THE AMINO ACID CONTENT AND NUTRITIVE VALUE OF THE PROTEINS OF COTTONSEED MEAL

BY

WILLIAM BARBOUR NEVENS

B. S. University of Wisconsin, 1914

THESIS

Submitted in Partial Fulfillment of the Requirements for the

Degree of

DOCTOR OF PHILOSOPHY

IN ANIMAL HUSBANDRY

IN

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THE PROTEINS OF COTTONSEED MEAL[1]

I. AMINO ACID CONTENT

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I. INTRODUCTION

One of the earliest references to the proteins of cottonseed meal was made by Ritthausen (1), who separated the proteins in the form of spheroids. Osborne and Voorhees (2) isolated a protein from cottonseed meal which had the nature of a globulin, being soluble in salt solutions, and comprised 42.3 per cent of the total nitrogen of the meal. Another protein (or proteins) was found to be insoluble in salt solutions but soluble in 0.2 per cent potash solution and amounted to 44.3 per cent of the total nitrogen of the meal. Two per cent of the total nitrogen was present in the form of water soluble proteose.

TABLE 1

Percentage of nitrogen in the different groups in various proteins

SOURCE	N AS AMMONIA	BASIC N	NON-BASIC N	N IN MgO PRECIPITATION	TOTAL N
Globulin, cottonseed	1.92	5.71	11.01		18.64
Globulin, wheat	1.42	6.83	9.82	0.28	18.39
Zein, maize	2.97	0.49	12.51	0.16	16.13
Hordein, barley	4.01	0.77	12.04	0.23	17.21

The distribution of nitrogen in various protein bodies was studied exhaustively by Osborne and Harris (3), who employed the modified Hausmann method (4). The following values are typical of their results.

[1] The results presented in this paper formed part of a thesis submitted to the Graduate School of the University of Illinois in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Animal Husbandry.

These investigators state that "This wide variation in the proportion of basic decomposition products of the various proteins . . . . raises important questions regarding their food value." Osborne (5) further found that the basic nitrogen of the globulin of cottonseed, as determined by precipitation with phosphotungstic acid, consisted of 3.46 per cent histidine, 13.51 per cent arginine and 2.06 per cent lysine.

The content of the mono-amino acids of the "edestin" of cottonseed meal was determined by Abderhalden and Rostoski (6) by the use of the Fischer ester method (7), and is as follows, calculated for dry, ash free edestin of cottonseed:

	per cent
Glycocoll	1.2
Alanin	4.5
Amino valerianic acid	Present
a-proline	2.3
Leucin	15.5
Glutamic acid	17.2
Aspartic acid	2.9
Phenyl alanin	3.9
Serin	0.4
Tyrosin	2.3
Tryptophane	Present

The quantitative determination of the amino acids of feedingstuffs by means of the Van Slyke method (8) was undertaken by Grindley and his co-workers (9), and a little later by Nollau (10). In Nollau's procedure, samples of the finely ground feeds were hydrolyzed with 20 per cent hydrochloric acid until the content of amino acid, as determined by the Van Slyke method, became constant. The material insoluble in hydrochloric acid was filtered off, the clear extract concentrated under diminished pressure and made up to a certain volume. The total nitrogen content of this extract was used as a basis for calculating the final results. In a report of the subsequent work of Grindley and co-workers (11), it is claimed that since Nollau filtered off the solid residue after hydrolysis of the feedingstuff and before making his total nitrogen determinations upon which the final calculations were based, that his results are not accurate since a part of the total nitrogen was undoubtedly discarded in the solid residue.

The heats of combustion of several vegetable proteins were carefully determined by Benedict and Osborne (12). The globulin of cottonseed was found to yield 5596 calories per gram, compared to 5358 calories per gram for the globulin of wheat and 5916 calories per gram in the case of the hordein of barley. In commenting upon their determinations, the investigators state that "many irregularities. . . . appear, which are doubtless due to the different proportion of the various amino acids which constitute the molecules of the different proteins."

It is evident from the foregoing discussion that our knowledge of the composition of the proteins of cottonseed meal is very incomplete. The globulin is the only protein of cottonseed which has been isolated in pure form and whose composition has been determined. The globulin, however, according to Osborne and Voorhees, contains only 42.3 per cent of the total nitrogen of the cottonseed. The character, identity and chemical composition of the remaining proteins are practically unknown, and it is evident from the data given above that our knowledge of the distribution of the nitrogen in the proteins of cottonseed meal is very meager indeed. The investigation of the distribution of the nitrogen in the proteins of cottonseed meal therefore constituted the object of this study.

II. METHODS EMPLOYED IN CHEMICAL ANALYSIS[2]

The method of analysis employed consisted of two main procedures. The first consisted of a series of extractions whereby the nonprotein together with a very small amount of protein was first removed, and following this, the proteins were extracted from the residual matter of the sample which consisted mostly of fiber. The second main procedure embraced the hydrolysis of the extracted proteins, and the analysis of the resulting solution

[2] The method of procedure here outlined is one which has been developed and perfected in this laboratory by Dr. H. S. Grindley, Mr. T. S. Hamilton and associates (9, 11, 33, 36 and unpublished manuscripts). The method of extraction preliminary to hydrolysis of the proteins has been developed entirely in this laboratory, while the actual determination of the nitrogen in the different groups follows closely the method of Van Slyke, but includes modifications perfected in this laboratory.

for certain amino acid and other groups according to the general method of Van Slyke (8).

The sample of cottonseed meal was prepared from good quality commercial meal, finely ground and passed through a 40-mesh sieve. Each sample taken for analysis weighed 15 grams and contained 1.0194 grams of nitrogen, or about 6 grams of protein.

In the first three extractions, which were carried out consecutively, cold anhydrous ether, cold absolute alcohol and cold 1.0 per cent trichloracetic acid were used. The samples of feeding stuff were placed in 500 cc. centrifuge bottles and 100 to 200 cc. of the reagents added. The bottles were placed on a shaking machine which rolled them back and forth continually. Usually two extractions were made each twenty-four hours, one extraction period being seven to eight hours and the other 15 to 16 hours in length. At the end of the extraction period, the sides of the bottles were washed down with the reagent, the bottles centrifuged and the clear supernatant liquid decanted. Usually six or seven extractions with each reagent were necessary.

The ether and alcohol extracts were filtered and any residues returned to the centrifuge bottles. After slight acidification with sulphuric acid, the ether and alcohol were evaporated and recovered and total nitrogen determinations made on the residue. The small amount of protein removed in the trichloracetic acid extracts was recovered by precipitation with colloidal ferric hydroxide (containing 5 per cent Fe_2O_3) in boiling solution. The precipitate was transferred to a digestion flask with 20 per cent hydrochloric acid, and total nitrogen determined in the filtrate.

The bulk of the proteins was removed from the residue remaining after extraction with 1.0 per cent trichloracetic acid by extraction, first, with dilute sodium hydroxide solution, then with 20 per cent hydrochloric acid followed by treatment with 5 per cent sodium hydroxide solution. The dilute sodium hydroxide solution used during the shorter period was a 0.2 per cent solution and that during the longer period a 0.1 per cent solution. These extracts were neutralized, acidified with hydro-

chloric acid and concentrated in vacuo to a small volume. An equal volume of concentrated hydrochloric acid was then added. The residues remaining after treatment with dilute sodium hydroxide solution were boiled for three minutes with 20 per cent hydrochloric acid. After cooling, the solution was filtered off, the residue washed and the procedure repeated once. The washings were evaporated to a small volume, an equal volume of concentrated hydrochloric acid added and the washings then combined with the main hydrochloric acid extract. The residues insoluble in hydrochloric acid were treated three times with 5 per cent sodium hydroxide solution using centrifuge bottles as in former extractions. After washing the residues nearly free from alkali, they were submitted to Kjeldahl analysis. The extracts and washings were acidified with hydrochloric acid, concentrated in vacuo and transferred to digestion flasks with an equal volume of concentrated hydrochloric acid.

The proteins precipitated by colloidal iron and the proteins removed by extraction with dilute sodium hydroxide, 20 per cent hydrochloric acid and 5 per cent sodium hydroxide were completely hydrolyzed by boiling for fifteen hours upon a combined electric plate and sand bath under reflux condensers. The resulting solutions were combined and analysis for the chemical groups characteristic of certain amino acids executed essentially as directed by Van Slyke (8), but with the use of minor improvements perfected in this laboratory.

III. DISCUSSION OF RESULTS

The results obtained by application of the method of chemical analysis outlined in the preceding section to eight portions of the same original sample of cottonseed meal are shown in the accompanying tables 2 and 3. Table 2 shows the values expressed in percentage of the total nitrogen present in the sample of feeding stuff when it was taken for analysis, while table 3 shows the same values expressed in percentage of the feeding stuff itself.

Two averages are included in the tables. The first is compiled by taking the average of all values obtained by analysis of the entire eight samples. The second average is obtained by

TABLE 2

Summary of the results of analysis of the proteins of cottonseed meal

(Results expressed in percentage of the total nitrogen in sample)

SAMPLE NUMBER	NONPROTEIN NITROGEN				RESULTS OF THE VAN SLYKE ANALYSIS										UNCHARACTERIZED NITROGEN LOST IN METHOD OF ANALYSIS						TOTAL
	Soluble in absolute ether	Soluble in absolute alcohol	In filtrate from colloidal iron	Total nonprotein nitrogen	Insoluble humin nitrogen	Soluble humin nitrogen	Ammonia nitrogen	Arginine nitrogen	Cystine nitrogen	Histidine nitrogen	Lysine nitrogen	Amino nitrogen in filtrate from the bases	Non-amino N in filtrate from the bases	Nonprotein N + results of Van Slyke analysis	Insoluble in strong alkali	Unadsorbed humin (filtered from solution during decomposition of phosphotungstate precipitate)	Soluble in amyl alcohol-ether mixture	In residue from solution of the bases	In residue from solution of the filtrate from the bases	Total nitrogen lost	Total nitrogen accounted for
C1	0.021	0.570	4.943[2]	5.534	2.609	3.462	9.455	18.569	0.961	6.095	3.871	38.495	4.079	93.130	0.220	2.403	0.906	0.206	0.017	3.752	96.992
								18.775	0.961	4.876	4.609	41.466	2.463	94.210		2.145	1.043	0.206	0.055	3.669	97.879
C2	0.089	0.618	4.870[2]	5.577	2.609	5.117	9.689	18.981	0.821	5.622	3.625	39.646	3.908	95.595	0.260	2.042	0.734	0.103	0.027	3.166	98.761
								19.119	0.983	7.038	2.494	41.432	2.955	97.013		1.081	1.078	0.326	0.051	2.796	99.809
C3	0.202	0.652	5.053[2]	5.907	2.492	5.459	9.929	18.295	1.035	7.966	3.471	39.157	2.348	96.062	0.302	1.867	0.614	0.240	0.079	3.102	99.164
								18.638	1.101	7.120	3.668	38.546	1.454	94.314		1.448	0.961	0.200	0.133	3.044	97.358
C4	0.109	0.614	5.531[1]	6.254	2.623	4.477	8.892	19.160	1.285	6.441	4.430	39.828[a]	0.161	93.551	0.233	3.011	0.841	0.243	0.130[a]	4.458	98.009
								17.635	0.961	8.038	4.510	39.828	0.161	93.379	—[b]	3.076	1.428	0.247	0.130	5.114	98.493
C5	0.081	0.506	5.245[1]	5.832	2.981	2.415	9.249	18.701	1.051	6.987	5.042	41.659	2.784	96.701		1.230	0.645	0.255	0.217	2.777	99.478
								16.339	1.051	10.181	3.881	42.257	2.577	96.763		1.153	0.736	0.601	0.082	3.002	99.765

C6	0.129	0.489	5.722[1]	6.340	2.930	2.650	9.318	19.580	0.963	6.568	4.780	41.659	2.504	97.292	0.685	1.021	0.642	0.445	0.096	2.889	100.818
								19.305	0.933	6.163	5.216	42.141	2.076	97.072		1.016	0.917	0.315	0.096	3.029	100.101
C7	0.081	0.420	6.097[1]	6.598	2.763	2.334	9.002	17.932	0.692	9.627	3.503	39.131	2.921	94.503	0.719	0.818	0.994	0.068	0.054	2.653	97.156
								18.041	0.722	9.074	3.697	39.276	3.253	94.760		0.725	1.057	0.124	0.076	2.701	97.461
C8	0.047	0.506	7.012[1]	7.564	2.772	2.746	9.764	19.799	0.810	5.657	5.845	43.023	3.095	101.076	0.589	1.384	1.049	0.151	0.054	3.227	104.303
								20.404	0.751	7.271	4.702	43.939	3.812	103.726		1.343	1.087	0.115	0.082	3.216	106.942
Average[3]....	0.095	0.547	5.559[1]	6.201	2.772	3.582	9.412	18.705	0.943	7.170	4.209	40.718	2.535	96.197	0.430	1.610	0.921	0.240	0.086	3.287	99.484
Average[4]....	0.125	0.545	5.436[1]	6.106	2.699	3.890	9.485	18.736	0.906	7.397	3.807	40.124	2.677	95.827	0.492	1.252	0.875	0.228	0.076	2.923	98.750

[a] Determination lost. Value from other half of sample substituted.
[b] Determination lost. Average of 7 determinations used.
[1] After first precipitation.
[2] After second precipitation.
[3] Average of all determinations.
[4] Average of complete samples C2, C3, C6, C7.

TABLE 3

Summary of the results of analysis of the proteins of cottonseed meal

(Results expressed in percentage of the feeding stuff)

SAMPLE NUMBER	NONPROTEIN NITROGEN				RESULTS OF THE VAN SLYKE ANALYSIS									UNCHARACTERIZED NITROGEN LOST IN METHOD OF ANALYSIS					TOTAL	
	Soluble in ether	Soluble in absolute alcohol	In filtrate from colloidal iron	Total nonprotein nitrogen	Insoluble humin nitrogen	Soluble humin nitrogen	Ammonia nitrogen	Arginine nitrogen	Cystine nitrogen	Histidine nitrogen	Lysine nitrogen	Amino nitrogen in filtrate from bases	Non-amino nitrogen in filtrate from bases	Insoluble in strong alkali	Unadsorbed humin (filtered from solution during decomposition of phosphotungstate precipitate)	Soluble amyl alcohol-ether	Residue from solution of bases	Residue from solution of filtrate of bases	Nonprotein N + results of Van Slyke analysis	Total nitrogen accounted for
C1	0.0014	0.0387	0.3360[2]	0.3761	0.1769	0.2352	0.6426	1.2620	0.0653	0.4142	0.2631	2.6157	0.2773	0.0150	0.1633	0.0616	0.0140	0.0012	6.3284	6.5835
								1.2760	0.0653	0.3314	0.3133	2.8180	0.1674		0.1458	0.0709	0.0140	0.0037	6.4022	6.6516
C2	0.0061	0.0420	0.3310[2]	0.3790	0.1773	0.3478	0.6585	1.2900	0.0558	0.3821	0.2464	2.6940	0.2656	0.0177	0.1388	0.0499	0.0070	0.0019	6.4966	6.7119
								1.2993	0.0668	0.4783	0.1695	2.8153	0.2009		0.0735	0.0733	0.0222	0.0035	6.5928	6.7830
C3	0.0137	0.0443	0.3435[2]	0.4015	0.1694	0.3710	0.6748	1.2433	0.0703	0.5414	0.2360	2.6610	0.1596	0.0205	0.1269	0.0418	0.0163	0.0054	6.5283	6.7392
								1.2666	0.0748	0.4839	0.2493	2.6193	0.0988		0.0985	0.0653	0.0136	0.0091	6.4094	6.6164
C4	0.0075	0.0417	0.3759[1]	0.4250	0.1783	0.3043	0.6043	1.3021	0.0873	0.4377	0.3011	2.7067[a]	0.0110	0.0158	0.2046	0.0572	0.0165	0.0089[a]	6.3579	6.6609
								1.1985	0.0653	0.5462	0.3065	2.7067	0.0110		0.2091	0.0971	0.0168	0.0089	6.3462	6.6939
C5	0.0055	0.0345	0.3563[1]	0.3963	0.2026	0.1641	0.6286	1.2709	0.0715	0.4749	0.3427	2.8310	0.1892	—[b]	0.0836	0.0439	0.0173	0.0147	6.5718	6.7605
								1.1104	0.0715	0.6919	0.2638	2.8715	0.1751		0.0784	0.0500	0.0409	0.0056	6.5758	6.7799

C6	0.0088	0.0332	0.3889[1]	0.4309	0.1991	0.1801	0.6333	1.3306	0.0655	0.4464	0.3249	2.8310	0.1702	0.0465	0.0694	0.0437	0.0302	0.0065	6.6120	6.8083
								1.3120	0.0635	0.4188	0.3545	2.8630	0.1411		0.0691	0.0623	0.0215	0.0065	6.5963	6.8022
C7	0.0055	0.0285	0.4144[1]	0.4484	0.1878	0.1586	0.6118	1.2186	0.0471	0.6543	0.2381	2.6590	0.1986	0.0489	0.0556	0.0675	0.0047	0.0037	6.4223	6.6028
								1.2260	0.0491	0.6167	0.2513	2.6690	0.2211		0.0493	0.0719	0.0084	0.0052	6.4398	6.6235
C8	0.0032	0.0344	0.4765[1]	0.5140	0.1884	0.1866	0.6635	1.3456	0.0551	0.3845	0.3972	2.9235	0.2104	0.0400	0.0941	0.0713	0.0103	0.0037	6.8689	7.0883
								1.3866	0.0511	0.4942	0.3196	2.9860	0.2591		0.0913	0.0739	0.0078	0.0056	7.0492	7.2678
Av[3]	0.0065	0.0372	0.3778	0.4214	0.1849	0.2434	0.6396	1.2712	0.0641	0.4873	0.2861	2.7669	0.1723	0.0292	0.1095	0.0626	0.0163	0.0059	6.5374	6.7609
Av[4]	0.0085	0.0370	0.3695	0.4150	0.1834	0.2644	0.6446	1.2733	0.0616	0.5027	0.2588	2.7265	0.1820	0.0334	0.0851	0.0595	0.0155	0.0052	6.5123	6.7110

[1] After first precipitation.
[2] After second precipitation.
[3] Average of all determinations.
[4] Average of complete samples C2, C3, C6, C7.
[a] Determination lost. Value for other half of sample substituted.
[b] Determination lost. Average of seven determinations used.

averaging the results secured in the analysis of the complete samples C2, C3, C6 and C7. It is believed that the latter average more nearly expresses the actual composition of the commercial cottonseed meal used, for the following reasons: (a) These samples, i.e., C2, C3, C6 and C7 show the best agreeing results throughout. The two parts of sample C1 agree well in the amount of arginine nitrogen, but show a considerable difference in the amounts of amino nitrogen, non-amino nitrogen and histidine nitrogen. The totals of the nonprotein nitrogen plus the protein nitrogen are considerably below the average of all the samples. In sample C4 the non-amino nitrogen is particularly low, this value being one of the principal factors contributing to the noticeably low nonprotein plus protein of this sample. Sample C5 is omitted from the average partly on account of the non-agreement of its arginine nitrogen and histidine nitrogen values. Of the latter values, one is 3 per cent above the average of all samples. (b) The second average, i.e., of samples C2, C3, C6 and C7, includes values which are most free from obvious errors. In making the determinations in the case of sample C4, two determinations were lost, and in the case of sample C5, one determination was lost. While the results obtained for sample C8 agree fairly well throughout with themselves and with the average, the results are consistently high, and it is excluded from this average on the grounds of the totals obtained, which are obviously too high.

Nonprotein nitrogen. The first section of tables 2 and 3 shows the amount of nitrogen removed in the preliminary extractions with absolute ether, absolute alcohol and trichloracetic acid. While the absolute ether in the cold is used primarily to remove the lipins, such as the oils, waxes, etc., it also dissolves various amounts of other substances, such as coloring matters, and at the same time a small amount of nitrogen. The thorough extraction with absolute alcohol following the treatment with absolute ether presumably completes the extraction initiated by ether. The alcohol removes somewhat more nitrogen than the extraction with ether. The bulk of the nonprotein nitrogen accounted for, about 89 per cent of the total, remains, however, in the tri-

chloracetic acid extracts after precipitation of the proteins by colloidal ferric hydrate and the removal of the precipitate by filtration. That the nitrogen determined in these latter extracts is not protein nitrogen is apparent from the work of Van Slyke, Vinograd, Vilchur and Losee (13), Hill (14), Wolff (15), and others.

It seems from the study of the character of the nonprotein nitrogenous constituents of feedingstuffs by Grindley and Eckstein (16) that the forms of nitrogen represented in this classification consist principally of those forms naturally resulting from the cleavage of the proteins upon hydrolysis and therefore could not interfere in the determination of the characteristic chemical groups of the proteins were they not removed in the preliminary extractions. Neidig and Snyder (17), who recently determined the proportion of nitrogen in the form of ammonia in the ether extracts and alcohol extracts of different kinds of silage, found that from 28.1 per cent to 100 per cent of the ether extract nitrogen consists of ammonia nitrogen, while from 14.2 per cent to 23.4 per cent of the alcohol extract nitrogen is yielded as ammonia nitrogen. This indicates that only a part of the nitrogen soluble in ether and alcohol would appear in the ammonia fraction were it not removed previous to hydrolysis. Therefore, the removal of the nonprotein nitrogen at this point avoids possible complications in the further prosecution of the analytical procedure, and it is believed that the accuracy of the further determinations has been increased over that of previous methods by the removal of the nonprotein nitrogen before hydrolysis of the proteins. The total amount of the nonprotein nitrogen present in cottonseed meal found by the method used amounted to 6.106 per cent of the total nitrogen contained in the feedingstuff.

Results of the Van Slyke analysis. It is a matter of common knowledge that one of the important sources of loss in the analysis of proteins by methods involving the employment of acid hydrolysis is the formation of an insoluble black substance called humin. The term melanin is also applied to this substance, on account of its supposed relationship or similarity to the

naturally occurring body pigments. The amount of humin formed in acid hydrolysis of the proteins is greatly increased by the presence of carbohydrates, as shown by Gortner and associates (18, 19), Hart and Sure (20), and Osborne, Van Slyke, Leavenworth and Vinograd (21). A part of the humin formed, however, remains in solution in the hydrochloric acid, and is termed soluble humin.

In these experiments the soluble humin which is adsorbed by the lime used in neutralizing the hydrolysate when determining ammonia nitrogen, carried with it a larger amount of nitrogen than the insoluble humin. The sum of the insoluble plus the soluble humin nitrogen found amounts to 6.589 per cent, which constitutes no inconsiderable error, since at present it is impossible to determine the character of the nitrogen discarded in this form.

It may be noted by referring to table 1 that the amount of soluble humin nitrogen in the first four samples is considerably greater than in the succeeding four. Possibly this is due to a slight variation in the analytical procedure. In the case of samples C1 to C4, inclusive, it was necessary to add 75 to 90 cc. of calcium hydroxide in order to neutralize the hydrochloric acid before distillation of ammonia. About 20 cc. in excess were then added. With the next four samples evaporation of the acid hydrolysate in vacuo was continued longer in order to drive off a greater proportion of the hydrochloric acid. In consequence, only 30 to 40 cc. of calcium hydroxide were necessary to effect neutralization, and in these cases only a small excess, about 10 cc., of calcium hydroxide was added. The hypothesis is put forward that the presence of a large excess of calcium hydroxide during the distillation of ammonia may result in the adsorption of some amino acid nitrogen which is incompletely removed in the subsequent washing of the sticky mass.

When it is recalled that the proteins of cottonseed meal form approximately 43 per cent of the feedingstuff, the amount of nitrogen in the humin resulting from the hydrolysis of these proteins, as determined in these experiments, is not excessive when compared to the amounts obtained in the hydrolysis of

pure proteins by Van Slyke (8), some of whose results are shown in the accompanying table.

The amount of nitrogen recovered as ammonia was quite constant in all the samples. Little can be said in regard to the significance of this fraction, aside from the fact that the proportion of the total nitrogen of cottonseed meal which appears as ammonia is quite in harmony with that of other feedingstuffs.

A particularly characteristic feature of the amino acid content of cottonseed meal is the remarkably high content of arginine. This is much higher than that found in any other feeding stuff so far examined, with the exception of peanuts, although it is not so high as that found in some other vegetable proteins. Van Slyke (8) found 27.05 per cent arginine nitrogen in edestin

TABLE 4

Amounts of humin nitrogen in pure proteins expressed in percentage of the total nitrogen of the protein

PROTEIN AND DESCRIPTION	HUMIN NITROGEN
	per cent
Gliadin from wheat	0.86
Edestin	1.83
Fibrin (Merck's)	3.43
Oxyhemoglobin ("pure, crystalized")	3.60
Dog's hair	7.35

while Nollau reported that hemp seed, peanuts, black walnuts, and hickory nuts have an arginine nitrogen content of more than 20 per cent.

In the Van Slyke procedure the only amino acids determined by direct analysis are arginine and cystine. Just how much importance may be attached to the results obtained for the latter is questionable, even though the values found in the different samples do not vary widely. These values, however, probably fall short of the true value, due to losses in the determination of cystine. Van Slyke (8) has shown that boiling cystine for sixteen hours with hydrochloric acid resulted in the conversion of one-half of its nitrogen into forms not precipitable by phosphotungstic acid. Since in the analytical procedure described above, hydrolysis of the proteins was carried out by

boiling them with 20 per cent hydrochloric acid for fifteen hours, it is probable that much of the cystine was destroyed during that reaction. If it is assumed that the correct value for cystine should be double that actually obtained, then the total nitrogen of the bases would amount to 31.75 per cent of the total nitrogen of the feedingstuff.

The values given for histidine and lysine are somewhat variable among the different samples. These variations are likely due in large measure to the indirect method used in their determination, since slight errors in any or all of the three direct determinations of arginine nitrogen, cystine nitrogen and the total nitrogen of the bases are doubtless all reflected at these points.

The content of mono-amino acid nitrogen of cottonseed meal is considerably less than that found in other feedingstuffs, possibly due to the greater proportion of the total nitrogen which is formed by the basic amino acids. One of the interesting features of the results of the Van Slyke analysis of the proteins of cottonseed meal is brought out in the summation of the ammonia nitrogen, the nitrogen of the bases, mono-amino acid nitrogen, and non-amino acid nitrogen, the four groups which represent the total content of strictly amino acid nitrogen as determined by this method. The sum of these is 83.132 per cent. While this sum is not so great as that in the case of some other feedingstuffs or of animal proteins, as determined by previous investigators employing the Van Slyke method of analysis, it is a much larger amount than it was possible to secure in most cases from comparable sources by the methods of isolation and purification employed by the earlier investigators. Thus the tabulations of Lusk (22), combining the results of Osborne and associates in this field, show the maximum amino acid content of zein of maize, to be 88.87 per cent and that of gliadin of wheat as 85.68 per cent, but in the majority of cases the sum of the nitrogen content of the amino acids actually isolated from vegetable proteins ranges from 50 to 65 per cent of the total nitrogen of the protein.

Uncharacterized nitrogen lost in analysis. In the various steps of the analytical procedure small amounts of nitrogen of unknown character are included in residues and solutions which are discarded. In general, these have been disregarded by workers in other laboratories, especially those losses occurring at points indicated in table 2 by the last four of the subheadings included under the heading "Uncharacterized nitrogen lost in analysis," but in this laboratory the nitrogen discarded at each of these steps has been determined. Under the above mentioned headings, it is shown that, on the average, only 0.492 per cent of the total nitrogen remains in the residues insoluble in strong alkali, or in other words 99.508 per cent of the total nitrogen of the feedingstuff is extracted as a result of the method employed, and that in individual cases as much as 99.78 per cent of the total nitrogen present was removed. As previous workers failed to isolate the proteins from the feedingstuff before hydrolysis, it is not established that part of the insoluble residue discarded in their methods did not include some nitrogen in the form of non-hydrolyzed protein although this does not seem highly probable. It is believed, however, that the nearly complete extraction of the proteins before hydrolysis lends to the accuracy of the method by facilitating hydrolysis and in reducing the amount of humin.

The largest item of loss occurs in the residue which remains after dissolving the precipitate of the bases in the amyl alcohol-ether mixture. This, presumably, is soluble humin which has not been adsorbed by the lime in the determination of ammonia, and fouls the solution at this point. This difficulty was also encountered by Menaul (23), who employed a preliminary precipitation with phosphotungstic acid in boiling solution for the separation of the humin and ammonia before the precipitation of the bases. In the present investigation, very little of the soluble humin appeared when the bases were precipitated, in most cases the precipitates being free from black particles. Washing with alternate portions of amyl alcohol-ether and water and then taking up the residue and washing thoroughly with water seemed to have little effect in reducing this source

of loss. A considerable portion of the nitrogen lost is soluble in the amyl alcohol-ether mixture, while smaller losses occur in the residues resulting from concentration of the solutions of the bases and filtered from the bases. Presumably, the second, third and fourth items of loss include some nitrogen which should be credited to the bases, but the character of this nitrogen was not determined. If these losses can be reduced, the total nitrogen of the bases of cottonseed meal may be found to be somewhat greater than the amount here reported.

Total nitrogen accounted for. Summation of the nitrogen found in the various fractions of the protein molecule together with that in the unavoidable losses in the procedure gives totals which average 98.75 per cent. While the use of the Van Slyke method of analysis has enabled others to account for as great a proportion of the nitrogen of feedingstuffs, it is doubtful, for reasons pointed out below, if their results give as accurate a picture of the distribution of nitrogen in feedingstuffs as is obtained by the procedure employed in the present investigation.

Physiological significance of the basic amino acids. Our knowledge of the physiological rôle of arginine and histidine has been enhanced by the studies of Ackroyd and Hopkins (24). Employing rations in which the nitrogen was provided in the form of hydrolyzed casein from which these two amino acids had been removed by precipitation according to the method of Kossel and Kutcher, it was found that rats receiving these rations declined rapidly in weight, but that when either amino acid was returned to the ration, loss in weight was prevented and some growth ensued. The investigators suggest that possibly either of these amino acids may be converted into the other by the animal body. It was further observed that when arginine and histidine are removed from the ration, the excretion of allantoin, which is the main end product of purine metabolism in the rat, was lowered. Subsequent experiments proved that the falling off of allantoin excretion was not due to lowered metabolism. From these observations and from the fact that the arginine, histidine and guanine molecules have similar structural

relationships, it was concluded that possibly one of the functions of arginine and histidine is to furnish the raw material for the purine metabolism of the animal organism.

The above conclusion regarding the importance of arginine in purine metabolism is given added weight by the findings of Myer and Fine (25) regarding the creatine content of muscle. Differences of as much as 2.5 per cent in the creatine content of muscle were noted as a result of feeding rations high and low in arginine.

That cystine plays an important part in nutrition has been brought out by several investigators, among them Osborne and Mendel (26). The latter obtained adequate growth by the addition of cystine to rations containing 9 per cent of casein, on which growth had been limited. Geiling (27), working in this laboratory, concluded that cystine seems to be necessary for the maintenance of adult mice. The importance of cystine to the animal organism is admirably set forth by Matthews (28).

In the intermediary metabolism of the body, that is, the metabolism of the tissue, sulphur probably plays a very important rôle. This is shown not only by the fact that it is absolutely necessary for the continued existence of the body, as necessary as nitrogen or any of the other elements, but also by the fact that it is one of the most labile elements of the protein molecule. No other element is split off from the proteins with greater ease than this. It is, indeed, the labile element *par excellence*. Moreover, cysteine, which is one of the amino acids, readily oxidizes itself. It is a reducing body. It oxidizes spontaneously and there are many points in its oxidation which strongly resemble the process of respiration. Thus the most favorable concentration of hydrogen ions for the oxidation of cysteine is the same as that in protoplasm; both cysteine and protoplasm are poisoned by many of the same substances, such as the nitriles, the cyanides, acids, and the heavy metals; their oxidations are catalyzed or hastened in the same manner by iron, arsenic and some other agents. For these reasons it has been suggested by Hefter and the author that there is more than a superficial connection between the oxidation of cysteine and the respiration of the cell.

The necessity of lysine for growth has been conclusively demonstrated by Osborne and Mendel (29). When gliadin of wheat, which contains only a minute amount of lysine, formed the sole source of protein in the rations of rats, the live weight of the animals was maintained over long periods, but normal growth could not be secured. When lysine was added to the rations, normal growth occurred. In other investigations (30), in which zein of maize was used as the source of the protein, it was found that a rat could be maintained at an almost constant weight of 50 grams for a period of one hundred and eighty-two days when tryptophane was added to the extent of 3 per cent of the zein. The further addition of lysine induced normal growth. Further study (31) of the necessity of lysine in the ration convinced these investigators that about 2 per cent of the protein of the ration must consist of lysine in order to promote normal growth in the rat. Osborne and Mendel (32) also demonstrated the necessity of lysine for the growth of chickens.

In view of the essential rôle which the basic amino acids play in nutrition as brought out above, it is reasonable to assume from a survey of the analytical results of cottonseed meal secured in this investigation that the proteins of this feedingstuff have a high nutritive value. The combined arginine and histidine content of cottonseed meal is greater than that of any other feedingstuff so far analyzed with the exception of the peanut. This feature alone is of great importance in view of the fact that arginine and histidine seem to be interchangeable in nutrition. While the lysine content cannot be said to be exceptional in any particular it seems apparent from the above discussion, that the combined proteins of cottonseed meal contain sufficient amounts of both cystine and lysine to render them adequate for nutrition.

Comparison with previous analyses of cottonseed meal. As shown in the introduction, there are but few determinations of the chemical composition of the proteins of cottonseed meal. The earliest studies were made upon one of the isolated proteins, the globulin or "edestin" of cottonseed, the results of which can not well be compared to analyses of the combined

proteins, since, as previously mentioned, the globulin contains but 42.3 per cent of the total nitrogen of cottonseed meal. In the accompanying table 5, the values secured by two previous investigators who made analyses of the combined proteins of cottonseed meal are brought together for comparison with those obtained in this investigation.

It is evident from the results presented that there is a general agreement between the three sets of values, but that there are considerable differences in several important particulars. As pointed out in the introduction, Nollau (10) calculated his results upon the total nitrogen content of the hydrolyzed solution after filtering off the solid residue insoluble in hydrochloric

TABLE 5

Distribution of nitrogen in cottonseed meal as determined by different investigators

(Results expressed in percentage of the total nitrogen of the feeding stuff)

INVESTIGATOR	HUMIN N	AMMONIA N	ARGININE N	CYSTINE N	HISTIDINE N	LYSINE N	AMINO N IN FILTRATE FROM BASES	NON-AMINO N IN FILTRATE FROM BASES	TOTAL N ACCOUNTED FOR
Nollau	6.27	14.06	12.77	2.74	7.57	1.94	45.02	7.49	97.48
Grindley	7.78	10.45	19.52	0.65	5.47	4.78	42.82	5.43	96.90
Nevens	6.58	9.49	18.74	0.91	7.40	3.81	40.12	2.68	98.75[1]

[1] Includes 9.03 per cent N removed in preliminary extractions plus uncharacterized nitrogen lost in method of analysis.

acid. This means that all of his calculations are too high, since a part of the nitrogen of the sample was undoubtedly discarded in the solid residue. The value of 6.27 per cent humin nitrogen reported by Nollau must, therefore, represent the soluble humin nitrogen, which is nearly as large a value as that obtained by the writer for the sum of the insoluble humin nitrogen plus the soluble humin nitrogen. The amount of soluble humin nitrogen found by the writer was but 3.89 per cent. Compared to the total humin nitrogen found by Grindley, et al. (9), the amount of total humin nitrogen as determined by the writer was 1.19 per cent less. The reduction of the humin nitrogen has no doubt been an important contributing

factor in the present investigation in securing somewhat higher values of the basic amino aids. In view of the known effects of acid hydrolysis of the proteins in the presence of carbohydrates, as already pointed out, it is reasonable to assume that the smaller amount of nitrogen discarded in the form of humin in these experiments than in those of Grindley may be attributed to the more complete separation of the proteins from the carbohydrates before hydrolysis.

The method of analysis of the proteins after hydrolysis by hydrochloric acid, as employed by Grindley et al., was similar to that employed by the writer, the main point of difference between the complete procedures being in the omission by the former workers of the extractions previous to hydrolysis. At just what point the 6.106 per cent of nonprotein nitrogen removed by the writer in the preliminary extractions might appear were it not so removed, is not clear. However, the sum of the ammonia nitrogen, amino nitrogen and non-amino nitrogen in the filtrate from the bases, obtained by Grindley et al, is 6.414 per cent greater than the sum of the corresponding values obtained by the writer, so it is possible that these three forms of nitrogen as reported by the former comprise some nitrogen not derived from the proteins as such.

It is evident from the table that the nitrogen of the bases as found by Grindley and his coworkers are in much closer agreement with those obtained by the writer than those reported by Nollau. The latter's figures for arginine are obviously too low, while his cystine values are more than four times as great as those of Grindley et al. and three times as great as those of the writer. Accordingly, the lysine nitrogen values as calculated by Nollau are correspondingly too low. The values for the total nitrogen of the bases as found by the three investigators in the order given in the table are as follows: 25.02 per cent, 30.42 per cent and 30.84 per cent respectively, the last being nearly 0.5 per cent higher than previous determinations.

The total nitrogen accounted for in the three reports is likewise shown to be 97.86 per cent, 96.90 per cent and 98.75 per cent. The greater amount in the last case is evidently due in part

at least, to the inclusion of the determinations of the uncharacterized nitrogen lost at points were unavoidable losses occur in the method of analysis. These losses were not determined by the first two investigators.

Comparison of the distribution of nitrogen in cottonseed meal with that in other feedingstuffs. A comparison of the results of analysis of the proteins of cottonseed meal, as discussed above, with those obtained by Hamilton, Grindley and Nevens (33) for alfalfa hay, oats and corn is of value in studying the relative nutritive value of the proteins of these feedingstuffs, as well as the applicability of the general method of analysis to feedingstuffs which vary widely in composition. In the analysis of oats and corn an additional preliminary extraction, which involves the use of hot trichloracetic acid, is employed to remove the starch. This extraction is not necessary in the case of cottonseed meal and alfalfa hay on account of the absence of starch in the former, as stated by Withers and Fraps (34), and the relatively small amount of starch in the latter.

The first point of interest in contrasting these feedingstuffs, as may be noted by reference to table 6, is their content of nonprotein nitrogen. Oats contain more than twice as much nonprotein nitrogen as cottonseed meal, while alfalfa hay contains more than three times as much. Hart and Bentley (35) found that 23.5 per cent of the nitrogen of alfalfa hay is present in a water soluble form, while Grindley and Eckstein (16) found a value of 28.4 per cent for the same feedingstuff.

The amount of total humin is greatest in the case of alfalfa, a natural result, since the proteins are more difficultly extracted from those feedingstuffs containing large amounts of crude fiber. The amount of humin in the case of corn is very small indeed, considering the high percentage of carbohydrates in this cereal, and compares very favorably with the amounts of humin resulting from the hydrolysis of pure proteins as shown in table 3. Cottonseed meal occupies a medium position in respect to the proportion of humin nitrogen.

The most striking difference between these four feedingstuffs is in their basic amino nitrogen content. Cottonseed

TABLE 6

Comparison of the distribution of nitrogen in cottonseed meal with that in other feedingstuffs

(Results expressed in percentage of total nitrogen of the feedingstuff)

FEEDING-STUFF	NONPROTEIN NITROGEN				RESULTS OF THE VAN SLYKE ANALYSIS										NITROGEN LOST IN METHOD OF ANALYSIS							TOTAL
	Soluble in absolute ether	Soluble in absolute alcohol	In filtrate from colloidal iron	Total nonprotein nitrogen	Insoluble humin nitrogen	Soluble humin nitrogen	Ammonia nitrogen	Arginine nitrogen	Cystine nitrogen	Histidine nitrogen	Lysine nitrogen	Amino acid N in filtrate from bases	Non-amino acid N in filtrate from bases	Total nonprotein + results of Van Slyke analysis	N in residue after treatment with strong NaOH	In alcohol precipitate of hot 2 per cent CCl_3COOH extract	Unadsorbed humin (filtered from solution during decomposition of bases)	Soluble in amyl alcohol-ether mixture	In residue filtered from solution of bases	In residue filtered from solution of filtrate from bases	Total nitrogen lost	Total nitrogen accounted for
Alfalfa hay	0.550	1.848	16.692	19.090	3.690	4.481	7.364	7.996	0.991	3.931	4.434	38.032	2.511	92.520	2.519		1.161	0.611		0.441	4.732	97.252
Oats	0.569	1.225	11.129	12.926	3.013	2.516	11.422	11.647	0.944	5.796	2.841	42.137	3.860	97.100	0.132	0.127	0.664	0.746	0.209	0.025	1.903	99.004
Corn	0.326	1.368	8.135	9.829	1.235	2.303	11.936	8.725	1.072	4.832	2.200	46.704	7.216	96.052	0.136	0.276	2.698	0.481	0.191	0.065	3.847	99.899
Cotton-seed meal	0.125	0.545	5.436	6.106	2.699	3.890	9.485	18.736	0.906	7.397	3.807	40.124	2.677	95.827	0.492		1.252	0.875	0.228	0.076	2.923	98.750

meal, as already indicated, is exceptionally high in arginine nitrogen, but it is also much higher in its total basic nitrogen content than the other three feedingstuffs, the values for the four feedingstuffs being: alfalfa hay, 17.412 per cent; oats, 21.228 per cent; corn, 17.529 per cent; and cottonseed meal, 30.846 per cent. The sum of the arginine nitrogen and histidine nitrogen is more than twice as great in the case of cottonseed meal as in the case of alfalfa hay and nearly twice as great as that of corn. From the considerations presented above regarding the biological significance of the basic amino acids, it would be logical to assume that these wide differences in the chemical composition of the proteins of different feedingstuffs indicate similar differences in their nutritive value, though probably not in corresponding degree. This point is mentioned in another paper in connection with the discussion of the results of the feeding experiment conducted for the purpose of studying the nutritive value of the proteins of cottonseed meal.

Alfalfa hay contains the smallest proportion of mono-amino acid nitrogen, possibly owing to its high content of nonprotein nitrogen while corn is exceptionally high in its content of both mono-amino and non-amino acid nitrogen.

The largest amount of nitrogen lost in the method of analysis occurs in the case of alfalfa, which is accounted for largely in the nitrogen remaining in the residues after the preliminary extractions have been completed. The next largest amount is in the case of corn, where the bulk of the loss is due to unadsorbed humin. The nitrogen is extracted very completely from both oats and corn. Cottonseed meal occupies a medium position with respect to the nitrogen lost in the analytical procedure.

The total nitrogen accounted for in the case of the various feedingstuffs is a point worthy of special note. The total is least in the case of alfalfa and greatest with corn. Here again cottonseed meal occupies a medium position. In this rather long method of analysis, which involves many extractions, concentrations, precipitations, filtrations and transfers, and which at some stages renders the proteins subject to putrefaction unless care

is taken, only 0.101 per cent of the total nitrogen originally present in the sample of corn was not accounted for, a very remarkable result indeed.

An examination of the results of individual analyses of the four samples of alfalfa hay and six samples each of oats and corn which were averaged to obtain the values shown in table 6, brings out the fact that the analytical results in the case of each of these feedingstuffs show, on the whole, less variability than the values for the eight samples of cottonseed meal shown in table 2. At least two factors operated to effect the difference. The analyses of the first three feedingstuffs mentioned were conducted by persons experienced in the manipulation and execution of the Van Slyke analysis and the analyses used for the averages were selected from a number of analyses. The analyses of cottonseed meal were made by the writer who had had no previous experience in the conduct of the Van Slyke method, and the analyses presented in table 1 are the entire results of the work. These considerations are strong evidence that the method of analysis here described is of general application to feedingstuffs and may readily be carried out.

Summary of the discussion of the results of the chemical analysis of the proteins. The accuracy of the determination of the amino acid content of the proteins of cottonseed meal has been increased over that of previous methods by the removal of the nonprotein nitrogen before proceeding with the hydrolysis of the proteins.

The accuracy of the determination has been still further increased by the reduction of the humin substances formed as a result of the hydrolysis of the proteins.

The amount of arginine nitrogen is much higher than that in most other feedingstuffs. The sum of the four basic amino acids is about 0.5 per cent higher than values previously found for cottonseed meal.

The method of extraction employed was found to result in the removal of 99.5 per cent of the total nitrogen present in the feedingstuff.

The sum of the ammonia nitrogen and amino acid nitrogen fractions is 83.132 per cent of the total nitrogen, an amount comparable to the sum of the same fractions previously obtained from pure vegetable proteins.

Of the total nitrogen originally present in the sample of cottonseed meal, 98.75 per cent was accounted for by summation of the fractions obtained at different stages in the method of analysis, a proportion greater than any previously reported for the same feedingstuff.

The complete method of analysis outlined in this paper is believed to be of general application to feedingstuffs and may readily be executed with successful results.

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THE PROTEINS OF COTTONSEED MEAL[1]

II. NUTRITIVE VALUE

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I. REVIEW OF THE PREVIOUS WORK ON THE NUTRITIVE VALUE OF THE PROTEINS OF COTTONSEED MEAL

Cottonseed meal and flour were found by Richardson and Green (1) to be satisfactory sources of protein for the growth of albino rats when these feeds furnished 18 per cent or more protein to the ration. Mendel (2) states that normal growth has been secured for considerable periods when the globulin of cottonseed was fed in suitable concentration, such concentration having been determined by Osborne and Mendel (3) as 18 per cent of the ration. The latter investigators (4) found that "Cottonseed flour forms a suitable adjuvant for the proteins of corn gluten," producing "satisfactory increments of growth" in chickens. In further studies of the value of certain proteins as supplements to corn gluten, these authors (5) demonstrated that the proteins extracted from cottonseed flour by sodium hydroxide solution were efficient supplements to the proteins of corn gluten for the growth of rats. The use of either the cottonseed globulin or the proteins precipitated from alkali extracts of cottonseed flour in an amount equal to 9 per cent of the ration resulted in "satisfactory growth" and when used to the extent of 6 per cent of the ration "considerable growth" was secured. This is interpreted as attesting the excellent quality of cottonseed proteins. McCollum and Simmonds (6) report the maintenance of body weights by rats fed a ration containing 6 per cent of protein derived from cottonseed.

[1] The results presented in this paper formed part of a thesis submitted to the Graduate School of the University of Illinois in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Animal Husbandry.

In studies of the relation of the quality of proteins to milk production, Hart and Humphrey (7) found an equality in efficiency of the proteins of gluten feed, oil meal, distillers' grains and cottonseed meal as supplements to the proteins of corn meal and alfalfa hay. In later experiments (8) of the same nature, cottonseed meal proteins proved less efficient than the proteins of gluten feed, oil meal and distillers' grains. In these experiments, however, the proteins of the feedingstuff tested formed but 40 per cent or less of the protein content of the ration, and the results were calculated upon the basis of the total nitrogen absorbed by the animals.

The digestibility of the proteins of cottonseed meal is stated by Fraps (9) to be 88.4 per cent in the case of steers and sheep; by Henry and Morrison (10) as 84 per cent, for choice and prime cottonseed meal; by Mendel and Fine (11), who employed dogs as experimental animals, as 67 to 75 per cent compared to 88 to 93 per cent for the proteins of meat; by Rather (12), using men as subjects, as 77.6 per cent in contrast to 96.6 per cent for the proteins of meat; and by Pomaski (13), who employed the gastric juice of the dog, as 99 to 100 per cent.

From a review of the literature, it is apparent that investigations upon the nutritive value of the proteins of cottonseed meal are quite limited in extent. In the majority of experiments cited, the investigators drew their conclusions from the maintenance of live weight, increase in live weight, state of health or combinations of these criteria. In most cases the amount of feed consumed is not recorded, so that it is impossible to judge whether or not the results secured were due to a failure of the animals to consume a sufficient amount of feed to cover their energy requirements. In but one series of experiments (7, 8), were the conclusions based upon metabolism studies. Hence, the further study of the nutritive value of the proteins of cottonseed meal constituted the object of the present investigation.

The toxicity of cottonseed meal

Before proceeding with the investigation, it was considered advisable to determine, so far as possible, whether or not the

toxic principle of cottonseed meal is associated with its proteins, and further, whether cottonseed meal would prove injurious to rats as has been found (14) in the case of many other species of animals.

From an examination of the literature, it would seem that there is but little basis for attributing the toxicity of cottonseed meal to its proteins. The assumption that the high protein content of cottonseed meal is responsible for its harmful effects (15) was denied by Dinwiddie (16), who maintains that this theory is not supported by a study of the recorded feeding tests. Withers and Brewster (17) attributed the toxic principle of cottonseed meal to a certain group of the protein molecule which contains loosely bound sulphur, but later work by Withers and associates (18) led them to conclude that the toxicity is due to the presence of "gossypol," a definite chemical compound soluble in ether and aniline. They believe "gossypol" may be changed to a nearly related substance "D-gossypol," the latter being insoluble in ether but soluble in aniline. When in alcoholic solution either of these compounds forms precipitates with the alcohol soluble proteins of wheat flour and of cottonseed meal. They reason that the reduction of the toxicity of cottonseed meal by heating may be due to the inability of the animal to digest the "gossypol" and "D-gossypol" protein compounds. The theory that gossypol is responsible for "cottonseed meal injury" is strengthened by the work of Alsberg and Schwartz (19).

In their series of feeding experiments with albino rats, Richardson and Green (1) and Osborne and Mendel (5) observed no toxic effects, but the cottonseed kernels themselves proved toxic.

In the light of the foregoing discussion, it seems very doubtful if the toxicity of cotton seed meal may be attributed to either its high protein content or to the character of the proteins which it contains. Further, it seems clear that commercial cottonseed meal of good quality may provide practically the entire nitrogenous components of the ration for albino rats over a considerable period of time with no injurious effects becoming manifest.

II. METHODS EMPLOYED IN STUDYING THE NUTRITIVE VALUE OF THE PROTEINS OF COTTONSEED MEAL

Object of feeding experiment. The object of this phase of the experiment was to study the nutritive value of the proteins of cottonseed meal and to compare their nutritive value with that of the proteins of corn and alfalfa hay for the growth of young albino rats. It was planned to feed rations containing a medium amount of protein, derived from the above mentioned sources, and by means of metabolism studies to determine the extent to which the proteins are utilized for maintenance and growth.

General plan of experiment. Young male albino rats in vigorous, healthy condition and having an initial weight of from 100 to 140 grams were employed. The metabolism periods were each seven days in length, two such periods following each other without intermission with each of the experimental rations tested. Before the first metabolism period and whenever the rations were changed, a three day preliminary or transition period, during which the ration to be employed during the metabolism period was fed, was inserted. It was planned to feed the animals as large amounts of the rations as they would consume, the daily feed allotment being slightly greater than the amount consumed.

The rats were placed in individual glass crystallizing dishes $7\frac{1}{4}$ inches in diameter and $3\frac{3}{4}$ inches in depth, inside measurements. The dishes were provided with weighted wire covers to which were attached large test tubes fitted with rubber stoppers and bent glass tubing, the latter extending downward through the wire cover. The test tubes were kept supplied with ammonia-free water. Large porcelain crucibles for receiving the feed were supported from the covers by means of wire frames. Crystallizing dishes of 60 mm. diameter were employed instead of the crucibles for rations containing alfalfa, which were very bulky. Ventilation was provided by means of a system of rubber tubes which conducted a current of compressed air to the bottom of each dish. From two to three sheets of filter paper, cut to fit the dishes, were placed in the bottom of each dish daily to absorb the urine.

Feces and urine were collected daily. In most cases the filter paper absorbed the urine completely, so that the feces were nearly always found dry. In a very few cases, particularly with rations containing alfalfa which resulted in the production of very bulky feces, there was evidently absorption of urine by the feces, so that it was necessary to extract the feces once or twice with hot acidified water before collecting them. The feces were preserved under 95 per cent alcohol acidified slightly with sulfuric acid. At the end of each seven-day metabolism period, the feces were transferred to large Kjeldahl flasks and digested according to the to the Kjeldahl-Gunning-Arnold method with sulfuric acid, sodium sulfate and mercury. The resulting solutions were transferred to 500 cc. volumetric flasks and aliquots taken for distillation.

After collecting the feces, the urine was extracted from the filter papers by washing with a stream of ammonia-free water acidified with sulfuric acid and held at nearly boiling temperature. The filter paper was thoroughly pulped and pressed out after each extraction by means of a glass rod. From four to six extractions were made, using 40 to 60 cc. of water each time, the sides and bottom of the dish also being thoroughly washed. The extracts were filtered through glass wool into 250 cc. volumetric flasks. The flasks were allowed to remain in the ice box over night. The solutions were then made up to volume at ice box temperature and transferred to 2.5 liter bottles which were kept in a cold storage room at a temperature of 5° to 10° C. until analyzed. About 0.5 gram of powdered thymol was employed as a preservative in each bottle in which the week's urine was collected. The composites were thoroughly mixed and aliquots measured out in the cold for total nitrogen determinations.

The feed was weighed daily into the crucibles and mixed with a little nitrogen-free water to the consistency of a thick paste. The following day the feed residues were scraped out and dried in the same oven and at the same temperature as the rations used. In some cases the animals scattered the feed from the crucibles about the metabolism dish. In such cases the eed

remaining in the metabolism dish at the time of collecting the excreta was carefully separated and added to the feed residues. When thoroughly dry the weight of the feed residues was determined and the amount of feed actually consumed during the seven-day period calculated. By previous tests in this laboratory it was found that the error involved in this calculation due to a difference in the moisture content of the residues and ration was less than 1 per cent, and further that the nitrogen contents of the residues and ration were identical (20).

Preparation of rations. In preparing the experimental rations, the starch used was first dextrinized by heating on the steam bath after the addition of cold water and a few crystals of citric acid. When ground corn formed one of the constituents of a ration, it was mixed with the starch and the starch of the mixture dextrinized. The other ingredients were then added, the agar being dissolved in boiling water and added at the boiling temperature. When necessary more hot water was added and the ingredients thoroughly mixed. The rations were dried on glass plates, placed above the steam bath, finely ground and dried in an oven at a temperature of about 40°C. After drying for several days, the rations were mixed, sampled for analysis and placed in tightly covered glass jars.

The nitrogen free ration consisted of the following:

	per cent
Salts	5
Butterfat	10
Sucrose	8
Starch	74
Agar	3

Water soluble vitamin, 150 mgm. of solids per 100 grams of ration.

The composition of the other rations is shown in table 1. The salt mixture used was compounded according to the formula of Osborne and Mendel (21), while the water soluble vitamin consisted of Osborne and Wakeman's (22) fraction II of the concentrated extract of the water soluble vitamin of brewers' yeast. The stock supply of the latter was prepared in the form of a water solution which was preserved by means of a small quantity of chloroform and kept in the ice box. The butter-

fat was obtained by placing fresh creamery butter in large beakers, heating to a temperature of 50° to 60° on the steam bath, centrifuging for an hour or until the fat became water clear, and then siphoning off the clear fat.

It was planned that all rations containing a protein feedingstuff should carry 10 per cent of protein (N × 6.25), but the actual content of protein was slightly higher, ranging from 10.38 per cent to 11.28 per cent, due to the fact that some of the constituents used in making up the ration had a slightly higher moisture content than the dried rations.

TABLE 1

Composition of experimental rations (expressed in percentage)

CONSTITUENT	RATION						
	1*	2	3	4*	5	6	7
Cottonseed meal	23.9			13.1		10.3	7.7
Corn		72.7		32.3	28.4		19.3
Alfalfa hay			63.5		40.6	35.7	29.3
Starch	55.1	6.3	18.5	33.7	13.0	36.0	25.7
Agar	3.0	3.0		3.0			
Sucrose	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Butterfat	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Salts	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Total nitrogen content	1.750	1.660	1.806	1.777	1.708	1.790	1.782

* Water soluble vitamin preparation added at the rate of 1.5 mgm. of solids per gram of ration to rations 1 and 4.

In preparing the rations in which two or more feedingstuffs were combined an effort was made to have each feedingstuff furnish an equal amount of digestible protein, using the coefficients of digestibility secured in period 2 as a basis for calculation, but keeping the total content of crude protein the same throughout the experiment, namely, 10 per cent.

The cottonseed meal used in the rations was a part of the same sample which was employed in the analytical study presented in a preceding paper. Through the courtesy of the Plant Breeding Division of the Agronomy Department of this university a

quantity of "high protein" corn containing 2.2 per cent nitrogen was secured, which made it possible to formulate a suitable corn ration containing 10 per cent protein. All of the feeding-stuffs used were in a finely ground condition before compounding the rations.

Accuracy of metabolism work with small animals. Since the accuracy of metabolism work depends in large measure upon the accuracy of the collection of the excreta, especially when such small amounts of nitrogen are involved as in the case of the smaller laboratory animals, several experiments were carried out to test the accuracy of the methods employed.

Each day during period 6, when 6 rats were receiving the same feed mixture, the paper residues remaining after extraction of the urine were collected in glass jars and placed in the ice box. At the end of the metabolism period, the entire mass of residues, including the glass wool used in filtration, was transferred to a 2 liter beaker and boiled for some time with about 1 liter of water acidified with sulphuric acid. The extracts were decanted and the procedure repeated, the acidified water being pressed out from the residues. The extracts were filtered through glass wool, evaporated on the steam bath and transferred to Kjeldahl flasks for total nitrogen determination. The paper residues also, together with the glass wool, were transferred to large Kjeldahl flasks, and total nitrogen determined. The results of these determinations are shown in table 2. The nitrogen extracted in the procedure described just above is assumed to be of urinary origin and is compared to the total amount of urinary nitrogen excreted during the week. It is shown that, as an average of 6 such determinations, the error in the collection of urine amounted to 2.0 per cent of the total nitrogen.

The nitrogen remaining in the paper residues which was not extracted by boiling with acidified water was assumed to be fecal nitrogen. In collecting the feces it was sometimes impossible to entirely remove the fecal matter from the filter papers, especially when the rations tended to cause a laxative condition. Such a condition was not a constant effect with any ration employed, but was more frequent with rations containing alfalfa.

With the latter rations, three filter papers were generally placed in each dish daily, while with the other rations two papers were used. Analysis of the filter paper showed that seven filter papers of the size used contained 1.06 mgm. of nitrogen. In making the calculations shown in table 2 it was assumed that the nitrogen originally contained in the filter papers was insoluble in the hot dilute acid employed in extraction and this has been deducted from the non-extractable nitrogen remaining in the paper residues. To the extent that such nitrogen is soluble in

TABLE 2

Test of the completeness of extraction of nitrogen from filter papers used as absorbents during one metabolism period

RAT NUMBER	EXTRACTABLE N IN PAPER PULP	N IN URINE COLLECTED	TOTAL URINARY N EXCRETED	ERROR IN COLLECTION OF URINE	NON-EXTRACTABLE N IN PAPER PULP	N IN FECES COLLECTED	TOTAL FECAL N EXCRETED	ERROR IN COLLECTION OF FECES	TOTAL N IN PAPER PULP RESIDUES	TOTAL* N OF EXCRETA FOR PERIOD	TOTAL ERROR IN COLLECTION
	mgm.	*mgm.*	*mgm.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	*per cent*	*mgm.*	*mgm.*	*per cent*
2	12.5	558.4	570.9	2.2	25.5	546.8	572.3	4.5	1143.2	38.0	3.3
3	15.9	716.3	732.2	2.2	25.8	833.7	859.5	3.0	1591.7	41.7	2.6
5	9.0	619.3	628.3	1.4	22.2	645.3	667.5	3.3	1295.8	29.2	2.3
6	13.2	541.0	554.2	2.4	20.1	615.8	635.9	3.2	1190.1	33.3	2.8
8	11.5	652.5	664.0	1.7	12.9	672.0	684.9	1.9	1348.9	24.4	1.8
9	18.3	764.3	782.6	2.3	28.0	602.0	630.0	4.4	1412.6	46.3	3.3
Average.....	13.4		655.4	2.0	22.4		675.0	3.3	1330.4	35.5	2.7

* After deduction of nitrogen contained in same number of clean filter papers.

hot dilute acid, however, it would tend to offset to a small degree the losses in the collection of urine, although the correction would not be in proportion to the variable total urinary nitrogen. The error in the collection of feces was found to be 3.3 per cent, using the average of six determinations, or, comparing the total nitrogen lost to the total nitrogen excreted in urine and feces during the week, the total error in the collection of both feces and urine is 2.7 per cent.

The results obtained in this test were applied to the metabolism data for period 6, the period during which this test was conducted,

to determine what effect the incomplete collection of the excreta has upon the utilization coefficients. It is evident that an error in the collection of the excreta is reflected directly in the nitrogen balance and in the percentage utilization. Using the average values given in table 2 of 13.4 mgm. of nitrogen representing uncollected urine and 22.4 mgm. of nitrogen representing uncollected urine and 22.4 mgm. of nitrogen representing uncollected feces during a seven day period, and applying them to the data for the individual animals during period 6 it is found that the percentages of utilization of absorbed nitrogen as given in the tables are approximately 2 per cent too high, while the percentages of absorbed nitrogen retained are slightly more than 3 per cent too high. Like results are obtained for period 7 during which the animals received the same ration as in period 6.

The completeness of the collection of urine was tested in still another way, that of the recovery of urea which was added in the form of a standard solution to the daily feed. Two mature rats were employed in the test. After the excretion of urinary nitrogen had been reduced to a nearly constant level by subsistence on protein free rations for seven days, known amounts of urea were added to the ration.

The excretion of this extra nitrogen was very prompt, as indicated by the results of the test as shown in table 3. In the case of rat 10, the results are somewhat difficult to interpret, owing to the fact that the consumption of feed decreased rapidly, evidently resulting in catabolism of small amounts of body protein to furnish energy for the body. By using the figures for the average excretion of nitrogen during the three days preliminary to the first urea day as the level of the endogenous nitrogen during the three urea days, the apparent recovery of the urea nitrogen amounted to 119 per cent. Similar results are obtained if the average nitrogen excretion during the preliminary and subsequent periods are employed. If it be assumed that, owing to a decrease in feed consumption below that of the energy requirements, the endogenous nitrogen should be taken as corresponding to that in the subsequent period, then the recovery of the urea nitrogen was approximately 100 per cent. Undue

emphasis should not be placed upon the results given by this animal, however.

With rat 11 more reliable data were obtained as it was not evident that the feed consumption was deficient in meeting the energy requirements. By using the average nitrogen excretion during both preliminary and subsequent periods as the level of the amount of body nitrogen excreted, the recovery of

TABLE 3

Test of the completeness of collection of urine by addition of urea to the ration

DAY	LIVE WEIGHT	FEED EATEN	UREA N ADDED	DAILY URINARY N
		Rat 10		
	grams	*grams*	*mgm.*	*mgm.*
1	182	10.5	0	35.6
2		10.5	0	24.0
3		10.5	0	23.5
4	178	6.1	42.1	74.6
5		6.1	42.1	83.0
6		6.1	42.1	76.0
7		4.6	0	35.9
8	167	5.6	0	35.8
		Rat 11		
1	176	7.2	0	42.8
2		7.2	0	30.2
3		7.2	0	39.8
4		7.7	56.5	82.8
5		7.7	56.5	101.8
6		5.4	0	37.5
7	164	5.4	0	29.3

urea nitrogen amounted to 95 per cent of that fed. If the average of the amounts of nitrogen excreted during days 3 and 6 be used as this level, then the recovery of urea nitrogen was practically 100 per cent.

It is evident from the data presented concerning the recovery of urea nitrogen that the method for the collection of urine as employed in these experiments, gives very nearly quantitative results.

Further tests of the metabolism method employed, which were performed in this laboratory and are described below, show that loss of ammonia due to bacterial decomposition of the urine does not occur to any appreciable extent.

In the first test, three portions of urine of 5 cc. each were measured out for total nitrogen determination. At the same time 5 cc. portions of urine were added to each of six metabolism dishes containing the usual number of filter papers. Three of these dishes were allowed to stand at room temperature in the metabolism laboratory, while the remaining three were placed in an oven at a temperature of about 40°C. At the end of twenty-four hours, the urine was collected from all six dishes in the same manner as employed in the metabolism work, i.e., by washing with hot acidified water. As a result of this test it was found that 5 cc. of urine contained 28.18 mgm. of nitrogen, while the amounts of nitrogen recovered from the dishes kept for twenty-four hours at room temperature and at 40°C. were, respectively, 27.61 mgm. and 27.54 mgm.

In the second of these tests, the urine from one rat receiving a constant amount of the same ration was collected daily. On the first, third, and fifth days the urine was collected at once by washing with acidified water in the usual manner. The urine was made up to a volume of 250 cc. and aliquots taken at once for total nitrogen determinations.

On the alternate days the urine was not collected at the end of the twenty-four hour period, but the filter paper was moistened and the dish allowed to stand another twenty-four hours in the metabolism laboratory before extraction in the usual maner. The rat meanwhile was transferred to a clean dish. At the end of the second day the urine was collected by washing as usual, made up to volume, and aliquots taken for total nitrogen determination. The results of the test are shown in table 4. It is evident that there was no appreciable loss of nitrogen due to bacterial decomposition even after the metabolism dishes had stood for two days.

How shall the nutritive value of proteins be compared? In attempting to compare the biological values of various feeding-

stuffs, it is first necessary to select a suitable basis for comparison. Several different methods for comparison are in use. As pointed out in the introduction one of the most common methods is to base conclusions upon the character of the growth secured, the principal index in such a case being the gain in live weight. Some of the data from table 1 of the appendix are brought together in table 5. These data were all obtained in periods 2 and 3. The rats consuming the cottonseed meal ration showed marked fluctuations in gain in live weight which can not be accounted for on the basis of a variable food intake. With the corn ration, there was a gain in weight by one rat in one period

TABLE 4

Effect of allowing metabolism dishes to stand twenty-four hours and forty-eight hours before the collection of urine

DAY OF EXPERIMENT	DAILY URINARY N WHEN COLLECTED AT END OF TWENTY-FOUR HOURS	DAILY URINARY N WHEN COLLECTED AT END OF FORTY-EIGHT HOURS
	mgm.	*mgm.*
1	57.9	
2		64.9
3	60.8	
4		67.3
5	63.2	
6		60.3
Average.....	60.3	62.1

only. The nitrogen of the ration, however, was being used by the body to a considerable extent, for on a nitrogen-free ration the same animals lost 16 to 19 grams in weight during a period of equal length, compared to 1 to 2 grams on the corn ration. Likewise, there was also a large variation in gains in weight by the rats receiving the alfalfa ration, the average gains of rats 7 and 8 being almost zero. It is probable that many factors other than the quality and amount of the protein consumed influence the gain in weight, such as the proportion of carbohydrates in the ration, amount of water drank, exercise, the proportions of gain which is protein or fat, etc. While interpretations based upon the gain in live weight may lead to reliable

conclusions in some instances, the adoption of such a criterion in the present case would certainly be a fallacious procedure.

It is a matter of common knowledge that all animals require protein food for keeping the body tissues intact, known as the maintenance requirement, and secondly, that growing animals need an additional quantity of protein for the construction of new tissue. If a standard for the comparison of the value of the proteins of feedingstuffs for growth is based simply upon the proportion of the nitrogen of the feedingstuff which is retained by the body, the values secured in such a manner are subject to gross errors. With such a method of computation the apparent value of the proteins for growth depends largely upon the nitrogen intake, or, in other words, upon the amount of feed eaten, and this in turn is subject to individual idiosyncracy and the palatability of the ration. As mentioned elsewhere, when the ration proves unsatisfactory, rats tend to eat less and less from day to day. By reference to table 5 it may be seen that both rats 5 and 6 ate less of the corn ration during period 3 than during period 2. Rat 5, during period 2, retained 16 per cent of the nitrogen absorbed, but during period 3, when the amount of feed consumed was evidently too little to maintain the animal's live weight, the nitrogen of the excreta was greater than the nitrogen intake, so that there was a loss of nitrogen from the body resulting in a negative value for the percentage of absorbed nitrogen retained. Similarly, the percentage of absorbed nitrogen retained by rat 6 falls from 23 per cent in period 2 to 9 per cent in period 3, a change which in this instance may also be attributed to a decreased food intake. Were the average percentage of the absorbed nitrogen retained by rats 5 and 6 taken as a measure of the utilization of the proteins of corn for growth, it would be a distorted picture of the facts.

There seem to be factors other than the amount of feed consumed which render the use of the percentage of absorbed nitrogen retained an unsatisfactory criterion of the utilization value of the proteins of feedingstuffs. As may be seen by reference to table 5, the percentage of nitrogen retained by rat 7 in periods 2 and 3 falls from 23 per cent to 8 per cent, and in the case of

rat 8 the percentage falls from 16 per cent in period 2 to 6 per cent in period 3. These violent fluctuations are not due entirely to a decreased nitrogen intake, for in the case of rat 7 the feed intake increased slightly during the second period. They are, however, associated with a slightly decreased digestibility, although there may be other causative factors.

TABLE 5

A comparison of three methods of expressing the utilization of proteins

RAT NUMBER	PERIOD	RATION	FEED CONSUMED DAILY	GAIN IN WEIGHT FOR PERIOD	UTILIZATION OF ABSORBED N FOR MAINTENANCE AND GROWTH*	ABSORBED N RETAINED*
			gm.	*gm.*	*per cent*	*per cent*
1	2	Cottonseed meal	9.52	11	63	31
1	3	Cottonseed meal	9.48	6	65	29
2	2	Cottonseed meal	8.66	9	64	21
2	3	Cottonseed meal	8.57	5	64	17
3	2	Cottonseed meal	11.51	12	71	44
3	3	Cottonseed meal	12.47	11	70	43
4	2	Corn	7.44	2	49	15
4	3	Corn	8.05	0	47	15
5	2	Corn	7.66	0	54	16
5	3	Corn	6.30	−1	43	
6	2	Corn	7.49	−1	55	23
6	3	Corn	6.35	−2	48	9
7	2	Alfalfa hay	9.33	6	62	23
7	3	Alfalfa hay	9.50	−5	57	8
8	2	Alfalfa hay	9.19	2	58	16
8	3	Alfalfa hay	8.20	−2	57	6
9	2	Alfalfa hay	11.42	3	67	22
9	3	Alfalfa hay	13.67	7	73	38

* For method of calculation of these percentages, see table 1 of the appendix.

Any suitable criterion used in feeding experiments for the comparison of the utilization of proteins for growing animals must necessarily consider the effect of the proteins in providing nitrogen for maintenance, for in growing animals these processes proceed concurrently. It is doubtful if the true protein requirement for the maintenance of a growing animal can be determined by feeding a ration containing protein, for Waters (23) has shown

that when young steers received a ration which just maintained their live weight some of the growth processes continued. Similar results were obtained by Aron (24).

Perhaps the nearest approach to the determination of the exact amount of nitrogen required for the maintenance of a growing animal is a study of the nitrogen excretion when the ration consists entirely of carbohydrates and this is being taken in an amount in excess of the body's energy requirement. Under such conditions the nitrogen excretion falls to a very low level, often to one third or less of that during starvation, as shown by Folin (25), Landergren (26), Cathcart (27) and Thomas (28). The amount of protein then being catabolized has been defined by Rubner (29) as the "wear and tear" quota of protein metabolism, which requires a "repair quota" of protein in the diet in order to replace it. A "growth quota" must be supplied the young animal in addition to the "repair quota" in order that growth may take place. Using dogs as experimental animals, Michaud (30) found, when protein in the form of casein or dog tissue was fed in amounts equivalent to the protein minimum after the metabolism had been reduced to this level, that there was no further loss of nitrogen from the body. Thomas (28) found, after the reduction of the nitrogen excretion by a carbohydrate diet to the minimum level, that nitrogen equilibrium could be restored by the ingestion of an amount of protein nitrogen in the diet equal to the amount of nitrogen being eliminated in the excreta.

On a nitrogen-free diet the amount of nitrogen excreted daily in the feces was about 1 gram, and this amount was not increased with a nitrogen intake of 3 grams furnished by a highly digestible protein. With diets producing a large bulk of feces he found that a greater proportion of digestive juices was eliminated, increasing the nitrogen content of the feces.

In the interpretation of the feeding experiments which follow it is assumed that the amount of protein required for body maintenance is a constant value for each individual at a given weight. Such an assumption is entirely in harmony with the theories of many investigators in the fields of both human and animal nu-

trition. Folin (25), as a result of his study of the different forms in which nitrogen is excreted on high and low protein diets, was led to formulate his theory of two distinct types of metabolism. The endogenous is most characteristically represented by the excretion of creatinine, which, "on a meat-free diet is a constant quantity, different for different individuals, but wholly independent of quantitative changes in the total amount of nitrogen eliminated." Folin's results have been substantiated by an immense amount of investigation concerning urinary creatinine, and his theory of protein metabolism is now almost universally accepted in its main essentials, although it has been necessary to modify this view slightly with our increased knowledge of the chemistry of the proteins.

The constancy of the protein minimum for the individual is accepted by Lusk, Thomas and others. That this minimum differs between individuals and is subject to slight variation due to environmental, temperamental and dietary changes, possibilities which are not precluded by Folin's theory, is brought out by Cathcart (31):

> As regards the uniformity of the protein minimum it may be definitely stated that there is no single minimum—common to all men and to all conditions. Rubner, Caspari and others also hold firmly to this opinion. Caspari quotes the work of Larguier des Bancels in 1903 in confirmation of this belief in the existence of mutiple protein minima. The facts that can be cited against a common minimum are many in number. Thus the caloric value of the diet given influences very materially the amount of nitrogenous material required, as is shown, for example, in the experiments of Voit and Korkunoff. Then, as Rubner has pointed out, the temperature influences quite markedly the course of protein metabolism. Finally, another factor of considerable importance may be mentioned, the activity of the organism.

In the sphere of animal nutrition, the constancy of the maintenance requirement for farm animals is recognized by Kellner, Armsby and Haecker. C. Voit and Kellner also proved conclusively that work production of varying intensity by farm animals does not increase the protein metabolism appreciably.

In this connection it should be stated that some of the current theories of protein metabolism are not in complete harmony with that just mentioned. Among these are the reversible reaction theory of Sherman (32), which seeks to account for the functions which the food protein serves in body maintenance by the assumption that the absorption of the amino acids liberated in digestion causes an increased concentration of these in the tissues which checks or even reverses the hydrolysis of tissue protein. This theory is hardly compatible with the known facts regarding the constancy of the endogenous metabolism, which has been found (33) to be uniform from hour to hour, as evidenced by the creatinine elimination, even during digestion and absorption of proteins. Absorption of the protein digestion products from the alimentary tract presumably occupies only a portion of the twenty-four hour period, so that even if the endogenous catabolism were inhibited by the increased concentration of amino acids, it would be only temporary, for it has been shown (34) that, in adult rats, protein feeding has only a very slight effect upon the amino acid concentration in the tissues. This known slight increase in the amino acid content of the tissues during digestion would not be of sufficient magnitude to inhibit the action of digestive enzymes when a digestion experiment is conducted in vitro. Further, it is unreasonable to assume that anabolism and catabolism of tissue proteins are simply reversible phases of the same reaction and that both these processes are promoted by the same enzyme. In the young growing animal protein feeding has been demonstrated (34) to increase considerably the amino acid content in the tissues. Were the endogenous metabolism inhibited entirely during the time this concentration is maintained, as must be assumed from the reversible reaction theory, then the catabolism of tissue protein per unit of weight in the young growing animal would be but a fraction of that of a mature animal.

Osborne and Mendel (35) explain the maintenance protein requirement upon the need of certain amino acids to serve special physiological functions, such as the formation of the active principles of the internal secretions and hormones. This theory

assumes, therefore, that when the animal is receiving a nitrogen-free ration, body tissue must be catabolized to furnish the essential amino acids, but that, on the other hand, when a ration containing a complete assortment of amino acids in sufficient amount is being consumed the endogenous metabolism is only a fraction of that on a non-nitrogenous ration, and that then only the catabolism of the internal secretions or the tissues which regulate metabolism would be affected. Under these conditions the muscles would scarcely be affected and the creatinine elimination would bear little relation to the endogenous metabolism. Moreover, the theory does not satisfactorily account for the effect of ammonium salts, mixtures of amino acids and single amino acids in partially supplying nitrogen for maintenance.

Since the plan of procedure and method of calculation employed in this investigation are dependent primarily upon the basic assumption that the endogenous metabolism of the animal organism is constant in character and amount for an individual at a given age and weight, an examination of the data obtained during all the metabolism periods was made in order to ascertain, if possible, whether this assumption is substantiated by the experimental results at hand. In making this examination, the data embodied in appendix table 1 were employed to obtain the first set of values shown in the column headed "As determined" under each "period" of table 6. These values were obtained by deducting the sum of the endogenous nitrogen and the metabolic nitrogen in the feces from the daily urinary nitrogen and calculating the percentage of the daily nitrogen intake which the remainder forms. The second column of values under each "period," headed "As calculated," was obtained in the same way as those in the first column, except that the average value for daily urinary nitrogen as determined with nine rats in period 1, i.e., 22 mgm. per 100 grams live weight, is used in calculating the "endogenous nitrogen" for periods 2 to 7, inclusive, instead of the individual values determined in the same period.

It is shown in table 6 that the individual daily urinary nitrogen values for rats 2, 5, and 9 are above the average, while those for

rats 3, 6 and 8 are below the average. Hence, for comparison, rats 2 and 3, 5 and 6, and 8 and 9 are arranged in pairs. The rats of each pair received the same rations throughout the experiment. It is natural to assume that two animals of the same age and weight and in a comparable nutritive condition will utilize the same ration with an equal degree of efficiency, subject of course to inherent individual variability. By reference to table 6, it may be noted that this holds true to a very great extent, although the natural variations to be expected in biological work of this kind are in evidence.

TABLE 6

The proportion of the daily intake of nitrogen above maintenance which appears in the urine (expressed in percentage)

RAT NUMBER	PERIOD 2		PERIOD 3		PERIOD 4		PERIOD 5		PERIOD 6		PERIOD 7	
	As determined	As calculated	As determined	As calculated	As determined	As calculated	As determined	As calculated	As determined	As calculated	As determined	As calculated
2	12.0	16.3	9.8	13.1	1.7	5.3	8.3	12.4	8.9	13.4	8.8	13.7
3	8.8	8.3	9.4	8.9	7.8	7.2	7.3	6.9	9.9	9.3	9.9	9.2
5	26.5	29.1	35.0	38.2	18.7	21.0	18.5	21.1	12.3	14.4	11.4	13.7
6	23.9	20.0	31.3	26.7	21.0	17.5	16.4	12.3	12.6	9.5	14.0	11.1
8	9.1	7.4	7.8	5.8	15.6	14.3	10.4	8.9	14.3	12.5	9.7	7.7
9	4.1	5.5	1.9	3.1	15.8	17.3	14.9	16.4	16.0	17.6	16.4	18.1

Considering first the values listed under the headings "As determined" in each period it is evident that there is a marked uniformity exhibited by the animals of each pair throughout the different experimental periods with but few exceptions. For example, it is shown that rats 2 and 3 eliminate in the urine about the same proportion of the nitrogen intake above maintenance "as determined," with the exception of periods 2 and 4. Rats 5 and 6 show quite uniform results throughout the entire six periods. Rats 8 and 9 do not very widely from each other in periods 4, 5 and 6. Moreover, certain rations seem to have a pronounced effect on these percentages. Rats 5 and 6 received the corn ration during periods 2 and 3, and the corn-

cottonseed meal ration during periods 4 and 5. With both of these rations, the proportion of the daily nitrogen intake eliminated in the urine was greater than with the other rations, but both animals behaved alike in this respect.

On the other hand, when the average values for the endogenous nitrogen of the urine are used in calculating maintenance, the variations in the percentages of the nitrogen intake above maintenance appearing in the urine of the animals of each pair are greatly exaggerated in 14 of the 18 cases involved, as shown under the headings "As calculated." In one of the four cases, that of rats 5 and 6, period 7, there is no change. In the other three cases, those of rats 2 and 3, period 4, and rats 8 and 9, periods 2 and 3, the spread is lessened slightly. In many cases the use of an average maintenance factor increases the spread between the animals of a pair as much as 200 per cent. These data seem to indicate quite conclusively that the endogenous metabolism is a function of the individual animal and that this is a definite and probably constant value under a given set of conditions. This is quite in harmony with the theory of Folin (25) respecting the constancy of endogenous metabolism. In calculating the results of these experiments, therefore, the use of individual maintenance values is evidently justifiable.

If, having determined the minimal "wear and tear" quota of an animal by appropriate metabolism experiments, feeding tests are then initiated to study the utilization of the proteins of feedingstuffs by that animal, it is possible to calculate the proportion of the nitrogen excreted in the urine which is of endogenous origin and likewise the amount of fecal nitrogen whose source is metabolic. This method follows closely that of Thomas (28) in calculating the biological values of foodstuffs. Such a criterion evaluates the proportion of the nitrogen of the food which is actually utilized by the animal in its metabolism, whether the animal is consuming an amount of protein which is not quite sufficient for it to maintain its live weight, or whether growth is permitted. The values obtained by applying this method of calculation to the data of metabolism periods 2 and 3 are shown in the last column of table 5. An inspection of these values

shows that this method overcomes some of the objections raised to the other methods. When the gain in live weight falls to zero or a little below, but at the same time it is evident that some of the nitrogen of the ration is being used by the animal, the percentage "utilization of the absorbed nitrogen for maintenance and growth" is lowered, but is, nevertheless, a very distinct positive value. With a slight decrease in food intake, there is usually a corresponding fall in the "utilization of absorbed nitrogen for maintenance and growth" coefficient, but this decrease is not so extreme as when the results are calculated upon the basis of the "absorbed nitrogen retained." Moreover, a decrease in the feed intake to just below the maintenance level does not result in a negative value. On the whole the "utilization of absorbed nitrogen for maintenance and growth" coefficients are much less variable than those of the "absorbed nitrogen retained." The former method is subject to a coefficient of variability of 8.4 per cent when all of the values obtained in the six metabolism periods are considered, and a mean is assumed for each ration. Similarly, the same values when calculated upon the basis of the percentage of "absorbed nitrogen retained" have a coefficient of variability of 27.7 per cent, a striking and important difference.

In this investigation, therefore, the endogenous metabolism of the experimental animals was studied during a metabolism period in which a nitrogen-free ration was fed, and this was followed by six metabolism periods in which the proteins of cottonseed meal were compared with those of corn and alfalfa hay.

III. DISCUSSION OF THE RESULTS

Metabolism of the rat on a nitrogen-free ration. During the first eleven days of the experiment the rats which had been consuming an ordinary stock ration were given nitrogen-free rations prepared as described above. On the fifth day collection of the feces and urine was begun, and continued for seven days. In order to check the results secured during the first period of the experiment, three of the rats were again placed on nitrogen-free

rations during period 6, after having received nitrogenous rations during the intervening time. The principal data of these trials are included in table 7. The rats lost slightly more than 2 grams of weight per head daily. For the first few days of the period the animals ate the ration in large quantities, but as they apparently found the feed unsatisfactory, they consumed smaller and smaller

TABLE 7

Metabolism of the rat when receiving a nitrogen free ration

RAT NUMBER	PERIOD	INITIAL WEIGHT	FINAL WEIGHT	AVERAGE FEED CONSUMED DAILY	DAILY URINARY NITROGEN	DAILY FECAL NITROGEN	DAILY URINARY N PER 100 GRAMS LIVE WEIGHT	FECAL NITROGEN PER 100 GRAMS FEED
		grams	*grams*	*grams*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	1	99	88	6.51	25	17	27	253
2	1	118	102	7.43	31	19	28	255
3	1	134	116	9.07	26	24	21	259
4	1	135	118	8.79	24	22	19	251
5	1	120	104	7.29	28	20	25	273
6	1	129	110	7.86	21	21	18	270
7	1	123	107	7.43	23	20	20	266
8	1	122	109	8.14	22	24	19	298
9	1	126	114	7.43	29	18	25	246
Average				7.78			22	264
1	6	113	102	6.10	22	19	20	306
4	6	135	130	9.02	16	20	15	223
7	6	118	105	4.76	24	17	21	357
Average				6.63			19	295

amounts from day to day, and a few of the animals scattered the feed from the containers at once upon being fed.

It was found that, while subject to some individual variation, the amount of urinary nitrogen per 100 grams of live weight is fairly constant, the average value of 22.4 mgm. obtained agreeing almost exactly with that found by Mitchell (20) in a large number of metabolism periods. From the results secured in period 6 it appears that there is a slightly less intense endogenous metabolism as the animal becomes older, as evidenced by a decreased

excretion of urinary nitrogen per 100 grams live weight. The slight increase in the case of rat 7 during period 6 as compared with period 1 is evidently due to a deficient food consumption during the former period, necessitating the catabolism of body protein to furnish energy. The individual values obtained in period 1 for urinary nitrogen per 100 grams live weight are used in the subsequent tables for calculating the utilization of the various rations, the values always being corrected to the average live weight of the particular animal during that period.

The quantity of fecal nitrogen per 100 grams feed when the rat is consuming a nitrogen-free ration is quite a constant factor, although this relationship seems to be affected somewhat by extremes in the amount of feed consumed, as may be noted in the case of rats 4 and 7, period 6. Perhaps a more potent factor in causing this fluctuation is the varying amount of filter paper eaten, as found by Mitchell (20) in an experiment in this laboratory in which rats during one period had no access to filter paper and during the other actually consumed some paper.

It is recognized that the calculation of the metabolic nitrogen in the feces by the method described is subject to an error when applied to a variety of rations. Were the content of crude fiber in all rations the same as in the synthetic nitrogen-free ration, the assumption that the metabolic nitrogen of the feces varies directly with the amount of feed consumed would be valid, but with rations varying as widely in the percentage of crude fiber as the corn and alfalfa rations, the adoption of such an assumption evidently leads to an error of undetermined magnitude. However, the method of correcting the absorbed nitrogen and the nitrogen balance, by the use of the factor for metabolic fecal nitrogen obtained on nitrogen-free rations, undoubtedly gives values nearer the truth than if no such corrections were made, since the actual metabolic fecal nitrogen on the experimental rations containing protein was very probably greater per 100 grams of food consumed than the factors used. In computing the amount of metabolic fecal nitrogen shown in the tables that follow, the values for fecal nitrogen per 100 grams feed eaten in

period 1 in the case of each animal are applied to the data for the same animals in later periods.[2]

If it is true, as seems probable from the meager data obtained, that the endogenous metabolism of the rat becomes less intense per unit of live weight as the animal approaches maturity, then in conducting investigations of the kind under consideration it would, no doubt, be advisable to introduce a metabolism period using a nitrogen free ration every six or eight weeks. With these data available it would be possible to make linear corrections for any changes in the requirement of the basal metabolism. In the tables showing the utilization of the proteins which follow, such corrections have not been attempted with the limited number of data at hand, but should the data secured with rats 1 and 4 be used for such a purpose, it would necessitate changes in the utilization coefficients given of not more than 1 to 2 per cent as a maximum.

Palatability of rations. The results obtained in the six metabolism periods during which nitrogenous rations were fed are summarized in table 8.

From an inspection of the figures giving the amounts of feed consumed daily it is evident that all rations containing cottonseed meal were readily consumed by the animals, attesting to the palatability of this feed, even when forming as much as 24 per cent of the ration. When corn was the sole source of protein in the ration the amounts of feed consumed were smaller than with any other ration. Ground corn seems to be less palatable to rats than whole corn for the latter is usually eaten readily. Perhaps another reason why less of the corn ration was consumed than the alfalfa ration, for example, is that the corn ration was much more digestible and had a higher calorific value, so that less of it was required to supply the energy requirements. The ration in which cottonseed meal and corn were combined was consumed in greater quantities than the corn ration but not so freely as the cottonseed meal ration. Alfalfa hay proved very palatable, as all rations of which it formed a part were readily eaten.

[2] These values, as well as those for urinary nitrogen, when being used for these calculations, were extended to one more decimal place than shown in table 7.

TABLE 8

The utilization of the proteins of cottonseed meal, corn and alfalfa hay; summary of results

RAT NUMBER	PERIODS	RATION	AVERAGE LIVE WEIGHT	FEED CONSUMED DAILY	ABSORBED NITROGEN	ABSORBED NITROGEN RETAINED	ENDOGENOUS NITROGEN	UTILIZATION OF ABSORBED NITROGEN.	N ABSORBED RETAINED
			grams	*grams*	*mgm.*	*mgm.*	*mgm.*	*per cent*	*per cent*
1	2 + 3	Cottonseed meal	98	9.50	124.8	54.5	25.3	64	30
2	2 + 3		109	8.62	107.1	38.2	30.4	64	19
3	2 + 3		127	11.99	169.6	91.3	28.0	71	44
Average			111	10.04	133.8	61.3	27.9	66	31
1	4 + 5	Cottonseed meal +	114	10.83	125.9	52.9	30.6	67	26
2	4 + 5	alfalfa hay	126	11.06	131.7	58.1	35.7	71	29
3	4 + 5		159	15.68	185.0	89.5	33.7	67	34
Average			133	12.52	147.5	66.8	33.3	68	29
2	6 + 7	Cottonseed meal +	136	9.82	124.3	45.6	38.2	63	21
3	6 + 7	alfalfa hay + corn	186	14.01	173.0	72.5	39.4	65	27
Average			161	11.92	148.7	59.0	38.8	64	24
4	2 + 3	Corn	118	7.75	118.9	34.6	22.8	48	15
5	2 + 3		108	6.98	105.9	24.7	27.1	49	8
5	2 + 3		115	6.92	104.5	34.1	20.3	52	16
Average			114	7.22	109.8	31.1	23.4	49	13
4	4 + 5	Cottonseed meal +	129	9.81	141.4	59.5	24.9	60	30
5	4 + 5	corn	118	8.30	127.8	43.6	29.2	59	21
6	4 + 5		124	8.11	120.2	48.9	22.3	60	28
Average			124	8.74	129.8	50.7	25.5	60	26
5	6 + 7	Cottonseed meal +	139	10.90	136.9	49.3	34.9	62	18
6	6 + 7	alfalfa hay + corn	141	11.20	146.8	64.8	25.1	61	28
Average			140	11.05	141.9	57.1	30.0	61	23
7	2 + 3	Alfalfa hay	104	9.42	99.4	37.2	21.4	60	16
8	2 + 3		103	8.69	92.9	33.7	20.0	58	11
9	2 + 3		121	12.55	128.1	61.2	29.4	70	30
Average			109	10.22	106.8	44.0	23.6	62	19
7	4 + 5	Alfalfa hay + corn	115	12.64	149.1	56.8	23.3	54	20
8	4 + 5		118	14.08	175.8	78.9	23.3	59	28
9	4 + 5		134	12.51	166.6	70.6	32.7	62	29
Average			122	13.08	163.8	68.8	26.4	58	26
8	6 + 7	Alfalfa hay + corn	142	11.81	152.6	65.0	27.0	61	26
9	6 + 7	+ cottonseed meal	154	13.06	184.0	76.1	38.0	62	29
Average			148	12.83	168.3	70.6	33.0	61	27

Utilization of proteins. In the summary of results shown in table 8 two methods of calculating the utilization of the proteins fed are included for comparison, although, for reasons discussed above, the second method, namely, the utilization of the absorbed nitrogen for both maintenance and growth, is employed in the discussion here.

The utilization of the nitrogen absorbed from a cottonseed meal ration containing 10 per cent of crude protein was found to be 66 per cent, using the average results of six metabolism periods with three rats. The utilization of the proteins of alfalfa hay was found to be only slightly less than that of cottonseed meal, namely, 62 per cent.

When these two feeds were combined in such proportion that each furnished about an equal amount of digestible protein to the ration, very interesting results were secured, indicating a slight supplementary effect of the proteins from these two sources. This effect was not pronounced, the utilization percentage being 2 per cent above that of cottonseed meal alone and 6 per cent above that of alfalfa hay alone. It is noted that during the periods when the cottonseed meal alfalfa hay ration was fed, greater quantities of feed were consumed and larger amounts of nitrogen were absorbed than with either the cottonseed or alfalfa hay rations alone, which may in some unknown way have operated in effecting a more efficient utilization of the nitrogen, although the same conditions hold true in the case of both groups of rats which received either the corn or the alfalfa ration during two periods and were then changed to rations containing proteins from both sources.

It was found that the proteins of corn were utilized the least efficiently of those of the three feedingstuffs compared. When corn was combined with cottonseed meal or with alfalfa hay the resulting utilization coefficients tended toward a mean of the utilization coefficients secured with these feedingstuffs when fed alone, but were nearer that of the feed other than corn. For example, the utilization coefficients found for the corn and cottonseed meal rations were 49 per cent and 66 per cent respectively, the mean of these two being 57.5 per cent, but

the utilization coefficient found for the cottonseed meal-corn ration was 60 per cent. Possibly this represents a slight supplementary relationship.

The results obtained with the ration in which cottonseed meal, corn and alfalfa hay were combined were remarkably uniform. Of the twelve values obtained with rats receiving this ration during two metabolism periods each, the lowest value was 57 per cent and the highest 67 per cent, the average of all being 63 per cent. The combination of the proteins from three different sources failed to indicate any farther supplementary effect of the proteins.

The high nutritive value of the proteins of cottonseed meal manifested by these experiments is in substantial accord with the conclusions of Richardson and Green (1), Osborne and Mendel (3, 4, 5, 6) and McCollum and Simmonds (6). They do not seem to be in harmony with the findings of Hart and Humphrey (8) who studied the utilization of the proteins of cottonseed meal for milk production, but since growth and milk production are dissimilar functions an absolute comparison of the results of the two experiments is not valid.

Correlation of chemical composition with nutritive value. In seeking for an explanation of the differences in the nutritive value of the proteins of these feedingstuffs based upon differences in their chemical makeup, it is evident first of all that their nutritive values do not vary so widely as the analytical data at hand would indicate. For example, the differences found between the utilization of the proteins of cottonseed meal and alfalfa hay was but 4 per cent, while from an examination of the data in table 5 of a preceding paper, it is apparent that cottonseed meal contains more than twice as much arginine nitrogen and nearly twice as much histidine nitrogen as alfalfa hay, while the latter contains more than three times as much nonprotein nitrogen as the former.

Several theories may be advanced in explanation of this apparent inconsistency. In the first place, alfalfa hay is shown to have a lower digestibility than either cottonseed meal or corn. There is no evidence to preclude the possibility that the char-

acter of the nitrogen absorbed from alfalfa hay differs qualitatively from that remaining in the undigested residues. Judging from the ease with which tyrosine is split off from proteins in tryptic digestion in vitro, it is possible that the absorbed nitrogen contains a greater proportion of amino acids essential to the body than the unabsorbed portion. Further, the sterochemical arrangement of the amino acids in the protein molecule may affect the extent to which the digestive enzymes are able to cause hydrolysis of the different proteins. A second consideration is the possible interchangeability of the various forms of nitrogen in nutrition, as already pointed out in the case of arginine and histidine. To what extent the nonprotein nitrogen of alfalfa hay is utilized in maintenance is problematical, but since there is no reason to doubt that the degradation products of crude protein are able to serve in this capacity, it is possible that a large part of the absorbed nonprotein nitrogen fulfills some of the requirements of the animal body.

It is reasonable to assign the higher content of the basic amino acids of cottonseed meal as the reason for its superiority over the proteins of alfalfa and corn. In the case of the last mentioned feedingstuff, there is the additional factor of a comparatively low lysine content to be considered, although from the studies of Osborne and Mendel concerning the lysine requirements for growth, a lysine content of 2.2 per cent of the protein appears to be ample for normal growth.

In the absence of further information respecting the character of the mono-amino acid and nonprotein nitrogen content of these feedingstuffs, a detailed picture of which the Van Slyke analysis does not include, correlations between the chemical composition and nutritive value of the proteins of feedingstuffs can proceed little beyond the realm of the functions and relationships of the basic amino acids.

Comparison of feed consumption with that of farm animals. It was noted during the course of this investigation that the rats consumed an enormous amount of feed in proportion to their live weights. In some few cases the amount of air dry feed eaten daily was equivalent to as much as 10 or 11 per cent of the

TABLE 9

Comparison of feed consumption by albino rats with that of farm animals

SPECIES OR BREED AND CLASS OF ANIMAL	LENGTH OF FEEDING PERIOD	FEEDS IN RATION	NUMBER OF ANIMALS FED	AVERAGE AGE	AVERAGE LIVE WEIGHT	FEED EATEN DAILY PER 100 POUNDS LIVE WEIGHT
	days			*days*	*pounds*	*pounds*
Percheron fillies*	28	Corn + oats + alfalfa hay	10	227	854	2.2
	28	Corn + oats + alfalfa hay	10	683	1484	1.8
Hereford steers†	35	Corn + linseed meal + clover hay	4		978	2.5
	28	Corn + linseed meal + clover hay	4		1466	1.5
Jersey heifers‡	30	Corn + bran + linseed oil meal + alfalfa hay	4	365	472	2.9
	90	Corn + bran + linseed oil meal + alfalfa hay	4	730	839	1.8
Holstein heifers‡	30	Corn + bran + linseed oil meal + alfalfa hay	4	365	656	2.4
	90	Corn + bran + linseed oil meal + alfalfa hay	4	730	1112	1.8
Swine§			174		38	6.0
			495		128	3.8
			105		320	2.4
Sheep**					45	2.1
					127	1.1
Albino rats	42	Cottonseed meal + corn + alfalfa hay + synthetic mixture	9		129††	8.4§§
	21 or more***	18 per cent protein	17		175 200††	4.6§§

* From Bul. 192, Ill. Agr. Exp. Sta.

† From Bul. 197, Ill. Agr. Exp. Sta. Data concerning "Full-feed lot."

‡ From Nebr. Agr. Exp. Sta. Unpublished manuscript. Data concerning "Heavy fed groups."

§ From Henry and Morrison, Feeds and Feeding, 15th ed. p. 569.

** Calculated from data of Weiske as quoted by Armsby, The Nutrition of Farm Animals, p. 432.

†† Grams.

§§ Grams per 100 grams live weight.

*** From Osborne and Mendel. Protein Minima for Maintenance. Jour. Biol. Chem., 1915, xxii, 241.

live weight. It seemed of interest to compare the feed consumption of albino rats with that of farm animals. Such a comparison is made in table 9. The tabulations of horses and cattle include in each case two entries of the same group of animals at different ages and weights. It is known that the horses and cattle were restricted in the amount of concentrates consumed but were offered roughage to practically the limit of their appetites. Hence a comparison of the feed consumption of these animals with that of the first group of rats, which were the ones concerned in this investigation, is warranted, but the data are indicative only. Data for the amount of feed consumed by the swine, sheep and second group of rats is not at hand.

It is evident from the data presented that the rat is a voracious eater, even when receiving rations comparable to those of farm animals. A rough approximation places the relative amounts of feed eaten by rats as about three times that of various breeds and classes of farm animals, if swine be excepted. The fact is also brought out, as has previously been noted by others, that the young animal consumes much more feed in proportion to live weight than when older and heavier.

Cottonseed meal probably not toxic to albino rats. None of the rations containing cottonseed meal seemed to exert any harmful influence upon the rats consuming it. Three of the animals received continuously for 7 weeks rations containing from 7.7 per cent to 23.9 per cent cottonseed meal with no evidence of toxic symptoms but remained in excellent nutritive condition. This observation is in agreement with those of Richardson and Green (1) and Osborne and Mendel (5).

SUMMARY OF THE DISCUSSION OF THE NUTRITIVE VALUE OF THE PROTEINS

Evidence is presented to show that metabolism experiments with the rat as a subject can be carried out with a high degree of accuracy.

Different methods of expressing the nutritive value of the proteins of feedingstuffs are discussed. The plan of employing the

results secured in a preliminary and final metabolism period during which the animal receives a nitrogen free ration, for the calculation of the percentage of the absorbed nitrogen utilized, is favored. In comparing the nutritive value of the combined proteins of the feedingstuffs cottonseed meal, alfalfa hay and corn, it was found that when one of these feeds furnished the sole source of protein in rations containing 10 per cent of crude protein, the utilization of the proteins for the growth of albino rats was, in the order in which the feedingstuffs are named, 66 per cent, 62 per cent and 49 per cent, respectively.

When rations containing these feedingstuffs, combined in various ways, but with each feed furnishing an equal amount of digestible protein, were fed, there was evident no clear cut supplementary effect of the proteins of one feed upon another, except in the case of the combination cottonseed meal and alfalfa hay, which showed a slight effect.

No symptoms of toxicity were noted as a result of feeding rations containing cottonseed meal over a period of seven weeks.

When suitable rations are provided, the albino rat consumes an enormous amount of feed in proportion to its live weight.

The writer desires to express his appreciation of the assistance of Dr. H. H. Mitchell in outlining the method used in this investigation and for many helpful suggestions. He is also indebted to Dr. H. S. Grindley for his encouragement and general supervision of the thesis problem.

APPENDIX TABLE 1

The utilization of the proteins of cottonseed meal, corn and alfalfa hay

RAT NUMBER	PERIOD	RATION	INITIAL WEIGHT	FINAL WEIGHT	DAILY FEED CONSUMED	(a) DAILY INTAKE OF N	(b) DAILY URINARY N	(c) DAILY FECAL N	(d) METABOLIC N IN FECES	(e) ENDOGENOUS N	(f) ABSORBED N RETAINED	(g) UTILIZATION OF ABSORBED N‡	(h) ABSORBED N RETAINED	(i) DIGESTIBILITY Ordinary coefficient	(j) DIGESTIBILITY Corrected coefficient
			grams	*grams*	*grams*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*per cent*	*per cent*	*per cent*	*per cent*
1	2	Cottonseed meal	92	103	9.52	166.7	71.8	62.2	24.1	24.4	56.8	63	31	63	77
2	2		104	113	8.66	151.6	70.7	62.2	22.1	30.4	40.8	64	21	59	74
3	2		121	133	11.51	201.4	74.3	68.3	29.7	26.8	88.5	71	44	66	71
Average												66	32	63	74
1	3	Cottonseed meal	103	109	9.48	165.8	68.8	68.9	24.0	26.1	52.1	65	29	58	73
2	3		113	118	8.57	149.9	67.0	69.2	21.9	30.4	35.6	64	17	54	69
3	3		133	144	12.47	218.1	82.2	74.1	32.3	29.2	94.1	70	43	66	81
Average												66	30	59	74
1	4	Cottonseed meal +	109	113	10.60	189.8	68.6	100.1	26.9	29.7	48.0	67	24	47	62
2	4	alfalfa hay	118	129	11.39	203.9	67.2	96.1	29.1	34.6	69.7	76	38	53	67
3	4		145	160	14.91	267.0	91.6	132.4	38.7	32.2	81.7	66	32	50	65
Average												70	31	50	65
1	5	Cottonseed meal +	113	121	11.06	198.0	77.40	90.9	28.0	31.4	57.7	66	28	54	68
2	5	alfalfa hay	129	133	10.73	192.1	80.00	93.0	27.4	36.7	46.5	66	19	52	66
3	5		160	173	16.45	294.5	99.30	140.6	42.7	35.1	97.3	67	35	53	67
Average												66	27	53	67

1	6	N-free.	113	102	6.10		21.5	18.7							
2	6	Cottonseed meal +	134	137	10.13	180.5	79.8	78.1	25.8	37.9	48.4	67	22	57	71
3	6	alfalfa hay + corn	182	188	14.47	257.9	102.2	119.1	37.5	39.0	74.1	64	26	54	69
												66	24	56	70
Average								73.3†			42.7	67		57	71
2	7	Cottonseed meal +	137	138	9.51	169.4	77.7	(65.5)*	24.3	38.5	(50.5)	(69)	19	(61)	(76)
3	7	alfalfa hay + corn	188	189	13.54	241.3	98.8	106.8	35.1	39.8	70.8	65	27	56	70
Average												62	23	57	71
4	2	Corn	117	119	7.44	123.4	80.3	28.5	18.6	22.7	33.2	49	15	77	92
5	2		108	108	7.66	127.2	81.7	30.1	20.9	27.1	36.3	54	16	76	93
6	2		116	115	7.49	124.3	70.4	32.4	20.2	20.4	41.7	55	23	74	90
Average												53	18	76	92
4	3	Corn	119	119	8.05	133.6	88.3	29.5	20.2	22.8	36.0	47	15	78	93
5	3		108	107	6.30	104.6	80.8	28.0	17.2	27.0	13.0	43	—	73	90
6	3		115	113	6.35	105.3	70.4	25.6	17.2	20.2	26.5	48	9	76	92
Average												46	12	76	92
4	4	Corn and cottonseed	122	131	9.95	176.8	80.4	61.1	24.9	24.3	60.2	60	31	66	80
5	4	meal	110	115	8.56	152.0	80.0	52.4	23.3	28.2	42.9	58	20	66	81
6	4		116	128	8.48	150.8	76.2	49.6	22.9	21.6	47.9	56	25	67	82
Average												58	25	66	81
4	5	Corn and cottonseed	131	135	9.67	171.9	83.5	53.9	24.2	25.5	58.7	59	29	69	83
5	5	meal	115	125	8.04	142.8	78.5	42.0	21.9	30.1	44.2	61	22	71	86
6	5		128	132	7.73	137.4	66.4	42.0	20.9	23.0	49.9	63	30	69	85
Average												61	27	70	85

APPENDIX TABLE 1—*Continued*

RAT NUMBER	PERIOD	RATION	INITIAL WEIGHT	FINAL WEIGHT	DAILY FEED CONSUMED	(a) DAILY INTAKE OF N	(b) DAILY URINARY N	(c) DAILY FECAL N	(d) METABOLIC N IN FECES	(e) ENDOGENOUS N	(f) ABSORBED N RETAINED	(g) UTILIZATION OF ABSORBED N‡	(h) ABSORBED N RETAINED	(i) DIGESTIBILITY	(j)
														Ordinary coefficient	Corrected coefficient
			grams	*grams*	*grams*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*per cent*	*per cent*	*per cent*	*per cent*
4	6	N-free.	135	130	9.02		16.3	20.1							
5	6	Cottonseed meal +	134	139	11.03	196.6	88.5	92.2	30.1	34.3	46.1	60	15	53	68
6	6	corn + alfalfa	133	143	10.68	190.3	77.3	88.0	28.9	24.4	53.9	60	24	54	69
Average												60	20	54	69
5	7	Cottonseed meal +	139	144	10.77	191.9	86.8	82.1	29.4	35.5	52.4	63	21	57	73
6	7	corn + alfalfa	143	148	11.72	208.9	86.8	78.2	31.7	25.8	75.6	62	33	63	78
Average												63	27	60	76
7	2	Alfalfa hay	103	109	9.33	171.0	61.0	91.4	24.8	21.3	43.4	62	23	47	61
8	2		103	105	9.19	165.8	62.5	91.1	27.4	20.0	39.6	58	16	45	62
9	2		116	119	11.42	206.3	65.4	122.5	28.1	28.8	46.5	67	22	41	54
Average												62	20	44	59
7	3	Alfalfa hay	109	104	9.50	171.5	63.4	102.4	25.2	21.4	30.9	57	8	40	55
8	3		105	103	8.20	148.0	55.9	88.7	24.4	20.0	27.8	57	6	40	57
9	3		119	126	13.67	246.9	68.4	136.3	33.7	30.0	75.9	73	38	45	58
Average												62	17	42	57

7	4	Alfalfa hay + corn	107	117	12.86	219.6	95.1	105.3	34.2	22.5	53.4	51	17	52	68
8	4		106	125	14.82	253.1	105.8	111.9	44.2	22.2	79.6	55	25	56	73
9	4		127	132	12.18	208.0	94.5	79.6	30.0	31.7	63.9	60	26	62	76
Average												55	23	57	72
7	5	Alfalfa hay + corn	117	122	12.41	211.9	89.4	95.3	33.0	24.0	60.2	57	23	55	71
8	5		125	129	13.34	227.8	88.0	101.5	39.8	24.4	78.1	62	30	55	73
9	5		132	140	12.84	219.3	97.5	76.1	31.6	33.3	77.3	63	32	65	80
Average												61	28	58	75
7	6	N-free.	118	105	4.76		23.8	17.0							
8	6	Cottonseed meal +	134	141	12.06	214.9	93.2	96.0	36.0	26.4	61.7	57	22	55	72
9	6	corn + alfalfa	147	1 57	13.55	241.5	109.2	86.0	33.3	37.2	79.6	62	30	64	78
Average												60	26	60	75
8	7	Cottonseed meal +	141	147	11.55	205.8	82.0	90.1	34.4	27.6	68.3	64	29	56	73
9	7	corn + alfalfa	157	160	12.57	224.0	106.6	75.8	31.0	38.8	72.6	62	28	66	80
Average												63	29	61	78

Method of calculation:

1. $a - b - (c - d) = f$
2. $\frac{e + f}{a - (c - d)} \times 100 = g$
3. $\frac{a - c - b}{a - c} \times 100 = h$
4. $\frac{a - c}{a} \times 100 = i$
5. $\frac{a - (c - d)}{a} \times 100 = j$

* A loss occurred during Kjeldahl digestion of feces.

† Based on amount of fecal nitrogen per gram of feed in Period 6.

‡ As calculated for both maintenance and growth.

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