg/flask) in incubations of rabbit caecal contents supplied with proteins, ribonucleic acid (RNA) and glucose. Standard errors are given in parentheses (D.F. = 3)*

Substrate	Total VFA	Molar proportions						CH_4	NH_3-N
		C_2	C_3	i-C_4	C_4	i-C_5	C_5		
Casein	1416	0·419	0·155	0·066	0·163	0·111	0·086	38·6	18·4
	(23·1)	(0·0066)	(0·0017)	(0·0022)	(0·0031)	(0·0031)	(0·0035)	(5·61)	(0·91)
Gluten	462	0·458	0·169	0·034	0·172	0·068	0·099	17·5	4·4
	(31·4)	(0·0198)	(0·0068)	(0·0043)	(0·010)	(0·0081)	(0·0095)	(3·63)	(0·41)
Gliadin	535	0·526	0·109	0·032	0·134	0·085	0·114	19·8	6·5
	(42·2)	(0·0192)	(0·0073)	(0·0056)	(0·0085)	(0·0075)	(0·0115)	(8·91)	(0·63)
Zein	119	0·410	0·114	0·036	0·255	0·126	0·059	8·2	1·5
	(26·1)	(0·0129)	(0·0098)	(0·008)	(0·0153)	(0·0115)	(0·0089)	(2·46)	(0·36)
Mucin	1475	0·604	0·169	0·017	0·148	0·022	0·040	56·4	12·8
	(15·8)	(0·0071)	(0·0026)	(0·0022)	(0·0042)	(0·0059)	(0·002)	(4·95)	(0·46)
RNA	700	0·787	0·047	—	0·127	0·018	0·021	94·1	14·0
	(23·8)	(0·0148)	(0·007)		(0·0063)	(0·0077)	(0·007)	(6·93)	(0·76)
Glucose	1257	0·512	0·212	0·007	0·240	0·002	0·027	112·5	−2·6
	(35·3)	(0·0075)	(0·0027)	(0·0013)	(0·0054)	(0·0021)	(0·0023)	(3·63)	(0·14)

* Data were corrected for amounts present in incubated blanks.

Table 4. *Initial and final concentration of substrates (mg/ml) solubilized in the incubation fluid*

Substrate	Time of incubation (h)	
	0	20
Casein	3·30	0·61
Gluten	0·10	0
Gliadin	0·31	0
Zein	0·24	0
Mucin	3·58	0
RNA	6·10	0
Glucose	6·08	0

No protein tested was completely solubilized at the beginning of the incubation. Solubility of gluten, gliadin and zein in the incubation fluid was poor (Table 4). At the end of the incubation no glucose was found in the culture supernatant and the difference in protein concentration between cultures supplied with proteins and incubated blanks was either small or nil. Concentration of orcinol-reactive compounds was the same in RNA-supplied and substrate-free cultures at the end of the incubation.

DISCUSSION

The rabbit is the best-studied caecum fermenter. Quantitative information on caecal nitrogen metabolism in rabbits, however, is scarce. In the current experiment, crude protein (N × 6·25) concentration was 272 mg/g of the digesta DM. Very similar concentrations of DM (257 mg/g) and crude protein (279 mg/g DM) in caecal digesta of 8-week-old rabbits were reported by Piattoni *et al.* (1995), and earlier by Hoover & Heitmann (1975). The main fraction of nitrogen in the caecum was the true protein, estimated by the Lowry method. True protein accounted for two-thirds of crude protein. The proportions of bacterial protein, protein of endogenous origin (enzymes and mucin secretions, cell desquamation), and undegraded dietary protein have yet to be defined. Non-ammonia, TCA-soluble nitrogenous compounds represent the second main fraction of nitrogen in the caecum. This fraction was composed of free amino acids and peptides. Because the amount of ninhydrin reaction was much greater after acid hydrolysis than before, it appears that peptides and not amino acids were the primary components in this ninhydrin-reactive material. For one of the rabbits, the concentration of free amino acids in the caecal content was only 7·4% of that in the small intestinal digesta (data not shown). Whether amino acids can be absorbed from the rabbit caecum and transported against the concentration gradient into the blood remains uncertain. Endogenous urea entering the caecum was detectable in the caecal contents, but its concentration was negligible (2·3 μg N/g). Higher urea concentrations, *c.* 19 μg N/g, were found in the caecum pouch of sheep (Feješ *et al.* 1982). The flux of endogenous urea from blood into the digestive tract of rabbits is high, representing half of the urea produced by nitrogen catabolism of the animal (Viallard 1984). Low concentration of urea in the digesta indicates that endogenous urea was immediately hydrolysed by

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caecal bacteria or by bacteria adhering to the intestinal wall, as also shown with rumen microbes (Cheng & Costerton 1979).

Presumably, undegraded dietary proteins entering the caecum are plant proteins of low solubility, i.e. prolamins and glutelins. Three proteins of this class were investigated in the current study. Gluten is the principal protein component of wheat endosperm, practically insoluble in water. Gliadin is a simple protein containing a high proportion of glutamine, one of the prolamins of wheat. Zein is a hydrophobic protein, the principal protein of maize. The solubility of these proteins in the incubation fluid was very low (Table 4). In contrast, casein is a phosphoprotein, readily soluble in diluted alkali. As expected, production of metabolites in cultures with insoluble plant proteins was smaller than from casein and mucin. Mucin is a glycoprotein, present in saliva, gastric juice and intestinal juice. Carbohydrate accounts for about 45% of its molecular weight (Merck Index 1989). The carbohydrate moiety of the nitrogen compounds influenced the fermentation pattern. Composition of fermentation end-products in cultures with mucin and RNA resembled to some extent end-products of glucose fermentation as (i) production of methane was significantly higher, and (ii) production of branched VFA was lower in the former cultures than in those supplied with pure proteins. A considerably higher acetate to propionate ratio in RNA-grown cultures may reflect differences between pentose (RNA) and hexose (glucose, mucin) fermentation. Purine and pyrimidine bases (i.e. products of nucleic acid decomposition) are rather stable under anaerobic conditions, as observed in several rumen experiments (reviewed by Smith 1975), thus probably did not contribute to the VFA formation. Ammonia accumulation in cultures with RNA, however, indicates that amino groups were deaminated at some stage of nucleic acid degradation. The fermentation of casein produced only 34% of the methane produced with glucose. In ruminal experiments, casein produced only 50% of the methane (Demeyer & Van Nevel 1979) and 32% of the gas (Cone & Van Gelder 1999) produced with carbohydrates. Fermentation of casein in current incubations yielded more VFA and less methane per g of substrate than fermentation of casein in the sheep rumen fluid in the experiment of Demeyer & Van Nevel (1979). This can be related to differences between an acetogenic (caecum) and methanogenic (rumen) type of fermentation, as suggested by Demeyer *et al*. (1989). The VFA formed in casein-supplied cultures contained 0·197 molar proportion of isovalerate and valerate, comparable to 0·198 molar proportion of leucine, isoleucine and lysine in casein amino acids.

Individual VFAs differ in their physiological significance for the host. Acetate is energy yielding and a precursor for lipogenesis and cholesterolgenesis. Propionate is gluconeogenic and a precursor of the synthesis of amino acids. Butyrate is the energy-yielding substrate of enterocytes. Branched VFA and valerate are growth factors of rumen cellulolytic bacteria (Dehority *et al*. 1967). In ruminants, branched VFA have a general positive influence on rumen microbial metabolism, and are sometimes used as a feed additive to improve feed utilization (reviewed by Andries *et al*. 1987). Similarly, phenylpropanoic acid, the product of phenylalanine deamination, greatly stimulated cellulose digestion by ruminal ruminococci (Stack & Cotta 1986). Ammonia released from N-containing compounds is the main nitrogen source for growth of caecal microbes. More than half of rabbit caecal bacteria utilized ammonia as the sole source of nitrogen (Forsythe & Parker 1985). The caecal fermentation of proteins and nucleic acids thus represents a supply of nutrients and growth factors which support optimal microbial growth, necessary for caecal organic matter digestion. In addition, caecal microbes convert macromolecular nitrogenous compounds, which would be lost in hard faeces, into volatile products, available to the host.

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REFERENCES

Andries, J. I., Buysse, F. X., Brabander, D. L. & Cottyn, B. G. (1987). Isoacids in ruminant nutrition: their role in ruminal and intermediary metabolism and possible influences on performances – a review. *Animal Feed Science and Technology* **18**, 169–180.

AOAC (1980). *Official Methods of Analysis*. Washington, DC: Association of Official Analytical Chemists.

Burroughs, W. N., Frank, A., Gerlaugh, P. & Bethke, R. M. (1950). Preliminary observations upon factors influencing cellulose digestion by rumen micro-organisms. *Journal of Nutrition* **40**, 9–24.

Cheng, K.-J. & Costerton, J. W. (1979). Adherent rumen bacteria – their role in the digestion of plant material, urea and epithelial cells. In *Digestive Physiology and Metabolism in Ruminants* (Eds Y. Ruckebush & P. Thivend), pp. 227–250. Lancaster: MTP Press.

Cone, J. W. & Van Gelder, A. H. (1999). Influence of protein fermentation on gas production profiles. *Animal Feed Science and Technology* **76**, 251–264.

Conway, E. J. (1957). *Microdiffusion Analysis and Volumetric Error*. 4th edition, pp. 248–278. London: Crosby Lockwood and Son.

https://doi.org/10.1017/S0021859699008345 Published online by Cambridge University Press

DEHORITY, B. A., SCOTT, H. W. & KOWALUK, P. (1967). Volatile fatty acid requirements of cellulolytic rumen bacteria. *Journal of Bacteriology* **94**, 537–543.

DEMEYER, D., DE GRAEVE, K., DURAND, M. & STEVANI, J. (1989). Acetate: a hydrogen sink in hindgut fermentation as opposed to rumen fermentation. *Acta Veterinaria Scandinavica* **86** (*Supplement*), 68–75.

DEMEYER, D. & VAN NEVEL, C. (1979). Protein fermentation and growth by rumen microbes. *Annales de la Recherche Vétérinaire* **10**, 277–279.

EMALDI, O., CROCIANI, F. & MATTEUZZI, D. (1979). A note on the total viable counts and selective enumeration of anaerobic bacteria in the caecal content, soft and hard faeces of rabbit. *Journal of Applied Bacteriology* **46**, 169–172.

FEJEŠ, J., VÁRADY, J. & BOĎA, K. (1982). Secretion of endogenous urea into the caecum in sheep (in Slovak). *Veterinary Medicine* (Czech) **27**, 75–80.

FORSYTHE, S. J. & PARKER, D. S. (1985). Nitrogen metabolism by the microbial flora of the rabbit caecum. *Journal of Applied Bacteriology* **58**, 363–369.

GIDENNE, T. (1992). Effect of fibre level, particle size and adaptation period on digestibility and rate of passage as measured at the ileum and in faeces in the adult rabbit. *British Journal of Nutrition* **67**, 133–146.

GIDENNE, T. (1997). Caeco-colic digestion in the growing rabbit: impact of nutritional factors and related disturbances. *Livestock Production Science* **51**, 73–88.

HERBERT, D., PHIPPS, P. J. & STRANGE, R. E. (1971). Chemical analysis of microbial cells. In *Methods in Microbiology*, Vol. 5B (Eds J. R. Norris & D. W. Ribbons), pp. 209–344. London: Academic Press.

HOOVER, W. H. & HEITMANN, R. N. (1975). Cecal nitrogen metabolism and amino acid absorption in the rabbit. *Journal of Nutrition* **105**, 245–252.

MERCK INDEX (1989). Merck Index. Rahway, USA: Merck & Co., Inc.

PIATTONI, F., MAERTENS, L. & DEMEYER, D. (1995). Age dependent variation of caecal contents composition of young rabbits. *Archives of Animal Nutrition* **48**, 347–355.

SMITH, R. H. (1975). Nitrogen metabolism in the rumen and the composition and nutritive value of nitrogen compounds entering the duodenum. In *Digestion and Metabolism in the Ruminant* (Eds I. W. McDonald & A. C. I. Warner), pp. 399–415. Armidale, Australia: University of New England Publishing Unit.

SPIES, J. R. (1957). Colorimetric procedures for amino acids. In *Methods in Enzymology*, Vol. III (Eds S. P. Colowick & N. O. Kaplan), pp. 467–477. New York: Academic Press.

SPREADBURY, D. (1978). A study of the protein and amino acid requirements of the growing New Zealand White rabbit with emphasis on lysine and the sulphur-containing amino acids. *British Journal of Nutrition* **39**, 601–613.

STACK, R. J. & COTTA, M. A. (1986). Effect of 3-phenylpropanoic acid on growth of and cellulose utilization by cellulolytic ruminal bacteria. *Applied and Environmental Microbiology* **52**, 209–210.

TWOMBLY, J. & MEYER, J. H. (1961). Endogenous nitrogen secretions into the digestive tract. *Journal of Nutrition* **71**, 453–460.

VIALLARD, V. (1984). Endogenous urea as a nitrogen source for microorganisms of the rabbit digestive tract. *Annals of Nutrition and Metabolism* **28**, 151–155.

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