at 330° quantitatively into carbon monoxide and water. Weight for weight, it is over twice as active as the most active alumina catalyst. The activity of thoria from the ignition of the nitrate is between that of titania and that of alumina; the catalyst gave nearly equal proportions of the monoxide and dioxide. The results obtained with titania and thoria are almost identical with those obtained by Sabatier.

Summary[9]

1. Data are presented to show that alumina may be selectively activated towards the two reactions through which formic acid decomposes by modifying the distance between the aluminum atoms.

2. The effect of the amount of water in the gas phase and of the spacing of the atoms in the solid catalyst upon the ratio of the decarboxylation and the dehydration reactions of formic acid is interpreted in terms of an extension of Langmuir's theory of the mechanism of reactions at surfaces.

3. The catalyst determines not only the proportion of the products of the reactions of formic acid but also determines the ratio of the temperature coefficient of the velocity of the two reactions involved.

4. Experimental evidence is presented which is not in accord with the hypothesis of selective adsorption as to the causation of reactions.

MADISON, WISCONSIN

[CONTRIBUTION FROM THE DEPARTMENT OF ANIMAL HUSBANDRY, UNIVERSITY OF ILLINOIS]

THE QUANTITATIVE DETERMINATION OF AMINO ACIDS OF FEEDS. II. THE AMINO ACIDS OF LINSEED MEAL, WHEAT BRAN, SOY BEANS AND RED CLOVER HAY

BY T. S. HAMILTON, N. UYEI, J. B. BAKER AND H. S. GRINDLEY

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The Van Slyke[1] method for the determination of certain amino acids is limited in its application to pure proteins, solutions of practically pure proteins or protein substances comparatively free from carbohydrates, fiber, fats, etc.[2,3,4] A method for the direct application of the Van Slyke method to the analysis of feeds has been designed in this Laboratory and applied to the determination of amino acids of oats, corn, cottonseed meal, and alfalfa by Hamilton, Nevens and Grindley.[5] The amino acids of

[9] The work described in this paper was supported in part by a grant from the Research Fund of the University of Wisconsin.

[1] Van Slyke, *J. Biol. Chem.*, **10**, 15 (1911); **22**, 281 (1915).

[2] Gortner, *ibid.*, **26**, 177 (1916). Gortner and Blish, THIS JOURNAL, **37**, 1630 (1915).

[3] Gortner and Holm, *ibid.*, **39**, 2477, 2736 (1917).

[4] Grindley and Eckstein, *ibid.*, **38**, 1425 (1916).

[5] Hamilton, Nevens and Grindley, *J. Biol. Chem.*, **48**, 249 (1921).

linseed meal, wheat bran, soy beans, and red clover hay by the same method are reported in the present paper. These feeds, together with those analyzed and reported in the paper mentioned above, represent most of the common sources of protein for the farm animal.

This investigation consisted of 6 complete analyses of a linseed meal containing 5.070% of nitrogen, 4 complete analyses of wheat bran containing 2.413% of nitrogen, 4 of a sample of Medium Early Yellow soy beans containing 6.791% of nitrogen, and 6 of a cured, air-dried red clover hay containing 1.984% of nitrogen. All feeds were ground so as to pass through a 60-mesh sieve and material equivalent to approximately 6 g. of protein was taken for each analysis. Thus, exactly 20 g. of linseed meal, 45 g. of wheat bran, 15 g. of soy beans and 50 g. of clover hay were taken for each analysis. The results are expressed in percentages of the total nitrogen of the feed. The averages of these analyses are in bold faced type in Table I.

Since the ability to obtain consistent results in repeated analyses of the same feed is of utmost importance in an analysis such as this, it may be stated that the results of the different analyses on each of the 4 feeds analyzed were, in general, more in agreement than those reported in an earlier paper.[5] The maximum variations from the average were, in general, very small. The percentage of nitrogen left in the residues after the series of extractions to which the sample is subjected was also very low. In the case of soy beans an average of only 0.135% of the total nitrogen remained, while the percentage in case of linseed meal was 0.409, of wheat bran was 0.743, and of clover hay was 4.929. The higher percentage of nitrogen left in the clover hay residue was to be expected on account of its high fiber content which prevents thorough extraction.

Part of the feeds reported in this paper have been similarly analyzed by previous investigators.

Nollau,[6] using the Van Slyke method, analyzed a sample of soy beans and wheat bran. Nollau's results and those reported by the present writers on the same feeds do not agree well. The differences are, in general, the same as those pointed out by Hamilton, Nevens and Grindley[5] when comparing their results with those obtained by Nollau on the same feeds. As pointed out at that time, the differences in results can be explained largely by the differences in procedures. Grindley and Slater,[7] using the Van Slyke method, reported (among others) results on the amino acids of a sample of white soy beans and considering the slight differences which may be due to difference in variety and the fact that these workers did not remove the non-protein nitrogen, the results here reported on yellow soy beans agree very well with those on the white variety.

In Table I are given the amino acid content of oats, corn, cottonseed meal and alfalfa, as obtained by Hamilton, Nevens and Grindley,[5] and the

[6] Nollau, *J. Biol. Chem.*, **21**, 611 (1915).

[7] Grindley and Slater, This Journal, **37**, 2762 (1915).

TABLE I

DISTRIBUTION OF NITROGEN IN THE COMMON FEEDS

Expressed as percentage of total nitrogen of the feed

Feed	Total basic N	Ammonia N	Humin N	Arginine N	Cystine N	Histidine N	Lysine N	Mono-amino acid N. Amino N in filtrate from bases	Proline, oxyproline, tryptophan, etc. Non-amino N in filtrate from bases	Ether-soluble N	Alcohol-soluble N	Non-protein N soluble in cold 1% $CCl_3.CO_2H$ in filtrate from colloidal Fe	N lost in method of analysis	Total
Bloodmeal[8]	28.11	5.85	3.95	9.16	0.69	8.53	9.73	56.57	4.42	..	..	...	..	98.80
Skim-milk[8]	22.99	11.09	5.93	8.67	1.25	4.87	8.20	51.89	5.60	..	..	...	..	97.50
Tankage[8]	27.85	6.58	4.40	14.15	1.28	4.94	7.48	52.39	7.27	..	..	...	..	98.49
Soy beans	**28.94**	**9.38**	**2.87**	**15.70**	**1.46**	**5.60**	**6.18**	**48.28**	**2.43**	**0.16**	**0.58**	**5.55**	**2.23**	**100.53**
Alfalfa	17.35	8.17	7.36	8.00	0.99	3.93	4.43	38.03	2.51	0.55	1.85	16.69	4.73	97.24
Cottonseed meal	31.03	9.41	6.30	18.71	0.94	7.17	4.21	40.72	2.87	0.10	0.55	5.56	3.29	99.83
Wheat bran	**24.02**	**9.17**	**6.86**	**11.99**	**0.81**	**7.32**	**3.90**	**38.07**	**3.59**	**0.04**	**1.23**	**15.26**	**2.27**	**100.51**
Linseed meal	**26.81**	**10.86**	**5.09**	**15.92**	**1.07**	**6.14**	**3.68**	**44.55**	**3.26**	**0.12**	**0.37**	**7.88**	**2.80**	**101.74**
Oats	21.23	11.42	5.53	11.65	0.94	5.80	2.84	42.14	3.86	0.57	1.22	11.13	1.90	99.00
Red clover hay	**15.40**	**7.35**	**13.09**	**6.86**	**0.87**	**5.05**	**2.62**	**39.82**	**3.28**	**0.62**	**1.57**	**11.29**	**6.87**	**99.29**
Wheat[8]	13.47	17.59	9.21	7.99	1.34	1.67	2.47	47.67	13.59	..	..	...	..	101.53
Corn	16.83	11.94	3.54	8.73	1.07	4.83	2.20	46.70	7.22	0.33	1.37	8.14	3.85	99.92
Barley[8]	16.55	15.16	8.79	9.46	1.26	3.64	2.19	45.81	13.84	..	..	...	..	100.15

[8] Grindley, *Proc. Am. Soc. Animal Production*, **1916**, 133.

amino acid content of the feeds reported in the present paper; in order to give a fairly complete list of the common sources of protein to the farm animal, the table also includes a summary of the analyses of tankage, bloodmeal, skim-milk, wheat and barley, reported by Grindley and associates.[8,9] The results on tankage, bloodmeal, and skim-milk should agree fairly well with results which would be obtained by the method used in this investigation, for the chief differences in the methods used consist in procedures not greatly affecting the results on these feeds, that is (1) the removal of the non-protein nitrogen, (2) the extraction of the starch, and (3) the absence of fiber during hydrolysis.

The feeds in Table I represent common sources of protein to the farm animal. We now know, however, that equal amounts of protein from different sources may not be equal in nutritive value. Many comparatively recent studies have demonstrated the importance of *quality* as well as *quantity* of the protein fed. We know, further, that the degree to which a certain protein or protein feed is utilized may be limited solely by the absence, or presence only in small quantities, of one or possibly more essential amino acids; for example, the presence of only minute quantities of lysine in gliadin of wheat, lysine and tryptophan in zein of corn, cystine in casein, etc. While our knowledge concerning the importance of all the various amino acids in the nutrition of animals is far from complete, we do know much concerning the requirements, of certain animals at least, for the group of basic amino acids, that is arginine, histidine, cystine and lysine.

Our knowledge of the physiological role of arginine and histidine has been greatly enhanced by the investigations of Ackroyd and Hopkins,[10] Geiling,[11] and Myers and Fine.[12] That cystine is essential for normal growth of the young white rat has been demonstrated by Osborne and Mendel[13] and more recently by Johns and Finks.[14] Geiling,[11] working with adult white mice, concluded that cystine was essential for their maintenance. Lewis[15] has recently shown that this amino acid is necessary for the maintenance of dogs. The necessity of lysine for growth has been conclusively demonstrated by Osborne and Mendel.[16]

In view of the essential role which the basic amino acids play in nutrition, it is of interest to compare the analytical results in this respect for the different feeds. The first column in Table I gives the total basic nitrogen,

[9] Grindley, Joseph and Slater, THIS JOURNAL, **37**, 1778 (1915).

[10] Ackroyd and Hopkins, *Biochem. J.*, **10**, 551 (1916).

[11] Geiling, *J. Biol. Chem.*, **31**, 173 (1917).

[12] Myers and Fine, *ibid.*, **21**, 389 (1915).

[13] Osborne and Mendel, *ibid.*, **20**, 373 (1915).

[14] Johns and Finks, *ibid.*, **41**, 379 (1920).

[15] Lewis, *ibid.*, **43**, 289 (1920).

[16] Osborne and Mendel, *ibid.*, **17**, 325 (1914); **20**, 351 (1915); **25**, 1 (1916).

representing a summation of the percentages of arginine, histidine, cystine, and lysine nitrogen. If the feeds were arranged in order of decreasing amounts of basic nitrogen, the order would be cottonseed meal, soy beans, bloodmeal, tankage, linseed meal, wheat bran, skim-milk, oats, alfalfa, corn, barley, red clover hay and wheat. In Table I, however, the feeds are arranged in order of their decreasing percentage of lysine nitrogen for the reason that, in case of those feeds presented, the limiting factor in their utilization is more probably lysine than arginine, histidine or cystine.

With our present meager knowledge concerning the physiological role played by the other amino acids as well as the functions of the non-protein constituents, a detailed picture of neither of which is given by present available methods, it would be futile to attempt an arrangement of feeds in the order of their true nutritive value from analytical results alone. Especially is this true since the order would probably vary, depending on the purpose for which the feeds were used, that is, growth, maintenance, milk production, etc. A strict correlation between chemical composition and nutritive value as indicated by biochemical methods will not be found until our knowledge concerning the two is much more advanced.

Summary

1. The Van Slyke method as previously described can be used with as high, or very nearly as high, a degree of accuracy in estimating the amino acids of feeds as in estimating the amino acids of pure proteins.

2. The results obtained by this method represent more accurately the true amino acid content of the mixed proteins of feeds than those obtained by methods in which extracts, containing only a part of the proteins present, are analyzed.

3. The average percentage of nitrogen left in the residues after the series of extractions to which the sample is subjected was, in case of soy beans, only 0.135% of the total nitrogen, in case of linseed meal, 0.409%, of wheat bran, 0.473% and of clover hay, 4.929%.

4. In addition to the amino acid content of linseed meal, wheat bran, soy beans, and red clover hay, the average results of the amino acid content of oats, corn, wheat, barley, alfalfa, cottonseed meal, bloodmeal, skim-milk and tankage, as determined by the Van Slyke method, are tabulated in order of their decreasing amounts of lysine nitrogen.

5. By further application of available methods for the estimation of other amino acids to hydrolyzed protein solutions, prepared in a manner similar to that described for this work, it may be possible to obtain further important knowledge concerning the nutritive value of the proteins of foods and feeds.

URBANA, ILLINOIS