

Effects of supplements on intake, rumen function and nutrient supply and growth in cattle eating alkali-treated oat straw

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1. Expt 1. Six 200 kg Hereford heifers fitted with rumen and abomasal cannulas were fed *ad lib.* on alkali-treated oat straw sprayed with urea and minerals, with no supplement (O), or 700 g rolled barley (B) or cottonseed meal (C)/d, in a 3 × 3 Latin square with two replicates.

2. Intakes of the basal diet were not significantly affected by the supplements. Intakes of digestible organic matter (OM) were 3135, 3325 and 3515 g/d on diets O, B and C respectively. Abomasal OM flow on diet C was 13 and 12% higher than on diets O and B respectively ($P < 0.05$) which was associated with a decrease in the proportion of OM intake apparently digested in the rumen ($P < 0.05$) and an increase in the proportion of abomasal OM digested in the lower gut ($P < 0.05$).

3. Total nitrogen and bacterial N flows at the abomasum were significantly higher ($P < 0.01$ and $P < 0.05$ respectively) on diet C than on diets O and B. Efficiency of bacterial N synthesis on diet C was 19 and 28% higher than on diets O and B respectively ($P < 0.05$).

4. Dry matter (DM) pool size in the rumen was greater on diet C than on diets O and B ($P < 0.05$).

5. Expt 2. Thirty-three 210 kg Friesian heifers were allocated to the same treatments as in Expt 1. Intakes of the basal straw diet were not significantly affected by the supplements. Intakes of digestible OM were 3.80, 4.16 and 4.34 kg/d on diets O, B and C respectively.

6. Rumen ammonia and plasma urea levels were significantly higher on diet C than on diets O and B ($P < 0.05$).

7. Live-weight gains were 679, 838 and 1051 g/d on diets O, B and C respectively ($P < 0.01$) and live-weight gain/MJ metabolizable energy intake was highest on diet C ($P < 0.01$).

8. It was concluded that the growth response to barley was attributable to the increase in energy supply, and that the additional response to cottonseed meal was attributable to greater protein absorption arising from greater abomasal flows of bacterial and dietary proteins.

In a previous experiment with steers given a basal diet of alkali-treated wheat straw, together with supplements, growth rates were higher when cottonseed meal was fed than when barley grain was fed (Spragg *et al.* 1982). This growth response was associated with an increase in diet digestibility, no increase in intake, and an increase in efficiency of utilization of calculated metabolizable energy (ME) intake.

When rumen degradable nitrogen (RDN) is non-limiting, protein supplements sometimes increase growth rates in ruminants given roughage diets. Possible reasons for growth responses are: (1) increase in voluntary intake of roughage (Egan, 1965; Kempton & Leng, 1979); (2) increase in efficiency of microbial protein synthesis (Hume, 1970); (3) increase in flow of undegraded dietary protein to intestines, supplying essential amino acids to the animal; (4) increase in non-specific energy supply to the animal; (5) increase in diet digestibility (Oldham *et al.* 1979); (6) increase in rumen contents (Egan, 1970); (7) increase in efficiency of acetate utilization caused by amino acids acting as precursors for gluconeogenesis (Trenkle, 1980).

The present study was undertaken to investigate more fully responses to protein and energy supplements, in order to identify mechanisms of animal responses to protein supplements. Two experiments were conducted with heifers given alkali-treated oat straw as the basal diet. A preliminary report was given by Spragg *et al.* (1984).

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Table 1. *Expts 1 and 2. Chemical composition (g/kg dry matter (DM)) of alkali-treated oat straw (O) and the two supplements, rolled barley (B) and cottonseed meal (C)*

Dietary treatment...	Supplement		
	O	B	C
DM (g/kg)	852	918	929
Chemical composition (g/kg DM)			
Organic matter	884	974	924
Nitrogen	18.3	21.3	73.2
Neutral-detergent fibre	688	138	339
Lignin	57	14	63

MATERIALS AND METHODS

Expt 1

Animals and management. Six Hereford heifers aged approximately 18 months (average live weight 200 kg), fitted with simple cannulas (64 mm diameter) in the rumen and abomasum (Hecker, 1974), were tethered in individual pens with water available *ad lib.* and continuous lighting. Animals were allocated to two groups of three heifers each, in a 3 × 3 Latin square with two replicates. Dietary treatments were: no supplement (O), rolled barley (B), cottonseed meal (C).

Diets and feeding procedure. The basal diet was oat straw milled through a 38 mm screen in a trailer-mixer. During mixing the straw was sprayed with a solution supplying (g/kg straw) 44 sodium hydroxide and 140 water, followed by a solution supplying (g/kg straw) 30 water, 13.7 N (as urea), 0.95 sulphur (as sulphuric acid), 1.5 phosphorus (as phosphoric acid) and (mg/kg straw) 4.3 copper, 0.1 cobalt after which it was sprinkled with hydrated lime supplying (g/kg straw) 2.0 calcium. Straw was mixed in batches of 250 kg and stored in hessian bags for at least 17 d before feeding. Animals were offered the basal diet of alkali-treated oat straw *ad lib.*

Supplements used were solvent-extracted cottonseed meal (C) and steam-rolled barley (B). Animals receiving supplements were given 700 g (air dry) once daily in a separate container. Chemical compositions of the basal diet and supplements are shown in Table 1.

Intakes of basal diet were recorded daily and samples of feed and feed residues were retained for analysis.

Basal diet and supplement degradabilities. Dry matter (DM) and N degradability for the basal diet and supplements were determined using nylon bags. Ground (< 1 mm) samples of supplements C and B (5 g) and alkali-treated straw (3 g) were placed in nylon bags measuring 80 × 160 mm with pore size 30 × 50 μm and were incubated in the rumen of heifers on diets C, B and O, in Expt 1, for periods of 0, 3, 6, 9, 12, 24 and 48 h, and also 72 and 96 h for alkali-treated straw. Immediately following removal from the rumen, bags were washed under running tap-water for 5 min, by which time the washings were clear; the bags were then dried at 80° overnight. Degradation rates of DM and N were calculated from weight changes in the content of the bags. Natural log residues were plotted *v.* time and manual curve peeling procedures used to identify soluble, fast-digesting and slow-digesting pools as described by Kempton (1980).

Marker infusion and digesta collections. The dual markers used were acid-detergent lignin (Goering & Van Soest, 1970) and CrEDTA. CrEDTA was prepared according to Binnerts *et al.* (1968) and infused into the rumen using a peristaltic pump. Animals had

a 21 d adaptation period to the diet before infusion, which started 5 d before digesta and faecal sampling. Rumen and abomasal digesta (85 ml per sample) were collected every 6 h over a 3 d period during which there were two 8 h intervals to allow a shift in sampling times. Therefore, 3-d composite samples from the rumen and abomasum each comprised twelve digesta samples, representing each even-numbered hour of the 24 h day. Rumen digesta were strained and acidified to pH 2. Digesta samples were stored at -10° . From the start of digesta sampling, faecal grab samples were taken three times daily for 5 d. Following digesta sampling, Foley urethral catheters were inserted into the bladder for urine collection over 4 d. Sufficient hydrochloric acid was added to maintain pH below 2; 1% daily samples were bulked and stored at -10° . After each collection period, marker infusion was terminated and eight rumen samples were taken over 26 h to determine fluid fractional outflow rates. On the final day of each period, rumen pool size was determined by manual emptying and weighing of rumen contents 3 h after feeding supplements. After mixing in a concrete mixer, a sample was taken for DM and pH determinations and the contents were returned to the rumen. The time taken for rumen emptying, sampling and return of digesta was about 30 min per animal.

Heifers were weighed between each sampling period.

Sample preparation and chemical analyses. Feed, rumen digesta, abomasal digesta and faeces were analysed for DM by drying to constant weight at 80° , organic matter (OM) by ashing at 550° overnight. Subsamples of rumen digesta, abomasal digesta and faeces were dried at 50° in a forced-draught oven before analysis for N by a microKjeldahl technique, and lignin by the method of Goering & Van Soest (1970).

Samples of abomasal digesta were centrifuged at 2400 g for 5 min to obtain liquid-rich fractions which analyses showed did not contain any acid-detergent lignin. Liquid-rich fractions, total abomasal digesta, urine and faeces were analysed for chromium by atomic absorption spectroscopy. Liquid-rich fractions were also analysed for DM, OM and N. Flows of abomasal digesta and faeces were calculated from the concentrations of Cr and acid-detergent lignin according to Faichney (1975). Faecal lignin output rather than feed lignin intake was used to calculate the daily intake of marker lignin, because digestion or solubilization of lignin occurs primarily in the rumen (Egan *et al.* 1975; Faichney, 1980). Daily intakes of marker lignin were 39.8, 37.9 and 43.4 g/d on treatments O, B and C respectively.

Acidified rumen samples collected during the experiment were used to isolate rumen bacteria, according to Hutton *et al.* (1971). Rumen fluid bacteria and total abomasal digesta were analysed for 2,6-diaminopimelic acid (DAPA) by ion-exchange chromatography using an amino acid AutoAnalyzer TSM (Technicon Instrument Corporation, Tarry Town, New York) following 24 h hydrolysis in 6 M-HCl at 110° under N_2 , using norleucine as an internal standard. The proportion of abomasal N present as bacterial N was calculated as mg DAPA/g N in digesta \times g N/mg DAPA in bacterial samples from individual animals. Volatile fatty acids (VFA) in acidified rumen samples were analysed using gas-liquid chromatography with a flame ionization detector (Hewlett Packard Model 402). VFA were separated on a 1.8 m long \times 2 mm i.d. column packed with 15% neopentyl glycol adipate and 2.5% H_3PO_4 on chromosorb W-AW (80-100 mesh). The internal standard was 3-methyl *n*-valeric acid. Non-acidified rumen samples were assayed for Cr and acid-detergent lignin. Mean retention times were calculated as the reciprocal of disappearance rate constants for the marker CrEDTA and rumen lignin pool (g)/lignin intake (g/h) for lignin. Rumen ammonia was determined by the method of Chaney & Marbach (1962).

Statistical analysis. Data were subjected to an analysis of variance for two 3×3 Latin squares. Interactions between treatments and squares were not significant and effect of treatment was tested against residual mean square (4 df). Treatment means were compared on the basis of least significant difference (Steel & Torrie, 1960).

Expt 2

Animals and management. Thirty-three yearling Friesian heifers (average live weight 210 kg) were housed in individual pens with water available *ad lib.* and continuous lighting. After a 14 d adaptation period, animals were allocated to three treatments (eleven heifers/treatment) on a live-weight basis, using restricted randomization from three live-weight groups. Live weights were recorded twice weekly over 76 d, and live-weight changes estimated by regressions of live weight *v.* time.

Diets and feeding procedure. The basal diet was the same alkali-treated oat straw as fed in Expt 1. It was offered *ad lib.* and accumulated food refusals were removed twice weekly. The treatments were the same as in Expt 1; daily portions of supplements were placed in feed buckets separate from the basal diet.

Sampling procedure. On days 36 and 77, 3 h after supplement feeding, samples of rumen fluid were collected from all animals, using a stomach tube. After measuring pH, rumen fluid was filtered, acidified to pH 2 and stored at -10° . Concurrently, blood samples were collected from a jugular vein in all animals, plasma was separated and stored at -10° . During days 71–75 inclusive, faecal samples were collected three times daily from freshly voided faeces and stored at -10° .

Chemical analyses. Feed and faecal samples were analysed for DM, OM, N, acid-detergent fibre (ADF), neutral-detergent fibre (NDF) and acid-detergent lignin as in Expt 1. Rumen ammonia and plasma urea were determined using the method of Chaney & Marbach (1962).

Statistical analysis. Values were analysed as a randomized block, and treatment effects were tested against residual mean square (30 df). Treatment means were compared on the basis of least significant difference (Steel & Torrie, 1960).

RESULTS

Estimated degradabilities of the basal diet and supplements are given in Table 2. The large proportion of soluble N in the basal diet was urea. In contrast there was much less soluble N in the supplements. The rate of disappearance of N from the fast-digesting pool was much more rapid from B than from C.

Expt 1

Animals remained in good health throughout. Intakes of straw DM, total DM and OM were not significantly different between dietary treatments (Table 3). Abomasal OM flow for diet C was 13 and 12% higher than for diets O and B respectively, these differences being significant ($P < 0.05$). The fraction of digestible OM (DOM) apparently digested in the stomach was significantly lower on diet C than on diets O and B ($P < 0.05$).

Total N intake was 106, 113 and 150 g/d on diets O, B and C respectively (Table 4). Although abomasal flow of N and N apparently digested post-ruminally did not differ significantly between diets O and B, N balance was significantly higher on diet B than on diet O ($P < 0.05$). Total abomasal N flow and N apparently digested post-ruminally was substantially higher on diet C than on diets O and B ($P < 0.05$). The proportion of bacterial N in total abomasal N did not differ significantly between diets, but the efficiency of bacterial N synthesis on diets B and C was 15 and 31% higher respectively than on diet O. N balance on diets B and C was substantially higher than on diet O.

Total VFA and rumen ammonia levels were higher on diets B and C than on diet O (Table 5), although not significantly so ($P > 0.05$). Liquid and solid retention times in the rumen were similar on the three treatments. Liquid-pool sizes did not differ significantly

Table 2. Expts 1 and 2. Percentage composition and half lives ($t_{\frac{1}{2}}$) for dry matter (DM) and nitrogen disappearance of material from that pool for alkali-treated oat straw (O) and the two supplements, rolled barley (B) and cottonseed meal (C), determined in nylon bags suspended in the rumen

Dietary treatment		Soluble pool	Fast-digesting pool		Slow-digesting pool	
		Size (%)	Size (%)	$t_{\frac{1}{2}}$ (h)	Size (%)	$t_{\frac{1}{2}}$ (h)
O	DM	17.7	52.1	10.4	30.2	133.3
	N	73.9	26.1	71.9	—	—
B	DM	15.4	64.5	9.1	20.1	41.3
	N	11.8	88.2	11.4	—	—
C	DM	14.2	32.3	6.5	53.5	53.3
	N	17.4	82.6	31.3	—	—

Table 3. Expt 1. Intake of dry matter and organic matter (OM) and digestion of OM in heifers eating alkali-treated oat straw (O) and the two supplements, rolled barley (B) and cottonseed meal (C)

Dietary treatment...	Supplements			SEM
	O	B	C	
Dry matter intake (g/d):				
Straw	5966	5588	5816	148.5
Total	5966	6230	6467	148.5
OM intake (g/d)	5348	5635	5818	133.0
OM leaving abomasum	2992 ^a	3041 ^a	3445 ^b	71.5
OM in faeces	2213	2311	2303	97.3
Apparent OM digestibility in whole tract	0.58	0.58	0.61	0.010
Proportion of digestible OM (DOM) apparently digested in stomach	0.75 ^a	0.78 ^a	0.67 ^b	1.97
DOM intake (g/d)	3135 ^a	3325 ^{ab}	3515 ^b	65.7

^{a, b} Mean values with different superscript letters differed significantly ($P < 0.05$).

between treatments and estimates based on CrEDTA did not differ significantly from values based on manual emptying 3 h after feeding supplements. The DM pool in the rumen on diet C was larger than that on diets O and B ($P < 0.05$).

Expt 2

Animals remained in good health throughout and readily consumed alkali-treated straw and supplements. No significant differences were recorded in either straw DM intake or total DM intakes (Table 6). DOM intake (DOMI) and DOM in DM (DOMD) were significantly higher on diet C than on diet O. Live-weight gain on diet C was 25% higher than on diet B which was 23% higher than on diet O, these differences being significant ($P < 0.01$). Diet C had higher live-weight gain/MJ ME intake than either diets O or B

Table 4. *Expt 1. Intake and digestion of nitrogen, abomasal flows of bacterial N and efficiencies of bacterial N synthesis in heifers eating alkali-treated oat straw (O) and the two supplements, rolled barley (B) and cottonseed meal (C)*

Dietary treatment...	Supplements			SEM
	O	B	C	
Total N:				
Intake from straw (g/d)	105.6	99.3	102.4	—
Intake from supplement (g/d)	0	13.7	47.6	—
Abomasal flow (g/d)	104.8 ^a	103.2 ^a	135.9 ^b	2.91
Urine output (g/d)	66.5 ^a	55.9 ^a	81.4 ^b	3.90
Faecal output (g/d)	37.9 ^a	42.1 ^{ab}	48.0 ^b	1.84
N balance (g/d)	1.2 ^a	15.0 ^b	20.6 ^b	3.80
Apparently digested post-ruminally (g/d)	66.8 ^a	61.2 ^a	87.9 ^b	4.01
Proportion of abomasal flow apparently digested	0.63	0.59	0.65	0.022
Bacterial N:				
Abomasal flow (g/d)	65.9 ^a	82.7 ^b	89.6 ^b	4.94
Proportion in total abomasal N	0.60	0.66	0.69	0.077
Bacterial N (g)/kg organic matter apparently digested in rumen	28.9 ^a	33.2 ^{ab}	37.9 ^b	1.20

^{a, b} Mean values with different superscript letters differed significantly ($P < 0.05$).

Table 5. *Expt 1. Concentrations of total volatile fatty acids (VFA), ammonia and pH in rumen fluid, retention time of CrEDTA, solid turnover time in the rumen and rumen pool size of heifers eating alkali-treated oat straw (O) and the two supplements, rolled barley (B) and cottonseed meal (C)*

Dietary treatment...	Supplements			SEM
	O	B	C	
Total VFA (mmol/l)	74.2	84.7	85.1	13.30
Rumen ammonia (mmol/l)	6.9	8.3	9.0	1.02
Rumen pH	7.0	6.9	6.9	0.08
Retention time (h):				
CrEDTA	14.7	14.2	12.8	0.81
Lignin	24.6	23.9	24.3	0.31
Pool size:				
Liquid (kg):				
CrEDTA	51.0	41.9	40.4	3.60
Manual emptying	46.1	42.8	48.5	1.84
Dry matter (kg):				
Manual emptying	5.10 ^a	5.19 ^a	5.88 ^b	0.211
Dry matter in rumen (%)	11.0 ^a	12.2 ^b	12.1 ^b	0.22

^{a, b} Mean values with different superscript letters differed significantly ($P < 0.05$).

Table 6. Expt 2. Dry matter intake (DMI), digestible organic matter intake (DOMI), digestible organic matter in dry matter (DOMD), calculated metabolizable energy (ME) intake, predicted and actual live-weight gains and rumen ammonia and plasma urea of thirty-three Friesian heifers eating alkali-treated oat straw (O) and the two supplements, rolled barley (B) and cottonseed meal (C)

Dietary treatment...	Supplements			SEM
	O	B	C	
DMI				
Straw (kg/d)	7.00	6.93	7.02	0.247
Straw (g/kg LW ^{0.75})	115.1	112.9	111.3	1.78
Total (kg/d)	7.00	7.57	7.67	0.247
DOMI† (kg/d)	3.80 ^a	4.16 ^{ab}	4.34 ^b	0.146
DOMD‡ (%)	54.3 ^a	55.0 ^{ab}	56.6 ^b	0.64
ME intake§ (MJ/d)	59.3 ^a	65.0 ^{ab}	67.7 ^b	2.34
Actual LW gain (g/d)	679 ^a	838 ^b	1051 ^c	33.2
Predicted LW gain (g/d)	730	830	875	—
LW gain/ME intake (g/MJ)	11.5 ^a	13.0 ^a	15.7 ^b	0.60
Rumen ammonia (mmol/l)	11.5 ^{ab}	10.1 ^a	13.3 ^b	0.91
Plasma urea (mmol/l)	4.3 ^a	4.0 ^a	5.2 ^b	0.16

LW, live weight.

^{a, b} Mean values with different superscript letters differed significantly ($P < 0.05$).

† DOMD × DMI.

‡ % lignin in feed OM × OM intake × 100

‡ % lignin in faeces OM × DM intake

§ DOMD × 0.156 × DMI.

|| Agricultural Research Council (1980).

($P < 0.01$). Rumen ammonia and plasma urea levels were higher for diet C than diet B ($P < 0.05$).

DISCUSSION

The objective of the present experiments was to gain a clearer understanding of the mechanisms by which protein supplements can increase growth rates in ruminants given low-quality roughage diets. RDN and S were made readily available for microbial growth by spraying the basal diet with urea and sulphuric acid. Rumen ammonia concentrations were > 6.9 mm in Expt 1 and > 10.1 mm in Expt 2. These were considerably in excess of levels of 1 mm and 3.6 mm which Schaefer *et al.* (1980) and Satter & Slyter (1974) respectively, reported to be effective for maximal microbial growth.

Supplements used in these experiments were assumed to supply approximately equal amounts of energy so that the main difference between them was the proportion of energy supplied as protein. The protein content of the straw used in the basal diet was very low (crude protein (N × 6.25) 29 g/kg DM) so that undegraded dietary protein flowing from the stomach would have been mainly from the supplements. Abomasal flows of N were similar on diets O and B (Table 4) which suggests that barley protein was completely degraded in the rumen. If the non-bacterial N is considered, it appears that rumen digestion of N in the basal diet was increased by the barley supplement. This is in accord with the observation that N in barley disappeared rapidly from nylon bags in the rumen (Table 2). Abomasal flow of N on diet C was substantially higher than on diets O and B, and this was contributed both by bacterial and non-bacterial N (Table 4). If it is assumed that the

non-bacterial N was largely undegraded dietary N, this would be in accord with the observation that N in diet C disappeared slowly from nylon bags in the rumen (Table 2).

The greater efficiency of bacterial N synthesis and greater flow of bacterial N on diet C than on the other two diets (Table 4) was probably attributable to the stimulatory effect on bacterial growth of amino acids from the C supplement. Whilst most rumen bacteria can grow with, or require, N in the form of ammonia (Allison, 1970), many bacteria are stimulated to grow faster in the presence of amino acids or a source of peptides (Bryant *et al.* 1959; Slyter & Weaver, 1977). Thus Hume (1970) found that casein stimulated efficiency of microbial growth in the rumen, and our observations appear to support this finding.

It is possible that supplement C also stimulated microbial activity by acting as a source of slow-release energy. For optimal microbial activity in the rumen, supplies of ammonia, peptides and other microbial nutrients should be synchronized with the supply of energy-yielding substrates. Urea sprayed onto the alkali-treated straw is likely to have synchronized RDN release in the rumen with intake of the basal diet, but the rate of energy release from fermentation of the basal straw diet may have limited microbial fermentation. Slow disappearance of DM from diet C held in nylon bags in the rumen (Table 2) suggests that C would act as a slow-release source of energy as well as amino acids.

Associated with stimulation of microbial activity by C, there was an increase in apparent OM digestibility in the whole tract, similar to that reported by Oldham *et al.* (1979) when they replaced urea with fish meal. There was also an increase in the proportion of OM digested post-ruminally (Table 3), which was partly attributable to the increase in N apparently digested post-ruminally (Table 4). Also associated with stimulation of microbial activity by C was an increase in rumen DM, similar to that reported by Egan (1970) when he added casein to a diet of oaten hay. It is possible that part of this was due to an increase in microbial mass.

Neither supplement B nor C had a significant effect on intake of the basal diet. This absence of a stimulatory effect on intake of roughage is consistent with all other experiments carried out in this laboratory (Kellaway & Leibholz, 1983). When claims have been made that protein supplements stimulate intake of low-quality roughages, the responses can usually be attributed to the supplements supplying RDN, S, energy or minerals (Hennessy *et al.* 1983; Lee *et al.* 1985). Because these nutrients can usually be supplied at lower cost than proteins, it is important for economic reasons to identify the components of protein supplements which do stimulate intake. The present experiment and others from this laboratory (Kellaway & Leibholz, 1983) have shown that when RDN, S and minerals are supplied in adequate amounts, protein supplements do not stimulate intake of low quality roughages.

Both supplements stimulated live-weight gain, to an extent which was predictable in terms of energy intake in the case of supplement B but not in the case of supplement C (Table 6). Indeed, with supplement C, live-weight gain per unit energy intake was significantly higher than that on the other two diets. It appears that the animals responded to the greater protein supply from C, although cattle above 200 kg live weight are considered not to require supplementary undegraded dietary protein (Agricultural Research Council, 1980). In the light of the substantial response which we recorded, it would seem appropriate to re-consider this recommendation.

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REFERENCES

- Agricultural Research Council (1980). *The Nutrient Requirements of Farm Livestock*, pp. 153. Slough: Commonwealth Agricultural Bureaux.
- Allison, M. J. (1970). In *Physiology of Digestion and Metabolism in the Ruminant*, pp. 456–473 [A. T. Phillipson, editor]. Newcastle upon Tyne: Oriel Press.
- Binnerts, W. T., Van't Klooster, A. Th. & Frens, A. M. (1968). *Veterinary Record* **82**, 470.
- Bryant, M. P., Robinson, I. M. & Chu, H. (1959). *Journal of Dairy Science* **42**, 1831–1847.
- Chaney, A. L. & Marbach, E. P. (1962). *Clinical Chemistry* **8**, 130–132.
- Egan, A. R. (1965). *Australian Journal of Agricultural Research* **16**, 451–462.
- Egan, A. R. (1970). *Australian Journal of Agricultural Research* **21**, 735–746.
- Egan, A. R., Walker, D. J., Nader, C. J. & Storer, G. (1975). *Australian Journal of Agricultural Research* **26**, 909–922.
- Faichney, G. J. (1975). In *Digestion and Metabolism in the Ruminant*, pp. 277–291 [I. W. McDonald and A. C. I. Warner, editors]. Armidale: University of New England Publishing Unit.
- Faichney, G. J. (1980). *Journal of Agricultural Science, Cambridge* **94**, 313–318.
- Goering, H. K. & Van Soest, P. J. (1970). *Forage Analysis. Agricultural Handbook* no. 379. Washington, DC: Agricultural Research Service, United States Department of Agriculture.
- Hecker, J. F. (1974). *Experimental Surgery on Small Ruminants*, pp. 322. London: Butterworths.
- Hennessy, D. W., Williamson, P. J., Nolan, J. V., Kempton, T. J. & Leng, R. A. (1983). *Journal of Agricultural Science, Cambridge*, **100**, 657–666.
- Hume, I. D. (1970). *Australian Journal of Agricultural Research* **21**, 305–314.
- Hutton, K., Bailey, F. J. & Annison, E. F. (1971). *British Journal of Nutrition* **25**, 165–173.
- Kellaway, R. C. & Leibholz, J. (1983). *World Animal Review* **48**, 33–37.
- Kempton, T. J. (1980). In *Recent Advances in Animal Nutrition*, pp. 28–37 [D. J. Farrell, editor]. Armidale: University of New England Publishing Unit.
- Kempton, T. J. & Leng, R. A. (1979). *British Journal of Nutrition* **42**, 289–302.
- Lee, G. J., Hennessy, D. W., Williamson, P. J., Nolan, J. V., Kempton, T. J. & Leng, R. A. (1985). *Australian Journal of Agricultural Research* **36**, 729–742.
- Oldham, J. D., Broster, W. H., Napper, D. J. & Smith, T. (1979). *Proceedings of the Nutrition Society* **38**, 128A.
- Satter, L. D. & Slyter, L. L. (1974). *British Journal of Nutrition* **32**, 199–208.
- Schaefer, D. M., Davis, C. L. & Bryant, M. P. (1980). *Journal of Dairy Science* **63**, 1248–1263.
- Slyter, L. L. & Weaver, J. M. (1977). *Applied Environmental Microbiology* **33**, 363–369.
- Spragg, J. C., Kellaway, R. C. & Kempton, T. J. (1982). *Proceedings of the Australian Society of Animal Production* **14**, 679.
- Spragg, J. C., Kellaway, R. C. & Leibholz, J. (1984). *Proceedings of the Australian Society of Animal Production* **15**, 750.
- Steel, R. G. D. & Torrie, J. H. (1980). *Principles and Procedures of Statistics*, 2nd ed. New York: McGraw Hill.
- Trenkle, A. H. (1980). In *Digestive Physiology and Metabolism in Ruminants*, pp. 505–522 [Y. Ruckebusch and P. Thivend, editors]. Lancaster: MTP Press.