THE BIOLOGICAL ASSESSMENT OF PROTEIN QUALITY: EFFECTS OF CONSUMPTION OF 'CRUDE FIBRE', NaCl AND BODY HAIR ON FAECAL NITROGEN EXCRETION IN THE RAT*

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In the biological assessment of protein quality it is important to consider the effects of the non-protein dietary components and other environmental factors on the excretion of those products of nitrogen metabolism which serve as criteria of the absorption and anabolism of the test protein. For example: when determining protein digestibility we must consider, among other things, the metabolic nitrogen output of the test animals, a value which can be estimated with a fair degree of accuracy on the basis of lean body-weight and dry-food consumption.[1] However, some of the earlier workers in this field have obtained results indicating that metabolic nitrogen output is also subject to the effect of certain indigestible dietary components, such as crude fibre.

PREVIOUS STUDIES

From a study of the relevant literature it appears that the first serious attempt at investigating the effects of indigestible food materials on metabolic nitrogen output was published in 1912 by Mendel and Fine,[2] who used dogs and studied the effect of the addition to a predominantly meat diet of specific quantities and combinations of agar, bone ash and disintegrated paper. According to the results, consumption of these materials invariably caused an increase in faecal nitrogen output. The above finding as well as observations made subsequently by himself and his co-workers[3,4] induced Mitchell[5] to list the dietary concentration of indigestible, non-nitrogenous material or 'roughage' as a factor of 'great importance' in respect of the excretion of metabolic nitrogen. ('The greater the concentration of such "roughage" the greater the fecal excretion of metabolic nitrogen per unit of dry matter consumed.') Mitchell[6] therefore considered it necessary that, for accurate estimation of metabolic N output, the standardization diet used in protein evaluation trials should contain 'the same concentration of indigestible ballast as the ration containing the food protein under test'. He also suggested[7] that consumption of high-fibre diets increases protein requirements on account of the stimulation of metabolic N output by such diets.

Following on the classical work of Mendel and Fine,[2] however, Adolph and Wu[8] investigated faecal nitrogen excretion (*a*) in rats on from low to very high dietary levels of ground filter paper, rice chaff, agar-agar and China clay (kaolin clay, eaten in large amounts by Chinese peasants especially during periods of famine), and (*b*) in human subjects on exceptionally high intakes of cabbage fibre. Convincing proof of an appreciable stimulation of faecal N output was secured only in the trials with agar-agar, this material being described as having 'laxative action'.

Funnell and her co-workers[9] studied the effect on a human subject of nitrogen-free bran fibre, and found that the daily addition of 3·5 G to a standard basal diet raised the faecal N output from 0·73 to 0·86 G per day. Normal bran caused an even greater rise in nitrogen output, viz. from 0·73 to 0·92 G in the same subject, presumably because of the poor digestibility of the nitrogenous components of highly lignified materials such as bran.[10]

Duckworth and Godden[11] recorded an increase in faecal N output as well as in faecal phosphatase activity in rats, caused by inclusion of paper pulp in a low-fibre basal diet. Moreover, differences observed by Kehar and Mukherjee[12] in faecal N excretion per 100 G dry food consumed between, on the one hand, cattle fed rations containing Izatnagar hay and oat straw (said to contain relatively low concentrations of indigestible material) and, on the other hand, cattle fed highly indigestible 'untreated' wheat straw, also suggested that indigestible food materials stimulate faecal N excretion. In their studies on rats Kehar and Mukherjee[12] found that, when a 15%-fibre diet was fed, faecal N per 100 G dry food consumed was 0·24 G, compared with 0·18 G for rats on a fibre-free diet.

In what appears to be the first attempt at a quantitative evaluation of the effect of crude fibre on faecal N excretion, Meyer,[13] as recently as 1956, fed 4 separate groups of young rats on equal quantities of a low-egg (4% defatted egg protein) diet which varied from one group to another in fibre (Alphacel) content from 0 through 5 and 15 to 30%. The faecal N output of the rats during a 7-day collection period was (group-to-group) 1·38, 1·44, 1·75 and 1·81 mg./G dry food consumed. Regression analysis of these data by me indicated an increase in faecal N output in mg./G food consumed amounting to approximately 0·015 × % fibre in the diet, the output at zero fibre being about 1·41 mg./G dry food consumed. Having done an experiment of the same type as that carried out by Meyer,[13] Hennig,[14] some 2 years later, recorded results showing a linear relationship between the faecal N output of rats per unit weight dry, N-free food consumed and the percentage 'protein-free' indigestible matter in the faeces. Since the latter parameter would correspond to what is normally understood under the term 'indigestible matter' in the diet, and since the relationship found was a direct one, Hennig's[14] results agreed with those of Meyer.[13]

In contrast, Mangold and Behm,[15] who made extensive investigations of the effects of cellulose (ground filter paper) and sawdust, fed at graded levels in protein-free diets to rats, rabbits and pigs, failed to demonstrate a clear upward trend in faecal N output per unit weight dry food consumed in spite of substantial increases in fibre intake. In fact, from the results obtained with rats it appears that those diets which contained from 8 through 10 and 15 to 20% cellulose, yielded slightly lower faecal N outputs per unit dry food consumed than the diets of lower cellulose content.

Recalculation of data obtained in yet another series of quantitative studies—those of Whiting and Bezeau,[16,17] who investigated the effects of pure cellulose preparations

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(Solka-Floc and Methocel) and of oat hulls on the faecal nitrogen output of pigs—consistently revealed a direct relationship between faecal N/G dry food consumed and the fibre content of the diet. However, in one study[16] the rise in faecal N/G food was found to be about 0·1 mg./1% increase in the Solka-Floc content of the diet, while, in the other study,[17] the rise was about 0·046 mg.

From a comparison of the relative effects of the cellulosal materials investigated, Whiting and Bezeau[17] concluded that some materials had a greater effect on faecal N output than others. The same conclusion could be drawn from results obtained by me.[18] Studying the effects of certain highly indigestible 'antidiarrhoeic substances', I could not show an increased faecal nitrogen excretion in rats (at 3 different dietary egg-protein levels) when from 10 to 20% of the dextrin component of the diet was replaced with kaolin or Apella (a kaolin-pectin mixture); but in the case of similar replacements with Arobon (a product prepared from the carob bean and containing only about 32% indigestible matter), there was a marked rise in N output above that of the control rats.

From the above review of available literature it seems clear that the work done in this field has often yielded conflicting results, and that there is reason for uncertainty as regards the qualitative and quantitative effect of indigestible dietary constituents on metabolic nitrogen excretion. Attempts at obtaining further information on the subject therefore appear to be justified.

Another aspect of the metabolic nitrogen output of the rat which appeared worthy of investigation was the possible effect of the NaCl content of the diet. Although no reference could be found to results showing a relationship between NaCl intake and metabolic N excretion, the possibility that such a relationship does exist could not be excluded. The matter was considered of special interest in respect of its obvious bearing on the validity of protein digestibility data obtained in rat trials with high-NaCl foodstuffs such as soup powders.

As regards the effect on metabolic N output of the third factor to be considered in this paper, viz. consumption of body hair (trichophagy), Schneider[19] wrote: 'The ingestion of hair may be incidental to the cleaning process, as the white rat cleans itself thoroughly each day. There is, however, reason to believe from the quantities of hair observed in the faeces, that the amount of hair increases as the amount of food is restricted.'

Consumption of body hair may be expected to increase faecal N output because of (*a*) the high N content as well as the relative indigestibility of hair and (*b*) the concomitant addition to the bulk of dry matter consumed. However, it was considered of interest to know whether or not hair consumption has a significant effect on faecal nitrogen output under the conditions prevailing in this laboratory in the routine testing of protein digestibility.

EXPERIMENTS

The experimental work consisted of one series of trials on the effect of cellulosal materials on faecal N excretion in the white rat (series A); another series (B) on the effect of NaCl intake; and a third series (C) on the effect of hair consumption. Since the general information bearing on the rats, the basal diet and the analytical and metabolic methods employed in this laboratory at the time of the trials have already been reported elsewhere,[1] the descriptions given here will be limited to specific details regarding each of the experiments conducted in the present study.

As a general rule all the experiments consisted of the feeding of the specific experimental diets to rats for a 7-day 'preliminary' period and a 10-day 'collection' period, food intake and faecal N output data being secured during the latter period and the basal experimental diet used being the protein-free diet, the composition of which was changed in one way or another to suit the particular objective in mind. Specific details are given in the following sections.

Series A

This series can be subdivided into two studies, one which was an investigation of the effect on faecal N output of purified bran when fed with 'low-egg' diet to rats varying in body-weight (and age) from 'low' to 'high' (experiment A1); and another (A2) in which the objective was comparable to that of the first, but the fibre component of the diet was pure cellulose, the rats were young animals of approximately equal age and weight, and the diet was either a 'low-egg' or a 'high-egg' diet.

Experiment A1. Three groups of 90 rats each (the rats weighing from 35 to 125 G) including equal numbers of males and females, were selected at random from rats available in the colony at the beginning of the trial. All 3 groups received defatted whole-egg protein in their diets at a 3·8% level, but these diets differed in respect of fibre (purified bran) content. One group received no bran, another about 4 parts of purified bran, and the remaining group about 6 parts of purified bran per 100 parts of diet, the bran replacing an equal weight of the dextrin component of the diet. (The exact crude fibre contents of the above diets were determined according to the method of Van de Kamer and Van Ginkel,[20] and were found to be, respectively, 0, 4·3 and 6·4% on a dry basis.) The weight of food offered to each rat per day was 10% of the body-weight.

The 'purified bran' used in this study was wheaten bran from which the nitrogenous and other non-fibre components had been removed as thoroughly as possible by boiling small batches of the raw material successively with 1·25% (w/v) H_2SO_4 for 30 min. and 1·25% (w/v) NaOH solution for 30 min., the liquid fractions being separated each time by filtration through an acid-resistant cloth and traces of acid and alkali being removed by washing with hot water.

Repeated extraction of the raw material gave a product which, when dried and ground, contained 95·2% crude fibre on a moisture-free basis. Expressed per 100 G crude fibre the product contained 96·5 mg. N. This nitrogen remained even after further extraction with acid and alkali. Particle size after grinding was such that the amounts retained per 100 G material by 20, 30, 40, 60, 80 and 100 mesh sieves were 1, 10, 31, 69 and 83 G, respectively.

An undesirable property of the purified bran was obviously the fact that the product contained a small

amount of nitrogen (96·5 mg./100 G fibre). The extent to which use of the product could lead to the appearance of irrelevant nitrogen in the faeces was therefore a matter to be considered. A preliminary crude-fibre balance trial with 10 young rats fed a 3·8% egg-protein diet containing the bran at a level to yield a crude-fibre content of 6·4% of the diet, indicated that for every 100 G of fibre consumed 81·2 ± 2·6 (standard error of mean) was excreted in the faeces. Assuming (*a*) that the N content of the excreted fibre was equal to that of the ingested fibre (96·5 mg./100 G) and (*b*) that the N in that weight of fibre which was digested by the rat was absorbed,* we can say that the amount in milligram of irrelevant N in the faeces of the rat would be $\frac{96{\cdot}5}{100} \times \frac{81{\cdot}2}{100} \times$ weight of fibre consumed. This formula was applied for the calculation of irrelevant N in the faeces of each rat. The irrelevant fibre nitrogen excretion thus calculated was then subtracted from the total faecal N value obtained by Kjeldahl analysis.

Experiment A2. Two main groups (*a* and *b*) of 60 weanling rats each were used. Individual body-weights varied in group *a* from 47 to 56 G and in group *b* from 49 to 62 G. Each group was divided on a weight and sex basis into 6 subgroups (I - VI and VII - XII) of equal mean weight and equal numbers (5 + 5) of males and females per subgroup.

The basal diet fed to group *a* contained egg protein at approximately a 6% level, and that fed to group *b*, egg protein at approximately a 10% level. To ensure that all of the diet offered to a rat each day was eaten, the feeding level of basal diet was set slightly below the usual 10% of body-weight, viz. at 9%.

In addition to the basal diets, the rats in the various subgroups received cellulose in the diet at approximately the following levels: 0% (groups I and VII); 4% (groups II and VIII); 8% (groups III and IX); 12% (groups IV and X); 15% (groups V and XI); and 18% (groups VI and XII). The cellulose used was Grycksbo Pappersbruk brand cellulose powder, recommended by the manufacturer for use in electrophoresis studies. This material was mixed into separate quantities of the respective basal diets at the desired levels. Each diet was then fed to the rats in the particular subgroup to which that particular diet had been allocated. The quantities fed were sufficient to ensure that from subgroups I and VI to subgroups VII and XII the basal diet intake level per unit body-weight remained constant and the cellulose consumption level per unit body-weight rose from zero to high.

Series B

The general approach in this series was to feed groups of weanling rats a basal diet of either about 5% or about 10% egg-protein content, and, in the case of each protein intake level, to grade the NaCl intakes of the rats upwards from one group to another. The series included 2 experiments, B1 and B2, each bearing on the question of faecal N excretion under a specific set of dietary conditions.

Experiment B1. A group of 50 weanling rats, ranging in body-weight from 46 to 58 G, was divided into 5 subgroups (I - V) of equal mean weight and of an equal number (5 + 5) of males and females. Each subgroup was then fed a 5% egg-protein basal diet in which some of the dextrin component was replaced with additional NaCl in a quantity sufficient to ensure a certain NaCl intake over and above that normally provided by the basal diet. The additional NaCl contents of the 5 diets allocated to the 5 subgroups were: 0% (group I); 2% (group II); 4% (group III); 6% (group IV); and 8% (group V).

In addition to the technical operations usually performed in the conducting of our balance studies, this experiment included measurements of the water consumption of each rat during the collection period. Water was therefore given from glass bottles fitted with burnished stainless-steel drinking pipes, water consumption data being secured by means of regular weighing of the bottles. These data were corrected for evaporation losses according to the loss from a bottle attached to an empty metabolism cage.

Experiment B2. The design and conditions of this experiment were similar to those of experiment B1, but experiment B2 was carried out at a later date and the egg-protein content of the diet was about 10%, this protein not being from the same sample of laboratory-prepared egg protein as that used in experiment B1.

Series C

In this series the total faecal N outputs of certain rats were compared with the outputs of other rats, the latter being measured after removal of body hair from the faeces. The series was completed in one trial (C1) including 5 different dietary conditions. These conditions differed from one another in respect of either the protein feeding level or the quality of the protein fed.

A group of 60 weanling rats was divided on a weight and sex basis into 10 subgroups of 3 males and 3 females each. The 10 subgroups were then allocated at random and their 10-day faeces collections treated as is shown in Table I.

TABLE I. ALLOCATION OF SUBGROUPS OF RATS USED IN STUDY OF EFFECT OF BODY HAIR CONSUMPTION ON FAECAL N EXCRETION

Subgroup	*Mean wt at beginning of expt in G*	*Protein and concentration of protein in diet (%)*	*Body hair removed from faeces excreted during collection period*
Ia	51·2	0%	No
Ib	51·7	0%	Yes
IIa	51·3	Wheat gluten (10%)	No
IIb	51·3	Wheat gluten (10%)	Yes
IIIa	51·7	Cooked soy-bean (10%)	No
IIIb	51·5	Cooked soy-bean (10%)	Yes
IVa	51·5	Casein (10%)	No
IVb	51·7	Casein (10%)	Yes
Va	51·5	Defatted whole egg (10%)	No
Vb	51·5	Defatted whole egg (10%)	Yes

Storage of the faeces in the usual manner (under concentrated H_2SO_4) during the collection period would have

*This assumption could be erroneous. Johnson *et al.*[23] have shown that the site of cellulose breakdown in the rat is the caecum, a segment of the intestinal tract generally not associated with absorption of proteinaceous materials. It would therefore probably have been more in line with current thinking to have assumed that all the N in the ingested fibre was excreted in the faeces. However, as will be shown at a later stage (Table IV), the highest fibre consumption level reached by any of the rats used in this experiment was 8·2 G/10 days. This means that at a maximum the amount of irrelevant N in the faeces from dietary fibre was about 8 mg. in a rat which excreted a total of 425 mg. faecal N. Comparison with the figure obtained for irrelevant N according to the method of estimation actually used (6·4 mg.) indicates that the error—if such it is—is negligible, viz. < 2 mg. N.

caused disintegration of the body hair present in the faeces, thus rendering separation of hair from faeces impossible. The daily collections from each rat were therefore pooled in a glass beaker containing 120 ml. 0·5% (w/v) H_2SO_4. Separation of body hair from faeces and sampling of the faeces for Kjeldahl analysis were done as follows:

The contents of the glass beaker (containing the entire 10-day collection) were washed quantitatively with distilled H_2O into the jar of a variable-speed blender of the Waring type. The contents of the jar were then agitated slowly at a speed and for a time sufficient to disintegrate faeces pellets but not the hair present in the faeces, the speed and blending time being kept constant from one sample to another. After blending the sample was transferred to a 1-litre volumetric flask, either quantitatively (when hair was not removed) or through a 20-mesh stainless-steel sieve (when removal of hair was intended). The contents of the flask were then made up to volume and mixed thoroughly. Suitable aliquots of from 150 to 250 ml. were measured out for Kjeldahl analysis by means of a measuring cylinder after the mixture in the flask had again been shaken vigorously for several minutes.

RESULTS AND DISCUSSION

The Effect of Crude Fibre Intake on Faecal N Excretion

The results obtained in experiment A1 are shown in Tables II - IV. The data considered in the investigation of the effect of crude fibre are (*a*) the lean body-weights of the rats; (*b*) the dry food and N intakes; and (*c*) the values obtained for faecal N excretion after deduction of the N content of the indigestible fibre consumed (see description of experiment A1).

To investigate whether or not the dietary crude fibre

TABLE II. FAECAL N EXCRETION DATA OF 90 RATS OF DIFFERENT WEIGHT FED A LOW-PROTEIN, FIBRE-FREE DIET

Lean wt at middle of collection period (*G*)	*Total dry food intake during collection period* (*G*)	*Fibre intake* (*G*)	*Faecal N* *N intake during collection period* (*G*)	*Faecal N* *output during collection period* (*G*)	*Faecal N* *Estimated fibre N in faeces* (*G*)	*Faecal N-faecal fibre N* (*G*)
41·5	41·5	—	0·2928	0·0828	—	—
47·0	47·4	—	0·3343	0·0881	—	—
53·0	43·3	—	0·3060	0·0940	—	—
52·0	36·2	—	0·2553	0·0851	—	—
58·5	42·8	—	0·3020	0·1284	—	—
65·5	43·2	—	0·3047	0·1455	—	—
71·5	51·8	—	0·3658	0·1546	—	—
71·0	47·0	—	0·3316	0·1543	—	—
77·0	45·6	—	0·3218	0·1308	—	—
84·5	60·4	—	0·4264	0·1767	—	—
93·0	71·1	—	0·5021	0·2268	—	—
94·5	75·4	—	0·5323	0·2214	—	—
97·5	55·9	—	0·3941	0·2050	—	—
105·5	64·8	—	0·4573	0·1815	—	—
40·0	40·1	—	0·2829	0·0890	—	—
44·0	33·5	—	0·2362	0·0837	—	—
54·0	44·0	—	0·3106	0·1052	—	—
53·5	56·4	—	0·3981	0·1420	—	—
62·5	51·3	—	0·3619	0·1382	—	—
62·0	46·6	—	0·3290	0·1552	—	—
70·0	71·2	—	0·5027	0·1880	—	—
78·0	75·6	—	0·5336	0·2356	—	—
80·5	86·6	—	0·6113	0·2543	—	—
84·5	69·9	—	0·4935	0·1802	—	—
98·0	90·3	—	0·6376	0·2710	—	—
93·0	63·9	—	0·4507	0·1981	—	—
108·0	95·6	—	0·6745	0·2739	—	—
108·0	67·3	—	0·4751	0·2368	—	—
38·0	33·7	—	0·2383	0·0948	—	—
41·5	44·2	—	0·3120	0·1030	—	—
45·5	47·5	—	0·3357	0·1120	—	—
57·0	49·8	—	0·3515	0·1237	—	—
54·5	44·9	—	0·3173	0·1199	—	—
63·0	41·0	—	0·2896	0·1199	—	—
59·5	52·4	—	0·3699	0·1917	—	—
64·5	46·4	—	0·3278	0·1231	—	—
71·0	51·3	—	0·3620	0·1640	—	—
78·5	54·4	—	0·3844	0·1669	—	—
71·5	51·2	—	0·3614	0·1503	—	—
92·0	64·0	—	0·4522	0·1619	—	—
99·5	68·9	—	0·4864	0·1765	—	—
93·0	57·7	—	0·4074	0·1637	—	—
99·0	55·0	—	0·3883	0·1718	—	—
103·5	84·3	—	0·5957	0·2252	—	—
35·0	35·4	—	0·2501	0·0913	—	—
39·5	41·5	—	0·2929	0·1053	—	—
46·5	50·5	—	0·3567	0·1123	—	—
48·5	35·4	—	0·2501	0·1004	—	—
54·5	47·3	—	0·3337	0·1611	—	—
59·0	49·7	—	0·3508	0·1578	—	—
61·0	50·3	—	0·3554	0·1634	—	—
70·5	59·2	—	0·4180	0·1625	—	—
71·5	77·1	—	0·5443	0·2222	—	—
76·5	49·4	—	0·3488	0·1424	—	—
84·0	67·0	—	0·4732	0·1991	—	—
90·0	98·3	—	0·6944	0·2991	—	—
101·5	94·5	—	0·6674	0·2757	—	—
105·5	92·6	—	0·6543	0·2495	—	—
111·5	99·7	—	0·7043	0·3068	—	—
110·5	114·6	—	0·8096	0·3435	—	—
35·0	35·9	—	0·2563	0·0854	—	—
41·5	38·1	—	0·2722	0·0985	—	—
42·5	40·2	—	0·2869	0·1049	—	—
47·5	30·7	—	0·2190	0·0778	—	—
53·5	45·2	—	0·3228	0·1201	—	—
53·5	37·8	—	0·2702	0·0997	—	—
63·5	53·9	—	0·3847	0·1280	—	—
67·5	63·8	—	0·4559	0·1672	—	—
69·0	52·9	—	0·3781	0·1652	—	—
78·5	55·5	—	0·3967	0·1973	—	—
82·0	59·6	—	0·4260	0·1941	—	—
88·0	81·8	—	0·5844	0·2490	—	—
88·5	65·6	—	0·4686	0·2060	—	—
94·5	74·8	—	0·5345	0·2163	—	—
103·5	74·6	—	0·5325	0·2621	—	—
35·0	32·8	—	0·2343	0·0877	—	—
36·5	37·3	—	0·2662	0·1140	—	—
44·0	35·0	—	0·2503	0·0935	—	—
49·5	42·7	—	0·3048	0·0795	—	—
52·5	54·5	—	0·3894	0·1310	—	—
56·5	46·8	—	0·3341	0·0930	—	—
63·5	43·8	—	0·3128	0·1110	—	—
70·0	60·8	—	0·4340	0·1933	—	—
78·0	74·5	—	0·5318	0·2040	—	—
77·0	87·0	—	0·6210	0·2227	—	—
89·5	79·1	—	0·5651	0·2392	—	—
89·5	89·4	—	0·6383	0·2879	—	—
100·5	100·3	—	0·7162	0·2762	—	—
110·5	104·8	—	0·7481	0·3179	—	—
106·0	109·9	—	0·7847	0·2850	—	—

TABLE III. FAECAL N EXCRETION DATA OF 90 RATS OF DIFFERENT WEIGHT FED A LOW-PROTEIN DIET CONTAINING BRAN FIBRE AT 4·3%

Lean wt at middle of collection period (G)	Total dry food intake during collection period (G)	Fibre intake (G)	Faecal N: N intake during collection period (G)	Faecal N: output during collection period (G)	Faecal N: Estimated fibre N in faeces (G)	Faecal N-fibre N (G)
37·5	32·3	1·389	0·2266	0·0754	0·0011	0·0743
43·0	32·8	1·410	0·2306	0·0937	0·0011	0·0926
47·0	28·7	1·234	0·2017	0·0789	0·0010	0·0779
55·0	39·6	1·703	0·2785	0·1125	0·0013	0·1112
60·5	45·0	1·935	0·3160	0·1320	0·0015	0·1305
60·0	40·9	1·759	0·2871	0·1217	0·0014	0·1203
71·5	43·2	1·858	0·3035	0·1205	0·0015	0·1190
68·5	43·6	1·875	0·3061	0·1520	0·0015	0·1505
74·5	49·5	2·128	0·3475	0·1567	0·0017	0·1550
84·5	79·6	3·423	0·5590	0·2086	0·0027	0·2059
90·5	52·3	2·249	0·3672	0·1655	0·0018	0·1637
94·5	90·6	3·896	0·6365	0·2838	0·0031	0·2807
104·5	88·3	3·797	0·6201	0·2897	0·0030	0·2867
104·0	60·7	2·610	0·4263	0·1893	0·0020	0·1873
40·0	33·8	1·453	0·2371	0·0884	0·0011	0·0873
45·0	43·9	1·888	0·3087	0·1075	0·0015	0·1060
46·0	47·7	2·051	0·3350	0·1287	0·0016	0·1271
51·0	49·4	2·124	0·3468	0·1131	0·0017	0·1114
63·5	53·5	2·300	0·3764	0·1409	0·0018	0·1391
62·5	59·7	2·567	0·4198	0·1814	0·0020	0·1794
69·0	53·9	2·318	0·3790	0·1799	0·0018	0·1781
69·0	46·9	2·017	0·3298	0·1666	0·0016	0·1650
79·0	66·2	2·847	0·4651	0·2090	0·0022	0·2068
87·5	75·8	3·259	0·5327	0·1996	0·0026	0·1970
97·0	101·0	4·343	0·7095	0·3654	0·0034	0·3620
101·5	109·6	4·713	0·7699	0·3013	0·0037	0·2976
103·5	107·7	4·631	0·7567	0·3288	0·0036	0·3252
114·5	91·2	3·922	0·6405	0·2762	0·0031	0·2731
38·5	34·5	1·484	0·2431	0·0799	0·0012	0·0787
40·5	41·2	1·772	0·2905	0·0966	0·0014	0·0952
41·5	41·3	1·776	0·2912	0·1030	0·0014	0·1016
52·0	39·1	1·681	0·2754	0·0986	0·0013	0·0973
58·0	53·9	2·318	0·3801	0·1377	0·0018	0·1359
57·5	47·8	2·055	0·3366	0·1351	0·0016	0·1335
64·0	68·2	2·933	0·4809	0·1634	0·0023	0·1611
66·0	53·8	2·313	0·3795	0·1558	0·0018	0·1540
72·0	54·5	2·344	0·3841	0·1532	0·0018	0·1514
78·5	48·6	2·090	0·3426	0·1435	0·0016	0·1419
76·0	36·9	1·587	0·2602	0·1085	0·0012	0·1073
83·0	57·8	2·485	0·4078	0·1990	0·0019	0·1971
88·5	54·7	2·352	0·3854	0·1759	0·0018	0·1741
100·5	47·3	2·034	0·3334	0·1899	0·0016	0·1883
101·5	43·1	1·853	0·3037	0·1780	0·0015	0·1765
110·5	62·9	2·705	0·4434	0·1926	0·0021	0·1905
34·5	30·7	1·320	0·2167	0·0890	0·0010	0·0880
41·5	43·1	1·853	0·3037	0·1126	0·0015	0·1111
45·5	43·5	1·870	0·3070	0·1141	0·0015	0·1126
50·0	53·5	2·300	0·3768	0·1357	0·0018	0·1339
55·5	46·6	2·004	0·3287	0·1380	0·0016	0·1364
56·5	37·0	1·591	0·2609	0·1205	0·0012	0·1193
60·5	54·0	2·322	0·3808	0·1380	0·0018	0·1362
69·5	47·8	2·055	0·3373	0·1517	0·0016	0·1501
70·5	50·8	2·184	0·3584	0·1850	0·0017	0·1833
72·5	55·3	2·378	0·3900	0·1631	0·0019	0·1612
98·0	84·9	3·651	0·5982	0·2835	0·0029	0·2806
89·5	72·3	3·109	0·5099	0·2164	0·0024	0·2140
97·0	82·4	3·543	0·5811	0·2572	0·0028	0·2544
103·5	69·7	2·997	0·4915	0·2764	0·0023	0·2741
103·5	76·0	3·268	0·5356	0·2397	0·0026	0·2371
97·0	84·6	3·638	0·5962	0·1921	0·0029	0·1892
37·0	29·8	1·281	0·2138	0·0722	0·0010	0·0712
38·5	35·8	1·539	0·2567	0·0813	0·0012	0·0801
40·5	29·8	1·281	0·2138	0·0900	0·0010	0·0890
49·0	43·0	1·849	0·3083	0·1050	0·0014	0·1036
54·5	37·8	1·625	0·2708	0·0991	0·0013	0·0978
60·5	40·5	1·742	0·2902	0·1161	0·0014	0·1147
62·5	52·1	2·240	0·3733	0·1370	0·0018	0·1352
69·0	46·0	1·978	0·3297	0·1283	0·0016	0·1267
74·0	57·9	2·490	0·4149	0·1713	0·0020	0·1693
74·0	47·1	2·025	0·3378	0·1430	0·0016	0·1414
77·0	49·2	2·116	0·3525	0·1552	0·0017	0·1535
84·0	56·1	2·412	0·4021	0·1691	0·0019	0·1672
88·0	44·0	1·892	0·3157	0·1467	0·0015	0·1452
91·5	71·5	3·074	0·5127	0·2158	0·0024	0·2134
108·5	94·5	4·064	0·6776	0·2446	0·0032	0·2414
36·5	33·0	1·419	0·2366	0·0798	0·0011	0·0787
39·5	29·6	1·273	0·2125	0·0702	0·0010	0·0692
41·0	30·7	1·320	0·2198	0·0830	0·0010	0·0820
49·0	43·0	1·849	0·3083	0·1210	0·0014	0·1196
54·0	36·9	1·587	0·2647	0·1152	0·0012	0·1140
56·5	51·2	2·202	0·3673	0·1690	0·0017	0·1673
64·0	41·6	1·789	0·2982	0·1251	0·0014	0·1237
63·5	48·2	2·073	0·3458	0·1131	0·0016	0·1115
71·5	75·1	3·229	0·5382	0·2158	0·0025	0·2133
74·0	65·3	2·808	0·4678	0·1922	0·0022	0·1900
83·5	70·9	3·049	0·5080	0·2246	0·0024	0·2222
92·0	79·1	3·401	0·5670	0·2324	0·0027	0·2297
98·5	87·9	3·780	0·6300	0·2665	0·0030	0·2635
104·0	114·7	4·932	0·8223	0·3318	0·0039	0·3279
95·5	88·3	3·797	0·6327	0·2783	0·0030	0·2753

content had an effect on faecal N output, over and above the effects of lean body-weight, dry-food consumption and egg-protein consumption, the faecal N output of each rat was first estimated on the basis of lean body-weight, dry-food intake and egg-protein N intake according to my equation,[22] the assumption being that the egg protein in the diet was 5% indigestible. These estimates were then compared with the 'experimental' values for faecal N, as is shown in Fig. 1. The rationale of this approach is that if the dietary fibre content had an effect on faecal N output, the experimental values would have tended to differ from the estimated values, the method of estimation not making allowance for effects other than those of the weight of dry food consumed, body size and egg protein consumed. Moreover, as it can be said that those rats which had the highest faecal N outputs were also the rats with the highest food consumption and, consequently, with the highest fibre consumption, this difference would have increased with an increase in faecal N output, thus causing an appreciable change in the 'least-squares' regression of 'experimental' values (Y) on estimated values (X) from 0% through 4·3% fibre to 6·4% fibre in the diet (Fig. 1).

From Fig. 1(a) it can be seen that there was good agreement between 'experimental' and estimated values at zero fibre in the diet, the intercept of the regression line being close to zero (4·4 mg.) and the regression coefficient being only slightly above 1, viz. 1·0176. This deviation of the regression coefficient from 1 could have been due to a slightly erroneous assumption as regards the magnitude of the indigestible fraction of the egg protein included in the diet. Further calculation revealed that if the indigestible fraction had been assumed as being 5·74% (as opposed to 5%) the regression coefficient would have been 1, and the intercept 3·9 mg. N.

However, the important question to be answered here

TABLE IV. FAECAL N EXCRETION DATA OF 90 RATS OF DIFFERENT WEIGHT FED A LOW-PROTEIN DIET CONTAINING BRAN FIBRE AT 6·4%

Lean wt at middle of collection period (*G*)	*Total dry food intake during collection period* (*G*)	*Fibre intake* (*G*)	*Faecal N* *N intake during collection period* (*G*)	*Faecal N output during collection period* (*G*)	*Estimated fibre N in faeces* (*G*)	*Faecal N-fibre N* (*G*)
42·0	30·8	1·971	0·2156	0·0781	0·0015	0·0766
43·0	34·2	2·189	0·2391	0·0928	0·0017	0·0911
51·0	34·8	2·227	0·2437	0·0824	0·0017	0·0807
55·0	53·6	3·430	0·3751	0·1501	0·0027	0·1474
61·5	51·6	3·302	0·3607	0·1160	0·0026	0·1134
60·5	46·4	2·970	0·3247	0·1284	0·0023	0·1261
67·0	36·8	2·355	0·2574	0·1090	0·0018	0·1072
74·0	50·5	3·232	0·3535	0·1400	0·0025	0·1375
73·0	42·9	2·746	0·2999	0·1669	0·0022	0·1647
80·0	43·2	2·765	0·3019	0·1507	0·0022	0·1485
93·0	70·1	4·486	0·4907	0·2222	0·0035	0·2187
97·0	81·0	5·184	0·5665	0·2767	0·0041	0·2726
105·0	77·2	4·941	0·5404	0·2295	0·0039	0·2256
108·5	68·2	4·365	0·4770	0·2079	0·0034	0·2045
42·0	41·1	2·630	0·2875	0·0902	0·0021	0·0881
46·5	47·1	3·014	0·3293	0·1073	0·0024	0·1049
47·0	43·9	2·810	0·3071	0·1090	0·0022	0·1068
48·5	34·2	2·189	0·2391	0·1189	0·0017	0·1172
56·5	41·4	2·650	0·2895	0·1171	0·0021	0·1150
59·5	39·5	2·528	0·2764	0·1200	0·0020	0·1180
71·0	55·6	3·558	0·3888	0·1709	0·0028	0·1681
70·0	38·4	2·458	0·2685	0·1394	0·0019	0·1375
86·5	91·1	5·830	0·6371	0·2430	0·0046	0·2384
87·0	89·6	5·734	0·6266	0·2550	0·0045	0·2505
96·5	84·0	5·376	0·5874	0·2676	0·0042	0·2634
91·5	61·8	3·955	0·4326	0·1728	0·0031	0·1697
112·0	120·5	7·712	0·8429	0·3702	0·0060	0·3642
120·0	128·7	8·237	0·9004	0·4245	0·0065	0·4180
34·5	27·1	1·734	0·1917	0·0706	0·0014	0·0692
39·5	33·3	2·131	0·2352	0·0884	0·0017	0·0867
43·5	37·7	2·413	0·2662	0·1135	0·0019	0·1116
47·0	32·2	2·061	0·2273	0·0983	0·0016	0·0967
52·5	36·5	2·336	0·2576	0·0977	0·0018	0·0959
65·0	55·8	3·571	0·3940	0·1675	0·0028	0·1647
59·0	37·3	2·387	0·2636	0·1056	0·0019	0·1037
62·0	57·9	3·706	0·4085	0·1829	0·0029	0·1800
71·0	47·7	3·053	0·3367	0·1529	0·0024	0·1505
75·0	53·8	3·443	0·3795	0·1587	0·0027	0·1560
72·0	52·8	3·379	0·3729	0·1608	0·0026	0·1582
82·0	57·6	3·686	0·4065	0·2016	0·0029	0·1987
84·0	52·9	3·386	0·3736	0·1964	0·0027	0·1937
94·0	68·5	4·384	0·4836	0·2177	0·0034	0·2143
105·5	70·8	4·531	0·4994	0·2130	0·0036	0·2094
103·5	55·9	3·578	0·3947	0·1759	0·0028	0·1731
35·5	35·5	2·272	0·2504	0·0832	0·0018	0·0814
42·0	42·4	2·714	0·2991	0·1030	0·0021	0·1009
49·5	37·3	2·387	0·2636	0·0957	0·0019	0·0938
51·0	48·5	3·104	0·3426	0·1301	0·0024	0·1277
56·0	41·7	2·669	0·2945	0·1412	0·0021	0·1391
70·0	51·1	3·270	0·3604	0·1567	0·0026	0·1541
66·5	56·3	3·603	0·3973	0·1829	0·0028	0·1801
71·5	44·3	2·835	0·3130	0·1179	0·0022	0·1157
78·5	81·8	5·235	0·5772	0·2675	0·0041	0·2634
87·0	95·2	6·093	0·6721	0·2665	0·0048	0·2617
102·5	89·8	5·747	0·6339	0·2879	0·0045	0·2834
109·0	94·2	6·029	0·6648	0·2679	0·0047	0·2632
101·0	80·5	5·152	0·5680	0·2465	0·0040	0·2425
34·5	30·6	1·958	0·2183	0·0658	0·0015	0·0643
36·0	25·5	1·632	0·1817	0·0669	0·0013	0·0656
48·0	29·6	1·894	0·2110	0·0684	0·0015	0·0669
50·5	35·9	2·298	0·2556	0·0772	0·0018	0·0754
51·5	38·0	2·432	0·2709	0·0973	0·0019	0·0954
63·0	52·5	3·360	0·3741	0·1353	0·0026	0·1327
61·0	48·7	3·117	0·3468	0·1690	0·0024	0·1666
68·0	57·3	3·667	0·4080	0·1929	0·0029	0·1900
74·0	62·0	3·968	0·4420	0·1790	0·0031	0·1759
79·5	59·1	3·782	0·4213	0·1678	0·0030	0·1648
80·5	57·5	3·680	0·4100	0·1704	0·0029	0·1675
87·5	94·3	6·035	0·6723	0·2396	0·0047	0·2349
89·0	71·3	4·563	0·5079	0·2198	0·0036	0·2162
96·0	71·3	4·563	0·5079	0·2112	0·0036	0·2076
104·5	93·4	5·978	0·6656	0·2738	0·0047	0·2691
35·5	28·9	1·850	0·2057	0·0707	0·0015	0·0692
39·0	32·1	2·054	0·2290	0·0713	0·0016	0·0697
47·0	43·8	2·803	0·3122	0·1083	0·0022	0·1061
45·0	39·9	2·554	0·2842	0·1412	0·0020	0·1392
53·5	34·2	2·189	0·2436	0·1082	0·0017	0·1065
58·0	50·6	3·238	0·3608	0·1549	0·0025	0·1524
65·5	51·7	3·309	0·3687	0·1596	0·0026	0·1570
68·5	59·4	3·802	0·4233	0·1733	0·0030	0·1703
70·0	53·9	3·450	0·3841	0·1735	0·0027	0·1708
82·0	72·6	4·646	0·5172	0·2007	0·0036	0·1971
81·0	92·0	5·888	0·6556	0·2579	0·0046	0·2533
84·0	77·9	4·986	0·5551	0·2485	0·0039	0·2446
94·0	88·7	5·677	0·6323	0·1968	0·0045	0·1923
104·0	88·0	5·632	0·6270	0·2436	0·0044	0·2392
104·0	119·0	7·616	0·8486	0·3318	0·0060	0·3258
112·5	93·9	6·010	0·6689	0·2999	0·0047	0·2952
106·5	62·3	3·987	0·4440	0·1764	0·0031	0·1733
112·0	104·6	6·694	0·7455	0·3355	0·0052	0·3303

is whether or not concordance between experimental and estimated values was poorer when a fibre-containing diet was fed than when no fibre was added to the diet; i.e. whether or not there was an appreciable difference between the regression line shown in Fig. 1(a) and those shown in Figs. 1(b) and 1(c). From Fig. 1(d), in which the 3 regression lines are shown together on one graph, it is apparent that the crude fibre content of the diet caused no marked change in the trends of the respective lines. The matter was also subjected to statistical scrutiny according to Steffens's[23,24] bivariate Student t-distribution test, and no significant difference was found ($P<5\%$, two-tailed) between the 0% fibre group and either the 4·3% or the 6·4% fibre group in respect of both the slopes and the intercepts of the relevant regression lines. The experiment therefore yielded no evidence in support of the view that crude fibre has an effect on faecal N excretion in the rat.

The data pertaining to the study of the effect of cellulose content of the diet at either the 6% or the 10% egg-protein level are listed in Table V. The crucial figures in this table are (*a*) the subgroup averages of the lean body-weights of the rats at the middle of the collection period; (*b*) the average N and fibre-free dry-food intakes during the 10-day collection period; (*c*) the average cellulose intakes during the collection period; and (*d*) the average faecal N excretions (expressed per G dry food consumed) during the collection period.

Since the subgroup averages in respect of lean weight, fibre-free food intake and N intake concorded closely from one subgroup to another at each given dietary protein level, the effect of the experimental variable (cellulose intake) could be assessed by comparing the faecal N outputs per gram fibre-free dry food consumed of the different subgroups at their respective cellulose and protein intake levels.

From an inspection of these data (faecal N/G fibre-

free dry food consumed) it became apparent that at neither the 'low-egg' nor the 'high-egg' protein feeding level was there an increase in faecal N output with an increase in cellulose intake. Surprisingly, these results tended to indicate a change in the opposite direction, viz. a slight decrease in N output with a rise in cellulose consumption, suggesting that the cellulose suppressed rather than stimulated metabolic N excretion. Moreover, statistical scrutiny by means of the T-test of Terpstra[25] revealed that the downward trend in the faecal N data was highly significant ($P < 1\%$) in the case of the 'high-egg' feeding level, although not so ($P > 10\%$) in that of the 'low-egg' feeding level. The above findings therefore suggest an effect of cellulose which is the opposite to that observed by Meyer,[13] Hennig,[14] and others,[16,17] who found in their studies that metabolic N output increased in a linear fashion with a rise in cellulose intake. My results are, however, in harmony with those of Mangold and Behm[15] who in their studies with rats obtained slightly lower values for faecal N output per gram dry food when dietary cellulose content was $\geqslant 8\%$ than when the cellulose content was $< 8\%$.

Although it was hoped that my investigations would assist in confirming the view that consumption of indigestible dry matter has a consistent and specific effect on metabolic N excretion; and would provide a quantitative basis for correction of metabolic N data in protein digestibility determinations in relation to the fibre content of the test diet, it must be concluded that neither the studies reported here nor the results reported by others can as yet serve as a basis for development of a general concept of the quantitative effect of indigestible dry matter on faecal N output. Rather, it would appear that this aspect of the assessment of

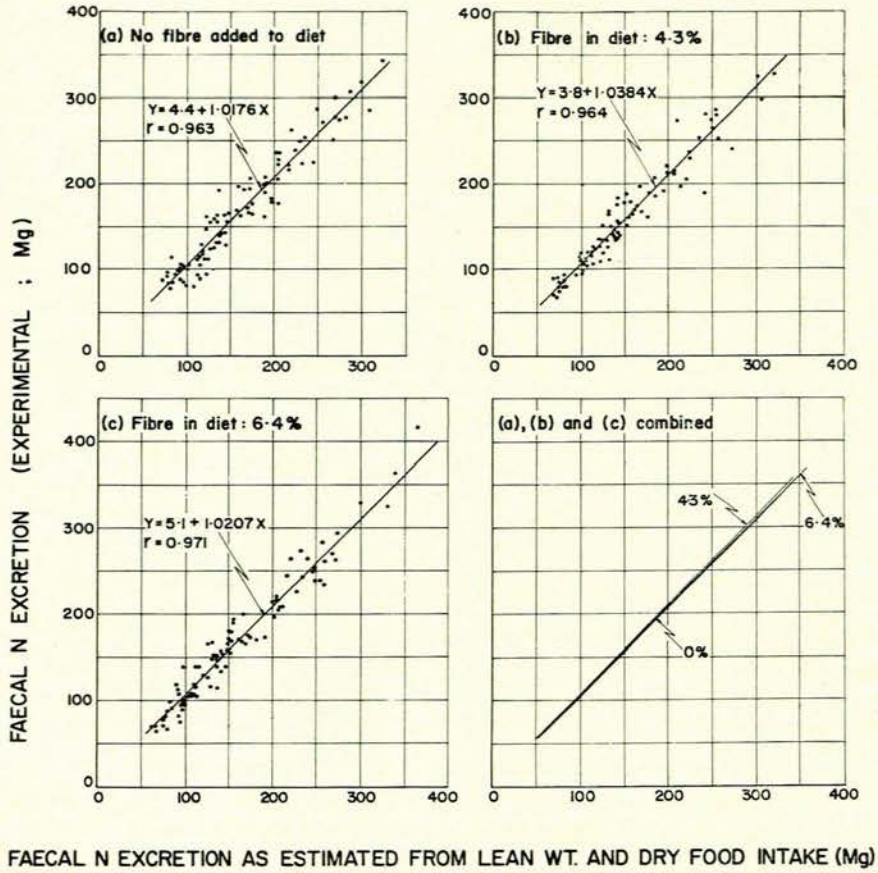

Fig. 1. Degree of concordance between data of faecal N excretion and estimates of faecal N excretion according to my equation[3] pertaining to rats fed diets containing purified bran fibre at certain levels.

TABLE V. DATA PERTAINING TO STUDY OF EFFECT OF DIETARY CRUDE FIBRE (CELLULOSE) CONTENT ON FAECAL N EXCRETION AT 'LOW' AND 'HIGH' EGG-PROTEIN INTAKE LEVELS

Rat group and % fibre content of food on dry basis	*Average lean wt of rats at middle of collection period (G)*	*Average intake per 10 days*				*Average faecal N output per 10 days (mg.)*	*Faecal N/G dry food in mg. (means ± SEM)*	*% digestibility ± SEM*
		Dietary N (G)	*Dry food (G)*	*Dry fibre (G)*	*Food plus fibre (G)*			
I (10 rats) (0%)	51·2	0·5523	46·0	—	46·0	120·0	2·61±0·10	96·2±0·801
II (9 rats)* (4·12%)	49·4	0·5537	45·9	1·9	47·8	124·4	2·71±0·09	95·6±0·688
III (10 rats) (7·91%)	50·6	0·5539	46·0	3·8	49·8	123·7	2·68±0·13	96·6±0·992
IV (10 rats) (11·45%)	50·4	0·5447	45·1	5·6	50·7	118·8	2·64±0·05	97·7±0·448
V (10 rats) (14·70%)	50·1	0·5429	44·9	7·42	52·3	117·6	2·62±0·08	98·4±0·656
VI (9 rats)* (17·8%)	51·4	0·5681	46·6	9·68	56·3	107·2	2·55±0·08	99·7±0·600
VII (10 rats) (0%)	60·5	0·9782	51·9	—	51·9	173·3	3·34±0·07	94·4±0·371
VIII (10 rats) (4·12%)	60·9	0·9957	52·4	2·16	54·6	166·9	3·20±0·10	95·6±0·557
IX (10 rats) (7·91%)	61·8	1·0118	52·8	4·32	57·1	178·3	3·37±0·06	95·2±0·243
X (10 rats) (11·45%)	61·1	1·0055	52·3	6·50	58·8	162·2	3·11±0·09	96·9±0·488
XI (10 rats) (14·75%)	62·8	1·0502	53·1	8·80	61·9	160·8	3·03±0·06	97·8±0·328
XII (10 rats) (17·80%)	60·7	1·0176	51·5	11·90	63·4	159·4	3·03±0·08	98·1±0·346

*One rat rejected during initial stage of experiment because of loss of appetite.

protein digestibility is one meriting thorough reinvestigation.

Scrutiny of the literature indicates a number of points which the prospective worker in this field might well observe. Firstly, some of the earlier results were secured under conditions which could have led to an increase in faecal N output which need not necessarily have been due to a metabolic effect of the dietary fibre consumed. Thus, for instance, in Mitchell's[3] experiments the collection of excreta was so arranged that both faeces and urine were passed onto 2 or 3 layers of filter paper, placed at the bottom of a 10-cm.-deep glass dish. It is of more than historical interest that Nevens,[26] a student of Mitchell's, had by then already recorded the fact that with the above arrangement '. . . particularly with rations containing alfalfa which resulted in the production of very bulky feces, there was evidently absorption of urine by the feces . . .'* It is reasonable to assume that, since the bulk of the faeces increases with an increase in fibre intake, and since such an increase in bulk is conducive to contamination of faeces with urinary N, the results secured according to some of the collection methods employed in the past could have created the erroneous impression that dietary fibre consistently increases metabolic faecal N output. A second point of interest is that some workers adopted the practice of oven-drying the faeces before grinding and sampling for N determination. It has been shown[27,28] that animal excreta lose appreciable quantities of N during oven-drying as well as during freeze-drying. It is conceivable that such losses could be relatively greater in the case of low-fibre droppings of small size, than in that of the bulkier droppings of a rat fed a high-fibre diet, thus erroneously indicating differences in faecal N output between 'low-fibre' and 'high-fibre' rats. A third factor which could be worthy of consideration is the N content of and the digestibility of the N in the fibrous material used. Although this factor cannot be of significance when the source of the fibre is purified cellulose, it could be important in the case of lignified materials such as straw, sawdust and seed hulls in which the nitrogenous components are protected against digestive attack, faecal N output thus being increased through the excretion of irrelevant undigested dietary N. Fourthly, the effect of indigestible matter could well depend upon the prevailing dietary conditions. In experiment A2 cellulose consumption depressed faecal N output significantly when the diet contained egg protein at the 10% level, but not significantly when the dietary protein content was 6%. Meyer,[12] using a 4% egg-protein diet, demonstrated an increase in faecal N output with increased cellulose intake. Finally, it would appear that the response of the intestinal tract to the feeding of indigestible matter could vary in accordance with the nature of the indigestible matter consumed. Reference has already been made to my earlier work[18] according to which a kaolin-pectin preparation and $BaSO_4$ had no effect on faecal N output while it could be shown that carob bean flour (Arobon) had a significant effect. The results of Adolph and Wu[1] and those of Whiting and Bezeau[17] are in harmony with this concept.

In spite of the rather confusing picture which has emerged from scrutiny of the various studies on the effect of indigestible dietary matter on faecal N excretion, it seems necessary to attempt answering the question whether or not the effects demonstrated in the present study were sufficiently great to be of practical importance in the determination of protein digestibility according to the balance method. In experiment A1 inclusion in the diet of purified bran up to a level of 6% had no appreciable effect on faecal N output, over and above that expected from consumption of a comparable weight of ordinary dry food matter. In experiment A2 cellulose had an effect in two respects, viz. (*a*) in at least one dietary context (10% egg-protein diet) cellulose suppressed the faecal excretion of N/G non-fibre dry food; and (*b*) at neither of the two protein levels studied (6% and 10% egg protein) did an increase in cellulose intake contribute towards increasing faecal N output, not even to the extent normally expected from increased consumption of an experimental diet. The results of experiment A2 therefore imply that an estimate of metabolic N—based, as is usually done, on the assumption that consumption of a specific weight of dry food matter induces excretion of a specific weight of metabolic faecal N—as well as the protein digestibility figure based on that estimate, would be higher than the true values when the diet contains cellulose. This point is illustrated by the digestibility data pertaining to the egg protein used in experiment A2, shown in the last column of Table V. The figures were calculated according to the usual definition of protein digestibility,[3] metabolic N being estimated from total dry food plus cellulose intake and lean body-weight according to my equation.

It will be seen that at both the 2 protein feeding levels the digestibility data show a slight rise with the rise in dietary cellulose content, this rise amounting roughly to about 0·2 digestibility units per 1% increase in the cellulose content. Since the fibre contents of the 10%-protein diets used in protein digestibility trials with human foods very rarely exceeded the 2% level, it therefore appears that the effect of cellulose as revealed in experiment A2 is of no practical significance in the testing of common human foods. This conclusion appears to be in harmony with the results of Meyer[12] which, although they show that, in Meyer's study, the cellulose increased faecal N output, nevertheless also indicate that the effect was a very small one, viz. in mg./G dry food consumed: about 0·015 × % fibre in the diet.

The Effect of Table Salt Intake on Faecal N Excretion

The protein contents of soup powders are usually in the order of 23% and the NaCl content often as high as 13%. A 10%-protein test ration made up for determination of the digestibility of the protein in soup powders of the above type will contain close on 6% NaCl. The extent to which a salt content of this magnitude is capable of changing the faecal N excretion of the test animal—and of thus having an effect on the result of the digestibility trial—can be gleaned from the results obtained in experiments B1 and B2 which are shown in Tables VI and VII.

It will be seen that at the 5% dietary protein level (Table VI) a change in dietary NaCl content had no appreciable effect on faecal N output per gram dry food consumed; statistical analysis according to Terpstra's

*It appears that Mitchell permitted Nevens to publish a description of the classical Mitchell method for protein evaluation before Mitchell himself published the same details some 3 years later.[3]

T-test[25] revealed no significant trend ($P > 16\%$; two-tailed) in the N excretion data, relative to a change in dietary NaCl content. There was, however, a more than 3-fold increase in water intake over the full range of NaCl intakes.

Surprisingly, the response in respect of faecal N output to an increase in NaCl intake was found to be different at the 10% dietary protein level (Table VII). In the latter case there was clearly a decrease in N output per gram dry food consumed over the range 0 - 8% NaCl in the diet. According to the T-test[25] this downward trend was statistically significant ($P < 1\%$; two-tailed), although, as can be seen from Table VII, the rats in the various treatment groups were equal in respect of body-weight, dry-food intake per unit lean body-weight, and N intake per unit lean body-weight. It would therefore appear that, unless this result was caused by some obscure experimental artefact, the NaCl, like the cellulose discussed above, suppressed faecal N output at the 10% protein level, but had no effect at the lower protein level investigated.

Although the causes of the effects of cellulose and NaCl on faecal N excretion observed in the above studies are by no means clear, the practical bearing of these findings on protein digestibility determinations is obvious. All non-protein dietary factors which have an effect on faecal N excretion can have an effect on the results of such determinations. If the effect tends towards decreasing faecal N excretion, there will be an increase in the digestibility figure and vice versa. Since, in the present study, N output decreased significantly with increased NaCl intake at the 10%-protein level, the digestibility figures obtainable from the experimental data may be expected to change in the opposite direction.

From the last column in Table VII it will be seen that there was in the NaCl study, as in the cellulose study, an upward trend in the digestibility figures* relative to an increase in dietary NaCl content. From an inspection of the figures it appears that up to the 6% NaCl level the rate of increase was about 0·4 digestibility units per 1% rise in dietary NaCl content, the rate falling to about 0·2 digestibility units at the 8% NaCl level. Although it was relatively small, the effect of NaCl content under the conditions of the studies reported here was in most cases about twice that of cellulose. It would therefore be judicious to view with caution digestibility figures obtained with experimental diets of a high NaCl content.

Consumption of Body Hair and Faecal N Excretion

Data pertaining to faecal N excretion *in toto* as well as after removal of body hair from the faeces of rats under 5 different dietary conditions are shown in Table VIII. These data were secured in experiment C1. The question to be answered is whether or not the removal of body hair from the faeces had a significant effect on the results of the determinations of faecal N excretion. Since the two rat groups under a given set of each of the 5 sets of dietary conditions correspond very closely in respect of lean weight, N intake and food intake, the above problem can be investigated on the basis of the results obtained for faecal N excretion per G dry food consumed (see penultimate column of Table VIII).

It will be seen that under 3 of the 5 conditions studied the values obtained after removal of the hair were from about 5 to 9% lower than the corresponding control values. Under the remaining 2 conditions the discrepancies were of approximately the same order of magnitude, but they pointed in an opposite direction, viz. higher values in spite of removal of the hair. Moreover, statistical scrutiny

*Calculated according to method described earlier in 'Discussion'.[1]

TABLE VI. EFFECT OF NaCl CONSUMPTION LEVEL ON H_2O CONSUMPTION AND FAECAL N EXCRETION PER G DRY FOOD CONSUMED AT A LOW (5%) DIETARY EGG-PROTEIN LEVEL

Rat group and % added NaCl in diet on air-dry basis	Average lean wt of rats at middle of collection period (G)	Average intake per 10 days: Dietary N (G)	Dry food (G)	NaCl (G)	H_2O (G)	Daily intake / Lean wt ×100: Dry food (G)	Dietary N (mg.)	Average faecal N output per 10 days (mg.)	Faecal N/G dry food in mg. (means ± SEM)
I (10 rats) (0%)	50·5	0·4563	50·2	0·161	60·2	9·94	90·4	134·3	2·68±0·05
II (10 rats) (2%)	48·8	0·4121	45·1	1·108	94·5	9·25	84·5	128·8	2·86±0·06
III (10 rats) (4%)	47·8	0·3870	43·1	1·978	127·0	9·01	80·9	112·9	2·63±0·10
IV (10 rats) (6%)	48·0	0·3796	42·0	2·817	162·1	8·77	79·2	106·5	2·54±0·11
V (10 rats) (8%)	46·3	0·3776	41·9	3·693	206·3	9·06	81·6	112·6	2·68±0·09

TABLE VII. EFFECT OF NaCl CONSUMPTION LEVEL ON H_2O CONSUMPTION, FAECAL N EXCRETION PER G DRY FOOD CONSUMED, AND PROTEIN DIGESTIBILITY AT A HIGH (10%) DIETARY EGG-PROTEIN LEVEL

Rat group and % added NaCl in diet on air-dry basis	Average lean wt of rats at middle of collection period (G)	Average intake per 10 days: Dietary N (G)	Dry food (G)	NaCl (G)	H_2O (G)	Daily intake / Lean wt ×100: Dry food (G)	Dietary N (mg.)	Average faecal N output per 10 days (mg.)	Faecal N/G dry food in mg. (means ± SEM)	Digestibility of egg protein (% ± SEM)
I (10 rats) (0%)	57·6	0·9706	55·1	0·1781	80·11	9·55	168·4	0·1708	3·10±0·11	94·9±0·556
II (10 rats) (2%)	57·7	0·9815	55·4	1·3832	134·3	9·61	170·2	0·1610	2·90±0·06	96·0±0·307
III (10 rats) (4%)	57·0	0·9599	54·5	2·5157	183·3	9·56	168·5	0·1512	2·78±0·09	96·7±0·483
IV (10 rats) (6%)	56·5	0·9587	54·4	3·6767	238·0	9·62	169·6	0·1440	2·64±0·10	97·4±0·556
V (9 rats)* (8%)	55·2	0·9278	53·3	4·7234	283·9	9·64	168·0	0·1470	2·76±0·13	96·6±0·678

*One rat accidentally injured during collection period.

TABLE VIII. DATA ON N PER G DRY FOOD CONSUMED AND PROTEIN DIGESTIBILITY FIGURES OBTAINED WITHOUT AND AFTER REMOVAL OF BODY HAIR FROM FAECES

Rat group (6 rats per group)	*Protein and concentration in diet*	*Faeces sifted (hair removed)*	*Average lean wt of rats at middle of collection period (G)*	*Average intake per 10 days**		*Daily intake / Lean wt × 100*		*Average faecal N output per 10 days (mg.)*	*Faecal N/G dry food in mg. (means ± SEM)*	*Digestibility of protein (% ± SEM)*
				Dietary N (G)	*Dry food (G)*	*Dry food (G)*	*Dietary N (mg.)*			
Ia	Nil	No	36·6	0·0306	22·9	6·28	8·4	44·9	1·95±0·03	—
Ib	Nil	Yes	34·8	0·0286	21·4	6·14	8·2	39·3	1·84±0·07	—
IIa	Gluten (10%)	No	42·5	0·5939	32·2	7·58	139·7	76·7	2·39±0·03	98·3±0·480
IIb	Gluten (10%)	Yes	42·6	0·6280	34·1	8·03	147·9	77·1	2·27±0·06	98·9±0·784
IIIa	Soy-bean (10%)	No	56·6	1·0167	56·6	10·00	179·6	180·6	3·19±0·04	94·3±0·481
IIIb	Soy-bean (10%)	Yes	58·2	1·0461	58·2	10·01	179·9	188·1	3·24±0·06	94·2±0·735
IVa	Casein (10%)	No	60·6	1·0798	60·7	10·04	178·7	144·3	2·39±0·05	99·0±0·745
IVb	Casein (10%)	Yes	60·3	1·0820	60·8	10·09	179·5	134·2	2·20±0·03	100·1±0·358
Va	Egg (10%)	No	64·4	1·1003	62·9	9·76	170·7	184·6	2·93±0·02	96·2±0·209
Vb	Egg (10%)	Yes	63·2	1·0638	60·8	9·62	168·3	195·2	3·21±0·07	94·6±0·799

*Calculated according to method mentioned in 'Discussion'.[1]

of the data by means of the Mann-Whitney U-test[29] revealed no significant differences in any of the 5 cases investigated, the probabilities of the occurrence of the observed differences under the nul hypothesis being: group I (protein-free diet) > 50%; group II (gluten) 50%; group III (soy-bean protein) > 50%; group IV (casein) 21%; and group V (egg protein) > 50%. It therefore appears that, although there can be no doubt that the removal of body hair from the faeces will increase the accuracy of faecal N data, the amounts of hair consumed by the rats in the study reported here were so small that significant effects on faecal N excretion could not be demonstrated.

Since the series of experimental diets used ranged in protein quality over the full scale, viz. from a protein-free diet to one containing egg protein at the 10% level, it can be said that experiment C1 was representative of most of the dietary conditions that can possibly be encountered in the routine testing of protein digestibility. As the elimination of body hair did not have a significant effect in any of the 5 trials, it seems reasonable to conclude that contamination of the faeces with body hair need not present a major technical hazard in protein digestibility determinations. It will, however, be judicious always to bear in mind Schneider's observation[19] regarding increased hair consumption when food supply is severely restricted, and to inspect the experimental animals regularly for signs of trichophagy with a view to eliminating its effect on the faecal N data.

SUMMARY

Investigations were made regarding the effects on faecal N output and the concomitant effects on protein digestibility data of (*a*) the crude fibre and (*b*) the NaCl contents of the experimental diet, and (*c*) consumption of body hair (trichophagy) by the experimental animal.

(*a*) Two experiments were done. In experiment 1 three groups of 90 rats each, were fed diets varying in respect of their contents of specially-prepared low-N wheaten bran fibre. Faecal N outputs, mean lean body-weights, dry-food intakes and N intakes were determined during a 10-day balance period following a 7-day preliminary period. In general the estimates of faecal N output concorded closely with the experimental data at each dietary fibre level. The experiment therefore yielded no evidence supporting the concept that crude fibre consumption stimulates metabolic N excretion.

In experiment 2 the effect of pure cellulose was investigated at 2 dietary egg-protein levels. For each protein level there were 6 cellulose levels, the latter ranging from 0% to about 18% over the various diets. The experimental animals were 120 young rats divided into 12 subgroups of 10 rats each. Each subgroup received a diet of specific protein and cellulose content for a 7-day preliminary and a 10-day balance period, experimental data being secured during the latter period. The results showed no increase in faecal N excretion per G dry, fibre-free food consumed relative to an increase in cellulose intake. At the 10% dietary protein level increase in cellulose intake suppressed faecal N excretion per G dry food consumed to a statistically significant degree, thus causing a concomitant rise in the digestibility figures for the egg protein to the extent of about 0·2 units per 1% rise in dietary cellulose content.

It appeared that the effect of crude fibre consumption on the results of protein digestibility determinations is relatively small, but that the effects of indigestible dietary ingredients on metabolic N excretion in general remains a subject meriting further investigation.

(*b*) The effect of NaCl intake was investigated at 2 dietary egg-protein levels. Five subgroups of 10 young rats each were fed a diet of a particular protein content, dietary NaCl content being varied from subgroup to subgroup. Experimental data were secured during the last 10 days of a 7 + 10-day balance study. It was found that H_2O consumption was trebled by a rise in dietary NaCl content of up to 8%, but that faecal N output per G dry food consumed was changed significantly by increased NaCl intake only at the higher dietary protein level.

The results suggested that it would be judicious to view with caution protein digestibility figures obtained with diets of high NaCl content.

(*c*) The effect of trichophagy was studied with the aid of 5 main groups of young rats, each consisting of 2 equal subgroups of 6 rats each, fed a diet of a specific protein value.

The study entailed a comparison of the faecal N data of the one subgroup with those of the other subgroup, the relevant data being secured in the one case after removal of body hair from the faeces and in the other without removal of hair from the faeces.

Separation of hair from faeces yielded N excretion data which were not significantly different from those obtained without removal of body hair. It was therefore concluded that trichophagy is not a major technical hazard in the routine determination of protein digestibility.

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