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A STUDY OF METHODS OF ESTIMATION OF METABOLIC NITROGEN

By E. B. FORBES, C. E. MANGELS, and L. E. MORGAN,
Ohio Agricultural Experiment Station

INTRODUCTION

The so-called metabolic nitrogen of the feces is that portion which has an origin other than as an undigested food residue. It consists of residues from the bile and digestive juices, of epithelium and mucus from the digestive tract, and of such products of bacterial activity as have been derived from digested or from digestible nitrogen.

Our reason for wishing to estimate this fraction of the nitrogen of the feces is that it is a factor which must be considered in the determination of the digestibility of protein—a matter of great importance in relation to practical animal and human nutrition.

The plan of this experiment was to feed a basal ration of corn alone to each of five pigs during the first period, and to add to this corn ration in subsequent periods nitrogenous supplements to be used in the comparison of methods. In the selection of these supplements it was our object to choose foods the protein of which would probably be entirely digestible. Those used were milk, blood albumen, and commercial dried egg albumen.

In the comparison of methods of metabolic-nitrogen estimation it was our object to determine which procedure would yield results representing these assumedly entirely digestible protein foods as being entirely digestible—that is, assuming the proteins of milk, for instance, to be entirely digestible, we made an effort to determine which method of estimation of metabolic nitrogen would assign to the protein of milk a digestion coefficient nearest to 100 per cent.

An experimental study involving so much assumption can not yield results of the highest value, but it was our hope that it might assist in the establishment of a useful conventional procedure.

The methods of metabolic-nitrogen estimation compared in this study were the acid-pepsin method, the acid-pepsin and alkaline-pancreatin method, and the alcohol, ether, hot-water, and cold-lime-water method suggested in 1888 by Jordan.[1]

The philosophy of the two methods first mentioned is that by the use of digestive enzyms the nitrogen which has been digested, absorbed, and returned to the feces may be separated from the indigestible nitrogen. In using either of these methods we assume that there is no further digestion, during the course of the estimation, of that part of the food protein which escaped digestion in the alimentary tract of the experimental subject. We have no means of proving the truth of this assumption.

The acid-pepsin method represents stomach digestion alone. The acid-pepsin and alkaline-pancreatin method more nearly follows the physiological process, in that intestinal digestion is also represented. The latter method naturally yields decidedly higher results.

In the Jordan method the treatment with solvents is designed especially for the purpose of washing out bile residues, protein cleavage products and mucin.

The exact procedures followed in the three methods are as follows:

ACID-PEPSIN METHOD

Weigh out 5-gm. samples of fresh feces from a weighing bottle; roll up in 9 cm. filter papers, and transfer to 200-c. c. volumeteric flasks. Add 100 c. c. of pepsin-hydrochloric-acid solution (made by adding 1.25 gm. of pepsin to each liter of 0.33 per cent hydrochloric-acid solution). Shake thoroughly and put into an air bath maintained at 38° to 40° C. Allow the digestion to continue for 24 hours. During the first 6 hours agitate by rotation once each hour; agitate again 1 hour before final removal from the air bath. Arrange funnels with 12.5 cm. fluted quantitative papers, and dry 100-c. c. volumetric flasks. Promptly at 24 hours from the time of starting the digestion remove the 200-c. c. flasks from the oven, cool, fill to the mark with cold distilled water, mix thoroughly, and filter. Determine the nitrogen in 100 c. c. of the filtrate. The result represents metabolic nitrogen.

ACID-PEPSIN AND ALKALINE-PANCREATIN METHOD

Weigh 1.5 to 2.5 gm. samples of fresh feces into 150 c. c. Jena beakers. Add 100 c. c. of acid-pepsin solution (1.25 gm. of pepsin to each liter of 0.33 per cent hydrochloric acid). Stir thoroughly with a glass rod and place in an air bath maintained at 38° to 40° C. Stir thoroughly once each hour for the first 8 hours. Allow the digestion to continue for exactly 24 hours. Filter immediately through 12.5 cm. fluted quanti-

[1] [Jordan, W. H.] Analytical and experimental methods. Protein digestion. *In* Maine Agr. Exp. Sta. Ann. Rpt. 1888, p. 197. 1889.

tative filters. Wash beakers, filters, and contents until free from acid, with water at a temperature of 40° C.

Return filters and contents to the proper beakers and treat with 100 c. c. of alkaline-pancreatin solution (1.5 gm. pancreatin in somewhat less than 1 liter of water; add 3 gm. of sodium carbonate (Na_2CO_3); dilute to exactly 1 liter, and mix thoroughly). Return the beakers to the bath and stir thoroughly. Allow to digest for exactly 12 hours. Filter immediately through fluted papers. Wash beakers, filters, and contents thoroughly and repeatedly with hot water, and allow to dry. Transfer the filters and contents to Kjeldahl flasks and determine the nitrogen in the usual manner. Subtract the result from total nitrogen of the feces; the remainder represents metabolic nitrogen.

JORDAN'S METHOD[1]

Weigh 2 to 3 gm. portions of fresh feces, and dry at 100° to 105° C.; transfer to extraction capsules and extract with ether for 16 hours. Transfer to 150-c. c. beakers and treat with 50 c. c. of boiling 95 per cent alcohol. Keep at boiling temperature for 10 minutes; decant the alcoholic extract through qualitative filters; wash several times with hot alcohol and once or twice with ether, by decantation. With a camel's-hair brush transfer the residue from the filter papers to the original beakers; add 50 c. c. of hot water and boil for 10 minutes; filter through the same papers used for the last filtration, washing with hot water, by decantation. Wash the residues from the filter papers back into the beakers with 50 c. c. of a saturated solution of calcium hydrate, and let stand for 6 hours; filter through the same filters last used; transfer all the material from the beakers to the filter papers; wash with lime water, and allow to drain. Transfer filter papers and contents to Kjeldahl flasks, and determine the nitrogen. Subtract result from total nitrogen of the feces; the remainder represents metabolic nitrogen.

EXPERIMENTAL PROCEDURE

The subjects of this experiment were five Yorkshire barrows of nearly uniform age and weight. The average weight at the end of the first period was 53.85 kgm., and at the end of the fourth, 59 days later, 84.42 kgm., the average daily gain in weight being 518 gm., or 1.14 pounds. They were confined in the metabolism crates illustrated in our previous publications.[2]

[1] Jordan, W. H., Op. cit. (Detailed specifications were not submitted in the original publication; the particulars as here stated were arbitrarily assumed.)

[2] Forbes, E. B., Beegle, F. M., and others. A chemical study of the nutrition of swine. Ohio Agr. Exp. Sta. Bul. 271, p. 224–261, 3 pl. 1914.

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The experimental periods were of 10 days' duration, separated by 7-day intervals during which were fed the rations of the periods to follow. The feces were marked with carmine.

Table I records the total amounts and nitrogen content of the foods consumed and feces produced.

Table II records the percentages of total nitrogen in the feces and of metabolic nitrogen, as estimated by the three different methods.

Table III records the coefficients of digestibility of the nitrogen of the foods.

TABLE I.—*Foods consumed and total nitrogen in foods and feces*

Period No. (10 days).	Pig No.	Foods consumed.		Nitrogen in foods.		Weight of feces.	Total nitrogen of feces.	
		Corn.	Supplements.	Corn.	Supplements.			
		Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Per cent.*	*Gm.*
I.....	1	18,200	.....	245.700	.....	6,357	0.995	63.252
	2	19,731	.....	266.373	.....	7,553	.889	67.146
	3	20,000	.....	270.000	.....	7,954	.989	78.665
	4	18,000	.....	243.000	.....	5,866	1.005	58.953
	5	16,800	.....	226.800	.....	5,645	1.000	56.450
			Blood albumen.		Blood albumen.			
II.....	1	16,200	730	215.298	83.987	5,737	.888	50.945
	2	18,000	810	239.220	93.191	6,144	.785	48.230
	3	19,000	855	252.510	98.368	6,710	1.006	67.503
	4	17,100	770	227.259	88.589	5,428	.892	48.418
	5	17,100	770	227.259	88.589	5,226	.943	49.281
			Skim milk.		Skim milk.			
III....	1	17,100	21,400	230.679	111.708	5,857	1.082	63.373
	2	19,000	23,800	256.310	124.236	6,566	.900	59.094
	3	19,000	23,800	256.310	124.236	6,936	1.059	73.452
	4	17,100	21,400	230.679	111.708	5,672	1.066	60.464
	5	17,100	21,400	230.679	111.708	5,529	1.060	58.607
			Egg albumen.		Egg albumen.			
IV.....	1	17,100	770	234.441	87.226	5,129	1.226	62.882
	2	19,000	855	260.490	96.854	5,847	.989	57.827
	3	19,000	855	260.490	96.854	6,263	1.346	84.300
	4	17,100	770	234.441	87.226	5,410	1.177	63.676
	5	17,100	770	234.441	87.226	5,371	1.070	57.470

TABLE II.—*Total and metabolic nitrogen of feces (per cent)*

Periods (10 days).	Pig No.	Total nitrogen.	Metabolic nitrogen.		
			Pepsin-hydrochloric acid method.	Pepsin-pancreatin method.	Jordan's method.
I	1	0.995	0.706	0.836	0.473
	2	.889	.619	.760	.418
	3	.989	.701	.834	.468
	4	1.005	.656	.816	.447
	5	1.000	.741	.858	.445
II	1	.888	.585	.749	.422
	2	.785	.581	.677	.404
	3	1.006	.773	.857	.446
	4	.892	.641	.738	.368
	5	.943	.712	.820	.380
III	1	1.082	.714	.831	.426
	2	.900	.590	.693	.347
	3	1.059	.739	.857	.392
	4	1.066	.704	.814	.416
	5	1.060	.691	.859	.420
IV	1	1.226	.802	.965	.525
	2	.989	.670	.761	.451
	3	1.346	.935	1.123	.599
	4	1.177	.729	.949	.565
	5	1.070	.742	.840	.433

TABLE III.—*Coefficients of digestibility of nitrogen*

Period No. (10 days).	Pig No.	Apparent digestibility.[a]	Pepsin-hydrochloric acid method.	Pepsin-pancreatin method.	Jordan's method.
I (corn)	1	74.26	92.52	95.89	86.49
	2	74.79	92.34	96.34	86.64
	3	70.86	91.52	95.43	84.65
	4	75.74	91.58	95.44	86.53
	5	75.11	93.55	96.47	86.19
II (blood albumen)	1	105.33	98.48	101.04	102.80
	2	112.96	106.22	102.27	109.18
	3	106.18	105.87	101.57	101.20
	4	107.58	106.22	102.26	102.45
	5	108.22	102.92	101.80	102.21
III (skim milk)	1	96.42	96.15	95.33	93.50
	2	104.44	99.42	96.61	98.34
	3	100.00	99.63	98.15	94.43
	4	95.97	99.01	96.62	94.81
	5	98.93	95.06	97.34	96.84
IV (egg albumen)	1	97.09	95.17	95.70	95.09
	2	108.10	101.34	96.08	103.45
	3	91.33	96.23	97.87	92.98
	4	92.20	94.84	98.12	98.25
	5	101.01	97.14	95.33	97.89

[a] On basis of total nitrogen of the feces.

CONCLUSIONS

The apparent digestibility of the protein of corn, based on the total nitrogen of the feces is about 75 per cent. On account of the existence in the feces of nitrogen of metabolic origin we know that the real digestibility is higher. The acid-pepsin method makes it appear that the real digestibility of the protein of corn is about 92 per cent, and the pepsin-pancreatin method about 96 per cent. Jordan's method gives appreciably lower figures, averaging 86 per cent.

The acid-pepsin method indicates that 70 per cent, the pepsin-pancreatin method 84 per cent, and the Jordan method 46 per cent of the nitrogen of the feces from corn is of metabolic origin.

All of the methods make the nitrogen of blood albumen appear more than completely digestible, even the apparent digestibility being over 100 per cent; thus, the feeding of blood albumen with corn seems to increase the digestibility of the corn protein to an extent more than sufficient to offset the incompleteness of digestibility of the protein of this supplement.

With skim milk the apparent digestibility varies from 95.97 to 104.44 per cent, the average being 99.15. With the acid-pepsin method three out of the five figures average 99.35. In previous work [1] five estimations by this method averaged 99.12. With the pepsin-pancreatin method the results were lower than with the acid-pepsin method. These low results on the supplementary food are reciprocals of the high results on the basal ration of corn.

The proteins of skim milk are made to appear more nearly completely digestible by the acid pepsin method than by the pepsin-pancreatin method or by the Jordan method.

With egg albumen the results varied considerably, but all were high. It would appear that raw, commercial, dried egg albumen is almost perfectly digested by swine.

Important inaccuracy seems to be inevitable in any determination of digestibility of supplementary foods in the usual way, by difference; and no other method seems more satisfactory. This applies equally to computations of real digestibility, and of apparent digestibility (based on total nitrogen of the feces).

The digestion coefficients for protein involved in the feeding standards of our reference works on animal production assume that the nitrogen of the feces is entirely an indigestible food residue. The rough measures afforded by the results of this study indicate that, as applying to the digestive capacities of swine, this assumption underestimates the digestibility of protein by about 20 per cent.

By way of interpretation of the individual variations in the digestion coefficients we would record the fact that pig 1, in Period II, manifested

[1] Forbes, E. B., Beegle, F. M., and others. Op. cit.

a pronounced dislike for the blood albumen. The acid-pepsin method indicates that this pig was less able than others to digest this foodstuff. Also, we would observe that, in response to most insistent demands for food, we gave to pig 5 in Period II a larger allowance of food per unit of body weight than was given to the other individuals. The digestion coefficient for blood albumen, as determined by the acid-pepsin method, with this pig also is low. Further, these two pigs, No. 1 and 5, had the lowest digestion coefficients, as determined by the acid-pepsin method, in the following period, No. III, where skim milk was fed.

In a study of the effects on metabolic nitrogen of storage of the feces in a frozen condition for 20 days, with and without the addition of thymol, compared with air-drying the fresh material, with and without thymol, no significant differences were observed which could be related to these methods of preservation.

In attempting to choose between these methods it seems to us that the acid-pepsin and the pepsin-pancreatin methods give results which are more nearly true than does Jordan's method, since the latter does not digest the bacteria, which may contain large proportions of the nitrogen of the feces and which presumably are more largely the product of digestible than of indigestible protein; but it is idle to attempt close comparisons of such conventional and inaccurate procedures. We have no accurate scientific basis for the determination of the digestibility of protein.