Utilization of low quality roughages: effects of urea and protein supplements of differing solubility on digesta flows, intake and growth rate of cattle eating oaten chaff

BY R. G. REDMAN, R. C. KELLAWAY AND JANE LEIBHOLZ

Department of Animal Husbandry, University of Sydney, Camden, New South Wales 2570, Australia

(Received 27 June 1979 – Accepted 9 June 1980)

1. Expt 1. Five 150 kg steers with ruminal, abomasal and ileal cannulas were given 3000 g oaten chaff daily plus pelleted supplement with no added nitrogen (diet A) or 50 g N/d as urea (diet B), casein (diet C), casein and formaldehyde-treated casein (HCHO-casein) (50:50 w/w; diet D) and HCHO-casein (diet E), in a 5×5 Latin square design. The basal diet and supplement were fed in eight equal increments at intervals of 3 h. Proportions of dry matter and organic matter digested in the stomach and whole tract were greater for diets B, C, D and E than for diet A. Total volatile fatty acid levels in the rumen and the proportion of acetic acid were lower, and the proportion of propionic acid higher on diet A than on the other diets. Rumen ammonia levels were lower on diets A, D and E than on diets B and C. N flows at the abomasum, ileum and rectum were lower on diet A than on the other diets; abomasal flows and apparent intestinal absorptions of amino acids were higher on diets D and E than on diets A, B and C. Efficiencies of bacterial protein synthesis were 15, 15, 14, 13 and 12 g bacterial N/kg OM truly digested in the stomach on diets A, B, C, D and E respectively.

2. Expt 2. Forty 300 kg steers were fed oaten chaff *ad lib*. plus twice the amount of the same pelleted supplements as in Expt 1. Intake of oaten chaff was 23 % higher with N supplements (diets B, C, D and E) than without (diet A). Live-weight gains were 356, 798, 843, 842 and 805 g/d on diets A, B, C, D and E respectively.

3. It was concluded that efficiency of bacterial protein synthesis was not limited by the supply of peptides and amino acids in the rumen, and that increases in amino acid availability in the intestines from feeding HCHO-casein did not increase food intake or live-weight gain.

Peptides and amino acids contribute 200-400 mg/g nitrogen incorporated into microbial cells in the rumen (Pilgrim *et al.* 1970; Nolan & Leng, 1972; Nolan *et al.* 1976) and in vitro studies indicated that the optimum value for non-protein-N to amino acid-N for microbial growth was 75:25 (Maeng *et al.* 1976). When casein replaced approximately half the N supplied by urea on a virtually protein-free diet fed to sheep, Hume (1970) found that there was an increased flow of microbial protein from the stomach.

These observations indicate that microbial growth in the rumen of animals eating a low protein diet may be restricted by the supply of peptides and amino acids, with a concomitant reduction in the rate of cellulose digestion in the rumen. Since the rate of digestion and retention time in the rumen are major determinants of the voluntary food intake of low quality roughages (Thornton & Minson, 1973) it is possible that their intake may be limited by the supply of peptides and free amino acids to rumen micro-organisms.

We investigated this possibility by supplying iso-nitrogenous amounts of urea, soluble protein (casein) or digestible by-pass protein (HCHO-casein) to steers fed oaten chaff.

EXPERIMENTAL

Expt 1

Animals and management. Five Friesian steer calves were fitted with simple cannulas in the rumen, abomasum and terminal ileum at 3 months of age. At 8 months of age and

			Supplements		
	<u>_</u>	<u></u>	Casein : for	maldehyde-tre	ated casein
	Control	Urea	100:0	50:50	0:100
Diet	Α	В	С	D	Е
Components					
Molasses	406	361	406	378	350
Oaten chaff	197	175	197	183	180
Maize flour	373	332		_	
Casein		→	373	174	—
Formaldehyde-treated casein		_		243	450
Urea	_	I I 2	—		
Calcium stearate	I 2	10	12	11	10
DM	919	910	930	925	920
Chemical composition					
Nitrogen	5.7	64.1	66.7	71.1	72.4
Ash	188	158	176	175	154
a-glucose polymers	435	462	54	51	53
Lignin	23	19	24	22	22
Amounts fed daily (g air dry)					
Expt I	336	378	336	360	387
Expt 2	670	754	670	720	776

Table 1. Expts 1 and 2. Components (g/kg air dry) and chemical composition (g/kg dry matter (DM)) of five pelleted supplements fed with oaten chaff to cattle together with daily amounts fed in the respective experiments

live weight 150 kg they were tethered in individual cages fitted with automatic feeders which delivered one-eighth portions of the daily feed at three hourly intervals.

Calves were allocated to the five dietary treatments according to a 5×5 Latin square design.

Diets and feeding procedure. The basal diet was oaten chaff containing (g/kg dry matter (DM)) 7.7 N, 228 α -glucose polymers, 51 ash, 47 lignin. The composition of the five pelleted supplements is given in Table 1. HCHO-casein was sprayed at the rate of 100 g formalin solution containing 15 g HCHO/kg casein DM, a concentration of HCHO selected to render the casein completely insoluble in the rumen and partly digestible in the intestines (Hemsley et al. 1973). The digestibility of the treated casein was measured in two sheep on constant daily intakes of oaten chaff by introducing 200 g/d over 5 d into the rumen cannulas. Casein digestibility, calculated from the change in faecal N excretion before and after introducing the casein, was 0.80. The daily intakes of diets C, D and E were designed to provide 250 g digestible casein/d. Diet B contained an iso-nitrogenous amount of urea and diets A and B contained maize flour to make them iso-energetic with the other diets. The amounts fed provided equal daily intakes of molasses, oaten chaff and calcium stearate from the five diets (Table 1).

In a preliminary period the five calves were given the basal diet *ad lib*. with pellets of diet A in eight equal portions at 3h intervals. Consistent intakes of 3 kg oaten chaff/d were recorded for all calves and intakes were kept at this level throughout the experiment. Each dietary period comprised a 2-week adjustment period followed by marker infusion over 8 d on the last 3 d of which digesta collections were made.

Marker infusions and digesta collections. Markers used were the ⁵¹Cr complex of ethylenediaminetetra-acetic acid (⁵¹Cr-EDTA) (Downes & McDonald, 1964) and ¹⁰³Ru-labelled tris-(1,10-phenanthroline)-ruthenium (ii) chloride (¹⁰³Ru-P) (Tan *et al.* 1971) and lignin. ⁵¹Cr-EDTA and ¹⁰³Ru-P were infused into the rumen at the rate of 113 μ Ci ⁵¹Cr and 23 μc^{103} Ru daily. Abomasal digesta samples were collected three times daily (0.5, 1.0 and 1.5 h respectively after three successive meals) and bulked; faecal samples were taken at the same times from the most recently voided material. Ileal digesta samples were collected once daily for 3 d and bulked. Digesta samples were stored at -10° .

After each collection period, marker infusion was discontinued and eight samples of rumen digesta were taken during the following 30 h. Half of each sample was acidified to pH 2 and all samples were stored at -10° .

Radioactivity measurements, chemical analyses and digesta flow calculations. Sub-samples of abomasal and ileal digesta were centrifuged at 2400 g for 20 min to obtain liquid-rich fractions and sub-samples of faeces were blended with water to form slurries. These slurry samples and samples of liquid-rich fractions and of total abomasal and ileal digesta were assayed for ⁵¹Cr and ¹⁰³Ru in a scintillation spectrometer (Model 3320; Packard Instruments Pty Ltd, Sydney).

Total digesta and faecal samples were analysed for DM, by drying to constant weight at 80°, OM by ashing at 550° overnight. Sub-samples of total digesta were freeze-dried before analysis for N by a micro-Kjeldahl technique, lignin by the method of Van Soest (1963), α -glucose polymers by the method of Macrae & Armstrong (1968). Total digesta samples were also analyzed for amino acids, including 2,6-diaminopimelic acid, by ionexchange chromatography using an amino acid Auto-Analyzer TSM (Technicon Instrument Corporation, Tarry Town, New York, USA) following 24 h hydrolysis in 6 M-hydrochloric acid at 136° using norleucine and guanidine as internal standards; corrections were made for losses of amino acids known to occur during this hydrolysis procedure. Liquid-rich digesta fractions were analyzed for DM, OM, N and α -glucose polymers.

Flows of abomasal, ileal and faecal digesta were calculated from the concentrations of two markers, using equations (1)–(3) from Faichney (1975). ⁵¹Cr-EDTA was used as the liquid marker throughout. The solid phase marker was ¹⁰³Ru-P in the first two periods and lignin in the remaining three periods; the change of marker was necessitated by the unexpectedly low specific radioactivity of ¹⁰³Ru in one batch of the marker. Comparisons between ¹⁰³Ru and lignin as solid phase markers were made with digesta samples from three animals. OM flow rates (g/d) in the three animals, based on ¹⁰³Ru and lignin respectively, were 1937 and 2008, 1756 and 1686, 1565 and 1672 for abomasal flows, 1536 and 1468, 1170 and 1162, 1283 and 1330 for ileal flows, and 1553 and 1512, 1149 and 1068, 1046 and 1000 for faecal flows.

Volatile fatty acids (VFA) in acidified rumen samples were determined by gas-liquid chromatography (F & M Scientific 402; Hewlett Packard Australia Pty Ltd, Sydney) using 3-methyl *n*-valeric acid as an internal standard. Ammonia in acidified rumen samples was determined by the method of Chaney & Marbach (1962). Non-acidified rumen samples were assayed for ¹⁰⁸Ru and ⁵¹Cr specific radioactivities and mean retention times were calculated as the reciprocal of disappearance rate constants for the two markers.

Bacterial N. 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. Bacterial samples isolated from rumen fluid samples collected from each of the five steers at the end of the last collection period contained 55.9, 61.2, 53.7, 40.9 and 53.7 mg DAPA/g N on diets A, B, C, D and E respectively; a mean value of 53.1 mg DAPA/g N was used throughout.

Expt 2

Animals and management. Forty Hereford steers aged approximately 12 months, and weighing an average of 288 kg live weight were used. Animals were housed in individual stalls with sawdust bedding and water available *ad lib*. They were allocated to five diets

on a live-weight basis, using restricted randomization. Live weights were recorded weekly over 57 d, and live-weight changes estimated by regressions of live weight v. time.

Diets and feeding procedure. The basal diet was the same oaten chaff as fed in Expt I. It was offered ad lib., accumulated food refusals being removed twice weekly. The five pelleted supplements were also the same as fed in Expt I, although larger amounts were fed (Table I) because of the higher roughage intakes of the larger animals. Daily portions of pellets were placed on top of the oaten chaff each morning, and generally eaten within I h.

RESULTS

Expt 1

Animals remained in good health throughout the experiment, and consumed all food offered. Digesta flows of OM at the duodenum, ileum and rectum were similar for the four diets given N supplements (diets B, C, D and E) (Table 2). On the control diet (A), faecal flows of OM were significantly greater than on the other diets; ileal and faecal flows followed the same trend but were less consistent as indicated by the larger standard errors. The proportion of abomasal flows of OM digested in the intestines tended to be lower on diets supplying soluble N supplements (diets B and C). Abomasal flow of α -glucose polymers was lower (P < 0.10) on the control diet than on the four N diets (diets B, C, D, E). Abomasal, ileal and faecal flows of N were significantly lower on the control diet than on diets B, C, D and E.

Abomasal N flows were 9-16 % higher than N intakes on diets B, C, D and E but 70 % higher on diet A, which indicated substantial N re-cycling on diet A (Table 3). The amount of N apparently digested in the intestines did not differ significantly between diets. Abomasal flow of bacterial N and efficiency of bacterial N synthesis did not differ between diets; bacterial N as a proportion of total abomasal N was significantly lower on diets D and E.

Abomasal flows of most amino acids were substantially higher on diets D and E than on diets A, B and C, particularly proline and glutamic acid (Table 4). On diets B and C, abomasal flows of amino acids were slightly higher than those on diet A. Amino acid analyses on ileal digesta samples were carried out on single pooled samples for each diet. For this reason, apparent intestinal absorptions (abomasal minus ileal flows) were not analysed statistically. Apparent intestinal absorptions of indispensable amino acids were similar on diets A, B and C, which were substantially lower than those on diets D and E. Apparent intestinal absorptions of dispensable amino acids were lowest on diets B and C, higher on diet A and highest on diets D and E. The proportion of abomasal flow apparently absorbed was higher with indispensable than with dispensable amino acids on all treatments.

VFA concentrations in rumen fluid from diets B, C, D and E were significantly higher than on diet A and this was associated with lower proportions of acetic and higher proportions of propionic acid on diet A (Table 5). Rumen ammonia levels on diets A, D and E were substantially lower than those on diets B and C (Table 5). Retention times for ¹⁰³Ru-P and ⁵¹Cr EDTA did not differ between treatments.

Expt 2

Animals remained in good health throughout the experiment, and readily consumed the pelleted supplements. Intakes of oaten chaff were similar for the four diets fed with N supplements (diets B, C, D and E) (Table 6), these intakes being an average of 23 % higher than on the control diet (diet A). Live-weight gains also were similar on diets B, C, D and E, and more than double that on diet A.

Calculated intakes of metabolizable energy (ME) were used to predict live-weight gains

		,	Supplements	•)	Statistical signific	ance of
			summind data				differences her	ween
	-		Casei	in:HCHO-¢	asein		means	
Diet*	Control A	Urea B	0:0 100:0	50:50 D	о: IO 0: I	SEM	F	٩
OM Trata (2/4)	2010	2010	1110	1910	TOT		1	1
Intake (g/u) Ahomasal flow (g/d)	1880	3102 1630	3141 1644	1016	3191 1655	1001		SN
Ileal flow (g/d)	1364	1373	1267	1285	1234	82.6	1	SN
Faecal flow (g/d)	1521	1292	1312	0611	1134	37.3	A > (BCDE)	10-0
Apparently digested (g/d) in	9, 11	6431	5071	1402	9631	9.101		3 0 -0
Scoulacu Small intestines	1 <u>14</u> 0 525	266 266	1641	474	421	15.5		SS
Tract	1616	1890	1829	1/61	2003	50.3	A < (BCDE)	10-0
Proportion intake apparently digested in stomach Tract	0.40	0.48	0-48 0-58	0.44 0.62	0.48 0.62	0-033	A < (BCDE) A < (BCDE)	0.02 0.001
Proportion of abomasal flow apparently digested in Small intestines	0.28	0-16	0.23	0.27	0.25	0.040	ÌI	SZ
a-glucose polymers Intake (g/d) Abomasal flow (g/d) Apparently digested in stomach (g/d)	829 29 800	858 42 816	701 40 661	702 55 647	704 47 657	7.0 7	A < (BCDE) (AB) > (CDE)	01.0
NS, Not significant. * For details. see Table 1.								

Nitrogen supplements and roughage utilization

† Where groups of treatment letters are shown in parentheses statistical tests were for combined means.

f bacterial synthesis in steers	
id efficiencies o	*
f bacterial N an	this minimum terrority
abomasal flows c	time and meters
nd digestion of N,	
3. Expt 1. Intake a	
Table 3	

348

		Ñ	upplement	2			Statistical signific	ance of
	ί		Caseir	:HCHO	casein		unierences ber	MOCI
	Control	Urca	0:00 100	50:50	001:0	SEM	F	A ,
Diet*	×	Ø	U	Ð	Щ			
Total N								
Intake (g/d)	24:9	47-2	45.4	48-6	51-0	l	I	I
Abomasal flow (g/d)	42.1	52.8	20.0	56.1	57-9	4.17	A < (BCDE)	0.05
Ileal flow (g/d)	0.11	15.0	18-7	8-61	21-7	1.18	A < (BCDE)	100.0
Faecal flow (g/d)	12.1	14-4	16.4	1.71	18.5	0.72	A < (BCDE)	100.0
Abomasal flow + intake	07-1	1.12	60.1	1.16	1.14	1	1	1
Apparently digested in small intestines (g/d)	1.18	37-8	31-3	36-3	36-2	4.38	I	SN
rroportion of accurate in the apparently digested in small intestines	0-74	0-72	0-62	0.65	0-63	££0.0	(AB) > (CDE)	10.0
Bacterial N								
Abomasal flow (g/d)	27-0	31-8	1.62	24.4	24.0	3-82	1	SZ
Proportion total abomasal N flow	0-65	0.00	0.58	0.45	0.42	0-065	(ABC) > (DE)	0.05
Bacterial N/kg OM apparently digested in stomach	21.6	20.6	19.4	17.4	15.6	3.42	1	SZ
Bacterial N/kg OM truly digested in stomacht	15.4	14 . 9	14.2	13-1	12.1	ł	1	ł
OM, organic matter; NS, not signi * For details, see Table 1. + When envire of restment letter	ificant.	i mara	entheee c	tatietical (tete were	for combi	and means	
‡ Equation no. 6 of Czerkawski (1	1978).			- monsman -				

eating oaten chaff plus supplements*

(Ministry of Agriculture, Fisheries and Food, 1975) which corresponded closely with actual gains.

DISCUSSION

Expt 1

Bacterial protein synthesis

Efficiency of bacterial protein synthesis was determined when iso-nitrogenous supplements of urea, soluble protein (casein) or digestible by-pass protein (HCHO-casein) were fed at three-hourly intervals. It was assumed that case in would increase the available pool of amino acids and peptides in the rumen. When solutions of casein were introduced into the rumen of steers, protein half-lives were found to be 5.6-21.5 min (Mangan, 1972) and 65 min (Broderick, 1978). Rates of proteolysis would have been slower in our experiment because the casein was fed as dry pellets, and solubilization of casein in McDougall's (1948) buffer at 38° takes approximately 12 min (R. G. Redman & R. C. Kellaway, unpublished results). Thus, it is reasonable to assume that the supply of amino acids and peptides in the rumen was indeed greater when casein was fed than when urea was fed. However, efficiencies of bacterial protein synthesis, as indicated by abomasal flows of bacterial N/kg OM truly digested in the stomach, were similar on the control, urea and casein diets (15.4, 14.9 and 14.2 respectively). It appears that the supply of amino acids and peptides was not limiting bacterial protein synthesis on this diet. If 60 % of bacterial N on the urea diet were derived from ammonia-N, 12.7 g N/d would have been supplied from amino acids and peptides arising from proteolysis of dietary protein and endogenous secretions. Of N recycled to the gut anterior to the duodenum 68-77 % was found to be endogenous protein rather than urea (Macrae et al. 1977). Measurements of endogenous protein in abomasal secretions (Harrop, 1974) indicate that this pathway would account for most of the endogenous protein recycled anterior to the duodenum, leaving little for secretion into the rumen. Thus, it appears that most of the amino acids and peptides in the rumen arise from dietary rather than endogenous sources. Dietary protein intake on the urea treatment was approximately 20 g/d of which 12.7 g/d may well have undergone proteolysis in the rumen. On semi-synthetic diets which were virtually protein-free, replacement of 100 mg/g urea N with casein N had no effect on efficiency of microbial N synthesis (Ben-Ghedalia et al. 1978) whereas replacement of 480 mg/g urea N with casein N increased abomasal flow of protein by 13 % (Hume, 1970). This may represent an upper limit to in vivo responses to soluble dietary proteins in terms of microbial protein synthesis.

The concentration of ammonia in the rumen which promotes maximal bacterial protein synthesis is approximately $4 \cdot I \mod / I$ (Mercer & Annison, 1976; Okorie *et al.* 1977). Ammonia concentrations were lower than this on the control diet $(2 \cdot I \mod / I)$ and on the treated-case diets $(2 \cdot 4 \mod 2 \cdot 2 \mod / I)$ on diets D and E respectively). On the control diet the low concentration of rumen ammonia was associated with a lower proportion of DM intake digested in the stomach (Table 2), a lower concentration of total VFA in the rumen and a lower value for acetic:propionic acid (Table 5) than on the other diets. Despite the lower rate of fermentation on the control diet, efficiency of bacterial growth was no lower than on other diets. DM intake was controlled at a similar level on all diets in Expt I. The more rapid rate of fermentation on all but the control diet would have facilitated higher DM intakes on these diets as indeed was found in Expt 2 (Table 6). Under these conditions it is possible that fractional outflow rates from the rumen would have been higher, recycling of microbial N lower, and therefore net efficiencies of microbial protein synthesis higher.

In contrast to the control diet, low levels of rumen ammonia on the treated casein diets did not reduce the extent of OM digestion. Higher levels of rumen ammonia on the urea

Table 4. Expt 1. Abomasal flows (F) (g/d) and apparent intestinal absorption (IA) (g/d) of amino acids in steers eating	outer cital pius supprenting
---	------------------------------

$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$						Suppl	ements						Statistical signic	ance of
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$		ſ						Casein:	нсно-с	asein	ſ		differences bet means‡	ween
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	Diet* A B C D D E IA F IA						{ s						į		ſ
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$						2	0:0	5	20	5	3	SEM	1.7	4
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	F IA IA <thia< th=""> <thia< th=""> <t< th=""><th>Diet*</th><th></th><th>₹.</th><th></th><th><u></u>д.</th><th>-</th><th><u>ں</u></th><th></th><th>д.</th><th></th><th>щ.</th><th></th><th></th><th></th></t<></thia<></thia<>	Diet*		₹.		<u></u> д.	-	<u>ں</u>		д.		щ.			
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	Indispensable amino acidsIndispensable amino acidsLysine165127185143181155198155196167163Lysine5517017111159716510015511097163Arginine1391061111175610015511097171Threonine11896105721167916813511497173Threonine726717511138111783147199123Methionine7267175111384117155119123Methionine1077511128411715511131054997Idel101731741551171571131054997Idel1017310587410587143188Seleucine107731058741059771054997Idel10173105874105741071501054997Jobeucine10773107074105741071501054143118Leucine107731058741057410597710749971054Mathionine101107730730730720720		l L	1	L L	₹	ן ה	I	L L	₹	L L	₹			
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	Lysine 165 127 185 143 181 155 198 128 225 167 163 Histidine 139 061 111 115 95 143 181 155 100 155 110 127 Arginine 139 061 111 115 95 119 127 Valine 99 75 115 71 115 99 125 119 127 Methionine 72 65 72 115 71 125 119 127 Methionine 72 65 117 125 119 127 Methionine 107 75 115 71 120 185 131 231 171 1263 191 228 Phenylatanic 107 75 115 77 4 105 47 155 113 193 143 118 Phenylatanic 107 75 115 77 4 105 47 155 113 193 193 143 188 $A \pm F$ 077 072 75 115 77 195 105 143 1054 997 $A \pm F$ 077 072 75 117 155 113 197 159 193 143 188 Aspartic 226 194 199 132 237 169 241 183 226 142 318 Aspartic 226 194 199 132 237 169 241 183 226 142 318 Serine 101 1011 75 109 132 237 169 241 183 226 142 318 Aspartic 226 194 199 132 237 169 241 183 226 142 318 Aspartic 226 194 199 132 237 169 241 183 226 142 318 Aspartic 226 194 199 132 237 169 241 183 226 142 318 Aspartic 226 194 199 132 237 169 241 183 226 142 318 Aspartic 226 194 199 132 237 169 241 183 226 142 318 Aspartic 226 194 199 132 127 171 103 192 Aspartic 226 194 199 132 127 167 193 193 193 192 Aspartic 226 194 199 132 237 169 241 183 226 142 319 Adamic 201 101 144 103 114 477 1156 157 92 165 117 917 920 1425 (1 Total 1082 785 111 442 103 114 477 1156 157 92 165 117 917 920 1425 (1 Total 1082 785 111 442 103 114 417 115 103 192 192 Cystine 82 65 102 72 127 117 670 1576 920 1423 (1 A+F 073 058 058 058 058 058 058 058 058 058 058	Indispensable ami	ino acids												
Histicine 55 410 57 67 475 74 42 86 57 071 000 <td>Histoline 55 41 56 40 54 45 74 42 86 57 070 121 Threonine 113 90 75 115 81 116 92 175 119 127 Value 99 75 115 81 117 116 93 145 112 86 97 113</td> <td>Lysine</td> <td>16.5</td> <td>12.7</td> <td>18.5</td> <td>14.3</td> <td>18.1</td> <td>15.5</td> <td>8-91</td> <td>12-8</td> <td>22.5</td> <td>16-7</td> <td>I-63</td> <td>SN</td> <td>1</td>	Histoline 55 41 56 40 54 45 74 42 86 57 070 121 Threonine 113 90 75 115 81 116 92 175 119 127 Value 99 75 115 81 117 116 93 145 112 86 97 113	Lysine	16.5	12.7	18.5	14.3	18.1	15.5	8-91	12-8	22.5	16-7	I-63	SN	1
	Argame 139 106 141 111 115 97 165 175 110 121 Threonine 113 95 105 72 113 68 135 119 121 Value 94 76 112 84 175 119 124 Value 94 76 112 84 115 69 95 134 135 119 124 Nolectione 72 107 75 117 155 191 274 997 16 Phenylalanine 1071 783 1070 782 1058 74 167 173 1054 997 16 IA+F 071 073 072 072 072 072 072 072 072 072 072 072 072 072 073 169 147 167 147 257 119 174 17 156 174 173 1	Histidine	5.2	4.1	5.6	40	5:4	4-5	7:4	4:2	8.6	5.7	02.0		10-0
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	Intronute 113 14 0 12 144 0 12 144 0 12 144 144 12 144 144 12 144 144 12 144 12 12 144 12 12 144 12 12 144 12 12 13 13 13 13 13 13 13 13 144 13 13 14 13 14 13 14 13 14 14	Arginine	6.E1	10.6	14.1	ŀII	.11 2	7-0	16·5	0.01	15.5	11-0	I-2I		0.05
	Walline 99 75 115 81 118 68 145 103 175 119 123 124 123 124 123 <td>Threonine</td> <td>8.11</td> <td>9.6</td> <td>10.5</td> <td>2 i 1</td> <td>9.11 1</td> <td>6.2</td> <td>16-8</td> <td>13.5</td> <td>14.0</td> <td>9.2</td> <td>I -42</td> <td></td> <td>10.0</td>	Threonine	8.11	9.6	10.5	2 i 1	9.11 1	6.2	16-8	13.5	14.0	9.2	I -42		10.0
$ \begin{array}{c cccc} \mbox{Methionine} & 72 & 62 & 70 & 57 & 68 & 52 & 84 & 70 & 86 & 69 & 058 & 048 & 001 \\ \mbox{Idencine} & 94 & 15 & 115 & 74 & 107 & 155 & 107 & 150 & 174 & 107 & 150 & 107 & 000 \\ \mbox{Leucine} & 107 & 75 & 115 & 74 & 106 & 47 & 155 & 113 & 197 & 263 & 197 & 269 & 097 & 000 \\ \mbox{Leucine} & 107 & 75 & 115 & 74 & 106 & 47 & 155 & 117 & 263 & 197 & 268 & 000 & 000 & 000 \\ \mbox{Leucine} & 107 & 75 & 115 & 74 & 106 & 47 & 155 & 117 & 263 & 197 & 263 & 0000 & 000 & 000 & 000 & 000 & 000 & 000 & 000 & 000 & 000$	Methionine 72 62 70 57 68 69 065 070 87 142 107 150 107 150 107 150 107 150 107 150 107 150 100 173 121 125 113 121 125 113 121 125 113 121 125 113 1233 123 123 <th< td=""><td>Valine</td><td>6.6</td><td>7.5</td><td>11:5</td><td>I.8</td><td>8.11</td><td>6.8</td><td>14-5</td><td>8.0I</td><td>17.5</td><td>6.11</td><td>1.27</td><td></td><td>10.0</td></th<>	Valine	6.6	7.5	11:5	I.8	8.11	6.8	14-5	8.0I	17.5	6.11	1.27		10.0
$ \begin{array}{c cccc} \mbox{Iscence} & 94 & 76 & 112 & 84 & 115 & 87 & 142 & 107 & 150 & 106 & 173 & 1007 & 120 & 100 & 174 & 1007 & 120 & 100 & 174 & 1001 & 1000 & 1001 & 10000 & 10000 $	$ \begin{array}{ccccc} & 9 & 4 & 7^{6} & 11'2 & 8'4 & 11'5 & 8'7 & 14'2 & 10'7 & 15'0 & 10'6 & 1'34 \\ \mbox{Phenylalanine} & 10'7 & 7'5 & 11'5 & 7'4 & 10'6 & 4'7 & 15'5 & 11'7 & 15'0 & 10'6 & 1'34 \\ \mbox{Phenylalanine} & 10'1' & 7'8' & 10'7 & 7'8 & 10'7 & 15'5 & 11'3 & 10'5'4 & 9'9'1 \\ \mbox{Total} & 101'1 & 7'8' & 10'7 & 7'8 & 10'5' & 7'5' & 11'3 & 10'5' & 9'9'1 & 10'7 \\ \mbox{Total} & 101'1 & 7'8' & 10'7 & 7'8 & 10'5' & 10'7 & 10'7 & 10'7 & 10'7 & 10'7 \\ \mbox{Dispensable amino acids} & 0'73 & 0'72 & 0'72 & 0'72 & 0'72 & 0'72 \\ \mbox{Dispensable amino acids} & 10'1 & 8'8 & 17'7 & 45'6 & 3'7 & 11'6 & 2'0' & 1'4' & 1'7 \\ \mbox{Serine} & 10'1 & 8'4 & 10'8 & 11'7 & 45'6 & 3'7 & 11'6 & 2'0' & 2'4'5 & 14'8 & 2'0'2 \\ \mbox{Serine} & 10'1 & 6'5 & 11'7 & 45'6 & 3'7 & 11'6 & 2'0 & 2'4'5 & 14'8 & 2'0'2 \\ \mbox{Clutamic acid} & 2'0 & 1'3' & 9'7 & 1'7 & 15'6 & 5'7 & 11'6 & 2'0 & 2'4'5 & 17'1 & 10'3 & 1'9'2 \\ \mbox{Clutamic acid} & 10'1 & 6'5 & 11'7 & 45'6 & 3'7 & 11'6 & 2'0 & 2'4'5 & 17'1 & 10'3 & 1'9'2 \\ \mbox{Clutamic acid} & 10'1 & 6'5 & 11'7 & 10'3 & 1'9' & 1'7' & 10'3 & 1'9'2 & 1'7' & 10'3 & 1'9'2 \\ \mbox{Clutamic acid} & 10'1 & 6'5 & 11'7 & 6'2 & 12'7 & 11'6 & 2'0 & 2'4'5 & 17'1 & 10'3 & 1'9'2 & 1'7' \\ \mbox{Clutamic acid} & 10'1 & 6'5 & 11'7 & 6'2 & 12'7 & 11'6 & 2'0 & 2'4'5 & 17'1 & 10'3 & 1'9'2 & 1'9'2 & 1'9'2 & 1'1'6 & 1'9'2 & 1'1'7 & 1'0'2 & 1'3'2 & 1'0'2 & 1'3'7 & 1'9'2 & 1'9'2 & 1'9'2 & 1'1'2 & 1'0'2 & 1'3'7 & 1'0'2 & 1'3'2 & 0'6'2 & 0'7'$	Methionine	7-2	, 2 6	0.1	5.7	9·8	5.5	8.4	<u>.</u>	9.9 8	6,9	0.85	(DE) > (ABC)	01.0
$ \begin{array}{c ccccc} \mbox{Lencine} & 162 & 125 & 171 & 120 & 185 & 131 & 171 & 263 & 191 & 228 \\ \mbox{Phenylalamine} & 107 & 75 & 117 & 126 & 137 & 155 & 117 & 163 & 197 & 1473 & 1054 & 997 \\ \mbox{Total} & 1011 & 783 & 1070 & 783 & 1058 & 761 & 1362 & 974 & 1473 & 1054 & 997 \\ \mbox{Dispensable amino acids} & & & & & & & & & & & & & & & & & & &$	Leucine 16^2 125 171 120 185 131 231 171 263 191 228 Phenylalanine $107^ 75$ 113^2 1070 78^2 1056 477 1555 1173 1070 78^2 1070 78^2 1070 78^2 1070 78^2 1070 78^2 1070 78^2 1070 78^2 1070 78^2 1070 78^2 1070 78^2 1070 78^2 1070 78^2 1070 717 60^2 2072 218^2 1142 218^2 1199 141 2177 427 2072 218^2 1199 1141 1177 466 220 447^2 221^2 1141 12^2 1141 12^2 1141 12^2 1141 12^2 1141 12^2 1141 12^2 110^2 12^2 110^2 12^2 110^2 12^2^2 <td>Isoleucine</td> <td>9.4</td> <td>7.6</td> <td>11:2</td> <td>8.4</td> <td>11:5</td> <td>8-7</td> <td>14-2</td> <td>2.01</td> <td>15.0</td> <td>9.0I</td> <td>1.34</td> <td></td> <td>10.0</td>	Isoleucine	9.4	7.6	11:2	8.4	11:5	8-7	14-2	2.01	15.0	9.0I	1.34		10.0
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	Phenylalamic 107 75 11.5 74 106 4.7 15.5 11.3 193 14.3 185 187 183 226 142 2318 177 185 226 142 2318 202 231 169 183 226 142 2318 202 231 2326 142 2318 202 202 202 202 202 202 202 202 202 246 222 141 202	Leucine	16.2	12.5	1.7.1	12.0	18.5	1.61	23·I	1.71	26.3	1.61	2.28		10-0
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	Total101:178:3107'078:2105'876'1136'297'4147'3105'4997/1 $IA + F$ 0~770~7730~720~720~720~720~720~72Dispensable amino acidsAspartic22:619:419:913:223:716'924:118'322:614'23:18Dispensable amino acids10018:419:913:223:716'924:118'322:614'23:18Serine100110:16:511'14:511'44:775:65'711'44'2'Foline10:16:511'14:511'41'746'632'047'824'214'2Clycine12:96:313:94:512'717'715'65'711'62'2'210'2Cystine15:711'41'31'31'31'31'3'1'2'710'31'9'21'4'1Cystine0.20'216'48'918'91'7'746'632'71'2'71'2'71'2'7Cystine0.216'48'918'91'7'716'51'7'71'6'61'7'71'2'71'4'21'1'6Cystine0.20'70'760'7'51'7'71'7'71'7'71'7'71'7'71'7'71'7'71'7'71'7'71'7'71'7'71'7'71'7'71'7'71'7'71'7'71'7'71'7'71'7'7 </td <td>Phenylalanine</td> <td>L-01</td> <td>7:5</td> <td>11-5</td> <td>7.4</td> <td>9.0I</td> <td>4-7</td> <td>15.5</td> <td>11.3</td> <td>19-3</td> <td>14.3</td> <td>1.85</td> <td></td> <td>10.0</td>	Phenylalanine	L-01	7:5	11-5	7.4	9.0I	4-7	15.5	11.3	19-3	14.3	1.85		10.0
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	Total	1.101	78-3	107-0	78-2	105-8	1.94	136-2	97.4	147-3	105.4	126-6		10-0/
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$		J	}]]]]]]]	}			
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	IA÷F	5	11.0	0	-73	•	<i>2L</i> .	0	-72	0	-72			
Aspartic 226 194 199 132 237 169 241 18^{-3} 226 142 318 NS $$ Serine 101 84 106 80 130 97 162 132 162 119 141 000 Glutamic acid 284 196 287 114 477 202 132 162 119 100 000 Glycine 127 167 177 466 327 117 128 177 466 320 478 202 000 000 Glycine 127 177 156 377 192 177 82 67 177 166 327 117 192 NS $$ 162 NS $$ 141 173 192 NS $$ 192 NS $$ 192 192 192 192 </td <td>Aspartic 22:6 19:4 19:9 13:2 23:7 16'9 24:1 18'3 22:6 14'2 3:18 17'1 defer 3:19 14'1 3:18 17'1 46'6 32:0 47'8 28'7 44'2 21'9 14'1 21'1 4'1'2 11'9 14'1 21'1 4'2'2 11'9 14'1 21'1 4'2'2 14'1 21'1 4'2'2 14'1 21'1 4'2'2 14'1 2'2'1 14'1 2'2'1 14'1 2'2'1 14'1 2'2'1 14'1 2'2'1 1'1'1 4'2'2 1'1'1 4'2'2 1'1'1 4'2'2 1'1'1 1'2'1'1 1'2'1'1 1'2'1'1 1'2'1'1'1'1'1'1'1'1'1'1'1'1'1'1'1'1'1'1</td> <td>Dispensable amine</td> <td>o acids</td> <td></td>	Aspartic 22:6 19:4 19:9 13:2 23:7 16'9 24:1 18'3 22:6 14'2 3:18 17'1 defer 3:19 14'1 3:18 17'1 46'6 32:0 47'8 28'7 44'2 21'9 14'1 21'1 4'1'2 11'9 14'1 21'1 4'2'2 11'9 14'1 21'1 4'2'2 14'1 21'1 4'2'2 14'1 21'1 4'2'2 14'1 2'2'1 14'1 2'2'1 14'1 2'2'1 14'1 2'2'1 14'1 2'2'1 1'1'1 4'2'2 1'1'1 4'2'2 1'1'1 4'2'2 1'1'1 1'2'1'1 1'2'1'1 1'2'1'1 1'2'1'1'1'1'1'1'1'1'1'1'1'1'1'1'1'1'1'1	Dispensable amine	o acids												
Serine 10.1 84 10.8 80 130 97 162 132 162 119 141 Proline 10.1 65 11.1 4.5 11.4 177 466 320 478 287 4.22 Proline 10.1 65 11.1 4.5 11.4 17 156 57 11.6 20 246 NS Glycine 12.9 6.3 139 4.5 127 17 156 57 11.6 20 246 NS Alanice 157 92 164 89 189 125 171 003 192 NS Alanice 0.2 0.2 14 14 13 13 13 13 1.8 177 9.9 1.9 NS Cystine 8.2 6.5 10.2 72 10.8 6.1 14.2 110 137 9.9 1.96 (DE) > (ABC) 001 Tyrosine 8.2 6.5 10.2 72 10.8 6.1 14.2 110 137 9.9 1.96 (DE) > (ABC) 001 Total 1082 785 1117 62.8 1211 670 1576 1075 1537 920 1425 (DE) > (ABC) 001 IA + F 073 0.56 0.55 0.68 0.60 0.559 Total amino 2093 1568 2187 1410 2269 1431 2938 2049 3010 1974 2240 (DE) > (ABC) 001 IA + F 073 0.56 0.55 0.68 0.66 0.559 Total amino 2093 1568 2187 1410 2269 1431 2938 2049 3010 1974 2240 (DE) > (ABC) 001 IA + F 075 0.64 0.63 0.70 0.66 0.559 Total amino 2093 1568 2187 1410 2269 1431 2938 2049 3010 1974 2240 (DE) > (ABC) 001 A + F 075 0.64 0.63 0.70 0.66 0.559	Serine 10:1 8:4 10.8 8:0 13'0 9'7 16'2 13'2 16'2 11'9 1'41 Clutamic acid 28:4 19'6 28'7 14'8 31'8 1'7'7 46'6 32'0 47'8 28'7 4'22 Proline 10:1 6'5 11'1 4'5 11'4 4'7 2'0'2 13'0 24'5 14'8 2'0'2 Clycine 12'9 6'3 13'9 4'5 12'7 1'7 15'6 5'7 11'6 2'0 2'46 Alanine 15'7 11'6 15'7 9'2 16'4 8'9 18'9 12'5 17'1 10'3 1'9'2 Cystine 8'2 6'5 10'2 7'2 10'8 6'1 14'2 11'0 13'7 9'9 1'36 (1 Tyrosine 8'2 6'5 10'2 7'2 10'8 6'1 14'2 11'0 13'7 9'9 1'36 (1 Cotal 108'2 78'5 111'7 6'2'8 121'1 6'7'0 15'7'6 10'7'5 15'3 9'2 1'3'6 (1 Tyrosine 8'2 6'5 10'2 7'2 10'8 6'1 14'2 11'0 13'7 9'9 1'36 (1 Cystine 0'2 0'2 0'2 0'2 0'2' 0'2'5 1'7' 9'2'0 14'2'5 (1 Tyrosine 8'2 6'5 10'2 7'2 10'8 6'1 14'2 11'0 13'7 9'9 1'36 (1 Tyrosine 8'2 6'5 10'2 7'2 10'8 6'1 14'2 11'0 13'7 9'9 1'36 (1 Tyrosine 8'2 6'5 10'2 7'2 10'8 6'1 14'2'1 10'0 13'7 9'9 1'36 (1 Total 108'2 7'8'5 11'7 6'2'8 12!'1 6'7'0 15'7'6 10'7'5 15'3 9'2'0 14'25 (1 Total amino 2'0'3 156'8 218'7 14'1'0 226'9 143'1 2'9'3'8 204'9 30'1'0 197'4 2'2'40 (1 A+F 0'75 0'6' 0'6' 0'7'0 0'7'0 0'6' 0'7'0 0'6' 0'7'0	Aspartic	22.6	19.4	6-6I	13.2	23-7	16-9	24-1	18.3	22.6	14-2	3.18	SN	1
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	Glutamic acid 284 19.6 287 14.8 31.8 17.7 46.6 32.0 47.8 28.7 4.22 10.1 6.5 11.1 4.7 20.2 130 24.5 14.8 202 02 226 27 11.6 127 11.6 202 245 14.8 202 0246 202 246 246 246 2202 246 202 2246 127 1102 1122 112	Serine	I.0I	8.4	10.8	8:0	13-0	L-6	I6-2	13.2	16-2	6.11	1:41		10.0
Proline1016511147102130245148202202Glycine1296313945127171565711620246NS-Alamine15771171565711620246NSAlamine15771171565711620246NSCystine0.20.21741171565711913799176NS-Tyrosine8.265100272108611422110137799176001Total1082785111762812116701576107515379201425001IA+F0730560560560580600599IA+F0730560530680600599IA+F075064063070059070059IA+F07506406307005618741240(DE) > (ABC)001IA+F075064063070059IA+F07506307006307005618742240(DE) > (ABC)001	Proline 10:1 6:5 11:1 4:5 11:4 4:7 20:2 13:0 24:5 14:8 20:2 13:0 24:5 14:8 20:2 13:0 24:5 14:8 20:2 13:0 24:5 14:8 20:2 13:0 24:5 14:8 20:2 14:8 20:2 14:8 20:2 14:8 20:2 14:8 20:2 24:6 57 11:6 2:4 6:1 14:2 11:0 17:1 10:3 17:9 12:9 6:2 0:2 </td <td>Glutamic acid</td> <td>28.4</td> <td>9.61</td> <td>28-7</td> <td>14.8</td> <td>31.8</td> <td>L-71</td> <td>46.6</td> <td>32.0</td> <td>47-8</td> <td>28-7</td> <td>4.22 }</td> <td>(DE) > (ABC)</td> <td>00.0 0</td>	Glutamic acid	28.4	9.61	28-7	14.8	31.8	L-71	46.6	32.0	47-8	28-7	4.22 }	(DE) > (ABC)	00.0 0
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	Glycine 12.9 6·3 13.9 4:5 12.7 17 15.6 5.7 11.6 2.0 2.46 Alamine 15.7 11.6 15.7 9·2 16·4 8·9 18·9 12.5 17'1 10·3 192 Cystine 0·2 0·2 1·4 1·4 1·3 1·3 1·8 0·2 0·2 0·2 1/3 1/9 1/19 1/19 1/9 1/19 1/16 1/9 1/16 1/12 1/1 1/10 1/10 1/10 1/10 1/10 1/12 1/1 1/12 1/12 1/1 1/12 1/12 1/12 1/12 1/12 1/12 <td>Proline</td> <td>10·1</td> <td>6.S</td> <td>I·II</td> <td>4:5 2</td> <td>11.4</td> <td>4.7</td> <td>20.2</td> <td>0.61</td> <td>24.5</td> <td>14•8 ·</td> <td>2.02</td> <td></td> <td>0.0 0</td>	Proline	10·1	6.S	I·II	4:5 2	11.4	4.7	20.2	0.61	24.5	14 • 8 ·	2.02		0.0 0
$ \begin{array}{llllllllllllllllllllllllllllllllllll$	Alanine 15.7 11.6 15.7 9.2 16.4 8.9 18.9 12.5 17.1 10.3 192 Cystine 0.2 0.2 1.4 1.4 1.3 1.3 1.3 1.8 1.6 0.2 0	Glycine	12-9	6.3	13-9	4-5	12.7	L-1	15-6	5-7	9.11	2.0	2:46	NS	1
Cystine 0.2 0.2 1.4 1.3 1.3 1.3 1.3 1.3 1.3 1.3 1.3 1.3 1.3 1.3 1.3 1.3 1.3 0.2	Cystine 0.2 0.2 0.2 0.2 0.2 0.2 0.2 0.2 0.2 0.2 1.3 1.3 1.3 1.3 1.3 1.3 1.3 1.3 1.3 1.3 0.2 0.2 0.2 0.2 0.2 0.2 1.3 1.3 1.3 1.3 1.3 0.2	Alanine	15.7	9.11	15.7	9.2	16-4	8 .9	18-9	12.5	1.71	10.3	1 ·92	SN	1
Tyrosine8.26.510.27.210.86.114.211.013.79.91.36(DE) > (ABC)0.01Total108.278.5111.762.8121.167.0157.6107.5153.792.014.25(DE) > (ABC)0.01IA + F0.730.560.550.680.600.559Total amino209.3156.8218.7141.0226.9143.1293.8204.9301.0197.422.40(DE) > (ABC)0.01acids209.3156.8218.7141.0226.9143.1293.8204.9301.0197.422.40(DE) > (ABC)0.01 $A + F$ 0.750.640.630.700.66187.4(DE) > (ABC)0.01IA + FNS, not significant.NS, not significant.0.630.700.660.66	Tyrosine 8·2 6·5 10·2 7·2 10·8 6·1 14·2 11·0 13·7 9·9 1·36 (1 Total 108 ³ 78·5 111·7 62·8 121·1 67·0 157·6 107·5 153·7 92·0 14·25 (1 IA + F 0·73 0·56 0·55 0·68 0·60 0·559 (1 14·2 <th< td=""><td>Cystine</td><td>0.7</td><td>0.7</td><td>1.4</td><td>1.4</td><td>£.</td><td>Г. Э</td><td>1·8</td><td>i.</td><td>0.7</td><td>0.7</td><td></td><td>NS</td><td>1</td></th<>	Cystine	0.7	0.7	1.4	1.4	£.	Г. Э	1·8	i.	0.7	0.7		NS	1
Total 108^2 78° 111° 62° 121° 67° 157° 107° 14° 26° 14° 26° 14° 26° 0.55° 0.68° 0.539° 14° 26° 0.73° 0.599° 14° 14° 226° 143° 293° 204° 301° 197° 22° 0° IA + F 0.75° 0.64° 0.63° 0.70° 0.66° 0.66° 0° IA + FNS, not significant.NS, not significant. 0.63° 0.70° 0.66° 0.66° 0.06°	Total 108^{2} $78\cdot5$ $111\cdot7$ $62\cdot8$ $121\cdot1$ $67\cdot0$ $157\cdot6$ $107\cdot5$ $153\cdot7$ $92\cdot0$ $14\cdot25$ (I IA + F 0.73 0.566 0.555 0.68 0.600 0.559 Total amino $209\cdot3$ $156\cdot8$ $218\cdot7$ $141\cdot0$ $226\cdot9$ $143\cdot1$ $293\cdot8$ $204\cdot9$ $301\cdot0$ $197\cdot4$ $22\cdot40$ (I acids $209\cdot3$ $156\cdot8$ $218\cdot7$ $141\cdot0$ $226\cdot9$ $143\cdot1$ $293\cdot8$ $204\cdot9$ $301\cdot0$ $197\cdot4$ $22\cdot40$ (I IA + F 0.75 0.64 0.63 0.70 0.66 0.66 0.66	Tyrosine	8:2	6.9	10-2	7.2	10-8	Q.I	14-2	0·11	13.7	6.6	1.36	(DE) > (ABC)	10.0
$ \begin{bmatrix} A + F & 0.73 & 0.56 & 0.55 & 0.68 & 0.60 & 0.559 & - \\ Total amino & 209.3 & 156.8 & 218.7 & 141.0 & 226.9 & 143.1 & 293.8 & 204.9 & 301.0 & 197.4 & 22.40 & (DE) > (ABC) & 0.01 \\ A + F & 0.75 & 0.64 & 0.63 & 0.70 & 0.66 \\ NS, not significant. & \\ \end{tabular}$	$ \begin{bmatrix} \mathbf{A} \div \mathbf{F} & 0 \cdot 73 & 0 \cdot 56 & 0 \cdot 55 & 0 \cdot 68 & 0 \cdot 60 & 0 \cdot 559 \\ \text{Total amino} & 209 \cdot 3 & 156 \cdot 8 & 218 \cdot 7 & 141 \cdot 0 & 226 \cdot 9 & 143 \cdot 1 & 293 \cdot 8 & 204 \cdot 9 & 301 \cdot 0 & 197 \cdot 4 & 22 \cdot 40 & (\mathbf{I} \\ \text{acids} & 209 \cdot 3 & 156 \cdot 8 & 218 \cdot 7 & 141 \cdot 0 & 226 \cdot 9 & 143 \cdot 1 & 293 \cdot 8 & 204 \cdot 9 & 301 \cdot 0 & 197 \cdot 4 & 22 \cdot 40 & (\mathbf{I} \\ \text{I} \mathbf{A} \div \mathbf{F} & 0 \cdot 75 & 0 \cdot 64 & 0 \cdot 63 & 0 \cdot 70 & 0 \cdot 66 \\ \end{bmatrix} $	Total	108.2	78.5	2.111	62·8	121.1	é-29	157-6	5-201	153.7	92.0	14-25	(DE) > (ABC)	10-0
Total amino acids $209\cdot3$ $156\cdot8$ $218\cdot7$ $141\cdot0$ $226\cdot9$ $143\cdot1$ $293\cdot8$ $204\cdot9$ $301\cdot0$ $197\cdot4$ $22\cdot40$ (DE) > (ABC) 0.01 1A + F 0.75 0.64 0.63 0.70 $0.66NS, not significant.$	Total amino 209:3 156.8 218.7 141.0 226.9 143.1 293.8 204.9 301.0 197.4 22:40 (I acids 0.75 0.64 0.63 0.70 0.66 0.66	IA ÷ F		EF.	°	- <u>5</u> 6	ò	55	°	Şê	0	ķ	0.559	I	I
acids $209\cdot3 \ 156\cdot8 \ 218\cdot7 \ 141\cdot0 \ 226\cdot9 \ 143\cdot1 \ 293\cdot8 \ 204\cdot9 \ 301\cdot0 \ 197\cdot4 \ 22\cdot40 \ (DE) > (ABC) \ 0.01$ $IA + F$ $0.75 \ 0.64 \ 0.63 \ 0.70 \ 0.66$ 0.66 NS, not significant.	acids $209.3 ext{ 156.8 } 218.7 ext{ 141.0 } 226.9 ext{ 143.1 } 293.8 ext{ 204.9 } 301.0 ext{ 197.4 } 22.40 (I)$ $ ext{ IA + F}$ 0.75 0.64 0.63 0.70 0.66	Total amino													
IA + F 0.75 0.64 0.63 0.70 0.66 NS, not significant.	IA + F 0.75 0.64 0.63 0.70 0.66	acids	509.3	156.8	218-7	141.0	536.9	143-1	293-8	204-9	301-0	197.4	22.40	(DE) > (ABC)	10-0
NS, not significant.		$\mathbf{IA} \div \mathbf{F}$	0	.75	Ö	द्वे	ò	63	0	<u>0</u> 2.	0	Ş			
	NS. not significant.		NS. DC	ot signific	ant.										
			+ Whe	ano orono	e of treat	mant lat	0 000 000	The second second		atoto ocor	tant tant.		- acmhine	and manage by	

‡ Relate to abomasal flows only.

Table 5. Expt 1. Concentrations of volatile fatty acids (VFA) and ammonia in rumen fluid, and mean retention times of 108 Ru-P and 51Cr-EDTA in the rumen of steers eating oaten chaff plus supplements*

		Su	pplements				Statistical signif	ficance
	~		Casei	n:HCHO-	casein		or differences be means	eiween
	Control	Urea	100:0	50:50	0:100	SEM	<i>F</i> †	P
Diet*	Α	В	С	D	Е			
Total VFA (тм	/l) 61	79	78	78	73	5.1	A < (BCDE)	0.02
VFA proportion	ıs							
Acetic	0.600	0.661	0.674	0.671	0.638	0.0135	A < (BCDE)	10.0
Propionic	0.260	0.197	0.158	0.169	0.206	0.0128	A > (BCDE)	0.01
iso-butyric	0.031	0.022	0.026	0.031	0.031	_	·	NS
n-butyric	0.080	0.089	0.100	0.090	0.093			NS
iso-valeric	0.018	0.013	0.051	0.010	0.027		—	NS
n-valeric	0.011	0.013	0.016	0.010	0.012		<u> </u>	NS
Ammonia (mmo	ol/l) 2·0	5.2	5.2	2.4	2.2	0.80	(ADE) < (BC)	0.02
Mean retention	times (h)							
¹⁰³ Ru-P	33.0	31.4	23.5	38.3	25.0	7:04	—	NS
⁸¹ Cr EDTA	21.3	22.0	20.8	28.3	19.4	3.45	—	NS

NS, not significant.

* For details, see Table 1.

† Where groups of treatment letters are shown in parentheses statistical tests were for combined means.

Table 6. Expt 2. Measured dry matter intakes (DMI), calculated intakes of metabolizable energy (ME) and net absorption of amino acid nitrogen (AAN), predicted and actual live-weight gains in 40 Hereford heifers eating oaten chaff plus supplements*

		S	upplement	ts			Statistical sign	ificance
			Casei	n:HCHO-	casein		means	Detween
	Control	Urea	100:0	50:50	0:100	SEM	F†	P
Diet*	Α	В	С	D	Е			
DMI (kg/d)								
Pellets	0.62	0.60	0.62	0.67	0.72		_	
Oaten chaff	5.21	6.72	6.70	6.96	6.69	0.218	$\dot{A} < (BCDE)$	0.001
Total	6.12	7.41	7.32	7.63	7.41	<u> </u>		—
Total (g/kg live wt ^{0.75}	°) 69∙0	83.3	83.7	88·o	82.9	2.52	A < (BCDE)	100.0
Calculated intakes and	absorptio	ns (/d)						
ме‡ (МЈ)	48.7	68.2	65.1	72·6	71.7		—	
Total N	50.9	105.4	101.1	110.0	113.3			
N intake/MJ ме	1.05	1.55	1.22	1.25	1.28	—		
AAN (g/d)§	36.1	38.9	39.3	58.3	54-2		_	
g AAN/MJ me	0.74	0.57	o∙60	0.80	0.76			
MJ me/kg DMI	8.0	9.2	8.9	9.2	9.7		_	
Predicted live-wt gain (g/d)	319	786	709	887	884		-	—
Actual live-wt gain (g/d)	356	798	843	842	805	68.7	A < (BCDE)	0.001

NS, not significant.

* For details, see Table 1.

† Where groups of treatment letters are shown in parentheses statistical tests were for combined means.

 \ddagger Digestible organic matter in dry matter (Table 2) $\times 0.156 \times DMI$.

§ Apparent absorption AAN/kg DMI (Tables 4 and 2) × organic matter intake × 0.75 (utilization factor; Roy et al. 1977).

|| Ministry of Agriculture, Fisheries and Food (1975).

and case in diets ($5\cdot 2$ and $5\cdot 2$ mol/l respectively) did not facilitate greater OM digestion in the stomach than on the treated case in diets.

The range of 12-15 g bacterial N/kg OM truly digested in the stomach reported here is lower than the mean of 20 g bacterial N/kg OM for thirty experiments, summarized by Czerkawski (1978), in which microbial N was measured in terms of DAPA. However, it corresponds with efficiencies determined on similar low quality roughages (Jackson *et al.* 1971; Kropp *et al.* 1977). Efficiencies calculated by the same method as we used (Czerkawski, 1978) were 12-14 g bacterial N/kg OM truly digested in the rumen of steers eating lowquality grass (Kropp *et al.* 1977), and these were associated with low dilution rates in the rumen ($2 \cdot 1 - 2 \cdot 2 \frac{9}{6}$ /h). A major factor which affects efficiency of microbial growth is rumen dilution rate, and the mean rate of $4 \cdot 5 \frac{9}{7}$ /h in the present experiment (reciprocal of 15Cr EDTA retention time in Table 5) is considerably lower than rates of $8 - 10 \frac{9}{7}$ /h which appear to be optimal (Owen & Isaacson, 1977). A possible reason for the relatively low dilution rates in our experiment was that the diet was fed as chaffed particles 10 mm long. Much less mastication and rumination, and therefore saliva production, could have occurred with this material than with long roughages (Balch & Campling, 1965).

N utilization

Abomasal N flow was 1.7 times the N intake on the control diet (Table 3) which indicates substantial N recycling on this diet. Studies with ¹⁵N-labelled urea indicated that only 8–18% of urea recycled to the digestive tract was degraded in the rumen (Nolan & Leng, 1972; Nolan *et al.* 1976). Other studies with ¹⁴C-labelled urea found that only 23–32% of N added to digesta between the mouth and duodenum could be accounted for as recycled urea N (Macrae *et al.* 1977). It appears that the balance of N recycled anterior to the duodenum can be accounted for by abomasal secretions (Harrop, 1974). In our experiment, if it is assumed that 27% of the N added to digesta between the mouth and duoden N pool, which is a relatively small proportion of the N intake of 25 g/d on the control diet. Indeed N was limiting the rate of fermentation on this diet as shown by the substantial increase in rumen fermentation when dietary N supplements were given.

N digestibility in the intestines was influenced by the microbial N:casein-N value. On diets A and B where bacterial N was 0.65 and 0.60 of abomasal N, apparent N digestibilities were 0.74 and 0.72 respectively, whereas on diets D and E where bacterial N was 0.45 and 0.42 of abomasal N, apparent N digestibilities were 0.65 and 0.63 respectively (Table 3). The relatively low digestibility of HCHO-treated casein in diets D and E was partly anticipated from the preliminary in vivo test which showed that the digestibility of treated casein between the rumen and rectum was 0.80. We attempted to compensate for this by feeding proportionately more N in diets D and E.

Mean abomasal flows and mean apparent intestinal absorptions of amino acids were 36 and 37 % higher respectively on diets D and E than on diets A, B and C. The proportions of glutamic acid and proline in abomasal digesta and apparently absorbed amino acids were greater on diets D and E than on diets A, B and C. This is related to the higher concentration of these amino acids in casein than in bacteria and confirms observations made by Faichney (1974), Sharma *et al.* (1974) and Williams & Smith (1976).

Expt 2

Production responses to N supplements

Intakes of oaten chaff with diets receiving N supplements were 21-26 % higher than on the control diet (Table 6). N intake/MJ ME was 1.05 on the control diet, which is con-

siderably less than the value of 1.25 adopted as a desirable value for rumen degradable N (RDN): ME intake (Roy *et al.* 1977). The consequences of low availability of RDN are reductions in digestibility and rate of passage (Campling *et al.* 1962). We found that in Expt I digestibility and rumen VFA levels were lowest on the control diet. It is likely that rate of passage was also lowest on this treatment and that this would have been a major factor limiting intake. N intake/MJ ME was in excess of 1.5 on all other treatments.

The substantial intake responses to urea in our experiment are similar to those reported by Campling *et al.* (1962) for oat straw. In our experiment 26 % of DM intake was α -glucose polymers of which one-third came from maize flour in the pellets and two-thirds from grain in the oaten chaff. It is possible that this highly-fermentable substrate enhanced efficiency of urea utilization as suggested by Egan (1975). However, Campling *et al.* (1962), Hemsley & Moir (1963) and Faichney (1965) all showed that addition of sucrose did not enhance urea utilization on straw diets. In contrast, Hennessy *et al.* (1978) found that intra-ruminal infusion of molasses (which avoids the palatability factor) increased considerably the effects of urea alone on digestibility and intakes of native pasture hay. Synergistic effects on intake of feeding highly fermentable substrates with urea are often confounded with palatability of the substrate. It would be useful to differentiate between effects of highly fermentable substrates on intake and rumen fermentation of low quality roughages supplemented with urea.

All N supplements fed in Expt 2 increased intake of oaten chaff to the same extent as urea. If abomasal flows of amino acids were indeed higher on HCHO-casein diets than on the other diets, as calculated in Table 6, there were no stimulatory effects on intake such as those reported by Egan (1975). The value for g digestible protein: MJ digestible energy (DE) below which duodenal infusion of casein increased voluntary intake in Egan's (1975) experiments with sheep was approximately 6.0. In Expt 2 it can be calculated from values in Table 6 that there were 5.7, 4.9, 5.8, 7.2 and 6.7 g digestible protein: MJ DE on diets A, B, C, D and E respectively. This may be interpreted to indicate that these animals should have increased their intake in response to the greater protein: energy on diets D and E. In fact they did not respond to the greater protein : energy, but did give a substantial response to urea-N which actually reduced the protein: energy ratio (4.9 on diet B). It is possible that the disparity between our results and those of Egan (1975) is due to species differences. The lowest voluntary intake per kg live-weight^{0'75} in our experiment was about double that in Egan's (1975) experiments, which conforms with observations of Playne (1970) that voluntary intake of low quality roughages per unit metabolic size is much greater for cattle than for sheep.

Predicted live-weight gains were very similar to actual live-weight gains which indicates that live-weight responses to N supplements can be explained entirely in terms of intake. The calculated values for amino acid N absorbed: MJ ME intake were all well in excess of requirements given by Ørskov (1977), and differences between treatments did not give rise to differences in efficiencies of energy utilization.

The authors wish to thank Professor E. F. Annison for helpful discussions throughout the project. They are very grateful to Mr C. Stimson and Mr R. Butchers for technical assistance. This study was supported financially by the Australian Meat Research Committee.

REFERENCES

Balch, C. C. & Campling, R. C. (1965). In *Physiology of Digestion and Metabolism in the Ruminant*, p. 108 [R. W. Dougherty, editor]. London: Butterworths.

Ben-Ghedalia, D., McMeniman, N. P. & Armstrong, D. G. (1978). Br. J. Nutr. 39, 37. Broderick, G. A. (1978). J. Nutr. 108, 181.

Campling, R. C., Freer, M. & Balch, C. C. (1962). Br. J. Nutr. 16, 115.

- Chaney, A. L. & Marbach, E. P. (1962). Clin. Chem. 8, 130.
- Czerkawski, J. W. (1978). J. Dairy Sci. 61, 261.
- Downes, A. M. & McDonald, I. W. (1964). Br. J. Nutr. 18, 153.
- Egan, A. G. (1975). Rev. Rur. Sci. 2, 135.
- Faichney, G. J. (1965). Aust. J. agric. Res. 16, 159.
- Faichney, G. J. (1974). Aust. J. agric. Res. 25, 583.
- Faichney, G. J. (1975). In Digestion and Metabolism in the Ruminant, p. 277 [I. W. McDonald and A. C. I. Warner, editors]. Armidale: University of New England Printing Unit.
- Harrop, C. J. F. (1974). J. agric. Sci., Camb. 83, 249.
- Hemsley, J. A. & Moir, R. J. (1963). Aust. J. agric. Res. 14, 509.
- Hemsley, J. A., Reis, P. J. & Downes, A. M. (1973). Aust. J. biol. Sci. 26, 961.
- Hennessy, D. W., Nolan, J. V., Norton, B. W., Ball, F. M. & Leng, R. A. (1978). Aust. J. exp. Agric. Anim. Husb. 18, 477.
- Hume, I. D. (1970). Aust. J. agric. Res. 21, 305.
- Jackson, P., Rook, J. A. F. & Towers, K. G. (1971). Proc. Nutr. Soc. 30, 1A.
- Kropp, J. R., Johnson, R. R., Males, J. R. & Owen, F. N. (1977). J. Anim. Sci. 46, 844.
- McDougall, E. I. (1948). Biochem. J. 43, 99.
- Macrae, J. C. & Armstrong, D. G. (1968). J. Sci. Fd Agric. 19, 578.
- Macrae, J. C., Wilson, S., Milne, J. A. & Spence, A. M. (1977). Proc. Nutr. Soc. 36, 77A.
- Maeng, W. J., Van Nevet, C. J., Baldwin, R. L. & Morris, J. G. (1976). J. Dairy Sci. 59, 68.
- Mangan, J. L. (1972). Br. J. Nutr. 27, 261.
- Mercer, J. R. & Annison, E. F. (1976). In Protein Metabolism and Nutrition, p. 397 [D. J. A. Cole, K. N. Boorman, P. J. Buttery, D. Lewis, R. J. Neale and H. Swan, editors]. London: Butterworths.
- Ministry of Agriculture, Fisheries and Food (1975). Tech. Bull. Min. Agric. Fish. Fd no. 33.
- Nolan, J. V. & Leng, R. A. (1972). Br. J. Nutr. 27, 177.
- Nolan, J. V., Norton, B. W. & Leng, R. A. (1976). Br. J. Nutr. 35, 127.
- Okorie, A. V., Buttery, P. J. & Lewis, D. (1977). Proc. Nutr. Soc. 36, 38A.
- Ørskov, E. R. (1977). Wld Rev. Nutr. Diet. 26, 225.
- Owen, F. N. & Isaacson, H. R. (1977). Fedn Proc. Fedn Am. Socs exp. Biol. 36, 198.
- Pilgrim, A. F., Gray, F. V., Weller, R. A. & Belling, C. B. (1970). Br. J. Nutr. 24, 589.
- Playne, M. J. (1970). Proc. Aust. Soc. Anim. Prod. 8, 511.
- Roy, J. H. B., Balch, C. C., Miller, E. L., Ørskov, E. R. & Smith, R. H. (1977). Proc. 2nd int. Symp. Protein Metabolism and Nutrition, The Netherlands, p. 126.
- Sharma, H. R., Ingalls, J. R. & Parker, R. J. (1974). Can. J. Anim. Sci. 54, 305.
- Tan, N. H., Weston, R. H. & Hogan, J. P. (1971). Int. J. appl. Radiat. Isot. 22, 301.
- Thornton, R. F. & Minson, D. J. (1973). Aust. J. agric. Res. 24, 889.
- Van Soest, P. J. (1963). J. Ass. off. agric. Chem. 46, 829.
- Williams, A. P. & Smith, R. H. (1976). Br. J. Nutr. 36, 199.