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The Nutrition of the Young Ayrshire Calf

4. Some Factors Affecting the Biological Value of Protein Determined by Nitrogen-Balance Methods

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The biological value of a protein as evaluated by the Thomas-Mitchell procedure (Mitchell, 1923-4) is affected to a considerable extent by the percentage of protein in the diet (Mitchell, 1923-4; Mitchell & Beadles, 1926-7; Hamilton, 1938). When the percentage is high, a larger proportion of the absorbed amino-acids is deaminated, the nitrogen appearing in the urine and the non-nitrogenous moiety ultimately being assimilated as a source of energy. It is only when the protein content of the diet is such that the demand by the tissues for amino-acids is greater than the supply, that maximal biological values are obtained. This inevitably entails partial protein deficiency in the animal and a submaximal rate of growth.

In the diet of the young growing rat the percentage of protein usually adopted for the determination of biological values is 8, whereas in adults percentages as low as 4-5 have to be used to ensure that tissue demand for amino-acids is greater than supply. Similar percentages were adopted in experiments with growing sheep and with growing cattle (Miller & Morrison, 1939, 1942; Harris & Mitchell, 1941; Swanson & Herman, 1943). Experiments designed to study the biological value of dietary protein in the calf during the first few weeks of life do not appear to have been conducted, and

information regarding the protein content of the diet that would render such experiments critical is not available. It is obvious that the very young calf in the first weeks of life is not physiologically comparable to the adolescent rat weighing 60–70 g. as far as protein requirement per kg. body-weight is concerned, especially as it has been shown by Soxhlet (1878) and by Moulton, Trowbridge & Haigh (1923) that early growth in cattle consists almost entirely of deposition of minerals and protein with comparatively little deposition of fat. Preliminary observations on the effect of the level of dried skim milk protein in the diet on its biological value have already been presented (Blaxter & Wood, 1951 b).

To obtain further information, N balances have been determined on calves given diets containing varying percentages of protein.

EXPERIMENTAL

Diets. The composition of the diets used is summarized in Table 1.

These diets were made twice weekly by methods previously described (Blaxter & Wood, 1951 a) and were stored at 5°.

Table 1. *Composition of the experimental diets*

Constituent	Diet no.		
	9	11	10
Dried skim milk (g./l.)	83·9	69·7	55·5
Lard, pure (g./l.)	41·8	41·9	42·1
Cod-liver oil (g./l.)	3·3	3·3	3·3
Glucose (g./l.)	—	15·4	30·8
Mineral mixture no. 2* (g./l.)	—	1·7	3·3
Lecithin (g./l.)	—	0·05	0·1
Calculated composition			
Cal./l.	772	744	716
Fat (%)	4·60	4·60	4·60
Protein in dry matter (%)	22·0	18·0	14·0
Protein calories as percentage of total calories	20·5	17·0	13·5

* See Blaxter & Wood (1951a)

Experimental animals and methods. Five Ayrshire bull calves were used. At least one N balance was determined on each. These calves were bought from the local market when 1–4 days old and were gradually accustomed to the diet over a period of 10 days before the experiments began. They were fed twice daily at 9 a.m. and at 7.30 p.m. Faeces and urine were collected using the equipment previously described (Blaxter & Wood, 1951a).

Dry matter, total N, ash and fat were determined in the faeces, and total N in the urine. The distribution of urinary N was determined in three balance experiments only.

Table 2 summarizes the experiments completed and shows the calves used. The results for the series of thirteen experiments, each lasting 12 days, shown in Table 2 permitted several direct comparisons of one diet with another. Results with very high intakes of diets nos. 9 and 10 were not, however, obtained and the results with diet no. 11 are therefore treated separately.

Table 2. *Arrangement of balance experiments and animals used (calves nos. 7, 8, 9, 11 and 12)*

Diet		
Type and no.	Amount given (l./day)	Calf no.
High-protein 9	2·4	11
	2·6	12
	3·8	7
	4·2	8
Medium-protein 11	3·4	11
	3·8	8
	4·2	9
	6·0	9
	9·0	9
Low-protein 10	2·4	12
	2·6	11
	3·8	9
	4·2	7

RESULTS

Digestibility of the diets. Table 3 summarizes the data obtained on the digestibility of the total dry matter, fat and total N of diet no. 9 (high-protein) and of diet no. 10 (low-protein). The mean digestibility of the dry matter, fat and protein tended to be higher when the diet high in protein was given. Statistical analysis of these results showed, however, that the differences were not significant.

Table 3. *Apparent digestibility for calves of diets nos. 9 and 10 at four levels of intake*

Amount given (l./day)	Dry matter		Total fat		Total N	
	Diet no. 9 (high-protein) (%)	Diet no. 10 (low-protein) (%)	Diet no. 9 (high-protein) (%)	Diet no. 10 (low-protein) (%)	Diet no. 9 (high-protein) (%)	Diet no. 10 (low-protein) (%)
2·4	93·1	86·8	89·6	81·5	87·8	64·6
2·6	95·7	90·8	96·3	86·9	91·3	84·0
3·8	92·9	91·5	92·4	87·7	86·2	82·6
4·2	93·8	95·1	91·9	91·6	91·7	91·5
Mean	93·88	91·05	92·55	86·92	89·25	80·67
Mean difference with its standard error (three degrees of freedom)	2·83 ± 1·73		5·63 ± 2·34		8·58 ± 5·08	

Table 4 summarizes the results obtained when diet no. 11 (medium-protein) was given. It shows that the apparent digestibility of the dietary fat and the apparent digestibility of the total N declined at the lowest levels of intake. This conclusion may also be made from the results with the low-protein diet given in Table 3. As far as the N digestibility is concerned it suggests that a constant fraction of the metabolic faecal N (Schneider, 1935) is present in the faeces of the young calf.

Table 4. *Apparent digestibility for calves of the medium-protein diet no.* 11
at five levels of intake

Amount given (l./day)	Dry matter (%)	Total fat (%)	Total N (%)
3·4	90·9	87·9	86·0
3·8	93·0	86·9	87·2
4·2	97·3	96·7	94·5
6·0	96·6	95·7	93·3
9·0	96·5	95·6	94·1

The percentage of N in the faeces tended to decline with increasing intake, but this was not statistically significant. Differences between the diets in percentage of N in the faecal dry matter were, however, statistically significant. The percentage of N in the dry faeces may be related to the percentage of protein in the diet by the equation

$$\text{N in dry faeces} = 1\cdot2 + 0\cdot3P,$$

where $P =$ the percentage of the total dietary calories present as protein. The intercept of this equation should represent the percentage N in the dry faeces when there is no N in the diet, that is the metabolic faecal N. The value of 1·2 g./100 g. faecal dry matter differs from the value of 2·0 g. as directly determined (Blaxter & Wood, 1951a). The errors involved, especially the assumption of linearity of the regression are, however, large and it is doubtful whether the difference is in fact real. From the variability in the N excretion in the faeces of these calves, ranging from 0·99 g. by calf no. 9 ingesting 4·2 l. of the medium-protein diet no. 11 to 2·73 g. by calf no. 12 ingesting 2·4 l. of the low-protein diet no. 10, it is clear that in the young calf N excretion in the faeces is not constant as in cattle or sheep fed on standard rations. This is largely due to varying degrees of alimentary disturbance in the calves, ranging from acute diarrhoea, when up to 60 % of the ingested N appears in the faeces, to mild digestive upsets, and it would appear that this factor of digestive disturbances is sufficient to prevent the demonstration of even comparatively large differences in the digestibility of diets unless many calves are used.

Urinary nitrogen and nitrogen balance. Table 5 summarizes the N-balance data. These show that for each diet an increase in the amount ingested was associated with very little change in the urinary excretion of N, but with a marked change in N balance. The relation between the intake of N expressed as apparently digested N and the urinary N was examined by analysis of covariance. One observation was omitted, that for the animal in negative balance on the low-protein diet. The analysis of variance is given in Table 6.

There were no statistically significant differences in the slopes of the individual regressions relating urinary N to the N intake apparently digested, but the mean regression was very highly significant statistically. There was a large difference between the urinary N excretion of the calves on the low-, medium- and high-protein diets, at the same level of N intake. The three equations relating urinary N to apparently digestible N were

$$UN_H = 0\cdot21 ADN_H + 5\cdot30, \qquad (1a)$$

$$UN_M = 0\cdot21 ADN_M + 3\cdot71, \qquad (1b)$$

$$UN_L = 0\cdot21 ADN_L + 2\cdot13, \qquad (1c)$$

Table 5. *Nitrogen balances of the calves*

Diet given (l./day)	Intake	Excretion		Balance
		Faeces	Urine	
		Diet no. 9 (high-protein)		
2·4	11·48	1·40	7·63	+2·45
2·6	13·23	1·15	7·85	+4·23
3·4	—	—	—	—
3·8	18·19	2·51	8·46	+7·22
4·2	—	—	—	—
6·0	—	—	—	—
9·0	—	—	—	—
		Diet no. 11 (medium-protein)		
2·4	—	—	—	—
2·6	—	—	—	—
3·4	13·77	1·93	6·17	+5·67
3·8	15·48	1·98	7·14	+6·36
4·2	17·80	0·99	6·82	+9·99
6·0	24·18	1·63	8·22	+14·34
9·0	37·68	2·21	11·47	+23·99
		Diet no. 10 (low-protein)		
2·4	7·69	2·73	5·76	−0·80
2·6	8·62	1·66	4·46	+2·50
3·4	—	—	—	—
3·8	12·20	2·11	3·97	+6·11
4·2	12·61	1·07	4·61	+6·93
6·0	—	—	—	—
9·0	—	—	—	—

Table 6. *Analysis of variance of the urinary nitrogen excretion of the calves expressed in g./day, including the covariance of urinary nitrogen on apparently digested nitrogen*

Component	Degrees of freedom	Estimated variance	Variance ratio (e^{21})
Joint regression of urinary N on apparently digested N intake	1	17·252	103·7***
Differences between regressions	2	0·035	N.S.
Differences between means	2	7·433	44·6***
Error	5	0·166	—
Total	10	—	—

*** Significant at $P < 0.001$. N.S.: not significant.

where *UN* represents urinary N excretion in g./day, *ADN* the apparently digested N and the subscripts H, M and L refer to the high-, medium- and low-protein diets.

The intercepts, 5·30, 3·71 and 2·13, represent the urinary N excretion when no N was given. These values do not necessarily represent the endogenous excretion of N, for in the region of negative N balance the excretion of N would increase, since a low N intake in these experiments must mean a low calorie intake. These regressions therefore may not be comparable to the equations of Allison (Allison & Anderson, 1945; Allison, 1948), based on observations in which adequate caloric intake was in general maintained. As the data used in the present study were all obtained in the region of positive balance, however, it is probable that they represent both an endo-

genous component and a constant 'basal deamination component', that is an amount of urinary N reflecting the higher amount of deamination that occurs on diets high in protein. This quantity should be independent of the dietary source of N.

The N balances were related to the N apparently digested in a similar analysis of variance, presented in Table 7. In this instance the data for the animal in negative balance were included.

Table 7. *Analysis of variance of the nitrogen balances of the calves expressed in g./day, including the covariance of nitrogen balance on the nitrogen apparently digested*

Component	Degrees of freedom	Estimated variance	Variance ratio (e^{2s})
Joint regression of N balance on apparently digested N	1	269·253	636·6***
Differences between regressions	2	1·447	3·4 N.S.
Differences between means	2	5·061	12·0*
Error	6	0·423	—
Total	11	—	—

*** Significant at $P < 0.001$. * Significant at $P < 0.05 > 0.01$. N.S.: not significant.

The differences between the individual regressions were not significant but the mean differences between the intercepts of the equation were significant. This is in agreement with the results obtained for urinary N, despite the inclusion of the one value in which the calf was in negative balance. The equations relating N balance to the apparently digested N intake were

$$NB_H = 0.81 ADN_H - 5.64, \qquad (2a)$$

$$NB_M = 0.81 ADN_M - 4.22, \qquad (2b)$$

$$NB_L = 0.81 ADN_L - 3.13, \qquad (2c)$$

where NB represents the N balance in g./day and the other terms have the same significance as for equation (1).

In view of the errors involved in the assumption that the results for an animal in negative balance can be included in the equation, the agreement between the two sets of equations is excellent. When this result is omitted the regression coefficient of N balance on N intake apparently digested is 0·79 and the values for the intercept -5.30, -3.71 and -2.31, that is the same as for the equation relating UN to ADN, except that the signs are changed. The equations based on all the data, however, have been used in the ensuing calculations as the justification for discarding the aberrant point cannot be tested at the present time.

From the above equations the results in Table 8 were calculated. They show that if equal quantities of gross energy are supplied, the storage of N falls as the protein content of the diet is reduced. For equal amounts of N apparently digested, however, the storage of N increases with decreasing protein content of the diet. This means that if a protein-free supplement is added to a basal diet, N retention will increase, the effect being to reduce the 'basal deamination component' associated with the higher

Table 8. *Storage of body nitrogen by the calves following ingestion of different amounts of nitrogen in diets with high, low and medium levels of protein (g./day)*

| | Storage of N when equal quantities of apparently digested N are given | | Storage of N when equal quantities of energy are given | |
| | N given | | Gross energy given | |
Type of diet	10 g.	20 g.	2500 Cal.	3500 Cal.
Low-protein	5·00	13·13	4·59	7·68
Medium-protein	4·01	12·04	5·53	9·43
High-protein	2·51	10·64	6·16	10·88

protein content of the basal diet. These relationships can be inferred from the data of Table 5, where comparisons can be made between animals receiving the same quantity of diets high or low in protein.

The body-weight gains of the calves reflect these differences in N retention. Thus, in the three calves given 3·8 l. of one of the three diets, the daily gains in body-weight were 360, 305 and 229 g. for the high-, medium- and low-protein diets respectively. In the calves given 2·6 l. of the high- or the low-protein diet, the daily gains were 155 and 54 g. respectively, whereas with only 2·4 l., the calf on the low-protein diet lost daily 46 g. of weight and the calf given the high-protein diet gained 18 g. For approximately equal intakes of energy, gains were smaller when the diets contained less protein.

A comparison of body-weight gains at equivalent protein intakes irrespective of total calorie intake, can similarly be made. Calf no. 12 ingesting 2·6 l. of diet no. 9 was ingesting about the same quantity of protein as calf no. 11 ingesting 3·4 l. of diet no 11 and calf no. 9 ingesting 3·8 l. of diet no. 10. The daily gains in weight were 155, 275 and 304 g. respectively. Similarly, the calf ingesting 3·8 l. of diet no. 9, gained 360 g. and the calf ingesting 4·2 l. of diet no. 11 gained 455 g. The intake of N of both animals was about the same. The body-weight gains, however, are subject to greater errors of estimation than are the N balances.

Biological values of the ingested protein. The biological value of a protein, as defined by Mitchell (1923–4), is given by the equation

$$BV = 100 \times \frac{NI - (UN - EN) - (FN - MN)}{NI - (FN - MN)},\qquad (3)$$

where NI = nitrogen intake, UN = urinary N excretion, EN = endogenous N excretion, FN = faecal N excretion, MN = metabolic component of the faecal N, BV = biological value. This equation may be rearranged by substituting the nitrogen balance (NB) for the necessary terms in the numerator of the equation and replacing the term ($NI - FN$) by the term ADN (apparently digested N). This gives the modification

$$BV = 100 \times \frac{EN + MN + NB}{ADN + MN}\qquad (3a)$$

Now it has already been shown that the N balance may be related to the amount of apparently digested N by a simple linear equation of the type used by Allison (1948) (see equations (2a), (2b) and (2c)). The biological value as defined by Mitchell (1923–4) can thus be determined by substituting in the above equation the linear equation

relating ADN to NB. It is also necessary to include values for excretion of endogenous N and of metabolic faecal N. These may, for the moment, be regarded as constants of 2·5 and 0·6 g. N respectively.

The biological value of the proteins of dried skim milk can thus be estimated from the equation

$$BV = 100 \times \left(\frac{2 \cdot 5 + 0 \cdot 6 + (0 \cdot 81 ADN - x)}{ADN + 0 \cdot 6} \right), \tag{4}$$

where x represents the intercept of the N-balance equation on the apparently digested N axis.

From this equation it is clear that the magnitude of the biological value obtained will depend on the value of this intercept; it will be greater when the intercept is small, as with the low-protein diets. It will also be determined by the amount of apparently digested N taken in, the biological value being greater the more N is apparently digested. This relationship, however, can only apply if the equation relating NB to ADN is linear. Little is known at the moment regarding the linearity of this equation at high levels of intake, but it has recently been shown by Blaxter & Wood (1951 c) that linearity of the equation still applies when sufficient whole milk is given to result in N balances of up to 25 g./day, that is equivalent to gains in body-weight of 910 g. and to an energy intake about 2·5 times the maintenance requirement.

The 'biological value' of a protein in an animal with a high capacity for N retention is thus a simple inverse function of the amount of diet consumed and a linear function of the intercept of the N-balance equation of Allison (1948). This in turn implies that the regression coefficient in the N-balance equation represents the approximate biological value attainable, expressed as a decimal. In fact it is too low a value, since the inclusion of the metabolic faecal N in equation (4) would increase the asymptote slightly.

Table 9. *Mean biological values of skim milk protein for calves determined by nitrogen-balance methods*

Amount of diet given (l./day)	Biological value		
	High-protein diet (%)	Medium-protein diet (%)	Low-protein diet (%)
2·4	40·0	—	51·2
2·6	54·8	—	61·6
3·4	—	74·5	—
3·8	64·6	68·1	87·9
4·2	—	78·0	80·0
6·0	—	80·2	—
9·0	—	80·2	—

It may be expected, therefore, that the biological values determined in the present experiments would be in agreement with the hypothesis presented above, and this is in fact shown in Table 9. For the high-protein diet, the biological values increased rapidly as the amount of diet given was increased from that allowing a daily gain of only a few g. to a quantity permitting a gain of 360 g. A similar increase is shown as well

for the diets of medium and of low-protein content. Errors are, however, attached to these estimates, and the values for metabolic faecal N and the endogenous excretion of N vary from animal to animal. Both quantities were estimated using the relationships between basal heat loss and endogenous metabolism, and faecal dry-matter excretion and metabolic N excretion (Blaxter & Wood 1951 a).

DISCUSSION

It is apparent that the biological value of a protein estimated by N-balance methods is by no means a constant. This statement, however, ignores one salient fact, that in order to measure the biological value of a protein, it is essential that the animal should not deaminate amino-acids to use the carbon-containing moiety as a source of energy in maintenance or in growth. A biological value, if it is to be a criterion of the balance of essential amino-acids making up a protein, must be determined under conditions whereby such a need for energy does not arise. Thus, despite the variability of the biological value, a valid estimate may be obtained under conditions where the possibility of deamination of the constituent amino-acids of a protein to provide energy is specifically excluded. It follows, however, that the use of diets of constant protein content would not result in comparable estimates of biological value. A low intake of the diet would depress this estimate, and the depression would be greater the higher the protein content of the diet.

The reason for this relationship between level of feeding and the utilization of dietary protein is to be found in the relative amounts of protein and energy needed for the growth and the maintenance of the animal. It is obvious that if the relative requirements for protein and energy are not the same for maintenance and for growth, then the requirement of a growing animal for protein will not be constant in relation to its energy requirement, but will vary according to the amount of growth. To study this aspect of the problem, the results from N-balance experiments have been analysed statistically.

First, the relation between the N balance of the young calf and its gain in body-weight was determined in N-balance experiments conducted in this laboratory. This information is shown in Fig. 1, and analysis of variance showed that the regression of N balance on body-weight was highly significant statistically ($P < 0.001$). The equation of the regression was:

$$NB = 0.0262G + 0.703, \tag{5}$$

where NB is the N balance in g./day and G the gain in weight in g./day. The intercept of 0.7 g. N, although only significantly different from zero at odds of 11:1, implies, that when there is no gain in weight storage of N still takes place, that is body-weight maintenance does not entail cessation of growth.

The regression coefficient of 0.0262 shows that for every gain of 100 g. body-weight the calf stores 2.62 g. N, or 16.5 g. protein. This may be interpreted to mean that the major part of the gain in body-weight in the young calf is a gain of flesh, for 16.5 g. protein would be expected to be present in about 80–85 g. muscle. The remainder must represent fat, bone and water. The energy value of the gain in body-weight can only be determined calorimetrically or by analysis of the carcass, but a first approxi-

mation can be made by statistical analysis of results obtained with diets containing ample protein and relating the digested energy intake to the gain in body-weight. The regression of body-weight gain (G) on calorie intake was found to be

$$G = 0.326\,DC - 577.5, \tag{6}$$

where DC represents the total number of Cal. ingested daily less the Cal. excreted in the faeces.
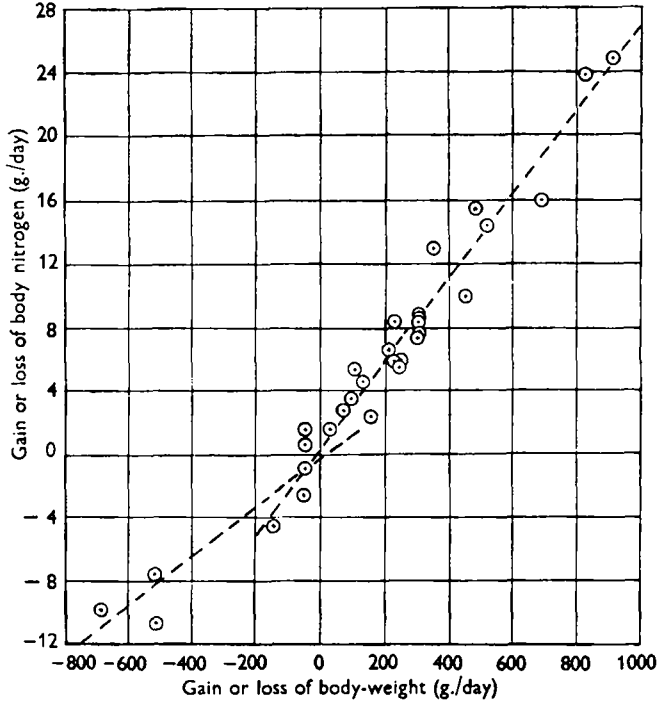

Fig. 1. Relation between body-weight gain or loss of calves and their gain or loss of body nitrogen.

Table 10. *Net protein requirements, uncorrected for losses in digestion or in metabolism, and digestible energy requirements of young calves weighing 30 kg., together with an estimate of the percentage of the total calories that must be present as protein in order to avoid deamination of dietary protein to meet energy requirements*

Gain in weight (g./day)	N retention (g./day)	Endogenous N (g./day)*	Total protein required by the tissues (g./day)	Digestible energy required (Cal./day)†	Percentage of the digestible energy needed as protein to avoid deamination
0	0·70	2·40	19·4	1572	6·9
100	3·32	2·40	35·7	1879	10·7
200	5·94	2·40	52·1	2186	13·5
400	11·18	2·40	84·9	2800	17·1
800	21·66	2·40	150·4	4028	21·1
1000	26·90	2·40	183·1	4642	22·2

    * 80 mg./kg. body-weight.
    † 52·4 Cal./kg. body-weight + 307 Cal./100 g. gain in weight.

This relationship was highly significant statistically, though, as may be inferred from Fig. 2 which shows the relation between gain in body-weight and the energy intake expressed per kg. body-weight, the variation about the regression was high. This variability is understandable since the errors involved in determining gain in weight and caloric intake are high; no account is taken of the loss of energy in the urine for diets high in protein or of the variation from animal to animal in energy requirements for maintenance or growth. The equation is only a generalization but it is probable that it represents the average actual relationship.
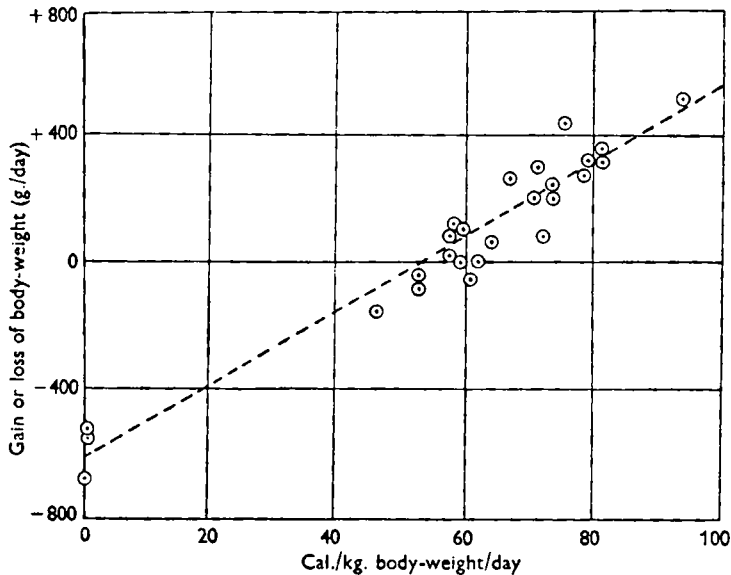

Fig. 2. Relation between the energy ingested by calves less that in the faeces, and the daily gain in body-weight.

The constants of the equation may be interpreted to show that in fasting the daily body loss is approximately 580 g. and that every additional Cal. is associated with a gain of 0·326 g. body-weight. By simple arithmetic it may be calculated that the calorie intake when there is no change in body-weight is 1771 Cal. and that each gain of 100 g. body-weight is associated with an intake of an additional 307 Cal. The former may be interpreted to represent the requirement of energy for body-weight maintenance, and the latter the requirement for body-weight gain. That these are essentially correct estimates is shown by the fact that the maintenance requirement per kg. body-weight would be 52·4 Cal. of 'digested' energy, a value in excess of the determined basal metabolism of 40–43 Cal./kg./day (Blaxter & Wood, 1951 *a*) by an amount of energy that may be accounted for by an activity increment of 25–30 %. Such an activity increment is extremely likely for an animal confined in a metabolism cage (Blaxter, 1948). Similarly, the value of 307 Cal. for the calorific value of the digested nutrients associated with 100 g. gain in body-weight is higher than that of the gain itself, as judged by comparison with the carcass analyses of Armsby & Moulton (1925).

It has previously been shown (Blaxter & Wood, 1951 *a*) that the endogenous N

metabolism of the calf is 80 mg./kg. body-weight. This value represents the animal's minimum requirement of N for maintenance of N equilibrium. From it, together with the results given in equations (5) and (6), an estimate of the requirements of protein and of digestible energy can be made. The protein requirements are net requirements in the sense that they represent only the amounts of protein which are stored or which are equivalent to the endogenous metabolism (Blaxter & Mitchell, 1948). With proteins of 100 % biological value and 100 % digestibility they represent minimal requirements of dietary protein that ensure no deamination of protein for energy purposes (Blaxter & Mitchell, 1948).

The results of the calculation are given in Table 10. The last column of the table shows that the percentage of the digested energy that must be present as protein increases with increasing rate of gain. These figures apply to proteins of 100 % biological value only; they would be higher if the biological value of the protein was less than 100. If an animal were given a diet containing 20 % of its digested energy as a high-quality protein, maximal biological values would not be attained unless the total intake was sufficient to result in a gain of at least 700 g./day. If the intake was smaller, the urinary excretion of N would relatively increase, since the protein given would be greatly in excess of the requirements at that particular rate of gain in weight.

The relation between the amount of N absorbed and the body balance of N has recently been studied extensively (Melnick & Cowgill, 1937; Harris & Mitchell, 1941; Allison & Anderson, 1945; Bricker, Mitchell & Kinsman, 1945; Hegsted, Tsongas, Abbott & Stare, 1946; Bricker & Mitchell, 1947; Barnes, Bates & Maack, 1946). Most, but not all, of these experiments were concerned with N balances below maintenance, and adequate energy was supplied. The experiments with growing rats with energy intakes above maintenance were mostly carried out, however, with diets containing a constant percentage of protein. Allison (1948) recently reviewed this work and concluded that the nitrogen-balance index (Allison, Anderson & Seeley, 1946) is 'some function of but not necessarily equal to the biological value of the protein source'. This nitrogen-balance index is not exactly equivalent to the regression coefficients of our equations (2a), (2b) and (2c), as it differs in the use of apparently digested N, and not truly digested N, as the independent variable. The meaning of this regression coefficient is, however, little altered, and it would appear that it does, in fact, represent the maximal biological value of the protein. That this relationship applies to intakes above maintenance in the calf is merely a reflexion of the intensity of its N metabolism, for in the young animal there is no indication of a limit to the capacity to store N at least up to a body-weight gain of about 1 kg./day. In mature animals the relationship of N balance to N intake is curvilinear above maintenance, because the capacity of the animal to store N is limited.

SUMMARY

1. Experiments in which five calves were given varying quantities of semi-synthetic, liquid diets containing high, medium and low percentages of dried skim milk protein are described.

2. The digestibility of the dry matter, fat and nitrogen tended to be lower when

low-protein diets were given, and the percentage of N in the dry faeces was much higher for the animals given the highest level of protein.

3. The urinary N increased at a constant rate with increasing intake of apparently digested N, irrespective of the amount of protein in the diet. The excretion of urinary N at any one level of intake was greatest for the diet containing the most protein.

4. The N balance also increased at a constant rate with increasing intake of apparently digested N, and the slope was independent of the N content of the diet. At comparable intakes of apparently digested N, storage was greater on the diet containing the smaller percentage of protein. At comparable intakes of energy, however, storage of N was greater for the diet high in protein.

5. The biological value of ingested protein was shown to be a simple inverse function of the amount of dietary N apparently digested and a linear function of the intercept of the equation relating N balance to apparently digested N, the magnitude of the intercept depending on the percentage of protein in the diet.

6. The slope of the regression of N balance on apparently digested N for a diet of constant composition appeared to be an approximation to the maximal biological value of the protein. This slope was comparable to the N-balance coefficient of Allison (1948). The biological value of the dried-milk proteins was thus found to be 81.

7. The reasons for these relationships are discussed, and it is pointed out that if the biological value of a food protein is defined as a description of N metabolism when there is no deamination of ingested amino-acids for provision of an energy source, then cognizance must be taken of the fact that the protein required is not a constant percentage of the energy requirement, but increases as the rate of growth increases. If diets of constant composition are used, they must be given at a level that ensures an excess of dietary energy.

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