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Relative retentions of the nitrogen of urea and groundnut in isoenergenetic diets for growing heifers

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1. Two experiments were conducted to compare the effect on nitrogen retention of three dietary levels of N given either as groundnut or as urea to growing heifers. Additions of maize starch or dextrose were made to the diets to equalize the inputs of digestible energy within and between experiments.

2. In the first experiment, in which the maximum level of N supplementation was 69·0 g/d, the response to additional N was linear, and was identical for both sources of N. Differences in faecal N between treatments were small; differences in urinary N were large and were entirely attributable to level of N intake.

3. In the second experiment, the maximum level of N supplementation was raised to 103·3 g/d. The response to additional N was again linear and identical for both sources of N; however, for a given level of N input, the amount of N retained was 4·6 g less than in Expt 1. This reduction in N retention may have been due to the change in the proportion of digestible energy derived from the fibrous components of the ration.

4. Live-weight changes calculated from the observed N retentions have been compared with published responses to the inputs of N and energy used in these experiments.

There is still much doubt about the response to be expected when non-protein nitrogen is substituted for protein in diets for ruminants. In comparison with conventional proteins, the efficiency of utilization of non-protein N has varied greatly between experiments (see review by Reid, 1953). In some instances, and especially with experimental diets rich in starch, the utilization of urea N appears to have been as high as the utilization of protein N (Loosli & McDonald, 1968). With many diets, more typical of those used on farms, the utilization of urea N appears to have been somewhat lower. Thus, the problem which remains is that of predicting the efficiency of utilization of non-protein N under particular circumstances.

To predict responses to particular inputs of N, consideration must be given to the interaction between inputs of both energy and N (Broster, Tuck & Balch, 1963; Elliott, Reed & Topps, 1964). The relationship between energy supply and protein requirement is complicated by the fact that, in addition to acting as a source of amino acids, protein can be utilized as a source of energy. If increasing amounts of protein are added to diets in which energy content is fixed and limiting to growth, the rate of gain and the N balance of growing animals receiving the diets continue to increase. The rate of gain is, however, lower than with diets in which energy is not limiting (Balch, 1967). There is little direct information about whether the response to increments of non-protein N declines in the same way as the response to protein when energy becomes limiting or whether the response is greater or less than that to protein. The present work was undertaken to calibrate and compare the responses to additional N as protein and as urea when energy is limiting and fixed. With a small number of animals available, a Latin square design was chosen and the response was measured in terms of N balance rather than live-weight change. In each experiment, three levels of each source of N were used to assess the curvilinearity of the responses. A supplement of inorganic sulphur was included in the diets, as this has improved the utilization of urea N in some work (Helmer & Bartley, 1971). Some preliminary results for the first experiment have been reported previously (Balch & Bines, 1968).

EXPERIMENTAL

Design of the experiments

Expt 1. A balanced 6 × 6 Latin square experiment was used to compare the effect on N balance of three levels of N given either as groundnut or as urea to six heifers. The heifers received a basal low-N ration consisting of 3·5 kg chopped barley straw and 4·5 kg of a concentrate mixture. The three control treatments were: 124 g groundnut meal and 589 g maize starch; 372 g groundnut meal and 408 g maize starch; 930 g groundnut meal. The additional amounts of N supplied were, respectively 9·2, 27·6 and 69·0 g. The three experimental treatments were 20, 60 and 150 g urea daily, each given with 680 g maize starch, supplying the same amounts of energy and respectively the same amounts of N as the control treatments.

Each 28 d experimental period consisted of 16 d during which rations were changed and the heifers were accustomed to the new rations, followed by 12 d during which the N balance was conducted.

Expt 2. A balanced 6 × 6 Latin square experiment was used to compare the effect on N balance of three levels of N given either as groundnut or as urea to six heifers. The highest level of N supplementation was increased by 50% compared with that in Expt 1. The digestible energy input was similar to that in Expt 1. The heifers received a basal low-N ration consisting of 3·5 kg chopped barley straw and 4·0 kg of a concentrate mixture. The three control treatments were: 116 g groundnut meal, 501 g dextrose and 865 g maize starch; 726 g groundnut meal and 661 g maize starch; 1307 g groundnut meal. The amounts of N supplied were, respectively 9·2, 57·3 and 103·3 g. The three experimental treatments were 20, 125 and 225 g urea daily, each given with 663 g dextrose and 865 g maize starch, supplying the same amounts of energy and respectively the same amounts of N as the control treatments.

Each 35 d experimental period consisted of 7 d for change-over of rations, 18 d for the heifers to become accustomed to the new ration and 10 d during which the N balance was measured.

Animals and housing

The heifers used in the two experiments were non-pregnant Friesians with a mean initial age of 19 months and mean live weights of 388 kg at the start of Expt 1 and of 383 kg at the start of Expt 2. The heifers were housed in individual standings in a metabolism house. Water and salt licks containing trace elements were available at all times.

Table 1. *Expts* 1 *and* 2. *Chemical compositions of the barley straw, concentrate mixtures, and groundnut*

		Dry matter (g/kg)	Ash	Crude fibre	Nitrogen	Gross energy (MJ/kg dry matter)
			(as g/kg dry matter)			
Expt 1:	Barley straw	848	60	447	8	18·9
	Concentrate	841	109	100	16	16·7
	Groundnut	881	59	46	90	20·0
Expt 2:	Barley straw	837	40	412	9	19·5
	Concentrate	849	124	117	14	16·7
	Groundnut	882	67	63	90	19·9

Foods

The barley straw was coarsely chopped. The composition of the concentrate used in Expt 1 was (per kg): molassed sugar-beet pulp 560 g, rolled barley 300 g, Molassine meal (The Molassine Co. Ltd, Greenwich, London) 100 g, mineral mixture 30 g and sodium sulphate 10 g; a supplement containing vitamins A and D was added at a rate of 1 kg/2000 kg mixture. The composition of the concentrate used in Expt 2 was (per kg): sugar-beet pulp 640 g, rolled barley 205 g, Molassine meal 115 g, mineral mixture 30 g and sodium sulphate 10 g; a supplement containing vitamins A and D was added at a rate of 1 kg/2000 kg mixture. The concentrate was given to the heifers as a loose mixture with the energy and N supplements indicated above. The chemical compositions of the foods are given in Table 1.

N balance

The total daily food allowance of each animal was given in two equal meals at 06.00 and 17.00 hours. Any uneaten food was removed and weighed immediately before the afternoon meal. All foods and supplements were sampled on alternate days; all except urea were dried in a forced-air oven at 100° and were then bulked for the duration of each collection period. Urine and faeces were collected separately by means of the harness and equipment described by Balch, Bartlett & Johnson (1951). Urine was collected in stainless-steel buckets containing approximately 100 ml glacial acetic acid. It was removed daily and weighed, and a sample was taken for analysis, mercuric chloride being added as a preservative. The samples for analysis were bulked for the duration of each collection period. Faeces were bulked for 48 h, then thoroughly mixed and two samples taken: one for N determination was stored under dilute sulphuric acid (low-N) and the other was dried in a forced-air oven at 100°. These faeces samples, in turn, were bulked for the duration of each collection period. The standings were washed daily with a small volume of hot water; the washings were collected, weighed and sampled for N determination and the value was added to the urinary N.

Chemical methods

Dried samples of foods and faeces were analysed for ash and crude fibre. N determinations were made on the acidified faeces, washings and urine samples and on the

Table 2. *Expt* 1. *Mean daily nitrogen intakes, outputs and balances and intakes of energy and water by six heifers given N at three levels, either as groundnut or as urea, in iso-energetic rations*

Ration	N intake (g)	N output (g)			N balance (g)	Energy digested (MJ)	Water intake (kg)
		Faeces	Urine	Total			
Low-urea	91·1	55·2^{ab}	27·7^{a}	82·9	8·2^{a}	77·0^{a}	28·3^{a}
Medium-urea	109·9	54·2^{a}	44·2^{b}	98·4	11·6^{a}	80·3^{a}	28·1^{a}
High-urea	151·9	56·3^{ab}	77·3^{c}	133·6	18·3^{b}	79·5^{a}	29·6^{a}
Low-groundnut	91·9	55·6^{ab}	27·0^{a}	82·6	9·2^{a}	78·7^{a}	29·3^{a}
Medium-groundnut	110·2	57·5^{b}	43·4^{b}	100·9	9·3^{a}	77·8^{a}	28·3^{a}
High-groundnut	155·9	62·3^{c}	74·6^{c}	136·8	19·0^{b}	84·9^{b}	27·9^{a}
SE		1·0	1·6		2·0	1·4	1·4

^{abc} Means in the same column with the same superscript are not significantly ($P > 0{\cdot}05$) different.

dried food samples. Urea was analysed for N without previous drying. All analyses were conducted according to standard methods (Association of Official Agricultural Chemists, 1965). The energy values of foods and faeces were determined in an adiabatic bomb calorimeter.

Statistical analyses

Statistical analysis of the results was based on methods described by Snedecor (1956); means were compared by a multiple range test (Duncan, 1955).

RESULTS

Expt 1

The N economies for the animals in Expt 1 are shown in Table 2. In spite of the large differences in N intake, the difference in faecal N between treatments was very small and was not significant ($P > 0{\cdot}05$) except for that with the high-protein treatment which was significantly ($P < 0{\cdot}01$) higher than all the others. Owing to this value with the high-protein treatment, there was a significant ($P < 0{\cdot}01$) total difference between the two sources of N, although at the low and medium levels of input this difference was not significant ($P > 0{\cdot}05$).

There were large differences between treatments in the amount of urinary N, but these were entirely attributable to levels of N intake, the differences between the two sources being non-significant ($P > 0{\cdot}05$). There was a small period effect on the amounts of urinary N, that in period 5 being significantly ($P < 0{\cdot}05$) higher than that in the other periods.

Thus, the N balances, as shown in Table 2 and Fig. 1, were not significantly ($P > 0{\cdot}05$) different for the two sources of N, at each of the three levels. There was a significant ($P < 0{\cdot}01$) effect of level of N intake on N balance, the relationship being linear for both sources of N. The attempt to equalize the amounts of energy digested with the six rations was not fully successful (Table 2). The energy intake at the highest level of groundnut supplementation was significantly ($P < 0{\cdot}05$) higher than that with the other five rations, which did not differ significantly ($P > 0{\cdot}05$) from each other.

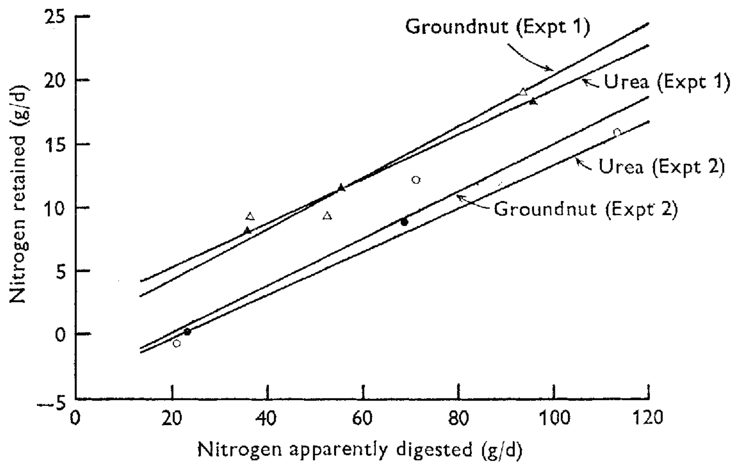

Fig. 1. Relationships between nitrogen retention and apparently digested N in heifers given groundnut (○, △) or urea (●, ▲) in isoenergetic diets in Expt 1 (△, ▲) and Expt 2 (○, ●).

Table 3. *Expt 2. Mean daily nitrogen intakes, outputs and balances and intakes of energy and water by six heifers given N at three levels, either as groundnut or as urea, in isoenergetic rations*

Ration	N intake (g)	N output (g) Faeces	N output (g) Urine	N output (g) Total	N balance (g)	Energy digested (MJ)	Water intake (kg)
Low-urea	82·7	59·6^{ab}	22·9^{a}	82·4	0·3^{a}	78·2^{ab}	25·3^{a}
Medium-urea	130·2	61·4^{abc}	60·0^{b}	121·3	8·9^{b}	79·5^{ab}	26·1^{a}
High-urea	178·8	62·8^{bc}	100·8^{c}	163·6	15·3^{b}	82·4^{b}	27·4^{a}
Low-groundnut	80·7	59·6^{ab}	21·8^{a}	81·4	−0·7^{a}	76·1^{a}	25·5^{a}
Medium-groundnut	129·2	58·1^{a}	58·9^{b}	117·0	12·2^{b}	80·8^{b}	24·9^{a}
High-groundnut	177·8	64·4^{c}	97·6^{c}	162·0	15·0^{b}	82·0^{b}	26·0^{a}
SE		1·2	2·3		2·4	1·4	1·4

^{abc} Means in the same column with the same superscript are not significantly ($P > 0{\cdot}05$) different.

The intakes of water (Table 2) did not differ significantly ($P > 0{\cdot}05$) between treatments: however, there were large and highly significant ($P < 0{\cdot}01$) differences between individual heifers, ranging from 23·6 kg to 34·8 kg daily.

Expt 2

The N economies for the animals in Expt 2 are shown in Table 3. As in Expt 1, large differences in N intake resulted in only small differences in the amount of faecal N; the only significant ($P < 0{\cdot}01$) difference in faecal N was again between the high- and medium-protein treatments. There was no significant ($P > 0{\cdot}05$) difference in faecal N between the two sources of N either total, or at any given level of N intake.

There were large differences between treatments in the amount of urinary N, but these were entirely attributable to highly significant ($P < 0{\cdot}001$) differences between levels of N intake, the differences between the two sources being non-significant ($P > 0{\cdot}05$). There was again a small period effect, the urinary N values being higher

Table 4. *Expts* 1 *and* 2. *Relationships between faecal and urinary nitrogen and N consumed and between N retained and apparently digested N in heifers given N supplements either as urea or as groundnut in isoenergetic rations*

	Regression equation	SE of b	SE of a	Correlation coefficient
	Expt 1			
Urea	$N_u = -45{\cdot}11 + 0{\cdot}81N_c$	0·06	6·31	$0{\cdot}96^{***}$
	$N_f = 52{\cdot}55 + 0{\cdot}02N_c$	0·03	3·73	$0{\cdot}18^{NS}$
	$N_r = 1{\cdot}85 + 0{\cdot}17N_a$	0·05	3·34	$0{\cdot}66^{**}$
Groundnut	$N_u = -38{\cdot}76 + 0{\cdot}73N_c$	0·04	4·37	$0{\cdot}98^{***}$
	$N_f = 45{\cdot}66 + 0{\cdot}11N_c$	0·04	5·44	$0{\cdot}52^{*}$
	$N_r = 0{\cdot}31 + 0{\cdot}20N_a$	0·04	2·78	$0{\cdot}76^{***}$
Combined	$N_r = 1{\cdot}09 + 0{\cdot}19N_a$	—	—	—
	Expt 2			
Urea	$N_u = -44{\cdot}35 + 0{\cdot}81N_c$	0·05	6·16	$0{\cdot}98^{***}$
	$N_f = 57{\cdot}49 + 0{\cdot}03N_c$	0·02	3·23	$0{\cdot}29^{NS}$
	$N_r = -3{\cdot}67 + 0{\cdot}17N_a$	0·05	3·55	$0{\cdot}69^{**}$
Groundnut	$N_u = -41{\cdot}70 + 0{\cdot}78N_c$	0·04	5·22	$0{\cdot}98^{***}$
	$N_f = 54{\cdot}07 + 0{\cdot}05N_c$	0·03	3·63	$0{\cdot}43^{\dagger}$
	$N_r = -3{\cdot}51 + 0{\cdot}18N_a$	0·05	3·53	$0{\cdot}72^{***}$
Combined	$N_r = -3{\cdot}58 + 0{\cdot}18N_a$	—	—	—

N_u = urinary N, N_f = faecal N, N_r = retained N, N_c = N consumed, N_a = apparently digested N.
a = intercept; b = regression coefficient.
^{NS}, not significant. *** $P < 0{\cdot}001$. ** $P < 0{\cdot}01$. * $P < 0{\cdot}05$. $\dagger$ $P < 0{\cdot}1$.

$(P < 0{\cdot}05)$ in the last three periods than in periods 2 and 3, but not different $(P > 0{\cdot}05)$ from those in period 1.

Thus, the N balances, as shown in Table 3 and Fig. 1, were again not significantly $(P > 0{\cdot}05)$ different for the two sources of N, at each of the three levels. There was a significant $(P < 0{\cdot}01)$ relationship between level of N intake and N balance, the relationship being linear for both sources of N.

Again, the attempt to equalize the amounts of energy digested with each of the rations was not fully successful (Table 3), there being a significant $(P < 0{\cdot}05)$ increase in energy intake with increasing N intake; however, there were no significant $(P > 0{\cdot}05)$ differences in energy intake between the two sources of N.

The intakes of water (Table 3) did not differ significantly $(P > 0{\cdot}05)$ between treatments, but again showed large and highly significant $(P < 0{\cdot}01)$ differences between individual heifers, ranging from 22·4 to 29·8 kg daily.

Regression equations were fitted to the results of the two experiments separately and are shown in Table 4. For both sources of N in both experiments there was a highly significant $(P < 0{\cdot}001)$ linear relationship between urinary N and N consumed, whereas faecal N was correlated significantly $(P < 0{\cdot}05)$ with N consumed only when groundnut was given in Expt 1. In both experiments there was a significant $(P < 0{\cdot}01)$ linear relationship between N retained and apparently digested N when urea was given, and this relationship was highly significant $(P < 0{\cdot}001)$ when the ration contained groundnut. Analysis showed no departures from linearity in any of the responses of N retention to apparently digested N and, within each experiment, the

Table 5. *Expts* 1 *and* 2. *Digestibilities of some constituents of the rations given to the heifers*

		Digestibility ratio		
Expt no.	Ration	Organic matter	Crude fibre	Crude protein
1	Low-urea	$0{\cdot}65^{a}$	$0{\cdot}52^{a}$	$0{\cdot}39^{aA}$
	Medium-urea	$0{\cdot}68^{b}$	$0{\cdot}56^{b}$	$0{\cdot}51^{cB}$
	High-urea	$0{\cdot}67^{b}$	$0{\cdot}55^{ab}$	$0{\cdot}63^{eC}$
	Low-groundnut	$0{\cdot}66^{ab}$	$0{\cdot}52^{a}$	$0{\cdot}40^{aA}$
	Medium-groundnut	$0{\cdot}66^{ab}$	$0{\cdot}54^{ab}$	$0{\cdot}48^{bB}$
	High-groundnut	$0{\cdot}67^{b}$	$0{\cdot}57^{b}$	$0{\cdot}60^{dC}$
	SE	0·005	0·01	0·01
2	Low-urea	$0{\cdot}63^{a}$	$0{\cdot}43^{a}$	$0{\cdot}28^{aA}$
	Medium-urea	$0{\cdot}64^{a}$	$0{\cdot}48^{b}$	$0{\cdot}53^{bB}$
	High-urea	$0{\cdot}66^{b}$	$0{\cdot}50^{b}$	$0{\cdot}65^{cC}$
	Low-groundnut	$0{\cdot}62^{a}$	$0{\cdot}44^{a}$	$0{\cdot}26^{aA}$
	Medium-groundnut	$0{\cdot}64^{a}$	$0{\cdot}51^{b}$	$0{\cdot}55^{bB}$
	High-groundnut	$0{\cdot}63^{a}$	$0{\cdot}52^{b}$	$0{\cdot}64^{cC}$
	SE	0·005	0·01	0·01

^{abcde} Means in each column, and within each experiment, with different superscripts are significantly different ($P < 0{\cdot}05$).

^{ABC} Means in each column, and within each experiment, with different superscripts are significantly different ($P < 0{\cdot}01$).

fitted regression lines were parallel and coincident; therefore combined regressions were fitted to the values from each experiment and are shown in Table 4. In comparing the results from the two experiments, the regression lines were found to be parallel but not coincident, there being a difference of about 4·6 g/d in N retention between the experiments for any given level of apparently digested N. N retention increased by 0·18–0·19 g per 1 g increase in apparently digested N.

The digestibilities of some constituents of the rations used in the two experiments are shown in Table 5. Within each experiment, digestibilities of organic matter and crude fibre tended to increase as N inputs increased, the effects being broadly similar for both N sources. However, the values were generally a little higher in Expt 1 than in Expt 2. There was a highly significant ($P < 0{\cdot}01$) increase in the digestibility of crude protein as the level of N supplementation increased, but the responses did not differ significantly with the source of N.

DISCUSSION

The results of these experiments show that, under the conditions of the experiments, there was no difference between groundnut and urea in the efficiency with which they were utilized by growing heifers as sources of N in the presence of a readily fermentable supply of energy and a source of inorganic sulphur. This finding confirms the ability of the rumen microflora to synthesize protein from non-protein N, under these conditions. After absorption from the gut, the products of digestion of N from the two sources were apparently used with equal efficiency, as there were no differences

Table 6. *Expts* 1 *and* 2. *Calculated live-weight changes and maximum potential live-weight changes set by nitrogen and energy contents of rations* (*Agricultural Research Council,* 1965) *given to heifers*

			Potential live-weight change (kg/d)	
Expt no.	Ration	Calculated live-weight change (kg/d) (N balance (g/d) × 31·25)	Limit set by N supply	Limit set by energy supply
1	Low-urea	0·26	0·15	0·35
	Medium-urea	0·36	0·70	0·45
	High-urea	0·57	> 1·20	0·39
	Low-groundnut	0·29	0·15	0·40
	Medium-groundnut	0·29	0·60	0·38
	High-groundnut	0·59	> 1·20	0·56
2	Low-urea	0·01	< 0	0·34
	Medium-urea	0·28	1·00	0·38
	High-urea	0·48	> 1·20	0·50
	Low-groundnut	−0·02	< 0	0·32
	Medium-groundnut	0·38	1·10	0·44
	High-groundnut	0·49	> 1·20	0·48

in either experiment in the urinary output of N from animals receiving a given level of N from the two sources.

Between the two experiments there was a difference in N balance of about 4·6 g/d at any given level of N intake. This difference could not be attributed to differences in the weights of the heifers used, as these averaged 432 kg in Expt 1 and 436 kg in Expt 2. However, it is possible that some other characteristic of the animals was involved, even though both groups were drawn from genetically similar stock. The difference in N balance was not attributable to a difference in digestible energy intake as this averaged 79·5 MJ/d in Expt 1 and 79·9 MJ/d in Expt 2, but could possibly have been due to differences in the source of energy, a larger proportion of which consisted of starch and dextrose in Expt 2. This explanation seems unlikely in view of the findings of Broster, Sutton & Smith (1968) that the energies of sucrose, dextrose and maize starch were each equivalent to the energy of a conventional concentrate in stimulating live-weight gain in heifers; N balance results (W. H. Broster, unpublished observations) do not dispute these findings. However, there is some doubt that energy sources are interchangeable at all levels (Tagari, Dror, Ascarelli & Bondi, 1964). Also, in studies specifically concerned with urea, there are several reports that urea N is utilized more efficiently when starch is included in the ration than when supplementary carbohydrate is in the form of cellulose; simple sugars were intermediate in their effect (Helmer & Bartley, 1971).

Another possible explanation of the discrepancy between the two experiments may lie in the fact that Expt 1 was conducted during the summer months, whereas Expt 2 was conducted during the winter when more energy would have been required to compensate for increased heat loss, thus reducing the amount available for live-weight gain. If this were so, in Expt 2, a higher urinary N output would be expected for a given apparently digested N intake, and this is confirmed by the observations.

There was a tendency for the intake of digestible energy to increase with increasing level of N intake. This was due to small refusals of the concentrate by some of the animals when receiving the lower-N treatments but, as the refusals occurred equally with groundnut and urea treatments, this observation does not detract from the main conclusion drawn from the results of the two experiments.

There was no evidence, in either experiment, of a diminishing response to additional increments of N, as has been reported by Broster, Tuck, Smith & Johnson (1969), in spite of giving, in Expt 2, more than double the amount (51 g/d) of digestible N at which the diminishing response was considered to become apparent. However, it should be noted that a reduced response to additional protein measured as live-weight gain can occur, even though no similar decline is seen in N balance (Broster *et al.* 1963). In an experiment of this nature, the short experimental periods make it impossible to assess accurately the effects of treatments on live-weight change. However, in Table 6, the observed N balances have been used to calculate possible live-weight changes using the approximate relationship

$$\text{live-weight change} = \text{N balance} \times 6{\cdot}25 \times 5.$$

The calculated live-weight changes have then been compared with the maximum responses which could have been expected from the respective inputs of N and energy according to published requirements (Agricultural Research Council, 1965). In both experiments, it is clear that when N intakes were lowest, they were limiting growth, but at the higher levels of N input, the potential live-weight gain was limited by the supply of energy and hence diminished responses per increment of N were to be expected.

In conclusion, it may be stated that urea can be used to supply up to at least 45 % of the N in rations for growing heifers provided a readily fermentable source of energy is supplied and provided urea intake is built up gradually. Under these conditions, growth rates can be expected to equal those achieved with conventional protein. However, without further experimental work, it would be dangerous to extrapolate this finding to lactating cows, in which the much higher demand for amino acids may exceed the synthetic ability of the rumen micro-organisms, either totally, or at least in terms of certain amino acids such as those containing sulphur.

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