[FROM THE DEPARTMENT OF ANIMAL HUSBANDRY OF THE UNIVERSITY OF ILLINOIS.]

THE QUANTITATIVE DETERMINATION OF THE AMINO ACIDS OF FEEDINGSTUFFS BY THE VAN SLYKE METHOD.

[SECOND PAPER.]

BY H. S. GRINDLEY AND M. E. SLATER
with the collaboration of H. C. ECKSTEIN AND J. C. ROSS.

Received October 4, 1915

Introduction.

In a previous paper[1] we have reported quantitative determinations of the amino acids of feedingstuffs made by the Van Slyke method. Additional results are presented in this paper. For easy reference and comparison, the analytical data for the three feeds—cottonseed meal, tankage, and alfalfa hay—previously reported are also included in Tables I and II, given below. The duplicate or triplicate results reported for the same feedingstuff were obtained from independent hydrolyses of the same sample. The figures for each individual constituent represents the average of two, three, or more determinations upon the same hydrolyzed solution.

Since the publication of our first paper, Nollau,[2] of the Kentucky Agricultural Experiment Station, has published a paper giving the amino-acid content of certain commercial feedingstuffs. On the whole, the results from the two laboratories do not agree well. In some determinations the results from the two sources are quite satisfactory, but in many cases the agreement is far from satisfactory. The lack of concordant results is due, in part at least, to differences in the details of procedure.

In the first place, Nollau removed the fat by extracting the finely ground feedingstuff with ether. In our work we did not remove the fat except in the case of tankage. It is impossible to say definitely, at the present time, how this difference in procedure would lead to differences in the amino-acid determinations.

In the second place, Nollau, in most of the feedingstuffs, filtered the hydrolyzed solution to remove the solid residue before concentrating under reduced pressure. The syrupy residue that was left after removing the excess of hydrochloric acid was dissolved in water, and the solution diluted to 250 cc. The total nitrogen determined in this solution was the basis for the calculation of the final results for the distribution of the nitrogen. By this method part of the total nitrogen of the feedingstuffs was removed and the quantity thus discarded was not determined. This procedure would lead to low humin-nitrogen figures and correspondingly high values for the remaining forms of nitrogen, considered on the basis of the total nitrogen of the feedingstuffs. Further, this method of analysis fails

[1] H. S. Grindley, W. E. Joseph and M. E. Slater, THIS JOURNAL, 37, 1778 (1915).
[2] *J. Biol. Chem.*, 21, 611 (1915).

to give results for the distribution of the total nitrogen in the feedingstuffs, and, therefore, such results cannot be used to calculate the nitrogen of the amino acids in terms of the feedingstuffs, since an undetermined part of the nitrogen of the samples was discarded.

By our method of procedure the hydrolyzed solution was not filtered before removing the excess of hydrochloric acid, or before determining the total nitrogen, the ammonia nitrogen, or the humin nitrogen. Therefore, we were able to calculate the nitrogen of the amino acids in per cent. of the feedingstuffs.

In the third place, there was evidently a difference in the details of the method for the determination of the organic sulfur in the two laboratories, for the cystine values of Nollau are two to four times greater than ours. These differences in the cystine values often, but not in all cases, make Nollau's lysine values considerably less than those obtained in this laboratory.

In the fourth place, the results for the nonamino nitrogen of the filtrate, as obtained in the two laboratories, clearly indicate a difference in the method of procedure in this determination. The nature of the difference is not evident at present.

Notwithstanding these marked discrepancies between the amino-acid determinations of the two laboratories, we feel confident from the results so far obtained that the Van Slyke method for the determination of the chemical groups characteristic of the amino acids of proteins will prove of much value as applied to the quantitative determination of the free and combined amino acids and amides of feedingstuffs when we become better acquainted with the details of the method, and succeed in adapting it to the specific conditions involved. At any rate, as yet, it is the only method that has been used for the determination of the amino-acid content of feedingstuffs, and, since it undoubtedly gives approximately quantitative results, it should, until some better method is developed, be used in gaining knowledge, that will aid us in applying to the economic and nutritive valuation of the common feedingstuffs, the fast accumulating results as to the nutritive value of the amino acids.

In this connection it may be well to call attention again to the fact that the object of the work reported in this paper and the preceding paper is to determine the free and the combined amino acids and the free and combined amides of feedingstuffs, and not merely the amino acids and the amides resulting from the hydrolysis of the proteins of the feedingstuffs.

Variations in the Percentages of Amino Acids in Feedingstuffs.

There are evidently marked variations in the free and combined amino-acid content of the common feedingstuffs. This variation can be clearly and readily seen from Table III, which gives the order of the feedingstuffs

according to their increasing content of the different forms of the nitrogen, determined by the Van Slyke method and expressed in percentage of the total nitrogen of the feedingstuff.

TABLE I.—THE NITROGEN OF THE AMINO ACIDS OF FEEDINGSTUFFS.

Results Expressed in Percentage of the Total Nitrogen of the Feedingstuffs.

Feedingstuff.	Ammonia N.	Humin N.	Argi-nine N.	Cys-tine N.	Histi-dine N.	Lysine N.	Amino N. in filtr. from bases.	Non-amino N. in filtr. from bases.	Total N. by summa-tion.
Blood meal.....	5.98	3.90	9.47	0.70	8.45	9.89	55.21	4.73	98.33
Blood meal.....	5.74	3.81	8.97	0.68	8.52	9.59	57.29	4.32	98.92
Blood meal.....	5.83	3.83	9.04	0.68	8.63	9.70	57.20	4.21	99.12
Average.....	5.85	3.95	9.16	0.69	8.53	9.73	56.57	4.42	98.80
Tankage.....	6.52	4.40	14.38	1.27	5.15	7.52	52.36	7.27	98.87
Tankage.....	6.56	4.50	14.18	1.24	4.76	7.64	52.57	7.17	98.62
Tankage.....	6.66	4.30	13.88	1.32	4.91	7.28	52.24	7.38	97.97
Average.....	6.58	4.40	14.15	1.28	4.94	7.48	52.39	7.27	98.49
Wheat.....	17.71	9.24	7.72	1.37	1.71	2.56	46.81	12.32	99.44
Wheat.....	17.48	8.98	7.89	1.33	1.77	2.44	48.50	13.88	102.27
Wheat.....	17.57	9.40	8.37	1.32	1.54	2.41	47.69	14.58	102.88
Average.....	17.59	9.21	7.99	1.34	1.67	2.47	47.67	13.59	101.53
Rolled wheat.....	17.02	9.08	8.10	1.64	3.29	2.42	47.89	13.86	103.30
Rolled wheat.....	17.05	8.99	8.29	1.67	3.12	2.53	47.51	14.04	103.20
Average.....	17.04	9.04	8.20	1.66	3.21	2.48	47.70	13.95	103.28
Barley.....	15.21	8.79	9.16	1.16	3.77	1.64	45.82	13.86	99.41
Barley.....	15.11	8.78	9.75	1.36	3.51	2.73	45.80	13.81	100.85
Average.....	15.16	8.79	9.46	1.26	3.64	2.19	45.81	13.84	100.15
Oats.....	12.98	9.94	11.46	1.18	4.42	3.36	51.27	7.85	102.46
Oats.....	13.13	9.93	11.37	1.14	4.22	3.61	52.17	7.94	103.51
Average.....	13.06	9.94	11.42	1.16	4.32	3.49	51.72	7.90	103.01
White soy beans..	10.15	6.56	12.74	0.66	5.88	6.08	49.71	8.66	100.41
White soy beans..	10.09	6.69	12.60	0.67	5.65	6.20	49.86	8.45	100.21
Average.....	10.12	6.63	12.67	0.67	5.77	6.14	49.79	8.56	100.35
Cottonseed meal..	10.46	7.65	19.33	0.62	5.28	5.81	42.61	5.74	97.50
Cottonseed meal..	10.30	7.78	19.49	0.65	4.90	4.33	42.04	5.30	94.79
Cottonseed meal..	10.59	7.91	19.74	0.67	6.23	4.21	43.80	5.26	98.41
Average.....	10.45	7.78	19.52	0.65	5.47	4.78	42.82	5.43	96.90
Alfalfa hay.....	8.46	15.54	7.72	0.79	7.39	4.08	43.88	10.14	98.00
Alfalfa hay.....	8.42	16.03	7.64	0.97	7.49	4.12	44.15	9.43	98.15
Average.....	8.44	15.79	7.68	0.88	7.44	4.10	44.02	9.79	98.14

The Humin-Nitrogen Content of Feedingstuffs.

From even a casual inspection of the results for the amino acids of feedingstuffs, given in Table I, it is evident that the humin-nitrogen results are unusually high, with the exception of those for blood meal and tank-

TABLE II.—THE NITROGEN OF THE AMINO ACIDS OF FEEDINGSTUFFS.

Results Expressed in Percentage of the Feedingstuff.

Feedingstuff.	Ammonia. N.	Humin. N.	Arginine. N.	Cystine. N.	Histidine. N.	Lysine. N.	Amino N in filtr. from bases.	Non-amino N in filtr. from bases.	Total N by summation.	Total N by analysis.
Blood meal.....	0.837	0.546	1.325	0.098	1.041	1.383	7.724	0.662	13.616	13.988
Blood meal.....	0.803	0.534	1.255	0.095	1.191	1.342	7.907	0.604	13.731	13.988
Blood meal.....	0.815	0.536	1.264	0.095	1.305	1.356	7.982	0.589	13.942	13.988
Average.....	0.818	0.539	1.281	0.096	1.179	1.360	7.871	0.618	13.762	13.988
Tankage.....	0.653	0.440	1.439	0.128	0.516	0.753	5.238	0.729	9.896	10.013
Tankage.....	0.657	0.451	1.420	0.124	0.477	0.765	5.264	0.718	9.876	10.013
Tankage.....	0.667	0.430	1.390	0.132	0.492	0.729	5.213	0.739	9.782	10.013
Average.....	0.659	0.440	1.416	0.128	0.495	0.749	5.238	0.729	9.854	10.013
Wheat.....	0.383	0.200	0.167	0.030	0.037	0.055	1.010	0.266	2.148	2.160
Wheat.....	0.378	0.194	0.171	0.029	0.038	0.053	1.048	0.300	2.211	2.160
Wheat.....	0.380	0.203	0.181	0.029	0.033	0.056	1.030	0.315	2.227	2.160
Average.....	0.380	0.199	0.173	0.029	0.036	0.055	1.029	0.294	2.195	2.160
Rolled wheat.....	0.276	0.147	0.131	0.027	0.054	0.040	0.774	0.225	1.674	1.620
Rolled wheat.....	0.276	0.146	0.134	0.027	0.050	0.041	0.770	0.228	1.672	1.620
Average.....	0.276	0.147	0.133	0.027	0.052	0.041	0.772	0.227	1.675	1.620
Barley.....	0.360	0.208	0.217	0.027	0.089	0.039	1.085	0.328	2.353	2.368
Barley.....	0.358	0.208	0.231	0.032	0.083	0.065	1.085	0.327	2.389	2.368
Average.....	0.359	0.208	0.224	0.030	0.086	0.052	1.085	0.328	2.372	2.368
Oats.....	0.285	0.218	0.252	0.026	0.097	0.074	1.126	0.173	2.251	2.196
Oats.....	0.289	0.218	0.250	0.025	0.093	0.080	1.146	0.174	2.275	2.196
Average.....	0.289	0.218	0.251	0.026	0.095	0.077	1.136	0.174	2.264	2.196
White soy beans..	0.579	0.374	0.727	0.037	0.335	0.347	2.834	0.494	5.727	5.702
White soy beans..	0.576	0.381	0.725	0.038	0.322	0.354	2.843	0.482	5.721	5.702
Average.....	0.578	0.378	0.726	0.038	0.329	0.351	2.839	0.488	5.727	5.702
Cottonseed meal..	0.702	0.514	1.298	0.041	0.355	0.390	2.862	0.385	6.547	6.694
Cottonseed meal..	0.692	0.522	1.309	0.044	0.329	0.291	2.824	0.356	6.367	6.694
Cottonseed meal..	0.711	0.531	1.326	0.045	0.418	0.283	2.941	0.353	6.608	6.694
Average.....	0.702	0.522	1.311	0.043	0.367	0.321	2.876	0.365	6.507	6.694
Alfalfa hay.....	0.221	0.408	0.203	0.021	0.194	0.107	1.160	0.248	2.553	2.628
Alfalfa hay.....	0.222	0.421	0.201	0.023	0.197	0.108	1.153	0.266	2.593	2.628
Average.....	0.222	0.415	0.202	0.022	0.196	0.108	1.157	0.257	2.579	2.628

age, as compared with those for the pure isolated proteins. The highest humin nitrogen reported by Van Slyke[1] for pure proteins was 3.6% in the case of ox hemoglobin. Hartley[2] reported 2.5% of humin nitrogen in euglobulin of ox serum. Our results for humin nitrogen in feedingstuffs, excepting blood meal and tankage, are from two to four and one-

[1] *J. Biol. Chem.*, **10**, 54 (1911).

[2] *Biochem. J.*, **8**, 543 (1914).

TABLE III.—THE ORDER OF THE FEEDINGSTUFFS ACCORDING TO THEIR INCREASING CONTENT OF THE DIFFERENT FORMS OF NITROGEN DETERMINED BY THE VAN SLYKE METHOD AND EXPRESSED IN PERCENTAGE OF THE TOTAL NITROGEN OF THE FEEDINGSTUFF.

Ammonia N.		Melanine N.		Arginine N.	
Blood meal	5.85	Blood meal	3.85	Alfalfa hay	7.68
Tankage	6.58	Tankage	4.40	Whole wheat	7.99
Alfalfa hay	8.44	White soy beans	6.63	Rolled wheat	8.20
White soy beans	10.12	Cottonseed meal	7.78	Blood meal	9.16
Cottonseed meal	10.45	Barley	8.79	Barley	9.46
Oats	13.06	Rolled wheat	9.04	Oats	11.42
Barley	15.16	Whole wheat	9.21	White soy beans	12.67
Rolled wheat	17.04	Oats	9.94	Tankage	14.15
Whole wheat	17.59	Alfalfa hay	15.79	Cottonseed meal	19.52
Histidine N.		**Cystine N.**		**Lysine N.**	
Whole wheat	1.67	Cottonseed meal	0.65	Barley	2.19
Rolled wheat	3.21	White soy beans	0.67	Whole wheat	2.47
Barley	3.64	Blood meal	0.69	Rolled wheat	2.48
Oats	4.32	Alfalfa hay	0.88	Oats	3.49
Tankage	4.94	Oats	1.16	Alfalfa hay	4.10
Cottonseed meal	5.47	Barley	1.26	Cottonseed meal	4.78
White soy beans	5.77	Tankage	1.28	White soy beans	6.14
Alfalfa hay	7.44	Whole wheat	1.34	Tankage	7.48
Blood meal	8.53	Rolled wheat	1.66	Blood meal	9.73

Amino N in the filtrate from the bases.		Non-amino-N in the filtrate from the bases.	
Cottonseed meal	42.82	Blood meal	4.42
Alfalfa hay	44.02	Cottonseed meal	5.43
Barley	45.81	Tankage	7.27
Whole wheat	47.67	Oats	7.90
Rolled wheat	47.70	White soy beans	8.56
White soy beans	49.79	Alfalfa hay	9.79
Oats	51.72	Whole wheat	13.59
Tankage	52.39	Barley	13.84
Blood meal	56.57	Rolled wheat	13.95
Diamino acid N.		**Monoamino Acid N.**	
Whole wheat	13.47	Cottonseed meal	48.25
Rolled wheat	15.54	Alfalfa hay	54.81
Barley	16.55	White soy beans	58.35
Alfalfa hay	20.10	Oats	59.62
Oats	20.39	Tankage	59.66
White soy beans	25.25	Barley	59.69
Tankage	27.88	Blood meal	60.99
Blood meal	28.11	Whole wheat	61.26
Cottonseed meal	30.42	Rolled wheat	61.65

half times greater than the result of Van Slyke for ox hemoglobin. Van Slyke found 7.42% of humin nitrogen in dog's hair.

The high humin results are undoubtedly due, in part at least, to the presence of soluble carbohydrates, judging from the researches of Gortner

and Blish[1] and of Maillard[2] and, possibly also to glycerol formed from fats by hydrolysis, judging from the researches of Maillard.[3]

Gortner and Blish have shown that the humin nitrogen of 0.5 g. of pure zein was increased from 0.46 to 1.84% by the presence of 0.50 g. of pure dextrose during the hydrolysis of the protein. They have further demonstrated that the humin nitrogen of 1 g. of pure gliadin was increased from 0.59 to 0.94 per cent. by the presence of 0.25 g. of pure dextrose, and from 0.59 to 2.30 per cent. by the presence of 2.0 g. of dextrose, during the hydrolysis. Gortner and Blish have also demonstrated that when tryptophane was boiled with mineral acid in pure solution no humin was formed, but when tryptophane was added to a protein, or when carbohydrates were present, an abundance of humin was formed. When an abundance of carbohydrate was present, they found that 86% of the tryptophane nitrogen remained in the humin-nitrogen fraction.

The experiments of Osborne, Van Slyke, Leavenworth, and Vinograd[4] have confirmed the results of Gortner and Blish in proving, first, that tryptophane when boiled with 20% hydrochloric acid in pure solution does not form humin, second, that tryptophane when boiled with 20% hydrochloric acid in the presence of glucose yields 86% of its nitrogen in the form of humin, and third, that the presence of glucose during hydrolysis increases the humin content of proteins. They found that the presence of glucose increased the humin content of lactalbumin from about 2.32 to 3.70%.

Maillard has apparently demonstrated that the amino acids, in general, readily react with the sugars in water solutions at temperatures from 100° to 150°, or below, to form humin-like substances that contain nitrogen. Further, this investigator claims that glycerol acts upon amino acids at increased temperatures, forming polypetids, and that a secondary product possessing humin-like properties is produced.

It is not clearly apparent from the publications of Maillard that such reactions as he maintains take place between amino acids and carbohydrates and between amino acids and glycerol would occur in strong acid solutions such as those used in the hydrolysis of proteins, but it seems probable that the humin substances would be produced under such conditions.

Unfortunately, for the good of the methods for the analysis of proteins, the conclusion of Gortner and Blish, that in all probability the humin nitrogen of protein hydrolysis has its origin in the tryptophane nucleus, is apparently not true, since humin contains in addition adsorbed nitrogen from other amino acids. That their conclusion is probably

[1] THIS JOURNAL, **37**, 1630 (1915).

[2] *Compt. rend.*, **154**, 66–68 (1912); and *Compt. rend. Biol.*, **72**, 599–601 (1912).

[3] *Compt. rend.*, **153**, 1078–1080 (1911); and *Compt. rend. Biol.*, **71**, 546–549 (1911).

[4] *J. Biol. Chem.*, **22**, 259 (1915).

not true seems apparent from the researches of Maillard. Further, we have obtained results that clearly indicate that, in addition to tryptophane, a number of other amino acids, when gently boiled with 20% hydrochloric acid for 24 to 36 hours in the presence of pure glucose give humin nitrogen. Preliminary experiments show that under the above treatment 4.7 and 6.3% of the total nitrogen of lysine and cystine, respectively, is separated as humin nitrogen. These results, when confirmed by experiments now under way, will be published in full.

From the results of Gortner and Blish, and from our experiments, it seems somewhat probable that tryptophane enters into chemical combination with carbohydrates as suggested by Gortner and Blish, and that the other amino acids are to a greater or less extent adsorbed by the humin substances formed by the action of mineral acids upon carbohydrates. The fact, that in the experiments of Gortner and Blish, the humin nitrogen of zein, a tryptophane-free protein, was increased from 0.46 to 1.84% by the presence of 0.50 g. of dextrose during the hydrolysis, indicates that adsorption of amino acids had resulted.

The researches of Gortner and Blish, Maillard, and the authors of this paper, therefore, apparently show that the high humin nitrogen results we have obtained in the direct analysis of feedingstuffs by the Van Slyke method are due, in large part, to the presence of soluble carbohydrates during the hydrolysis of the proteins.

It is also possible that the high humin nitrogen which results during the analysis of feedingstuffs is due, in part, to the presence of cellulose which mechanically prevents the complete hydrolysis of the proteins. The results for the humin nitrogen given in Table III seem to indicate that such may be the case. Experiments are now being made to determine the completeness or incompleteness of the hydrolysis of the proteins of feedingstuffs containing relatively large amounts of crude fiber.

The high results for humin nitrogen obtained in this work constitute a source of error in the direct application of the Van Slyke method to the analysis of feedingstuffs, since, on the average, from 8 to 10% of the total nitrogen of the feedingstuffs is separated in the humin, which is an unknown mixture of secondary products, formed, probably, from a number of the amino acids resulting from the hydrolysis. Judging from the results for alfalfa hay, this error is greater for the roughages than for the concentrates. However, it is to be hoped, that further studies will make it possible either to reduce the quantity of nitrogen separated as humin, or to determine the quantities of nitrogen of the several amino acids represented in the humin fraction.

Summary.

1. The results here reported confirm the conclusion previously drawn, namely, that the Van Slyke method for the determination of the chem-

ical groups characteristic of the amino acids of proteins can be applied directly to the quantitative determinations of the amino acids of feedingstuffs with at least a fair degree of accuracy.

2. The results which we have obtained for the quantitative determination of amino acids in feedingstuffs, on the whole, do not agree well with those recently published by Nollau. In some determinations the results from the two sources are quite satisfactory, but in many cases the agreement is far from satisfactory. The lack of concordant results is probably due in the main to differences in the details of procedure in the experimental work.

3. The results reported in this paper show that there are pronounced variations in the free and combined amino-acid content of the common feedingstuffs expressed in percentage of the total nitrogen. There are also wide and marked variations in the distribution of the nitrogen of the free and combined amino acids in the feedingstuffs, expressed in percentage of the feedingstuff.

4. It seems probable that the high results for humin nitrogen obtained in the direct analysis of feedingstuffs by the Van Slyke method are due, in part, to the presence of soluble carbohydrates during the hydrolysis of the proteins. It also seems probable that the high humin nitrogen which is obtained in the analysis of feedingstuffs may be due, in part, to the presence of cellulose, which mechanically prevents the complete hydrolysis of the proteins.

5. The high results for humin nitrogen constitute a source of error in the direct application of the Van Slyke method to the determination of the free and combined amino acids and amides of feedingstuffs.

URBANA, ILL.

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF COLUMBIA UNIVERSITY AND THE HARRIMAN RESEARCH LABORATORY, No. 257.]

THE EFFECT OF SODIUM CHLORIDE UPON THE ACTION OF INVERTASE.

BY H. A. FALES AND J. M. NELSON.

Received September 15, 1915.

The object of the work described in this paper is the study of the influence of sodium chloride upon the hydrolysis of cane sugar by invertase. The great susceptibility of the action of invertase to small amounts of acids and alkalies has been observed by Kjeldahl,[1] O'Sullivan and Tompson,[2] and others.

Sörensen[3] made an important advance when he showed that the ac-

[1] *Meddelelser fra Carlsberg Laboratorit*, 1, 337 (1881).

[2] *J. Chem. Soc.*, 57, 835 (1890).

[3] *Biochem. Ztg.*, 21, 131–304 (1909).