

The Nutrition of the Young Ayrshire Calf

7. The Biological Value of Gelatin and of Casein when given as the Sole Source of Protein

BY K. L. BLAXTER (IN RECEIPT OF A SENIOR AWARD OF THE
AGRICULTURAL RESEARCH COUNCIL)

AND W. A. WOOD

Hannah Dairy Research Institute, Kirkhill, Ayr

(Received 27 March 1951)

Mature ruminants, by virtue of the extensive microbial population of their digestive tracts, are probably almost independent of any variation in amino-acid composition of their protein supply. Degradative and synthetic processes in the rumen result in mixtures of amino-acids qualitatively similar, despite marked differences in the dietary mixture given, reaching the tissues. Such independence is contingent upon a vast microbial population. It could be assumed that during the short period after birth when rumen development is negligible the young calf would require certain amino-acids in its diet. The work presented in this paper was designed to test the assumption that the young ruminant animal is as dependent on a dietary source of amino-acids as simple-stomached animals. For this purpose diets containing gelatin or casein were given to calves.

It has been known since the experiments of Edwards & Balzac (1832) that gelatin is not a sufficient source of protein, and the concept of essential amino-acids probably commenced with Escher's (1876) experiments with gelatin supplemented with tyrosine. The biological value of gelatin itself has not been determined, but a preparation of pork cracklings containing 33.5 % collagen and 25.7 % elastin gave a value with rats of 25 (Mitchell, Beadles & Kruger, 1927). Casein is a fairly adequate source of protein for the rat, having a biological value of 69 (Kon, 1928; Beadles, Quisenberry, Nakamura & Mitchell, 1933; Kik, 1938; Hughes & Hauge, 1945). The amino-acids limiting the utilization of casein have been shown to be methionine and cystine (Beadles *et al.* 1933; Kik, 1938). Table 1 summarizes the amino-acid composition of these two proteins and the percentage deficit of amino-acids calculated according to the method of Block & Mitchell (1946-7) using dried whole egg as a standard. By using dried whole egg as a standard it is not intended to imply that this protein can be used as a standard for measurement of the amino-acid requirements of the calf. The table is merely meant to indicate the large differences between the composition of gelatin and casein and a protein that has been considered quite adequate in rat nutrition.

From Table 1 it can be seen that casein is deficient in cystine and that gelatin, owing to its very high content of glycine, is an extremely unbalanced protein besides being devoid of tryptophan and deficient in tyrosine, cystine, methionine and the leucines.

Table 1. 'Essential' amino-acid composition of gelatin and casein, and the percentage deviation from the corresponding values for whole-egg proteins

Amino-acid	Amino-acid content (%)			Percentage deviation from corresponding values for whole-egg proteins	
	Gelatin	Casein	Whole egg	Gelatin	Casein
Arginine	8.7	4.2	6.4	+ 36	- 34
Histidine	0.9	3.0	2.1	- 57	+ 43
Lysine	5.8	7.9	7.2	- 19	+ 10
Tyrosine	0.7	6.9	4.5	- 84	+ 53
Tryptophan	0.0	1.2	1.5	- 100*	- 20
Phenylalanine	2.1	5.6	6.3	- 66	- 11
Cystine	0.1	0.3	2.4	- 96	- 87*
Methionine	0.8	3.5	4.1	- 78	- 15
Cystine + methionine	0.9	3.8	6.5	- 86	- 42
Threonine	2.0	4.1	4.9	- 59	- 16
Leucine	3.1	9.9	9.2	- 66	+ 8
Isoleucine	1.7	6.5	8.0	- 79	- 19
Valine	2.8	6.7	7.3	- 62	- 8
Glycine	24.0	2.1	—	—	—

* First limiting amino-acid.

EXPERIMENTAL

Plan of experiment

Four young Ayrshire calves were used as experimental animals. They were purchased in the market at the age of 2-4 days and were gradually accustomed to the diet used in the preliminary period. The plan of the experiment is given in Table 2.

Table 2. Treatment of experimental animals

Calf no.	Diet given (l./day)	Period				
		Adjustment (6 days)	Preliminary (12 days)	First experimental (12 days)	Recovery (8 days)	Second experimental (12 days)
16	4.8	D.S.M.	D.S.M.	Casein	D.S.M.	Gelatin
17	4.4	D.S.M.	D.S.M.	Casein	D.S.M.	Gelatin
18	4.8	D.S.M.	D.S.M.	Gelatin	D.S.M.	Casein
19	4.4	D.S.M.	D.S.M.	Gelatin	D.S.M.	Casein

D.S.M.: dried skim milk.

It will be noted that each calf received both the casein and the gelatin diet following a period during which it had been given dried skim-milk as the source of protein. The composition of the three diets is shown in Table 3.

The diets were prepared according to methods previously described (Blaxter & Wood, 1951*a*), the glyceryl monostearate and egg lecithin being used as emulsifying agents. Daily supplements of DL- α -tocopheryl acetate were given throughout. During the periods in which casein and gelatin were given, 19 ml. of a yeast extract prepared by the method of Macrae, El-Sadr & Sellers (1942) and 2 mg riboflavin were given

daily. All diets were prepared at intervals of 2–3 days and stored at 5° in an immersion cooler. At low temperatures the gelatin diet was a solid jelly. It was given, as were all the diets, in a liquid form at 37°. In order to minimize difficulties in the interpretation of the results each calf was given a constant allowance of the diet throughout the experiment. Calves nos. 16 and 18 were given 4.8 l./day and calves nos. 17 and 19, 4.4 l.

Table 3. *Composition of the three diets*

Ingredient	Diet		
	D.S.M.	Gelatin	Casein
Lard (g/l.)	42.2	42.7	42.7
Glucose (g/l.)	38.1	65.0	65.0
Spray-dried skim milk (g/l.)	47.4	—	—
Casein* (g/l.)	—	—	18.9
Gelatin† (g/l.)	—	17.7	—
Arachis oil (g/l.)	3.3	3.3	3.3
Vitamin A‡ (i.u./l.)	6600	6600	6600
Vitamin D‡ (i.u./l.)	660	660	660
Yeast extract (ml./calf/day)	—	10	10
Mineral mixture no. 4§ (g/l.)	3.8	10.6	—
Mineral mixture no. 5 (g/l.)	—	—	10.6
DL- α -tocopheryl acetate (mg/calf/day)	50	50	50
Cholesterol (g/l.)	—	0.1	—
Lecithin (g/l.)	—	0.3	0.3
Glyceryl monostearate (g/l.)	—	0.7	0.7
Riboflavin (mg/calf/day)	—	2	2
Fat (%)	4.6	4.6	4.6
Nitrogen (%)	0.266	0.263	0.266
Cal./l. (calculated)	771	772	772
Cal. present as protein (percentage of Cal.)	11.9	11.9	11.7

D.S.M.: dried skim milk.

* The casein was commercial light white casein purchased from Messrs Prideaux Milk Foods Ltd.

† The gelatin was the highest grade commercial gelatin purchased from Messrs Richard Hodgson and Sons Ltd.; it was very light in colour and was free from elastin and heavy-metal impurities.

‡ Contained in the arachis oil.

§ Mineral mixture no. 4 was the same as that used in previous experiments (Blaxter & Wood, 1951*a, b*).

|| Mineral mixture no. 5 had a lower phosphorus content to allow for the phosphorus present in the casein.

The calves were harnessed for the collection of urine and faeces, and were confined to metabolism crates as previously described (Blaxter & Wood, 1951*a*). Nitrogen metabolism was studied in each animal and included partition of the urinary nitrogen as well as determination of nitrogen balances. Respiratory-exchange determinations were made using the apparatus and technique of Blaxter & Howells (1951). Records of the health, gain in weight, pulse rate and general well-being were also kept. The analytical methods used have been described by Blaxter & Wood (1951*a, b*).

RESULTS

Body-weight changes

Fig. 1 shows the mean changes in body-weight on transference of the calves from the dried skim-milk diet to the casein or gelatin diet. Table 4 summarizes these data, expressed as daily gains or losses in weight, calculated by linear regression analysis.

All gains in weight of the calves when they were receiving the dried skim-milk diet or the casein diet were very low indeed. This was clearly due to the low level of protein in the diet, for, in earlier experiments (Blaxter & Wood, 1951*c*) in which a similar diet containing more dried skim-milk protein and less glucose was given in equivalent amounts, far greater gains in body-weight occurred.

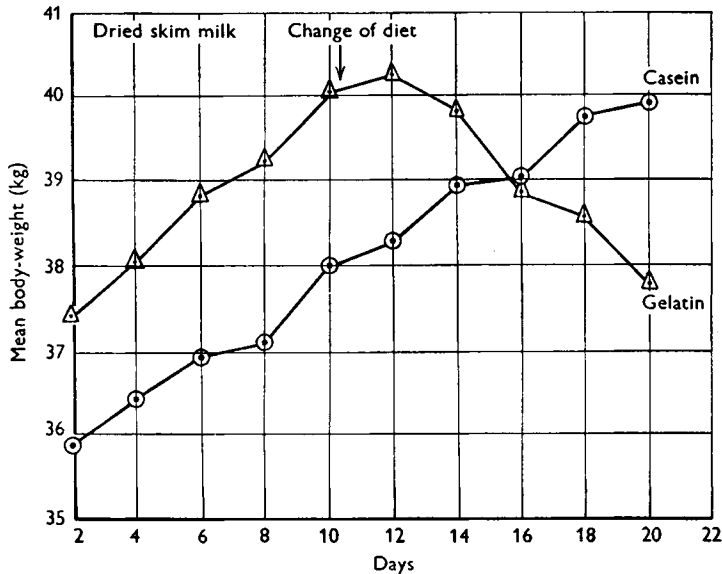


Fig. 1. The effect of diets containing dried skim-milk proteins, casein or gelatin on the mean body-weight of the calves. \odot , calves given first the dried skim-milk diet and then the casein diet; \triangle , calves given first the dried skim-milk diet and then the gelatin diet.

Table 4. Mean gains or losses of the calves in body-weight in g/day

Calf no.	Initial weight (kg)	Period			
		Preliminary (D.S.M. diet)	First experimental	Recovery (D.S.M. diet)	Second experimental
16	35.40	+248	+257 (casein)	+250	-212 (gelatin)
17	36.30	+210	+204 (casein)	+255	-239 (gelatin)
18	36.45	+377	-189 (gelatin)	+298	+200 (casein)
19	32.65	+234	-443 (gelatin)	+320	+204 (casein)

D.S.M.: dried skim milk.

Apart from the recovery period, each calf that received the higher daily allowance of milk gained more than its pair-mate, or, when the gelatin diet was given, lost less. The mean change in weight resulting from an increase in the allowance of diet from 4.4 to 4.8 l. can be evaluated from Table 5.

It will be noted from Table 5 that the animals given the casein diet gained less weight than those given the dried skim-milk diet, and that when these same animals were given the gelatin diet they could not maintain their body-weight and lost severely. Statistical analysis of their weight changes showed that the loss in weight with the gelatin diet was very highly significantly different from the gains that

occurred when dried skim-milk diet or casein diet was given. The difference in gain between calves given the casein diet and those given the dried skim-milk diet was not significant.

Table 5. *Mean daily changes in weight of the calves in g/day, classified according to experimental treatment*

Level of feeding (l./day)	Protein source		
	Dried skim milk	Casein	Gelatin
4.4	+ 222	+ 204	- 341
4.8	+ 313	+ 278	- 201
Mean	+ 268	+ 241	- 271

During the recovery period the two calves that had been given the gelatin diet and had lost considerable weight gained more than those that had been given the casein diet in the preceding period. The animals previously given casein gained 252 g, those that had previously received gelatin, 307 g. The difference was significant at odds of 16 : 1, suggesting that the calf compensates for periods of malnutrition. Such a conclusion, however, may not be correct for, judging by the calves' appearance, a slight dehydration occurred during the periods when gelatin was given, and the more rapid gain probably reflects an increase in tissue hydration which took place on transference to the dried skim-milk diet.

The digestibility of the diets

The mean apparent digestibility of the dry matter, fat, and total nitrogen as well as the calculated 'apparent digestibility' coefficients of the dietary energy are given in Table 6. The standard errors of the means were derived from analysis of variance.

Table 6. *Summary of apparent digestibility coefficients of the diets with their standard errors of the mean*

Diet	Dry matter	Total fat	Total nitrogen	Calories
D.S.M.	90.7 ± 1.48	84.3 ± 2.63	79.6 ± 1.94	84.4 ± 2.56
Casein	87.5 ± 1.48	77.4 ± 2.63	77.4 ± 1.94	84.6 ± 2.56
Gelatin	85.4 ± 1.48	76.2 ± 2.63	63.5 ± 1.94	82.2 ± 2.56

D.S.M.: dried skim milk.

The digestibility of all the dietary components was low. This was probably due to the low level of protein in the diet, as is shown graphically in Fig. 2, where results for previous experiments involving dried skim-milk (Blaxter & Wood, 1951 a-c) are combined with those obtained in the preliminary period of the present experiment. It will be noted that a decrease in the percentage of protein derived from dried skim milk led to a marked decline in the apparent digestibility of the fat and protein of the diets. Part of this decline was possibly due to the presence of a metabolic component in the faecal excretion of both fat and nitrogen.

The apparent digestibility of both the fat and dry matter was reduced when the

animals received the gelatin diet. On statistical analysis, however, the effects were only significant at a value of P between 0.1 and 0.05. The apparent digestibility of the total nitrogen of the gelatin diet was, however, significantly smaller than that of the dried skim-milk diet. The true digestibilities of the nitrogen were 90.5, 87.3 and 85.3 for the dried skim-milk, casein and gelatin diets, respectively. These results are of some interest, for it has been concluded by Morris & Wright (1935),

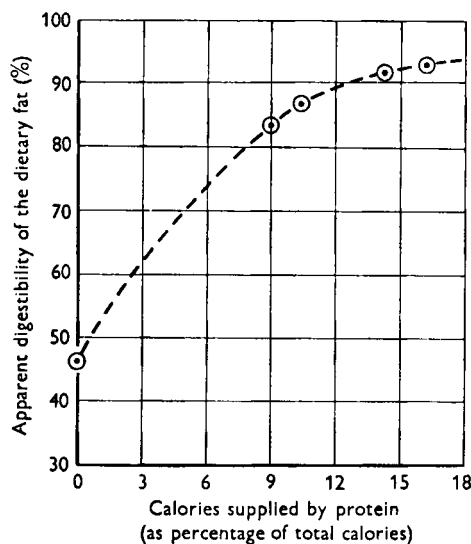


Fig. 2. The relation between the protein content of the diet and the apparent digestibility of the dietary fat.

Hutchinson & Morris (1936) and Swanson & Herman (1943) that a low apparent digestibility is inevitably associated with a high biological value. To quote Morris & Wright: 'It is not unreasonable to assume that absorption from the gut might be stimulated when inadequate rations are fed.' The true and apparent digestibilities of the dietary nitrogen in the present experiment, which refer to proteins of both high and low biological value (*vide infra*) do not confirm such a relationship. The cause of the high biological value in the experiments quoted above is in fact the low digestibility of the protein and consequent lower plane of protein nutrition rather than vice versa as the above workers have inferred.

The last column of Table 6 shows the percentage of the calorie intake that did not appear in the faeces. These values are low because the digestibility of fat was low. Nevertheless, the calculated number of calories apparently digested by any calf was higher than its basal metabolism determined at the same time (*vide infra*). In only one 2-day subperiod of the experiment was the apparent uptake of dietary energy from the gut low enough to be exceeded by the directly determined basal metabolism, and this subperiod for calf no. 19 during the period in which gelatin was given has been omitted in calculating the mean results.

Energy metabolism and pulse rate

The heat production of the calves, calculated from their respiratory exchange, was used to calculate the endogenous metabolism, using the previously determined factor of 1.90 mg endogenous nitrogen/basal Cal. (Blaxter & Wood, 1951*a*).

Table 7. *Heat production of the calves determined 15 h after feeding, calculated from respiratory-exchange determinations*

Calf no.	Diet	Heat production	
		(Cal./24 h)	(Cal./kg/24 h)
16	D.S.M. (1)	1704	45.47
	Casein	1818	45.33
	D.S.M. (2)	1881	45.75
	Gelatin	1974	47.34
17	D.S.M. (1)	1713	44.66
	Casein	1830	44.85
	D.S.M. (2)	1431	37.69
	Gelatin	1805	43.98
18	D.S.M. (1)	1771	43.84
	Gelatin	1962	49.91
	D.S.M. (2)	1828	46.69
	Casein	2010	47.75
19	D.S.M. (1)	1590	44.54
	Gelatin	1230	39.66
	D.S.M. (2)	1706	51.08
	Casein	1680	47.67
Mean	D.S.M. (1)	—	44.63
	Casein	—	46.40
	Gelatin	—	45.22
	D.S.M. (2)	—	45.05

D.S.M.: dried skim milk. (1) preliminary period; (2) recovery period.

Table 7 summarizes the heat production of the calves.

Two low values were encountered: one for calf no. 17 in the recovery period, the other for calf no. 19 in the period in which gelatin was given. In both instances there had been a short history of anorexia or diarrhoea with a concomitant reduction in the amount of dietary energy apparently digested. In view of the instability of the calf's heat production during inanition (Blaxter & Wood, 1951*b*) such an occurrence is quite understandable. The mean heat production of the calves given 4.8 l. of the diet each day was 46.52 Cal./kg/24 h and of those given 4.4 l./day, 44.50 Cal./kg/24 h. The difference between these values was statistically significant, confirming previous findings (Blaxter & Wood, 1952) that the plane of nutrition affects the heat production of the calf when it is determined 15 h after the last feed. In this connexion it should be noted that the present mean figures for basal metabolism were greater than those observed when calves were on nitrogen-free diets and subsequently subjected to short fasts (43.1 Cal./kg/24 h (Blaxter & Wood, 1951*a*)), this again being due to the effect of the previous calorie intake.

Pulse rates were recorded throughout the experiment twice daily with an 11 h

interval coinciding with feeding times. The daily records tended, however, to be variable. The mean data are shown in Table 8.

Table 8. *Mean pulse rates of the calves*

Calf no.	Level of feeding (l./day)	Pulse rate (beats/min)		
		D.S.M. diet	Gelatin diet	Casein diet
16	4.8	109.8	111.5	100.0
18	4.8	106.5	124.0	115.2
17	4.4	88.8	116.5	100.2
19	4.4	90.7	111.2	103.2
Mean	—	98.9	115.8	104.4

D.S.M.: dried skim milk.

Pulse rates were always much higher when the gelatin diet was given than when casein was the protein source, and higher on the casein than on the dried skim-milk diet. Analysis of variance of these data showed that the dietary differences were significant ($0.01 < P < 0.05$). It was also found that the mean pulse rates were inversely correlated with the changes of body-weight, and with the biological values of the three proteins (*vide infra*). The reasons for this relationship are dealt with later. The difference in mean pulse rate between calves given 4.8 l. and those given 4.4 l. of the diets was also statistically significant, the means being 111.1 and 101.8, respectively.

Nitrogen metabolism

The mean data relating to the nitrogen balances of each calf are presented in Table 9.

Table 9. *Mean nitrogen balances of the calves*

Calf no.	Level of feeding (l./day)	Diet	N intake (g/day)	Faecal N (g/day)	Urinary N (g/day)	N balance (g/day)
16	—	D.S.M. (1)	12.75	2.68	4.43	+5.64
	4.8	Casein	12.63	2.46	6.64	+3.53
	—	D.S.M. (2)	12.12	3.53	5.97	+2.62
	—	Gelatin	12.54	3.37	12.48	-3.30
17	—	D.S.M. (1)	11.69	2.06	4.14	+5.48
	4.4	Casein	11.66	3.17	6.12	+2.37
	—	D.S.M. (2)	11.10	4.61	4.72	+1.78
	—	Gelatin	11.13	2.79	9.27	-0.92
18	—	D.S.M. (1)	12.75	2.06	3.67	+7.01
	4.8	Gelatin	12.85	4.26	10.81	-2.22
	—	D.S.M. (2)	12.12	2.37	5.51	+4.24
	—	Casein	12.49	2.61	5.51	+4.37
19	—	D.S.M. (1)	11.69	3.12	4.51	+4.05
	4.4	Gelatin	10.98	5.89	9.46	-4.37
	—	D.S.M. (2)	11.10	4.01	4.07	+3.01
	—	Casein	11.67	2.98	4.39	+4.32
Mean, irrespective of feeding level	—	D.S.M. (1)	12.22	2.48	4.19	+5.55
	—	Casein	12.11	2.81	5.66	+3.64
	—	Gelatin	12.10	4.30	10.50	-2.70
Standard error of mean		—	—	±0.477	±0.449	±1.62

D.S.M.: dried skim-milk. (1) preliminary period: mean of five 2-day subperiods, except for calf no. 19 where value is the mean of four 2-day subperiods; (2) recovery period: mean of two 2-day subperiods.

Analysis of variance showed that there were no significant differences due to treatment as far as faecal nitrogen excretion was concerned, the high mean value for gelatin being largely due to one high value obtained with calf no. 19. The effect of diet on urinary nitrogen excretion was very highly significant ($P < 0.001$) as was the effect on nitrogen balance ($P < 0.01$). The effect on urinary nitrogen excretion is shown in Fig. 3. This was largely due to the very high urinary nitrogen excretion of the calves given gelatin. Nevertheless, the difference in the excretion of urinary nitrogen between the calves given casein and those given dried skim-milk was statistically significant at odds of 17 : 1.

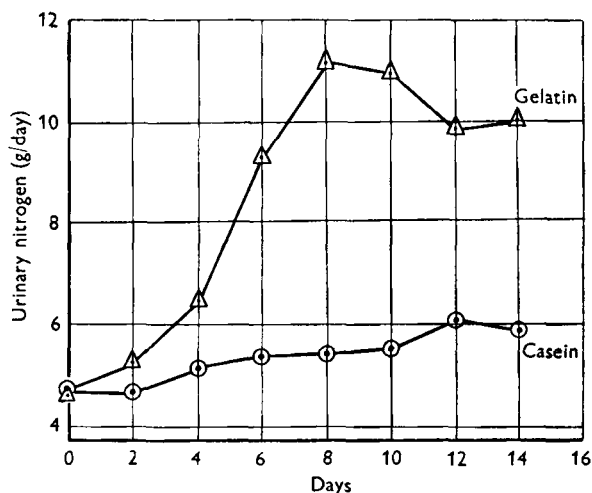


Fig. 3. The effect of transference from a diet containing dried skim-milk proteins to diets containing casein or gelatin on the mean excretion by calves of nitrogen in the urine. The excretion at 0 days represents the excretion of nitrogen when the dried skim-milk protein diet was given.

All balances were of course low, since all the diets were protein-deficient irrespective of the nutritional adequacy of the protein sources employed. It is of some interest, however, that despite this partial deficiency of protein the animals given dried skim-milk or casein gained in weight, though at a low rate. When the nitrogen balances of the calves were related to their gains in body-weight the following equation was found:

$$NB = 0.0128G + 0.898,$$

where NB is the nitrogen balance in g/day and G is the gain in weight in g/day. This regression was statistically highly significant ($P < 0.01$). The slope of the equation indicates that a gain in body-weight of 100 g corresponds to a gain of 1.28 g nitrogen. This value was much lower than the value obtained as the result of a similar analysis of results obtained with diets containing 14% or more protein (Blaxter & Wood, 1951c), where the following equation applied:

$$NB = 0.0262G + 0.703.$$

In this instance a 100 g gain in body-weight corresponds to a gain of 2.62 g nitrogen.

The difference between these two estimates (2.62 ± 0.122 less 1.28 ± 0.127) is statistically highly significant, indicating that the animals given the low-protein diets in the present experiment stored considerably less nitrogen/100 g body-weight gain than those given rations less deficient in protein. A larger part of the gain in weight of the former animals must, therefore, have been body fat. This was confirmed by statistical analysis of the body-weight gains of the calves in relation to the energy content of their diets. It was found that more energy was required per 100 g gain in body-weight with the calves given low-protein diets. The regression equations were:

$$\text{normal diets} \quad G = 0.326DC - 577.5,$$

$$\text{low-protein diets} \quad G = 0.2283DC - 439,$$

where G represents the gain in body-weight in g/day and DC the total energy intake (Cal./day) less the energy excreted in the faeces (Cal./day). The regressions were both statistically highly significant ($P < 0.001$ for the equation relating to normal diets and $0.01 > P > 0.001$ for that relating to the low-protein diets). The reciprocals of these equations were calculated by transformation of the original regression equations, the regression coefficient of G being the reciprocal of the regression coefficient of DC . These were:

$$\text{normal diets} \quad DC = 3.07G + 1771,$$

$$\text{low-protein diets} \quad DC = 4.38G + 1921.$$

The regression constants in these two equations represent the 'digested Cal.' required per g gain in body-weight, and the intercepts of the equation represent the theoretical maintenance requirements of the calves in terms of digested Cal./day. The statistical analysis of these results is given in Table 10.

Table 10. *The statistical significance of the inverse regression functions showing that the calves given low-protein diets required more energy/100 g gain in body-weight than those given diets normal in protein*

Type of diet	No. of observations	'Digested Cal./g gain*	Maintenance requirement in 'digested Cal./day*
Normal protein	22	3.07 ± 0.131	1771 ± 97
Low protein	8	4.38 ± 0.474	1921 ± 129
Difference	—	1.31 ± 0.492	150 ± 162

* The standard errors were calculated by analysis of variance using gain in weight as the independent variable.

There was no significant difference between the two estimates of maintenance requirement. The mean estimate is in agreement with the maintenance requirement determined by respiration calorimetry (Blaxter, 1952). The difference between the two estimates of energy required per g of gain in body-weight was statistically significant. This agrees with the data presented earlier. The data do not permit the calculation of the calorific value of the retained material, since the fat, water and ash values associated with the gains are not known.

Biological value of the proteins

Biological values were calculated from the nitrogen balances and the basal energy exchanges of the calves, making the assumption (Blaxter & Wood, 1951*a*) that the endogenous metabolism of nitrogen in the calf is proportional to its basal heat loss (Table 11).

Table 11. *Calculated biological values for the calves of dietary protein*

Calf no.	Dried skim milk	Casein	Gelatin
16	89.4	72.0	19.6
17	91.6	74.4	39.7
18	97.3	80.6	29.3
19	90.7	88.1	4.4
Mean	92.3	78.8	29.5

Dried skim milk had the highest biological value, that for casein was intermediate and for gelatin the lowest. The differences between the means were statistically highly significant. The high value obtained for calf no. 19 when given casein is anomalous. It was this animal that showed the greatest loss of nitrogen when given the gelatin diet, and the subsequent high value obtained when casein was given may reflect the increased retention of nitrogen following severe depletion. Though this may be an explanation of the high value, it does not provide a reason for omitting it in calculating the mean.

It is of some interest to compare these values with those obtained for other animals. Table 12 shows such a comparison with values summarized by Block & Mitchell (1946-7).

Table 12. *Biological value of proteins for man*, rat* and calf*

Protein	Value for			Relative values (D.S.M. = 100)		
	Calf	Rat	Man	Calf	Rat	Man
Dried milk	92	84	74	100	100	100
Casein	79	69	58	85	82	78
Gelatin	29	25	—	32	30	—

D.S.M.: dried skim milk.

* From Block & Mitchell (1946-7).

The values obtained with calves were all higher than those obtained with rats, which were in turn higher than those obtained with men. As shown in the second part of Table 12, the relative values obtained when dried skim milk was taken as equal to 100, were, however, very similar, irrespective of species. It appears, therefore, that the nutritive values of proteins as diverse in amino-acid content as gelatin and casein are relatively the same in the calf as in simple-stomached animals.

The reason why the biological values determined with calves were higher than those determined with other species was probably that the level of protein in their diets led to a greater degree of protein deficiency than occurred in rat or man. The results do not imply a species difference in the nutritive value of the three proteins used, but rather they indicate that the experiments with rats, calves and men are not necessarily comparable with regard to the degree of protein undernutrition employed.

The distribution of urinary nitrogenous metabolites

The mean results obtained from analysis of the urine for nitrogenous metabolites are given in Table 13. These means refer to the last 6 days of each period except for calf no. 19 when given the gelatin diet and calf no. 18 when given the casein diet. A single sample of urine in these periods had to be rejected and the mean daily

Table 13. *Summary of mean daily excretion by the calves of urinary metabolites during the last 6 days of each period†*

Nitrogenous metabolite	Diet			Standard error of difference between means
	D.S.M.	Casein	Gelatin	
Urea and ammonia	2010·8	2997·8	6326·8	± 378***
Albumin	128·8	143·5	149·9	± 33·4 N.S.
Amino-N	268·9	282·0	711·5	± 82·7***
Creatinine	407·8	378·4	384·0	± 16·5 N.S.
Creatine	296·0	330·9	499·3	± 54·7*
Purine base	93·6	146·0	252·1	± 27·0*
Uric acid	69·9	70·4	50·0	± 4·2*
Allantoin	599·1	518·7	530·6	± 12·5 N.S.

D.S.M.: dried skim milk.

† Last 4 days for calf no. 19 on gelatin diet and calf no. 18 on casein diet.

* Significant at $P=0\cdot05$.

*** Significant at $P=0\cdot001$.

N.S.: Not significant.

excretion was measured over 4 days. Albumin nitrogen, creatinine nitrogen, allantoin nitrogen and the total purine nitrogen were unaffected by the dietary source of protein. There was a small decline in the excretion of purine nitrogen as uric acid accompanied by an increase in excretion as purine bases. These changes, however, involved only small quantities of nitrogen and were not sufficiently large to result in any marked change in the total excretion of purine. The major part of the increase in urinary nitrogen excretion was in the urea, ammonia and amino-nitrogen fractions. These three fractions alone accounted for nearly 90% of the increased nitrogen excretion by the calves when given the gelatin diet. Creatine nitrogen also increased when either the gelatin or the casein diet was given.

These results are quite compatible with the contention that there was an increase in the deamination of dietary amino-acids when the calves were given either gelatin or casein as a sole source of protein. That this increase was due to failure of the protein to supply essential amino-acids and not due to a shortage of dietary energy is shown, first, by the fall in uric-acid excretion, since an increase occurs in energy starvation (Blaxter & Wood, 1951*b*) and, secondly, by the fact that with the exceptions previously mentioned, the basal metabolism of the calves was not lower than in the preliminary period when dried skim milk was given. A fall in the basal metabolism of the calf would have occurred had energy starvation ensued (Blaxter & Wood, 1951*c*). The constancy of creatinine elimination and of purine elimination despite such large changes in nitrogen metabolism, suggests that the endogenous metabolism of Folin (1905) does represent a reliable base-line for use in such studies.

General observations

All calves tended to become a little weak, as judged by a diminished playfulness in their metabolism cages and their general lethargy. Hair loss was considerable throughout, increasing in severity when the casein diet was given. As previously mentioned, mild alimentary disturbances were invariably present. When the calves were given the gelatin diet, they refused some of it after 8–10 days, and they tended to become dehydrated. On these occasions more water was added to their feed and in only one instance (calf no. 19) was it necessary to resort to the injection of a normal salt solution to restore tissue hydration. When the gelatin diet was given, pigmentation of the tongue developed in this calf, and the whole oral cavity became a dark magenta colour. Nicotinic-acid deficiency was suspected in this tryptophan-deficient calf, but no additional nicotinic acid was given in view of the metabolic relationships between tryptophan and nicotinic acid. There was no response to additional riboflavin (2 mg/day) and the condition slowly improved as the experimental period progressed. A similar condition, though not so severe, was noted in calf no. 16 when transferred to the gelatin diet. The exclusion of nicotinic acid from diets containing large quantities of casein does not produce any abnormalities in young calves (Johnson, Wiese, Mitchell & Nevens, 1947).

At slaughter nothing abnormal was noted in the carcasses of the calves. Serums were collected at this time and the serum proteins determined by sodium-sulphate fractionation. A non-protein fraction and three protein fractions were obtained using three concentrations of Na_2SO_4 (0, 159, 192 and 380 g/l.). These fractions differ from those proposed by Howe (1921), and were used as a result of fractionation studies on cattle serums by the method of Majoor (1946, 1947). Table 14 summarizes the results.

Table 14. *Concentration of serum-protein fractions of the calves at slaughter*

Na_2SO_4 concentration (g/l.)	Fraction of serum protein	(g/100 ml.) Calf no.				Mean	Normal calf given adequate colostrum
		16	17	18	19		
0–159	' γ globulin'	1.84	1.09	1.15	1.05	1.26	2.14
159–270	' $\alpha + \beta$ globulin'	1.84	1.81	1.65	1.59	1.72	1.85
Above 270	Albumin	1.97	2.29	1.67	1.94	1.97	2.64
	Total	5.67	5.19	4.57	4.62	4.95	6.61

Results obtained with a normal calf aged 6 days given cow's whole milk are included for comparison. The serum-protein concentration was low in all the experimental calves, the reduction being in both the globulin and the albumin fraction. In adult cattle the total serum-protein concentration is approximately 7–8 g/100 ml., this higher figure being accounted for mainly by a greater concentration of globulins. The low globulin content of the blood of the present calves may be partly accounted for by lack of proteins transmitted from the dam in the first few hours of life, but the low albumin content is clearly related to the inadequate level of protein nutrition.

DISCUSSION

Since the young calf can store body nitrogen and grow when given a protein known to be of fair nutritive value for the rat and yet will lose body nitrogen and weight when given an inadequate protein, it obviously is dependent on its diet for at least a part of its amino-acid supply. That it may be as dependent as the rat or man is demonstrated by the close similarity between the biological values obtained for the three different species for a good protein (dried skim milk), a fair protein (casein) and a very poor protein (gelatin). These results, to our knowledge, are the first demonstration that farm ruminants require dietary sources of amino-acids for metabolic functions involving nitrogen metabolism. The results apply, however, only to the calf reared under conditions in which development of an adult rumen function is unlikely to have occurred, and thus, considered together with the fact that the adult ruminant does not react in a similar manner, provide additional confirmation of Zuntz's (1891) hypothesis of rumen function.

This finding has a number of implications; it raises the question of the protein values of the calf meals and gruels used for calf rearing; it implies that the utilization and requirement of the young calf for a number of other dietary essentials may differ markedly from the utilization and requirement of the adult (cf. Wiese, Johnson, Mitchell & Nevens, 1947, Johnson *et al.* 1947), and it implies that during the early part of the calf's life, when rumen function has commenced but has not reached its maximal synthetic capacity, the calf may still be liable to dietary shortages of amino-acids.

The fact that losses in weight occurred when gelatin was given suggests that tryptophan—the first limiting amino-acid of gelatin—is required for maintenance of nitrogen equilibrium and body-weight. Where such a loss in weight occurs on a diet sufficient in energy it is obvious that the metabolism of the animal must increase. The higher pulse rates found when gelatin was given suggest that an increase in heat production did occur. That this was due to the increase in the heat increment of feeding and not to an increase in basal metabolism is implied by the fact that the basal metabolic rate was not significantly greater when the calves were given gelatin. The conclusion that the heat increment of feeding is greater in the animal given a diet deficient in essential amino-acids is in agreement with the general conclusions of the other workers, as recently reviewed by Kleiber (1945-6).

SUMMARY

1. Experiments have been made in which four calves were given dried skim-milk proteins, casein or gelatin as the sole source of protein. These diets were adequate in other respects but contained only 11.9 % of their total energy as protein and were thus deficient in protein.
2. The mean daily changes in body-weight of the calves were +268, +241 and -271 g for the dried skim-milk, casein and gelatin diets respectively.
3. There were no significant differences between the diets in respect of the apparent digestibility of dry matter, fat, nitrogen or calories, but the digestibility of the dietary fat was related to the protein content of the diet.

4. Basal heat production determined after 15 h fasting was not affected by the dietary differences. Pulse rates were 98.9, 104.4 and 115.8 when protein was supplied by dried skim milk, casein and gelatin, respectively. These differences were statistically significant.

5. Urinary nitrogen excretion rose when the calves were transferred from the dried skim-milk diet to the casein diet and was greatly increased when gelatin was given. The mean daily nitrogen balances were: dried skim milk +5.55, casein +3.64 and gelatin -2.70 g. The differences between these balances were statistically significant.

6. It was shown that the storage of nitrogen/g gain of body-weight was much lower in calves given protein-deficient diets than in those given sufficient protein. The protein-deficient animals required more energy/g gain in body-weight. It was therefore concluded that their gains of body-weight contained less protein and more fat than those of calves given more normal diets.

7. The biological values of the three proteins were 92.3 for dried skim milk, 78.8 for casein and 29.5 for gelatin. These values were higher than those found with rats, probably as a result of a relatively lower level of protein nutrition with the calves. When the biological values were related to dried skim milk taken as 100, there were no differences between the biological values obtained on casein and on gelatin as between calf, rat and man.

8. There was no significant change in the excretion of creatinine nitrogen, purine nitrogen or protein nitrogen. There was, however, a small change in the distribution of the urinary purine. Excretion of urea, ammonia, amino-nitrogen and creatine, however, increased when casein or gelatin rather than dried skim milk was given.

9. The results of the experiment are briefly discussed in relation to the hypothesis that the microflora of the digestive tract plays an important part in the protein metabolism of ruminants. It is concluded that, unlike the adult ruminant, the young calf is as dependent on its diet for a source of essential amino-acids as man, dog, or rat.

Our thanks are due to Mr D. Paterson for his care of the experimental animals, and to Miss C. Samson and Miss G. Breckenridge for their technical assistance.

REFERENCES

- Beadles, J. R., Quisenberry, J. H., Nakamura, F. I. & Mitchell, H. H. (1933). *J. agric. Res.* **47**, 947.
 Blaxter, K. L. (1952). *Brit. J. Nutrit.* **6**, 12.
 Blaxter, K. L. & Howells, A. (1951). *Brit. J. Nutrit.* **5**, 25.
 Blaxter, K. L. & Wood, W. A. (1951*a*). *Brit. J. Nutrit.* **5**, 11.
 Blaxter, K. L. & Wood, W. A. (1951*b*). *Brit. J. Nutrit.* **5**, 29.
 Blaxter, K. L. & Wood, W. A. (1951*c*). *Brit. J. Nutrit.* **5**, 55.
 Blaxter, K. L. & Wood, W. A. (1952). *Brit. J. Nutrit.* **6**, 1.
 Block, R. J. & Mitchell, H. H. (1946-7). *Nutr. Abstr. Rev.* **16**, 249.
 Edwards, W.-F. & Balzac (1832). *Ann. Sci. nat.* **26**, 318.
 Escher, T. (1876). *Vjschr. naturf. Ges. Zürich*, **21**, 36.
 Folin, O. (1905). *Amer. J. Physiol.* **13**, 117.
 Howe, P. E. (1921). *J. biol. Chem.* **49**, 93.
 Hughes, C. W. & Hauge, S. M. (1945). *J. Nutrit.* **30**, 245.
 Hutchinson, J. C. D. & Morris, S. (1936). *Biochem. J.* **30**, 1695.
 Johnson, B. C., Wiese, A. C., Mitchell, H. H. & Nevens, W. B. (1947). *J. biol. Chem.* **167**, 729.
 Kik, M. C. (1938). *Bull. Ark. agric. Exp. Sta.* no. 352.
 Kleiber, M. (1945-6). *Nutr. Abstr. Rev.* **15**, 207.

- Kon, S. K. (1928). *Biochem. J.* **22**, 261.
Macrae, T. F., El-Sadr, M. M. & Sellers, K. C. (1942). *Biochem. J.* **36**, 460.
Majoor, C. L. H. (1946). *Yale J. biol. Med.* **18**, 419.
Majoor, C. L. H. (1947). *J. biol. Chem.* **169**, 583.
Mitchell, H. H., Beadles, J. R. & Kruger, J. H. (1927). *J. biol. Chem.* **73**, 767.
Morris, S. & Wright, N. C. (1935). *J. Dairy Res.* **6**, 289.
Swanson, E. W. & Herman, H. A. (1943). *Res. Bull. Mo. agric. Exp. Sta.* no. 372.
Wiese, A. C., Johnson, B. C., Mitchell, H. H. & Nevens, W. B. (1947). *J. Dairy Sci.* **30**, 87.
Zuntz, N. (1891). *Pflüg. Arch. ges. Physiol.* **49**, 483.

Observations on the Vitamin Metabolism of the Common Fowl

1. The Partition of Free and Combined Riboflavin in the Tissues of the Laying Hen

By W. BOLTON

Poultry Research Centre, West Mains Road, Edinburgh 9

(Received 9 April 1951)

A considerable amount of work has been carried out on the estimation of the minimal requirements for riboflavin by laying hens, both for egg production and for hatchability, but comparatively little is known of the part of riboflavin in egg production.

Common & Bolton (1946) and Common, Rutledge & Bolton (1947) showed that the riboflavin content of the blood serum of immature pullets receiving injections of oestrogens increased to between ten and twenty times the pre-treatment value. In the latter paper they demonstrated that differences of the same order existed between serums of normal laying and non-laying hens.

Hertz, Dhyse & Tullner (1949) confirmed for plasma the increase in riboflavin following oestrogen treatment, and showed that this increase was greater when the diet contained adequate riboflavin than when a diet poor in riboflavin was given.

It has also been shown that the increase in serum riboflavin evoked by oestrogen treatment consists entirely of an increase in the free vitamin, the two naturally occurring combined forms, flavin-mononucleotide (FMN) and flavin-adenine-dinucleotide (FAD) being unaffected (Bolton, 1950). Examination of the blood serums of laying hens and of the albumen and yolk of their eggs showed that almost all the riboflavin in the blood serum was in the free form and that 85% of the riboflavin in the egg was also present as the free vitamin in both albumen and yolk (Bolton, 1951).

It therefore became of interest to ascertain the partition of riboflavin in the oviduct, since this organ forms the link between the blood-stream and the egg. This paper sets out the results obtained from an investigation of the partition in two parts of the oviduct, the magnum, where the yolk of the egg receives its layers of albumen, and the shell gland. Three other tissues were also examined: liver, kidney and heart. Liver and kidney are known to be rich in the vitamin, and from previous work (Bolton, 1947) the concentration in all three is known to reflect the level of intake by young