CCXXXIX. THE DIGESTIBILITY OF DIETARY PROTEIN IN THE RUMINANT.

II. THE DIGESTIBILITY OF PROTEIN FOLLOWING A PROLONGED FAST, WITH A DETAILED STUDY OF THE NITROGEN METABOLISM.

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IN the preceding paper [Hutchinson & Morris, 1936] it was shown that the faecal N could be divided into three fractions, (a) a constant fraction, (b) the true metabolic faecal N and (c) a fraction dependent on the N content of the diet. Only the first and second fractions were dealt with. The present publication deals specifically with the third fraction.

In a series of publications on the nutritive value of proteins for milk production Morris & Wright [1933, 1934] noted that, when foods containing protein of high biological value were ingested, a lowered N digestibility was found. This general relationship is shown in Fig. 1. Further work has confirmed the results [Morris & Wright, 1936]. Moreover, in another series of experiments designed to determine the nutritive value of proteins for maintenance [Morris & Wright, 1935] a similar general relationship was obtained. When this observation was first noted the authors wrote: "At first sight it seemed possible that this might be due to normal variations in digestibility . . . , but the possibility must be considered that the body itself can to some extent adjust the assimilation of N from, or re-excretion of N into the gut according to its needs. . . . It is not unreasonable to assume that absorption from the gut might be stimulated when inadequate rations were fed." They also suggested an investigation into the effect of varying levels of food intake on digestibility.

The immediate object of the present series of experiments was to determine whether the same food would show an altered apparent digestibility when fed at the same level to an animal under circumstances in which the requirements of that animal for essential amino-acids varied markedly.¹ This was carried out by feeding a low-N diet to an animal and following this with a period of prolonged starvation until the animal was in marked N debt. When a large amount of N

Fig. 1. Relation between biological value and faecal nitrogen.

¹ In previous work it has been shown that the biological value of proteins for ruminants depends chiefly on their contents of lysine and tryptophan.

had been lost, a protein of high biological value was added to the basal diet. This ration was continued until the N deficit had been reduced to minimum amounts. Any alteration in the faecal N excretion could then at once be observed.

Experimental method.

Two cows were used, both having terminated their period of lactation about 30 days before the commencement of the experiment. The rations fed are shown in Table I. Bean meal was selected as the protein of high biological value since it

Table I. *Details of daily rations for both animals.*

Period	Ration	N intake g.	Energy intake as lb. starch equivalent
1	1 lb. maize 12 lb. straw 2.25 lb. starch 200 ml. linseed oil	25.67	6.61
2	Starvation	—	—
3	1 lb. maize 12 lb. straw 3.75 lb. bean meal 200 ml. linseed oil	92.65	6.84
4	1 lb. maize 12 lb. straw 1.25 lb. bean meal 1.65 lb. starch 200 ml. linseed oil	47.32	6.84
5	Same as period 3	92.65	6.84
6	1 lb. maize 12 lb. straw 1 lb. gelatin 0.5 lb. starch 400 ml. linseed oil	92.65	6.80

has been shown [Morris & Wright, 1933] that for milk production it has a fairly high value, about 60, while Morris [1934] has shown that the % N as lysine and tryptophan is relatively high. The basal ration of straw and maize remained constant throughout the feeding periods, but the starch intake was varied with the addition of the beans in order to keep the energy content of the diet steady throughout the complete experiment. A final period in which gelatin replaced the bean meal was included.

DISCUSSION OF RESULTS.

(a) *Nitrogen balance.*

The N balances for each period are shown in Table II. It will be seen that before feeding the first high protein diet the two animals were in N debt to the extent of 394 g. and 697 g. respectively. At the commencement of the second period of high protein the debts were only 75 g. and 282 g. N. The former is minimum and can be ignored, but the latter is definitely high. It may be noted that, whereas cow 1 had a fairly large positive balance on the second period of low-N diet, cow 2 registered a loss of N. This is peculiar since cow 2 was in N debt to a far greater extent during this period of low-N diet than cow 1. From the balance figures it is obvious, however, that, in the first period of high protein, requirements were high, whereas in the second period for cow 1 they were minimum. With cow 2 the N requirements were minimum about midway through the second period of high protein feeding.

Table II. *Nitrogen balances.*

Period	Intake g.	Output g.	Balance g.	Total loss g. N.	Total loss g. N
Cow 1.					
1. Low-N	154.02	254.30	- 100.28	394.37	75.07
2. Starvation	0.00	294.09	- 294.09		
3. 1st high-N	926.50	670.51	+ 255.99		
4. Low N	851.76	788.45	+ 63.31		
5. 2nd high-N	741.20	482.78	+ 258.42	—	—
6. Gelatin	1019.15	787.73	+ 231.42	—	—
Cow 2.					
1. Low-N	231.03	424.73	- 193.70	697.26	282.08
2. Starvation	0.00	503.56	- 503.56		
3. 1st high-N	1667.70	1225.39	+ 442.31		
4. Low-N	615.16	642.09	- 27.13		
5. 2nd high-N	1111.80	816.71	+ 295.09	—	—
6. Gelatin	370.60	274.04	+ 96.56	—	—

(b) Faecal nitrogen excretion.

Daily faecal N excretions are shown in Fig. 2, and the corresponding figures for the digestibility during each period are shown in Table III. Following the

Fig. 2. Faecal excretion.

very low output during starvation, there is a rise in faecal N in the first period of high protein feeding. The level is, however, not significantly higher than that found during the ingestion of the low-N diet, and the digestibility of bean meal is 90 %. With cow 1 the period on low-N can be subdivided into two portions. The first is that in which bean meal shows a digestibility of 100 % and the second where the faecal N rises markedly and the digestibility falls to about 73 %. With cow 2 the digestibility falls immediately to about 70 % in the low-N

Table III. *Digestibilities of bean meal and gelatin.*

Period	Intake of N added to basal diet g.	Output of faecal N g.	Basal output of faecal N g.	% digestibility
		Cow 1.		
1st high-N	66.98	36.34	29.68	90.0
Low-N (a)	21.65	24.85	29.68	100.0
Low-N (b)	21.65	35.58	29.68	72.8
2nd high-N	66.98	43.07	29.68	80.0
Gelatin	66.98	30.36	29.68	99.0
		Cow 2.		
1st high-N	66.98	32.00	26.29	91.5
Low-N	21.65	32.50	26.29	70.1
2nd high-N (a)	66.98	34.77	26.29	87.3
2nd high-N (b)	66.98	49.64	26.29	65.1
Gelatin	66.98	25.55	26.29	100.0

period. When the high protein diet is again resumed the digestibility of the bean meal shows a figure of 80% with cow 1, whereas the decrease below the level of the first high-N period only occurs midway through the period with cow 2. With gelatin the digestibility rises once more to 100%.

The total weight of faeces and the percentage dry matter are of interest (Table IV). Comparing Tables III and IV it will be seen that an increase in

Table IV. *Amount of faeces and dry matter excreted.*

Period	Wt. of faeces (wet) g.	Wt. of faeces (dry) g.	Dry matter %
	Cow 1.		
1st high-N	15,475	3021	19.5
Low-N (a)	13,286	2222	16.7
Low-N (b)	18,591	2528	13.6
2nd high-N	20,630	2569	12.4
Gelatin	14,357	2170	15.1
	Cow 2.		
1st high-N	11,677	2118	18.1
Low-N	14,150	2381	16.8
2nd high-N (a)	16,125	2387	14.8
2nd high-N (b)	20,583	2855	13.9
Gelatin	12,688	2005	15.8

faecal N corresponds to an increase in total faecal weight, with a very slight increase, if any, in the amount of dry matter excreted. The faecal N varies directly with the moisture content of the faeces, the digestibility of total dry matter remaining constant. This fact was noted by Titus [1927]. It appears that the apparent digestibility of any foodstuff is not related to the digestibility of the total dry matter ingested, but varies rather with the moisture content of the faeces.

The low digestibility of the N given in the low protein period in cow 2 is presumably due to a temporary irregularity, since the water content of the faeces in this period was not high, nor was the faecal weight. The error of the experiment is naturally larger when a low-N diet is fed.

(c) *Nature of the faecal nitrogen.*

Having obtained evidence that with the same food different apparent digestibilities could be observed according to the requirements of the animal, it seemed

desirable to investigate the nature of the excess faecal N. Dried samples of the faeces which had been collected throughout the experiment were therefore analysed for total N before a more detailed examination. The results were unexpected, for the whole of the excess N was found to be lost on drying at 180° for 1 hour. The samples of faeces obtained during the first high-N, the second high-N and the gelatin periods were analysed. The results are summarized in Table V,

Table V. *Loss of nitrogen on drying faeces at 180° for 1 hour.*

Period	% digestibility	No. of samples analysed	Mean % loss on drying	Standard deviation
Cow 1.				
Gelatin	99.0	6	2.86	7.09
1st high-N	90.0	10	9.60	7.75
2nd high-N	80.0	8	25.97	9.34
Cow 2.				
1st high-N	91.5	8	2.52	9.27
2nd high-N (a)	87.3	5	7.94	7.40
2nd high-N (b)	65.1	4	19.22	4.03

the mean percentage loss on drying being recorded side by side with the percentage digestibility. It will be seen that as the digestibility falls the percentage loss rises. Considerable variations were found between individual samples, as is apparent from the relatively high standard deviations. The differences between the first and second periods are not statistically significant, between the second and third periods they are barely significant, but between the first and third periods they are definitely significant—the probability of such differences occurring by chance being less than 1 in 100.

From these facts it seems safe to conclude that the excess N excreted during the later periods of high-N feeding is composed of compounds which are (a) volatile or decomposed at 180°, or (b) transformed by heating at this temperature into nitrogenous bodies which cannot be estimated by the Kjeldahl method, as, for example, compounds containing the pyrrole ring structure.

(d) *Total urinary nitrogen.*

No detailed study of the changes in N metabolism following prolonged periods of both protein and total starvation has been undertaken in the past. Benedict & Ritzman [1927] make a brief reference to one or two results with starving steers. They found that for the first two days after feeding there was a marked fall in total N excretion together with a fall in both total and percentage urea and creatine N. The amino-acid N and hippuric acid N, on the other hand, increased markedly, whilst only a very slight percentage increase in ammonia N was noted. These changes were particularly noticeable after the first period of re-feeding. In the present experiment a detailed study of re-alimentation in the ruminant is presented.

Fig. 3 shows the urinary N throughout each feeding period, together with the results of the previous protein and total starvation periods. Two facts are evident from the results. The urinary N during the period of high protein feeding immediately following starvation is only slightly less than that found during starvation. As with Benedict's steers the first feed caused a marked fall in urinary N, with a rise in the succeeding days. Also the output in this period is higher than in the succeeding high protein period. Calculation of the percentage utilization of the absorbed N clearly illustrates this difference. With the first high-N diet the N excretions due to the food intake are 18.0 and 15.2 g. respec-

tively for the two cows (obtained by subtracting the endogenous N of the period before the starvation from the average daily excretion). The total absorbed N is 86 g. daily, which gives utilizations of 80 and 83 %. In the second high-N period the nitrogen excretions were 4.7 g. and 4.6 g. respectively. With a true intake of 65 g. N daily there are utilizations of 93 % with both animals. There are two possible explanations of this result. Either the caloric require-

Fig. 3. Urinary excretion.

ments immediately following starvation may be higher than the normal requirements, resulting in the deamination of amino-acids for energy purposes, or the ability of the animal to retain absorbed N may be somewhat impaired following a prolonged period of undernutrition.

The second fact of importance is that the urinary excretion in the second low-N period is lower than that in the first, before the starvation. This is interesting since the N intake in the second period is 16 g. higher daily, being supplied in the form of bean meal, a protein which was found to have a fairly high biological value for milk production [Morris & Wright, 1933]. From the very low N intake during the first low-N diet and from the maintenance requirements of the cow [Morris & Wright, 1935] it has been assumed that the urinary N excretion during this period is endogenous N. Since the addition of a fairly large amount of bean meal protein causes a decrease in N excretion, it appears that there is some evidence, in the ruminant, for Sherman's [1920] theory of decreased endogenous N with addition to the diet of a protein of high biological value.

With gelatin, owing to deficiencies of certain essential amino-acids, the urinary N excretion is higher than with bean meal.

(e) *Urinary partition of nitrogen.*

From a study of the N partition in the urine (Table VI) it will be seen that ingestion of food after a period of starvation causes marked alterations. Whereas

Table VI. *Average daily urinary partition of nitrogen following starvation.*

Figures in brackets represent percentages of total N.

Ration	Total N g.	Urea N g.	Ammonia N g.	Amino-acid N g.	Preformed creatinine N g.	Creatine N g.
Cow 1.						
Starvation	39.70	24.00 (60.3)	2.20 (5.6)	0.20 (0.5)	1.33 (4.7)	0.65 (2.3)
1st high-N	30.70	9.90 (32.2)	7.20 (23.4)	1.00 (3.3)	2.17 (7.1)	0.36 (3.6)
Low-N	10.41	1.36 (13.1)	2.57 (24.7)	0.42 (4.0)	1.63 (15.6)	0.18 (1.7)
2nd high-N	17.27	5.00 (28.9)	3.08 (17.8)	0.34 (2.0)	1.67 (9.7)	0.49 (2.8)
Gelatin	35.28	17.32 (49.1)	10.78 (30.5)	1.20 (3.4)	1.91 (5.4)	0.36 (3.2)
Cow 2.						
Starvation	40.60	24.40 (60.0)	4.22 (10.4)	0.28 (0.7)	1.60 (5.5)	0.49 (1.7)
1st high-N	36.08	15.84 (43.8)	8.30 (23.0)	0.92 (2.6)	2.25 (6.2)	0.15 (0.4)
Low-N	16.86	4.64 (27.5)	3.44 (20.4)	0.36 (2.0)	2.01 (11.9)	0.10 (0.6)
2nd high-N	25.86	9.38 (36.3)	4.68 (18.0)	1.14 (4.4)	2.39 (9.2)	0.31 (1.2)
Gelatin	42.96	—	—	—	—	—

the percentage of urea N falls the percentages of the other products increase markedly. These results confirm the findings of Benedict & Ritzman [1927].

It is of interest to note the difference between the partitions on the low and high protein (2) diets when the protein requirements are high. An increase is found in both total and percentage urea N excretions, whereas little change can be seen in the ammonia and amino-acid excretions. The preformed creatinine excretion remains constant in amount although the percentage falls on changing to the high protein ration. The creatine output increases in both total and percentage amounts. These results, except for the ammonia N excretion, agree closely with the results obtained by Folin [1905] on man.

When, however, the ingested protein cannot be used for tissue production purposes, as in the first high protein period and the gelatin period, the partition products are altered. Marked increases in total urea, ammonia and amino-acid N are found. As regards percentage output, the urea N alone shows a marked increase, the ammonia and amino-acids remain fairly constant, while the creatinine decreases.

From the complete series of results it appears that, with the ruminant, increased deamination leads to an increase in the total amounts of urea, ammonia and amino-acid N excreted. With the creatine no definite conclusions can be drawn, and it may be concluded that deamination has little effect on the urinary excretion of creatine. On the other hand, when the ingested protein is being used for tissue anabolism, a definite increase in creatine output occurs. Similarly during tissue catabolism, as in the starvation results, a marked increase in creatine output is found. It is evident that creatine excretion in the ruminant is controlled by cellular activity and is in no way affected by that portion of the ingested protein which is deaminated either to supply energy needs or because an excess of protein has been ingested in the diet.

(f) *Sulphur excretion.*

It has been shown in the previous paper [Hutchinson and Morris, 1936] that during starvation the N : S ratio of the excreted products is, after deducting the endogenous excretion, the same as that of muscle tissue. In the present paper the effect of feeding following a fast is studied, i.e. the changes which occur in the S excretion when anabolism is in progress. Wilson [1933] has shown that, with the one-stomached animal, S excretion during tissue catabolism and S retention during anabolism precede N excretion and retention. During starvation (i.e.

tissue catabolism) S excretion appears to precede N excretion, the N : S ratios being less than that for body tissue.

With the ingestion of food the system is reversed, anabolism replacing catabolism, and retentions of N and S replacing losses. The effect on the N : S ratio is shown in Table VII.

Table VII. *The effect of a high protein diet following starvation on the daily N : S ratio in the urine.*

Animal	Daily N : S ratio.								
	Day 1	2	3	4	5	6	7	8	9
Cow 1	3.8	6.4	5.3	6.1	8.2	7.3	5.7	3.0	4.5
Cow 2	3.8	9.6	11.0	10.4	11.8	12.1	9.0	7.9	6.4...

The first feed causes a marked decrease in excretion of urinary N with little change in the S output, thereby giving a N : S ratio very much lower than that found during starvation. This is followed by an increase in the ratio, showing that S is being retained in advance of N. This period, lasting about 7–8 days, is then followed by one in which N retention increases, with a concomitant decrease in the N : S ratio in the urine. It is evident that, with the ruminant, as with the one-stomached animal, S metabolism precedes N metabolism.

The N and S intakes and outputs together with the N : S ratio of the material retained are shown in Table VIII. The results show clearly that short experiments

Table VIII. *Nitrogen and sulphur excretions.*

Ration	True intake		Excreted		Retained		N : S of retained material
	Nitrogen g.	Sulphur g.	Urinary N g.	Urinary S g.	Nitrogen g.	Sulphur g.	
Cow 1.							
1st high-N	83.38	8.74	30.70	5.80	52.68	2.94	17.8
Low-N	31.55	4.78	10.41	4.20	21.14	0.58	36.4
2nd high-N	74.12	8.74	17.27	3.52	56.85	5.22	10.9
N : S ratio of total N and S retained in all three periods. Mean					43.56	2.91	15.0
Cow 2.							
1st high-N	84.50	8.74	36.08	4.58	48.42	4.16	11.6
Low-N	35.50	4.78	16.86	4.60	18.64	0.18	103.6
2nd high-N	81.65	8.74	25.86	5.40	55.79	3.34	16.7
N : S ratio of total N and S retained in all three periods. Mean					40.95	2.56	16.0

with ruminants may lead to entirely wrong conclusions. It will be seen that with cow 1 the N : S ratio of the retained material in the first period of high protein, and in the period of the low protein ration, shows that N is retained over each complete period to a greater extent than S, whereas in the last period the position is reversed. With cow 2 the first high protein period shows a greater retention of S whilst the low protein ration gives an extremely high N : S ratio, the result of an intense retention of N. Taking the mean of all three periods, however, it will be noted that the retained material has a N : S ratio very close to that of body tissue. It is clear that, using the ruminant as the experimental animal, correct conclusions can only be drawn with long experiments.

(g) *Biological values.*

Much work has been done in the evaluation of proteins for the growth of the young, chiefly using the rat as experimental animal. So far there has been no record of any attempt to find the biological value of proteins for growth, weight

increase or tissue repair *in adults*. The present series of experiments affords an opportunity to study this problem. During the periods on low N intake and starvation the animals suffered severe losses of body tissue, chiefly protein. This is readily seen from the large N deficit at the end of the fast. During the period of high protein intake and in the subsequent periods the retention of N was high. In those periods following the fast there is, therefore, opportunity for the study of the nutritive value of the proteins ingested. As in all studies of the biological value for production, it must be remembered that the results are for both production and maintenance, since no method has as yet been evolved which can separate these two functions.

The results for the proteins used in the present experiment, bean meal, straw and maize, are shown in Table IX. It will be noted that the results for the two

Table IX. *Biological values for tissue repair.*

$$\text{B.V.} = \frac{I - (F_t - F_m) - (U_t - U_e)}{I - (F_t - F_m)}$$

Period	Protein level %	Intake	F_t	F_m	U_t	U_e	B.V.
Cow 1.							
1st high-N	18	92.65	36.34	29.65	30.70	12.70	80
Low-N	7	47.50	31.40	29.65	12.40	12.70	100
2nd high-N	18	92.65	43.07	29.65	17.28	12.70	94
Gelatin	18	92.65	31.69	29.65	36.05	12.70	74
Cow 2.							
1st high-N	18	92.65	32.00	26.25	36.08	20.91	82
Low-N	7	47.50	32.53	26.25	16.86	20.91	100
2nd high-N	18	92.65	42.06	26.25	24.42	20.91	95
Gelatin	18	92.65	25.55	26.25	42.96	20.91	76

I = intake. F_t = total faecal N. F_m = metabolic faecal N, obtained from the period of low N intake before starvation. U_t = total urinary N. U_e = endogenous urinary N obtained from period before starvation.

animals are in remarkably close agreement. The fall in value between the low protein period and the second high protein period is due mainly to the decrease in apparent digestibility, giving an increase of 10 g. in the daily faecal N. The fairly high biological value of the gelatin-containing diet, i.e. 75, can be understood when it is remembered that although the gelatin is deficient in tryptophan the straw protein has 15% tryptophan [Morris, 1934]. This will, in part, make up for the gelatin deficiency.

The most important point, however, is the fact that an 18% protein level can give, for growth of tissue, a biological value of 95, very little less than the figure obtained with a 7% level. Clearly this type of experiment would be of value in estimating the nutritive properties of any type of protein or non-protein compound for growth purposes.

It is of interest to compare the results obtained with ruminants, in which beans formed the major portion of the diet, with those obtained with the one-stomached animals. In the latter, beans have a fairly low biological value owing both to low digestibility and low content of cystine. With the ruminant this experiment affords further evidence that cystine, if essential for ruminant nutrition, is only essential in minimum amounts. This fact has already been shown for maintenance [Morris & Wright, 1935].

SUMMARY.

1. Experiments were performed on cows to discover the effect on N metabolism of re-alimentation following a prolonged fast.
2. The faecal N excretion shows that the apparent digestibility of a protein varies according to the requirements of the animal and also according to the biological value of the protein ingested. The higher the biological value of a protein the lower is the digestibility. Similarly, the greater the requirement of the animal for protein the higher is the digestibility.
3. The excess N excreted during those periods when the apparent digestibility was low was found to be lost on heating at a temperature of 180°.
4. The urinary N shows that, immediately following prolonged starvation, there is poor utilization of absorbed N, owing either to increased demand for energy, or to impaired ability to retain absorbed N.
5. N partition products in the urine show that during active tissue anabolism a marked increase in creatine output occurs, whereas no alteration takes place when the absorbed N is deaminated and excreted.
6. S excretion and the N : S ratio show that in tissue anabolism S is retained in advance of N.
7. Calculation of the biological value of the ingested protein showed that, following a prolonged fast, an 18 % protein level had a value for tissue growth very little less than a 7 % level.

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