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The protein requirement of the ruminant calf

4.* Nitrogen balance studies on rapidly growing calves given diets of different protein content

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1. Twelve Friesian bull calves were weaned at 5 weeks and reared to approximately 18 weeks of age on diets consisting mainly of concentrates containing 209 (HP), 169 (MP) or 130 (LP) g crude protein/kg dry matter. Hay was removed from the diet when the calves reached 10 weeks of age.

2. In relation to metabolic body size, daily dry-matter intake rose rapidly until the calves were about 9 weeks old and then tended to level off at between 80 and 100 g dry matter/kg$^{0.73}$.

3. After adjustment for treatment differences in concentrate intake, the live-weight gains of calves given diets HP or MP were significantly greater, both from 1 to 12 weeks and from 1 to 16 weeks of age, than those of calves given diet LP.

4. Digestibility trials made twice on each calf, when given solely the concentrate diet and when live-weight gain was about 1 kg/d, showed no differences in dry-matter digestibility, but the apparent digestibility of crude protein increased with the protein content of the concentrate mixture. The true digestibility coefficient of dietary protein was estimated to be 0·880, with no difference between diets. Metabolic faecal nitrogen excretion was estimated to be 3·43 g N/kg dry matter ingested.

5. At the mean intake of digestible energy/kg$^{0.73}$, N balance was significantly greater with diets HP and MP than with diet LP. Endogenous urinary N excretion was estimated to be 220 mg/kg$^{0.73}$ and the apparent biological value of protein was 0·851 when diet LP was given.

6. The results confirm the importance of a balanced relationship between energy and protein intakes in the diet of the rapidly growing calf. It is concluded that the crude protein content of the diet can be reduced to below 172 g/kg dry matter, and possibly as low as 153 g/kg, for calves of 122 kg live weight to achieve weight gains of 1 kg/d, when given an all-concentrate diet. This lower protein level would be expected to result in the maximum efficiency of utilization of the dietary energy and protein.

From previous work (Stobo, Roy & Gaston, 1967*a*) it was concluded that no advantage was obtained by using a concentrate containing more than 159 g crude protein/kg (187 g/kg dry matter) for Friesian heifer calves weaned at 5 weeks, but that growth rate was depressed when the diet contained only 121 g crude protein/kg (143 g/kg dry matter). From nitrogen balance trials with Ayrshire and Shorthorn bull calves (Stobo, Roy & Gaston, 1967*b*) it was established that slightly greater amounts of N were retained when diets containing 196 g protein/kg (231 g/kg dry matter) rather than 122 g/kg (144 g/kg dry matter) were given, but in those trials the mean growth rate was only 0·48 kg/d.

As part of a general study of the protein requirement of the ruminant calf, the present experiment was planned to investigate the utilization of dietary N by Friesian bull calves gaining weight rapidly, when given diets containing different levels of crude protein. The diets were designed to supply protein at levels ranging from that

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Table 1. *Composition of the concentrates used throughout the experiment and their chemical analysis*

	Diet		
	HP	MP	LP
Ingredient (kg):			
Flaked maize	40·0	47·5	55·0
Crushed oats	20·0	23·75	27·5
Molassine meal*	10·0	10·0	10·0
Extracted decorticated groundnut meal	15·0	9·38	3·75
White fish meal	5·0	3·12	1·25
Dried skim milk	10·0	6·25	2·5
Vitamin supplement†	0·25	0·25	0·25
Mineral supplement‡	1·0	1·0	1·0
Chemical analysis:			
Dry matter (g/kg)	859	835	834
Composition of dry matter (g/kg)			
Crude protein	209	169	130
Crude fibre	54	46	56
Ether extract	20	21	21
Ash	53	46	40
Nitrogen-free extract	664	718	753
Gross energy (MJ/kg dry matter)	18·13	18·22	18·18

* Contained 750 g molasses and 250 g peat moss/kg (Molassine Co. Ltd, Greenwich, London, SE 10).
† Contained 529 200 μg retinol and 11 025 μg cholecalciferol/kg.
‡ Contained 174 g calcium, 114 g phosphorus, 300 g sodium+chloride, 1·8 g copper, 0·3 g cobalt, 0·51 g manganese, 0·25 g iodine, 7·5 g sulphur and 2·4 g iron/kg.

which had previously been found to be limiting in protein for maximum growth (Stobo *et al.* 1967*a*), to that which provided more than adequate amounts of protein.

METHODS

Plan of experiment

The experiment was made during the winter of 1962 to study the performance, digestibility of the diets and N utilization in rapidly growing calves given concentrates designed to contain 200, 160 or 120 g crude protein/kg air-dried material (treatments HP, MP and LP respectively). Twelve Friesian bull calves were allocated at random to one of the three treatments.

Diets

The composition of the concentrate mixtures, given in the form of a loose mix and the results of their chemical analysis are shown in Table 1. On analysis, all three diets had a lower protein content than was planned.

Calves

The calves were given 7 kg colostrum or allowed to suckle their dam during the first 2 d of life and were then given a milk substitute containing 200 g vegetable fat and 300 g milk-protein/kg dry matter (Roy, Stobo, Gaston & Greatorex, 1970) offered at the maintenance level (Roy, Shillam, Hawkins & Lang, 1958). The calves were weaned abruptly at 5 weeks of age. They were housed in individual pens on wooden slatted floors with no bedding. Live weight was recorded at weekly intervals from the date of birth.

Concentrates, hay and water were offered *ad lib.* from 1 week of age and intakes of concentrates and water were recorded daily and that of hay was recorded weekly. Hay was removed from the diet when the calves reached 10 weeks of age.

Digestibility and N balance trials, involving 7 d collection periods, were made twice on each calf, the first period beginning after 12 weeks of age, once the calf had been gaining weight at a rate of 1 kg/d for between 1 and 2 weeks. The second collection period began about 4 weeks later, provided that live weight was still increasing at about 1 kg/d.

Analytical methods

Samples of the concentrates used throughout the experiment were taken at intervals and bulked for analysis by the methods outlined previously (Stobo, Roy & Gaston, 1966). In addition, samples of the concentrates given during each digestibility trial were taken daily and the dry-matter and N content determined on the bulked 7 d sample. N was determined in acidified urine and faeces, and a sample of faeces was dried to constant weight at 100° to determine the dry-matter content.

RESULTS

Food intake

The mean intake of concentrates rose from 0·5 kg/d in the week before weaning to 1·2 and 1·8 kg/d respectively in the succeeding 2 weeks. Thereafter the consumption of dry food continued to rise, but the rate of increase became slower after about 8 weeks of age. Consumption of dry food from 1 to 16 weeks of age tended to be less when diet MP was given than when calves were offered either diet HP or LP, but this difference was not significant because of the considerable variation between calves on the same diet.

The relationship in the first 16 weeks of life between dry-matter intake (DMI) per unit of metabolic body-weight ($W^{0\cdot 73}$, kg), ($DMI/W^{0\cdot 73}$) and age is shown in Fig. 1. Curves were fitted to the values for each calf after exclusion of the values obtained during the 1st week of life, during which the calves were given a fixed quantity of milk but no dry food. There were no significant differences between diets in the relationship between $DMI/W^{0\cdot 73}$ and log age. The combined relationships for each diet and the combined relationship for all the diets were:

Diet HP $DMI/W^{0\cdot 73} = 92\cdot 8 \log A - 11\cdot 9$ (SD = 15·2),

Diet MP $DMI/W^{0\cdot 73} = 58\cdot 1 \log A + 14\cdot 9$ (SD = 10·8),

Diet LP $DMI/W^{0\cdot 73} = 68\cdot 0 \log A + 12\cdot 1$ (SD = 10·7),

Combined regression $DMI/W^{0\cdot 73} = 73\cdot 0 \log A + 5\cdot 0$ (SD = 12·9),

where $DMI/W^{0\cdot 73}$ is dry-matter intake/$W^{0\cdot 73}$ (kg) expressed as g/d and A = age (weeks).

Only the regression line calculated from the combined equation is shown in Fig. 1.

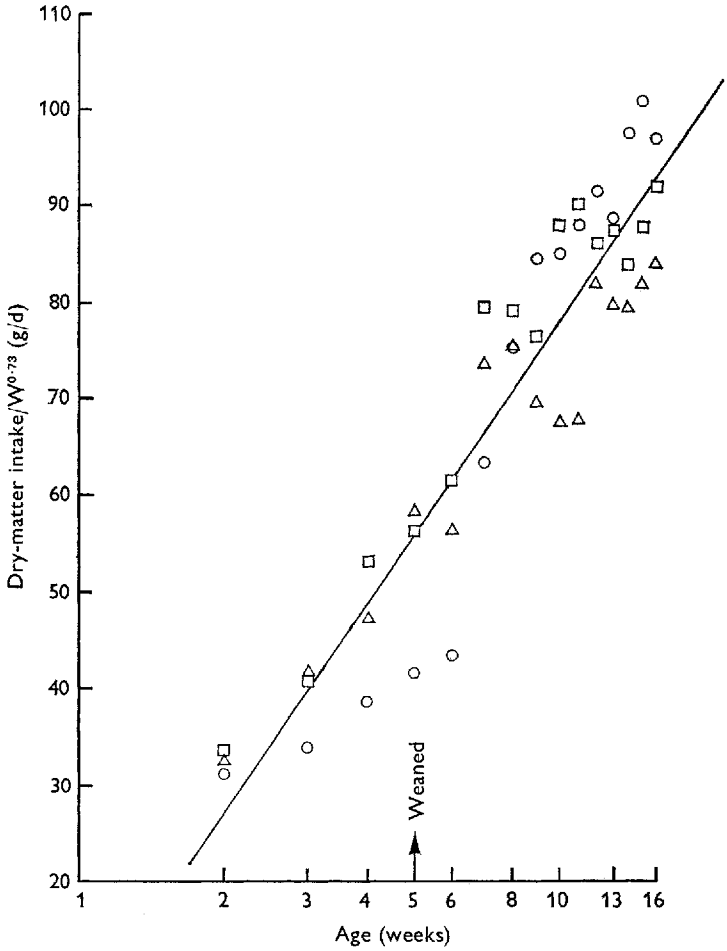

Fig. 1. Daily dry-matter intake/W$^{0.73}$ in relation to age (log scale) of calves weaned at 5 weeks of age and given *ad lib.* concentrates containing (○) 209 (HP), (△) 169 (MP), or (□) 130 (LP) g protein/kg dry matter.

Performance

Details of the performance of the calves from 1 to 12 and from 1 to 16 weeks of age are presented in Table 2. The initial live weight of the calves given diet MP tended to be lower than that of calves on the other two treatments. This factor was responsible for the significant difference in milk intake between calves on treatments MP and LP, since the amount of milk substitute was allocated in relation to live weight at the start of any particular week. Similarly, intakes of concentrates and water, and consequently live-weight gains, tended to be lower for calves on treatment MP, but differences between treatments were not significant. However, when live-weight gains for the period 1–12 weeks were adjusted for differences between treatment groups in total concentrate intake and initial live weight, calves given diets HP and MP gained weight significantly faster than the calves given diet LP. A similar significant difference occurred in adjusted live-weight gain for the period 1–16 weeks of age. This effect is

Table 2. *Effect of the protein content of the concentrate mixture on the performance of calves weaned at 5 weeks of age*

(Mean values with their standard errors)

	Diet			SE of a treatment mean	Significance of difference between diets		
	HP	MP	LP		HP *v.* MP	HP *v.* LP	MP *v.* LP
Crude protein content of concentrate (g/kg dry matter)†	209	169	130				
No. of calves	4	4	4				
Live wt at 1 week (kg)	45·9	41·4	47·5	2·0	—	—	—
Intake of diet (kg)							
Milk substitute (4 d to 5 weeks)	171·2	156·5	177·8	5·9	—	—	*
Concentrates (1–12 weeks)	107·6	98·2	124·5	11·5	—	—	—
(1–16 weeks)	208·7	175·8	215·1	20·1	—	—	—
Hay (1–10 weeks)	2·4	5·0	3·7	1·8	—	—	—
Water (1–12 weeks)	253·9	204·0	273·4	31·4	—	—	—
(1–16 weeks)	465·1	355·5	445·3	50·9	—	—	—
Live-wt gain (kg/d)							
1–12 weeks	0·65	0·58	0·66	0·07	—	—	—
1–16 weeks	0·77	0·64	0·71	0·08	—	—	—
Adjusted live-wt gain (kg/d)							
1–12 weeks‡	0·65	0·68	0·56	0·02	—	**	**
1–16 weeks§	0·73	0·74	0·65	0·02	—	*	*
Food conversion ratio, 5 to 16 weeks (kg food/kg wt gain)	2·94	3·35	3·77	0·21	—	*	—

* Significant at $P < 0·05$. ** Significant at $P < 0·01$. *** Significant at $P < 0·001$.

† Mean value of samples taken throughout the feeding trial.

‡ Adjusted for differences between treatment groups in mean consumption of concentrates ($b = 0·005 \pm 0·001$***), and live wt at 1 week ($b = 0·010 \pm 0·003$*).

§ Adjusted for differences between treatment groups in mean consumption of concentrates ($b = 0·004 \pm 0·0003$***).

Table 3. *Effect of the protein content of the concentrate mixture on the digestibility of the diet by rapidly-growing calves*

(Mean values with their standard errors)

	Diet			Pooled SE of mean	Significance of effect		
	HP	MP	LP		Diet	Period	Interaction, diet × period
Protein content of concentrate (g/kg dry matter)†	213	172	132				
No. of calves	4	4	4				
No. of collection periods	8	8	8‡				
Mean age at start of collection period (d)	103	123	114‡	7	—	***	—
Mean live wt (kg)	119·2	122·0	127·3‡	6·5	—	***	—
Dry-matter intake (g/d)	2966	2871	3409‡	233	—	***	—
Crude protein intake (g/d)	647	491	438‡	58	HP > LP*	**	—
Apparent digestibility coefficient							
Dry matter	0·814	0·811	0·801‡	0·015	—	*	—
Crude protein	0·785	0·750	0·714‡	0·011	**	—	—
Digestible energy intake (MJ/d)§	44·8	43·3	50·4‡	3·6	—	***	—

* Significant at $P < 0·05$. ** Significant at $P < 0·01$. *** Significant at $P < 0·001$.

† Mean value of samples taken during the collection periods.

‡ Includes one estimated missing value (see p. 119).

§ Individual values calculated from digestible dry-matter intake by the regression technique of Moir (1961).

confirmed by the difference which occurred in food conversion rate (kg food/kg weight gain) after weaning, diet LP being used significantly less efficiently than diet HP.

Digestibility of the diets and N balance of the calves

Significant differences occurred between periods, as shown in Tables 3 and 4, but, as there were no significant diet × period interactions, the results for both periods were combined for each diet.

Digestibility (*Table* 3). Calves given diet MP tended to be older, and those given diet LP tended to be heavier, when the digestibility trials were started, but these differences were not significant. In this experiment, live-weight gains of 1 kg/d were not achieved until the calves weighed approximately 100 kg, the mean live weights at the start of the first digestibility trial being 99·7, 104·9 and 106·3 kg for calves given diets HP, MP and LP respectively. One calf given diet LP contracted clinical pneumonia just before it was due to start its second digestibility trial; this animal was removed from the experiment and missing values were calculated for use in the statistical analysis of the results.

Despite a tendency towards a higher DMI during the digestibility trials by calves given diet LP, the apparent digestibility coefficient of dry matter was 0·01 lower in these calves. However, no relationship could be established in the present experiment between DMI and the apparent digestibility of the dry matter. As shown in Table 1, there was very little difference in the energy value of the diets.

Estimates were made of the digestible energy intake of each calf during the collection periods by use of the regression equation obtained from Moir (1961) in which

$$Y = 19{\cdot}54x - 0{\cdot}803,$$

where Y = digestible-energy content of diet (kJ/g dry matter) and x = dry-matter digestibility expressed as a coefficient. As expected, there was no significant difference between treatments in digestible-energy intake.

The apparent digestibility of crude protein (Table 3) was found to differ significantly between all three treatments, there being an increase in digestibility with increase in protein content of the diet. After adjustment for differences between treatments in intake of crude protein, the apparent digestibility of crude protein was still significantly lower for calves on diet LP, but there was no significant difference between the other two treatments.

N metabolism (*Table* 4). Despite differences in the amounts of N ingested (NI), the excretion of N in the faeces did not differ between treatments, and thus, as planned, the intake of apparently digested N (ADN) was significantly greater for calves given diet HP than for those given diet MP or LP. However, the calves given diet HP excreted in their urine 0·53 of the ADN, compared with 0·40 and 0·35 for those given diets MP and LP respectively; consequently, the proportion of NI that was retained was significantly lower when diet HP was given. There was no significant difference between treatments in the amount of N retained, although there was a marked tendency for N balance to be higher as the protein content of the diet increased.

The relationship between N balance (NB, g/d) and the intake of digestible energy/

Table 4. *Effect of the protein content of the concentrate mixture on nitrogen utilization by rapidly growing calves*

(Mean values with their standard errors)

	Diet			Pooled SE of mean	Significance of effect		
	HP	MP	LP		Diet	Period	Interaction, diet × period
Protein content of concentrate (g/kg dry matter)†	213	172	132				
No. of calves	4	4	4				
No. of collection periods	8	8	8‡				
Metabolic body size ($W^{0.73}$, kg)	32·7	33·3	34·3‡	1·3	—	***	—
Live-wt gain (kg/d)	0·96	0·92	1·08‡	0·05	—	*	—
Digestible energy intake (MJ/$kg^{0.73}$ per d)	1·37	1·30	1·46‡	0·10	—	*	—
N intake (g/d)	103·6	78·5	70·1‡	9·2	HP > LP*	**	—
Faecal N (g/d)	22·0	19·4	19·7‡	1·9	—	*	—
Apparently digested N (g/d)	81·6	59·1	50·4‡	7·6	*	**	—
Urinary N (g/d)	43·4	23·7	16·7‡	3·6	**	*	—
N balance (g/d)	38·2	35·5	33·7‡	4·6	—	*	—
(mg/g N intake)	361	451	473‡	22	*	—	—
(g/kg live-wt gain)	38·9	38·8	30·9‡	3·2	—	*	—
Adjusted N balance (g/d)§	38·6	39·3	29·5‡	2·5	{HP > LP, MP > LP*}		
Apparent biological value of protein	0·604	0·768	0·851‡	0·019	***	—	—

* Significant at $P < 0.05$. ** Significant at $P < 0.01$. *** Significant at $P < 0.001$.
† Mean value of samples taken during the collection periods.
‡ Includes one estimated missing value (see p. 119).
§ Adjusted for differences between treatments in mean intake of digestible energy/$W^{0.73}$.

unit metabolic body-weight ($DE/W^{0.73}$, kJ/d) was similar for all three treatments, but there were significant differences between treatments in the intercept. The combined equations, used for the adjustment of N balance, were:

$$\left.\begin{array}{ll}\text{Treatment HP} & NB = 0.0484DE/W^{0.73} - 27.9\\ \text{Treatment MP} & NB = 0.0484DE/W^{0.73} - 27.2\\ \text{Treatment LP} & NB = 0.0484DE/W^{0.73} - 37.1\end{array}\right\} (\text{SD} = 6.87).$$

When N balance was adjusted for differences between treatments in mean intake of $DE/W^{0.73}$, the adjusted N balance was significantly lower in calves given diet LP than in those on either of the other two treatments.

The quantity of N retained per kg live-weight gain tended to be greater with diets HP and MP than with diet LP, but differences were not significant. These values were in close agreement with those obtained by Stobo *et al.* (1967*b*) for calves given diets with protein contents similar to those used in the present experiment.

Significant relationships were established between NB and ADN for each treatment separately. The equations, together with their residual standard deviations, were:

$$\begin{array}{lll}\text{Treatment HP} & NB = 0.569\ ADN - 8.23 & (\text{SD} = 5.204)\ (P < 0.001),\\ \text{Treatment MP} & NB = 0.547\ ADN + 3.17 & (\text{SD} = 1.853)\ (P < 0.001),\\ \text{Treatment LP} & NB = 0.848\ ADN - 9.48 & (\text{SD} = 4.752)\ (P < 0.01).\end{array}$$

The difference between slopes of these regressions just failed to reach significance, but there was a significant difference ($P < 0.01$) in position of the regression lines. No marked improvements in the relationships were obtained by attempting to fit curvilinear relationships to the values, or when both variables were expressed in relation to $W^{0.73}$. The relationship between NB and ADN is therefore shown in Fig. 2. In addition to the twenty-three observations plotted, treatment mean values for both NB and ADN, after they have been adjusted to the mean intake of $DE/W^{0.73}$ (1.37 MJ $DE/W^{0.73}$) are shown. The slope of the regression line that is given is that for treatment LP, but the position of the line has been drawn to pass through the adjusted mean values for NB and ADN for this treatment. Since the adjusted NB of calves on this treatment was significantly lower than that on either of the other two treatments, this regression line can be regarded as indicating the response in NB to increments of ADN when protein intake was limiting.

The over-all relationship between log urinary N excretion (log UN, g/d) and NI (g/d) was significant at $P < 0.001$ and was:

$$\log UN = 0.0064\ NI + 0.8652 \quad (\text{SD} = 0.1123).$$

The intercept of this equation, equivalent to a daily output of 7.33 g UN, may be used as an estimate of endogenous urinary N excretion. This amount of UN is equivalent to 220 ± 42 mg $N/W^{0.73}$.

The over-all relationship between ADN/kg dry-matter intake (ADN_{DMI}) and NI/kg dry-matter intake (NI_{DMI}) was significant at $P < 0.001$ and is shown in Fig. 3. This relationship was:

$$ADN_{DMI} = 0.880\ (\pm\ 0.029)\ NI_{DMI} - 3.43 \quad (\text{SD} = 0.82).$$

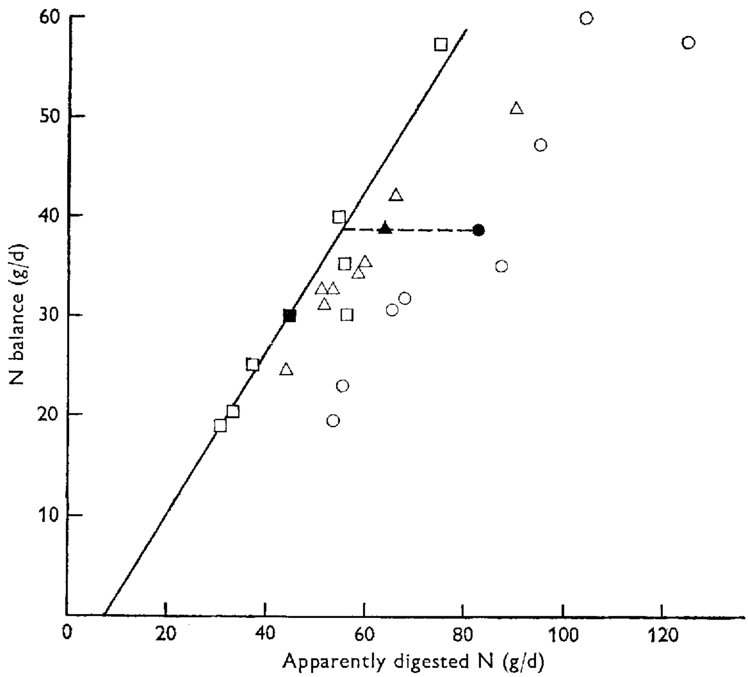

Fig. 2. Relationship between nitrogen balance and apparently digested N intake in rapidly growing calves given concentrate diets containing (○) 213 (HP), (△) 172 (MP) or (□) 132 (LP) g protein/kg dry matter. The regression line represents the relationship for diet LP, when protein is limiting. ●, ▲ and ■ show the mean values for N balance and apparently digested N when both variables have been adjusted to the mean intake of digestible energy/$W^{0.73}$.

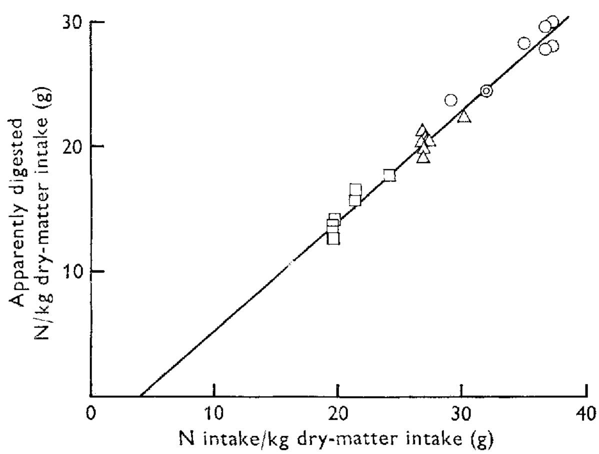

Fig. 3. Relationship between apparently digested N/kg dry-matter intake and N intake/kg dry-matter intake in rapidly growing calves given diets containing (○) 213 (HP), (△) 172 (MP), or (□) 132 (LP) g protein/kg dry matter.

Since there was no difference between treatments in the slope or position of the relationship, this equation shows that the true digestibility coefficient of protein in all three diets was 0·880. The intercept of this equation may be used to give an estimate of the metabolic faecal N excretion, of $3·43 \pm 0·82$ g N/kg dry matter ingested.

The values obtained for endogenous urinary N and metabolic faecal N excretion were used to calculate the apparent biological value (BV) of protein for each digestibility and balance trial. As shown in Table 4, the apparent BV of dietary protein was 0·851 when diet LP was given, and significant reductions in apparent BV occurred as the crude protein content of the diet increased.

DISCUSSION

The effect of protein level in the concentrate on performance of the calves in the present experiment was obscured because calves given diet MP tended to have a lower birth weight and their intakes of dry matter were below those of calves given either of the other two diets. However, adjustment for differences between treatments in concentrate consumption and live weight at 1 week revealed that live-weight gain was significantly lower when the protein content of the diet was only 130 g/kg dry matter, thus confirming previous observations (Stobo *et al.* 1967*a*). Moreover, the efficiency with which concentrates were converted into live-weight gain was reduced when diet LP was given, as indicated by the significant increase from 2·94 to 3·77 kg in the amount of food required per kg live-weight gain between diets HP and LP, shown in Table 2. A similar observation was made in a previous experiment (Stobo *et al.* 1967*a*) when calves were given diets similar to those used in the present experiment at *ad lib.* levels; however, when in that experiment concentrate intake was restricted to 2 kg/d, the protein content of the concentrate had no effect on food conversion rate.

The tendency towards a lower rate of increase in DMI/$W^{0·73}$ with age, when calves were given diet MP, reflects a fall in dry-matter intake that occurred between 8 and 11 weeks of age. There was no obvious reason for this, although the possibility of a subclinical infection or metabolic disorder affecting food intake cannot be ruled out. However, it is perhaps pertinent that a reduction in dry-matter intake was reported by Kay & MacLeod (1968) when a diet containing 170 g crude protein/kg dry matter was compared with similar diets containing 200 g or 140 g crude protein/kg dry matter, and we have observed a similar tendency in a previous experiment (Stobo *et al.* 1967*a*). The results shown in Fig. 1 are in close agreement with the findings of Johnson & Elliott (1969), who reported an increase in voluntary daily dry-matter intake with age, which, for calves weaned at 45 d and receiving an all-concentrate diet, levelled out at approximately 90 g/$W^{0·73}$ by 18 weeks of age.

The finding that apparent digestibility of crude protein decreased with a reduction in crude protein content of the diet confirms previous observations in the ruminant calf (Brown, Lassiter, Everett, Seath & Rust, 1958; Whitelaw, Preston & Ndumbe, 1961; Stobo *et al.* 1967*b*; Kay & MacLeod, 1968). However, the finding that there was no difference between treatments in either the slope or position of the relationship between ADN_{DMI} and NI_{DMI}, and the high correlation ($r = 0·99$) between these two variables, indicates that a large proportion of the N appearing in the faeces was of metabolic origin. The value of 3·43 g N/kg dry matter ingested, obtained for metabolic faecal N excretion is identical with the value reported from another experiment by Stobo & Roy (1966), in which calves between 75 and 225 kg live weight were given all-concentrate diets limiting in protein. These values agree closely with the value of 3·7 g/kg dry matter ingested, reported by Harris & Loosli (1944), but are lower than the value assumed for ruminant calves (Agricultural Research Council, 1965) of 5·0 g N/kg dry matter ingested. There is, however, evidence of an increase in metabolic faecal N excretion as the fibre content of the diet increases (Stobo, 1964), and in veal calves metabolic faecal N excretion has been reported as being only 1·9 g/kg dry matter ingested (Roy, Gaston, Shillam, Thompson, Stobo & Greatorex, 1964; Roy *et al.* 1970).

Our estimate of endogenous urinary N excretion is almost 0·5 higher than the value accepted by the Agricultural Research Council (1965), which was based on a study of published estimates obtained from experiments in which cattle were kept for several days or weeks on diets containing relatively little protein. Under such conditions, animals tend to maintain a constant body-weight or to lose weight. Since the output of urinary N declines rapidly within a few days of changing to a protein-deficient diet (Blaxter & Wood, 1951), our results may reflect the higher 'maintenance requirement' of rapidly growing calves. High labile N reserves occur when growth is rapid (Munro, 1964; Allison & Wannemacher, 1965) and thus the rate of turnover of body protein reserves is likely to be higher in the rapidly growing animal.

The results of the N balance trial did not give a clear indication of the protein level necessary for maximum N retention until the values were adjusted to the mean value of digestible energy intake/$W^{0.73}$. However, after these adjustments had been made, the results were in agreement with our previous observation (Stobo *et al.* 1967*a*) that maximum live-weight gains can be made by calves when the crude protein content of the concentrates is between 143 and 187 g/kg dry matter.

The relationship between N balance and the intake of ADN for calves given the low-protein diet can be used to estimate the protein requirement of the calf, since the amount of N retained by calves receiving this diet was limited by the intake of ADN, an observation that is confirmed by the high value of 0·851 for the apparent BV of protein. Thus, originating from the diet containing 132 g protein/kg, a decrease or increase in the intake of digestible N at the same level of dietary energy intake (i.e. a reduction or increase in crude protein content of the concentrate) would be expected to cause corresponding reductions or increases respectively in N balance.

Extrapolation of the regression relating N balance to ADN for diet LP gives a value of 11·18 g/d for ADN when N balance is zero. This is equivalent to 330 ± 156 mg ADN/$W^{0.73}$ required to maintain the calf in N equilibrium, and this amount would be required to replace the endogenous urinary N excretion and a small metabolic faecal N loss. In an earlier experiment (Stobo *et al.* 1967*b*) with calves of 85 kg live weight given an all-concentrate diet containing 144 g crude protein/kg dry matter, but with a daily digestible energy intake of only 1·09 MJ/$W^{0.73}$, the relationship between N balance and ADN was:

$$\text{NB} = 0{\cdot}815\ \text{ADN} - 5{\cdot}5 \quad (\text{SD} = 1{\cdot}066).$$

In that experiment the ADN requirement for N equilibrium was 6·75 g/d, equivalent to 270 ± 53 mg ADN/$W^{0.73}$. This apparent lower maintenance requirement for ADN/$W^{0.73}$ at reduced energy intakes is in keeping with the view already expressed (see p. 124) that the rate of turnover of body protein reserves is increased in animals with a rapid rate of growth.

A reduction in protein content of the diet from 213 to 172 g protein/kg dry matter did not affect N balance at a constant digestible-energy intake, whereas a further reduction to 132 g protein/kg resulted in a smaller amount of N being retained. It follows, therefore, that there must be some minimum level of dietary protein between 132 and 172 g/kg at which maximum N retention still occurs. Above such a level of protein, the supply of protein is no longer limiting and the supply of dietary energy becomes the limiting factor for growth. It is possible to estimate the point at which maximum use is made of both dietary protein and energy by extrapolation of the line joining the mean values for N balance and ADN for diets HP and MP to the point at which this extrapolated line meets the regression line passing through the mean value for N balance and ADN for calves given diet LP (see Fig. 2). The point of intersection represents a N balance of 39 g/d and an intake of ADN of 56 g/d. For calves on this experiment at a mean live weight of 122 kg, receiving a digestible-energy intake of 1·36 MJ/$W^{0.73}$, an intake of 56 g/d digestible N would therefore be expected to be the minimum intake that would produce the maximum N retention. These levels of digestible energy and digestible N would be provided by a diet containing 153 g crude protein/kg dry matter. This estimate is not precise, however, as it does not take into account the errors associated with the N balance and ADN at the mean digestible energy intake, nor does it allow for the fact that a certain amount of curvature is almost inevitable near the point of intersection of the lines.

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