the sodium sulphate and added to a large volume of water. The pinkish colored precipitate was filtered off and dried. It showed a melting point of 145–7° and weighed 3.82 grams or 82 per cent. of the theoretical. On recrystallizing from boiling water a very small amount of a dark red substance was gotten rid of and the *m*-brombenzoic acid was obtained in pure condition, m. 154°. Analysis: calculated for $C_7H_5O_2Br$, Br 39.80; found, Br 39.97.

CHAPEL HILL, N. C., February 25, 1909.

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THE CHEMISTRY OF ANIMAL FECES.

FIRST PAPER.—A COMPARISON OF THE ANALYSIS OF FRESH AND AIR-DRIED FECES.

BY A. D. EMMET AND H. S. GRINDLEY.

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It has been the usual custom in agricultural chemical work to prepare feces, and feeding stuffs high in water content, for analysis by first air-drying them and then grinding the resulting residue or product until it will pass through a sieve having 1 mm. openings. It is highly desirable, however, to ascertain, if at all possible, the chemical composition of the fresh, undried sample. This procedure was found to be possible in the case of meats which contained as high as 76 per cent. of water.[1] In metabolism experiments with men, the fresh undried feces have been examined for fatty matter by Long[2] with his paper coil method. In this laboratory[3] it has been found that fresh human feces can be kept safely with thymol together with a reduced temperature for several days, then, after compositing and thoroughly mixing, the resulting fresh sample can be analyzed with a high degree of accuracy. In nutrition experiments with swine, cattle, sheep, and horses, the feces are of a very different physical composition than in the case of the carnivora, being abundant in carbohydrate materials which ferment easily, and they are also composed of coarser undigested particles, and are, therefore, apparently much more difficult to sample. However, in view of the fact that in our experiments, thus far, the feeds have been either ground or chopped to a medium fineness before feeding, it seemed plausible to undertake a comparative chemical study of the composition of the fresh and air-dried feces. The added value of the results, in determining the coefficients of digestibility of the various constituents and the nature of the metabolic changes, upon the fresh substance seemed well worth studying.

Considerable work has already been done along this line by several

[1] Grindley and Emmett, THIS JOURNAL, **27**, 658 (1905).

[2] THIS JOURNAL, **28**, 704 (1906).

[3] Howe, Rutherford, and Hawk, *Proc. Am. Soc. Biol. Chem.* (Feb.), 1909.

investigators in agricultural chemistry, mainly, however, in comparing the loss of nitrogen only. The nature of these losses resulting in the air-drying have been directed mainly to those occurring in ensilage and manure, during this operation. It has been found, that generally there is quite an appreciable loss of nitrogen during the air-drying. This was especially true in the case of animal feces if the feeds were high in protein materials. This loss of nitrogen in drying may be due either to the formation of volatile ammonium salts, resulting from the fermentation processes which may take place in substances rich in carbohydrates, or to the actual breaking down of the protein and non-protein compounds themselves, provided the samples are not properly preserved and protected. Kellner,[1] in working with clover ensilage, found that of two portions of the expressed juice, one dried directly at 100° and the other with hydrochloric acid, the former lost 45.8 per cent. more nitrogen. With Kozai and More[2] he found that in analyzing fresh ensilage directly by the Wilfarth-Kjeldahl method and comparing these results with those made upon drying at 70–80° and then at 98°, there was a loss of nitrogen, amounting to 3.2 to 23.3 per cent., depending apparently upon the kind of ensilage. Morgen[3] found in making a study of the effect of air-drying beet and maize ensilage with and without hydrochloric acid, that the acid treatment prevented an appreciable loss of nitrogen, 10–20 per cent., in the case of beet-leaf ensilage but that it had no effect on the maize samples. Woll[4] found in testing the hydrochloric acid method with fourteen samples of maize ensilage and air-drying at a temperature of 60°, that in eight instances the samples contained less nitrogen when treated with acid, than when dried without, while in five samples they had about the same percentage content of nitrogen in both cases. König[5] followed a somewhat different procedure in estimating the losses on air-drying. He compared, like Kellner, the nitrogen content of the fresh samples with those after air-drying. Silage from grass, lupines, red clover, and beet chips were examined. No acid was added during the drying. The nitrogen in the fresh samples was estimated by his sulphuric acid method, which is used by many at the present time. It consisted, briefly, in adding a definite quantity of the sample to some strong acid and stirring the whole till the mass became thoroughly disintegrated. A weighed amount of the resulting mixture was then taken for the regular nitrogen determination. The results showed that there was a loss of total nitrogen during the air-drying amounting to from 2 to 3 per cent. on the average, but in one

[1] *Landw. Vers. Stat.*, 32, 37.
[2] *Ibid.*, 39, 105.
[3] *Journ. Landw.*, 36, 300.
[4] *Landw. Vers. Stat.*, 36, 161.
[5] *Ibid.*, 38, 230.

sample of grass ensilage, it was as high as 13.0 per cent. König also applied his method to animal feces, using, however, as a further preventive, dilute sulphuric acid in the air-drying. He found that there was a loss of total nitrogen in one case of 14 per cent. and in another of 20 per cent. Frear and Holter,[1] working in Armsby's laboratory, made a thorough study of the losses resulting from air-drying maize ensilage, and steer and sheep manures. They also considered the matter of preserving feces with carbon disulphide. The fresh products were analyzed by König's method. The air-drying was done at 60° both with and without hydrochloric acid. These investigators found: first, that König's method, while cumbersome, gave excellent duplicate results with the fresh material; second, that carbon disulphide was a good preservative; third, that there was considerable danger from loss of nitrogen in air-drying animal feces either with or without hydrochloric acid, being from 11 to 8 per cent., respectively, when the percentage content was as high as 0.5; and, fourth, that, it is very desirable to estimate the nitrogen upon the fresh substance, if accurate chemical data are to be sought.

Experimental.

In our laboratory the procedure of preparing the samples of dung is as follows: (*a*) For the fresh feces, the 24-hour collections are weighed daily, and a definite portion is then taken from the well-mixed sample. This fraction is transferred to a thymoled vessel, having an air-tight cover. A small portion of the powdered thymol is sprinkled over the mass and the whole carefully mixed. The vessel is then placed in a refrigerator, kept at about 2°. At the end of the weekly experimental period, the several daily fractions are combined and thoroughly mixed. The portion, taken for chemical study, is placed in a thymoled vessel and properly labeled. In cases of necessity, the mixing is done in a bowl with a six-bladed chopping knife. (*b*) For the air-dried feces, a portion of the composite fresh sample is transferred to a tared vessel having a large flat surface. It is then placed in an automatic electric oven, the temperature of which never goes above 60°, or below 50°. In the case of the particular samples reported herein, the drying was done at approximately 60°, before the above oven was made. After drying in the usual manner, weighing and sampling, these air-dried feces were analyzed in exactly the same way as the fresh samples.

The chemical constituents which were determined directly in these samples were: Total nitrogen, fat, ash, phosphorus, moisture, and crude fiber. The nitrogen was estimated by means of the Gunning-Kjeldahl-Jodlbauer method; the ash, by the usual method as applied in plant analysis; the phosphorus, in the ash residues, by the gravimetric method; the moisture in a steam-electric oven at 102°, and the crude fiber on the residue, left from the fat extraction, by the official method, using beakers instead of flasks and also Lincoln's cup arrangement with silk covering for the filtration of the acid digest. In the case of the crude fiber determination, a modification in the technic of the method has been made necessary and consequently these data will not represent as far as duplicates are concerned, the best results in all cases for the fresh material. The fat was determined by means of both ether and carbon tetrachloride.[2] In this report, however, the latter values are alone given since the crude

[1] Report Pennsylvania State College, 123, 1891.

[2] Bryant, THIS JOURNAL, 26, 568.

fiber determinations were made upon the residue from these extractions. A subsequent paper will present results of this particular study upon the solvent action of these two reagents.

The samples of the fresh and air-dried feces, which were analyzed directly and compared, were obtained in connection with extended digestion experiments with swine in coöperation with William Dietrich of this station. Two Berkshire pigs, designated as A and B, were used and fed in each case in exactly the same manner throughout a period of five months. The rations were changed at specific times to suit the purposes of the experiment. For example, ground corn was fed at the start, then a definite proportion of middlings was added, later, one of cracklings, then the middlings and cracklings were taken out and a certain percentage of tankage was substituted and so on throughout the entire experiment. Analyses of the samples from the first two periods are given here in detail, and in addition samples from a third period are given to aid in the comparison of the loss on drying as given in Table No. 1.

A Comparison of the Losses on Drying.—Table No. 1 represents the data for the air-dried and fresh feces and shows the differences which resulted from determining the so-called moisture or more accurately the gross loss on drying. For the air-dried samples, the percentage loss on air-drying at 60° is given and also the further loss obtained on heating these dried residues at 102°. The sum of these two, both calculated to the fresh basis, should be comparable to the percentage loss found upon heating the fresh samples directly at 102°.

It will be seen from the table, that in every instance there is a greater loss in the case of the air-dried feces samples, ranging from 0.11 per cent. in samples 2398 and 2400 to 1.00 per cent. in samples 2379 and 2397, and averaging for all eight tests a loss of 0.49 per cent. This greater loss is in agreement with the usual belief and expectation, and while small and insignificant in some of the cases, it is quite appreciable in others, and shows the desirability of making a direct analysis of the fresh products. It will be of interest to note the effect of these various differences in connection with the study of the comparative chemical composition of the fresh and air-dried feces.

A Comparison of the Methods of Analyzing Fresh and Air-dried Feces.—In Table No. 2, the chemical composition of the fresh and air-dried feces is given in duplicate, calculated in per cent. of their respective fresh substance. The same general arrangement of the data is followed as in Table No. 1, as regards the animals and the kind of feces, but it will be noticed that the data for the respective air-dried samples follow, those for the fresh in each case. In studying the composition of the fresh samples, it will be seen that for the moisture, fatty matter, ash, protein, phosphorus, and total carbohydrate constituents, the duplicates agree quite well. This fact is especially true in the case of the moisture, ash, protein, and carbohydrates.

From the composition of the fresh dung, these data show a range in the duplicates: for the moisture, from 0.01–0.28 per cent.; for the fatty matter, from 0.01–0.19 per cent.; for the ash, from 0.01–0.09 per cent.; for the protein, from 0.00–0.08 per cent.; for the phosphorus, from 0.04–0.05 per cent.; and for the carbohydrates, from 0.06–0.30 per cent. Now, if these various figures be considered in per cent. of their respective totals, the corresponding percentage errors will be as follows: 0.0 and 0.4 per cent. for the moisture; 0.2 and 3.3 per cent. for the fat; 0.2 and 2.2 per cent. for the ash; 0.0 and 1.3 per cent. for the protein; 1.2 and 7.5 per cent. for the phosphorus; and 0.3 and 1.7 per cent. for the carbohydrates. The averages of all the four experiments show the percentage errors to be 0.1 for the moisture, 2.3 for the fatty matter, 1.2 for the ash, 0.7 for the protein, 6.0 for the phosphorus, and 1.0 for the carbohydrates.

Again, comparing the duplicates in the case of the air-dried feces, the percentage differences are: from 0.00–0.19 for the moisture; from 0.03–0.12 for the fatty matter;

TABLE 1.—A COMPARISON OF THE LOSSES ON DRYING ANIMAL FECES BY THE AIR-DRIED AND DIRECT METHODS.

(Calculated in per cent. of fresh substance.)

Serial number.				Loss on heating by air-drying.				Difference. Increase due to air-drying method.
Fresh.	Air-dried.	Description.	Feed used.	First at 60°. Per ct.	Second at 102°. Per ct.	Total. Per ct.	Fresh direct at 102°. Per ct.	Per ct.
2378	2396	Pig A, digestion experiment No. 1	Ground corn	65.43	2.47	67.90	67.61	+0.29
2398	2400	Pig A, digestion experiment No. 2	Ground corn, middlings	67.67	1.42	71.09	70.98	+0.11
2379	2397	Pig B, digestion experiment No. 1	Ground corn	62.05	2.20	64.25	63.25	+1.00
2399	2401	Pig B, digestion experiment No. 2	Ground corn, middlings	65.25	1.69	66.94	66.09	+0.85
2407	2409	Pig A, digestion experiment No. 3	Ground corn, middlings, cracklings	69.70	2.30	71.00	70.48	+0.52
2422	2424	Pig A, digestion experiment No. 4	Ground corn, tankage	67.82	2.22	70.04	69.74	+0.30
2408	2410	Pig B, digestion experiment No. 3	Ground corn, middlings, cracklings	64.27	1.93	66.20	65.90	+0.30
2423	2425	Pig B, digestion experiment No. 4	Ground corn, tankage	63.98	1.92	65.90	65.35	+0.55

TABLE 2.—CHEMICAL COMPOSITION OF ANIMAL FECES.

(Calculated in per cent. of the fresh substance.)

Serial number.	Description.	Experiment number.	Moisture.	Fatty matter.	Ash.	Protein.	Phosphorus.	Carbohydrates.		
								Crude fiber.	Nitrogen-free extract.	Total.
2378–1	Pig A, fresh	1	67.61	5.27	4.10	4.90	0.63	...	...	17.49
2378–2	Pig A, fresh	1	67.62	5.10	4.00	4.90	0.59	...	...	17.79
	Average		*67.62*	*5.19*	*4.05*	*4.90*	*0.61*	...	...	*17.63*
2396–1	Pig A, air-dried	1	7.22	14.65	11.38	13.84	1.75	18.89	32.27	51.16
2396–2	Pig A, air-dried	1	7.03	14.62	11.41	13.92	1.73	19.04	32.25	51.29
	Average		*7.13*	*14.64*	*11.40*	*13.88*	*1.74*	*18.97*	*32.26*	*51.22*
2398–1	Pig A, fresh	2	70.84	3.87	3.83	4.82	0.70	6.22	9.72	15.94
2398–2	Pig A, fresh	2	71.12	3.94	3.82	4.76	0.65	5.60	10.11	15.71
	Average		*70.98*	*3.91*	*3.83*	*4.79*	*0.68*	*5.91*	*9.91*	*15.82*
2400–1	Pig A, air-dried	2	4.66	12.19	12.34	15.43	2.24	19.32	33.82	53.14
2400–2	Pig A, air-dried	2	4.78	12.03	12.33	15.36	2.08	19.24	34.18	53.42
	Average		*4.72*	*12.11*	*12.34*	*15.40*	*2.16*	*19.28*	*33.00*	*53.28*
2379–1	Pig B, fresh	1	63.28	5.67	4.34	6.62	0.63	8.35	11.11	19.46
2379–2	Pig B, fresh	1	63.23	5.86	4.25	6.59	0.67	...	...	19.40
	Average		*63.26*	*5.77*	*4.30*	*6.61*	*0.65*	*8.35*	*11.11*	*19.43*
2397–1	Pig B, air-dried	1	5.80	14.19	10.59	16.23	1.66	20.38	31.15	51.53
2397–2	Pig B, air-dried	1	5.80	14.24	10.45	16.29	1.63	20.32	31.27	51.59
	Average		*5.80*	*14.22*	*10.52*	*16.26*	*1.65*	*20.35*	*31.21*	*51.56*
2399–1	Pig B, fresh	2	66.10	4.24	3.99	6.01	0.69	6.69	12.38	18.97
2399–2	Pig B, fresh	2	66.09	4.25	4.00	5.93	0.64	6.50	12.59	19.09
	Average		*66.10*	*4.25*	*4.00*	*5.97*	*0.67*	*6.60*	*12.43*	*19.03*
2401–1	Pig B, air-dried	2	4.93	12.98	11.13	16.77	1.80	18.25	34.14	52.39
2401–2	Pig B, air-dried	2	4.79	12.91	10.86	16.74	1.80	18.53	34.37	52.90
	Average		*4.86*	*12.95*	*11.00*	*16.76*	*1.80*	*18.39*	*34.25*	*52.64*

from 0.01–0.27 for the ash; from 0.03–0.08 for the protein; from 0.00–0.16 for the phosphorus; and from 0.06–0.51 for the carbohydrates. The percentage errors range in these cases: for the moisture from 0.0–2.9; for the fat from 0.3–1.3; for the ash from 0.0–2.4; for the protein from 0.1–0.6; for the phosphorus from 0.0–7.4; and for the carbohydrates from 0.1–1.0. Averaging these variations the percentage deviation is 2.0 per cent. for the moisture, 0.6 per cent. for the fat, 1.0 per cent. for the ash, 0.4 per cent. for the protein, 2.4 per cent. for the phosphorus, and 0.6 per cent. for the carbohydrates.

In the final analysis, then, it is seen that of the two methods, the analyses of the fresh undried feces show that the greatest errors are in the fat and phosphorus determinations, while in the air-dried feces, the errors fall upon the moisture and phosphorus determinations. This, then, by excepting the phosphorus from consideration, places the two methods upon a par, with perhaps some points in favor of the first—the fresh —inasmuch as any variation in moisture is bound to influence to some extent the percentage of the other constituents.

Further, to study this question more in detail, the data from the analysis of the air-dried samples have been calculated back to the original fresh basis. In so doing, both moisture values in Table 1 have been used, that is, the data have been calculated upon the basis of the total loss upon air-drying plus that due to the subsequent loss on heating at 102°, and upon the basis of the direct loss upon heating the fresh samples at 102°. In this manner, the two sets of data for the air-dried feces, are directly comparable with the results which were obtained by analyzing the undried fresh samples. Therefore, we should have a direct comparison of the two methods of analysis which on the one hand will show differences due to the methods themselves, and on the other, differences which are in part influenced by the variations found upon air-drying. Table No. 3 gives these data.

In this table, it will be seen that the data for the fresh feces are given first; below these are the values for the air-dried samples calculated to the same moisture content as the fresh feces, and beneath these, are the air-dried products calculated to their own moisture content, obtained directly. In comparing the two direct methods of analyses, it will be seen that the air-dried feces are, as a rule, lower for each of the respective constituents that was determined directly, water of course being omitted from consideration. The only exception to this statement is in the case of the determination of fatty matter for samples 2399 and 2401–B. Upon close inspection, the differences in the corresponding constituents are comparatively slight, being perhaps most pronounced for samples 2379 and 2397–B, where the variation in the ash is 0.30 per cent. and in the protein 0.44 per cent. Aside from these two incidents, the percentages are as close as duplicates generally run with such complex substances.

However, it will be of interest as was stated above to calculate the data for the air-dried feces to the original fresh substance and also to the same water content as that for the fresh undried feces. These values are designated in the table as direct for the former and indirect for the latter. The data indicate, as would be expected, that where the differences resulting from the losses on drying were small or large, there were corresponding proportional variations. In every case, the fat for 2401–A being excepted, it will be seen that the results obtained indirectly approach more nearly to those for the fresh samples. On the whole, it may be stated that in this condition the data for the two methods, the air-dried indirect and the fresh direct, are as close as could be expected. Furthermore, these facts would tend to suggest that the differences in the losses, resulting from the drying of the samples, were due primarily to mechanical errors in the handling and the stirring of the products during the air-drying, and that direct moisture determinations on the fresh feces were the more

TABLE 3.—CHEMICAL COMPOSITION OF ANIMAL FECES.

(Calculated to the original fresh substance.)

Serial number.	Description.	Experiment number.	Moisture.	Fatty matter.	Ash.	Protein.	Phosphorus.	Carbohydrates.		
								Crude fiber.	Nitrogen-free extract.	Total.
2378	Pig A, fresh, direct	1	67.62	5.19	4.05	4.90	0.61	..	...	17.63
2396–A	Pig A, air-dried, indirect	1	67.62	5.11	3.98	4.84	0.61	6.61	11.23	17.84
2396–B	Pig A, air-dried, direct	1	67.90	5.06	3.94	4.80	0.60	6.56	11.14	17.70
2398	Pig A, fresh, direct	2	70.98	3.91	3.83	4.79	0.68	5.91	9.90	15.81
2400–A	Pig A, air-dried, indirect	2	70.98	3.69	3.76	4.69	0.66	5.88	10.34	16.22
2400–B	Pig A, air-dried, direct	2	71.09	3.68	3.74	4.67	0.66	5.85	10.31	16.16
2379	Pig B, fresh, direct	1	63.26	5.77	4.30	6.61	0.65	8.35	11.06	19.41
2397–A	Pig B, air-dried, indirect	1	63.26	5.56	4.11	6.34	0.65	7.94	12.14	20.08
2397–B	Pig B, air-dried, direct	1	64.25	5.41	4.00	6.17	0.63	7.72	11.82	19.54
2399	Pig B, fresh, direct	2	66.10	4.25	4.00	5.97	0.67	6.60	12.41	19.01
2401–A	Pig B, air-dried, indirect	2	66.10	4.61	3.92	5.97	0.64	6.55	12.21	18.76
2401–B	Pig B, air-dried, direct	2	66.94	4.50	3.82	5.83	0.62	6.39	11.90	18.29

TABLE 4.—COEFFICIENTS OF DIGESTIBILITY OF THE CONSTITUENTS IN THE FEEDS CONSUMED.

Serial number.	Calculated from	Experiment number.	Total dry substance.	Total organic matter.	Fatty matter.	Ash.	Protein.	Phosphorus.	Carbohydrates. Crude fiber.	Carbohydrates. Nitrogen-free extract.	Carbohydrates. Total.
			GROUND CORN.								
2378	Pig A, fresh feces, direct	1	87.1	86.7	19.5	..	80.6	64.7	..	..	91.7
2396–A	Pig A, air-dried feces, indirect	1	87.1	86.8	20.8	..	80.9	64.7	77.8	93.6	91.6
2396–B	Pig A, air-dried feces, direct	1	87.2	86.9	21.5	..	81.0	65.0	78.0	93.6	91.7
2379	Pig B, fresh feces, direct	1	86.5	86.2	18.0	..	76.9	65.4	74.2	94.3	91.5
2397–A	Pig B, air-dried feces, indirect	1	86.5	86.3	21.0	..	77.0	65.4	75.5	93.7	91.3
2397–B	Pig B, air-dried feces, direct	1	86.9	86.7	23.1	..	77.6	66.3	76.2	93.9	91.6
			GROUND CORN AND MIDDLINGS.								
2398	Pig A, fresh feces, direct	2	87.3	87.6	51.9	25.5	84.4	74.6	77.0	93.3	91.3
2400–A	Pig A, air-dried feces, indirect	2	87.3	87.6	54.7	26.8	84.7	75.4	77.1	93.1	91.1
2400–B	Pig A, air-dried feces, direct	2	87.3	87.7	54.8	27.1	84.8	75.4	77.2	93.1	91.1
2399	Pig B, fresh feces, direct	2	86.8	87.2	53.6	30.9	82.7	77.7	77.1	92.7	90.7
2401–A	Pig B, air-dried feces, indirect	2	86.8	87.2	49.7	32.3	82.7	78.7	77.3	92.8	90.8
2401–B	Pig B, air-dried feces, direct	2	87.1	87.6	50.9	34.0	83.2	79.2	77.9	93.0	91.0

reliable. Especially does this seem true, when it is borne in mind that the duplicates in these cases and in many others that have been run in this laboratory, have been found to agree very well within themselves. To get a further insight into the values of these two methods, it will be of interest to ascertain the actual coefficients of digestibility in both cases. Table 4 gives the coefficients of digestibility for the feeds consumed: ground corn, for experiments No. 1, and ground corn and middlings, for experiments No. 2.

In Table 4, the coefficients of digestibility are calculated to the same three forms as in Table 3, namely: the fresh undried feces, direct; the air-dried feces, direct; and the air-dried feces, indirect. These data show that in the case of the total dry substance, the total organic matter, the protein, the nitrogen-free extract, and the total carbohydrates, there are but slight differences between the three sets of values. Further, they indicate that when the data for the air-dried samples are calculated to the indirect form, they approach so nearly the values for the fresh feces that they are practically identical with them. The coefficients of digestibility for the other constituents compare quite favorably, when it is remembered that the fatty matter is so complex and difficult to determine, that the percentage content of the ash and phosphorus is small in both the feeds and the feces, and that the method of determining crude fiber is quite empirical. In some instances, however, it will be seen that even under these conditions, the values for the phosphorus and crude fiber are exceedingly close when the data for the air-dried samples are calculated to the indirect form and compared with the fresh feces. On the whole, it would seem that the differences in the coefficients of digestibility which do exist between the respective constituents for the fresh and the air-dried feces are due: first, in the extreme cases, primarily to the more or less crude methods of analysis which have not, as yet, been sufficiently perfected, and second, in general, to the errors in the technique of preparing and handling the samples during the process of air-drying.

Conclusions.

From the comparisons which have been made and reported, and also from the results of several hundred duplicate determinations which have been made in this laboratory upon fresh feces from man, swine and cattle it seems safe to state:

1. That, the fresh undried feces of swine and cattle can be analyzed directly, easily and satisfactorily for the usual constituents—protein, fat, moisture, ash, carbohydrates and phosphorus.

2. That, in the case of swine, the losses on air-drying seem to have been in part due to mechanical errors, and not to a loss of any one constituent in particular.

3. That, in cases where there is any danger of loss of nitrogen or the products of fermentation, this method is recommended in preference to either the air-drying process or König's nitrogen method, but only in cases where the feeds consumed have been ground or chopped to a medium fineness.

4. That, it is hoped investigators in animal nutrition will coöperate and give this method a fair test, since it must be evident that in analyzing the fresh undried dung there are comparatively unlimited opportunities

for studying the nature of the metabolic and undigested products which cannot be done with any degree of satisfaction upon the air-dried materials.

URBANA, ILL.

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD COLLEGE.]

SOME AZO DYES FROM *p*-AMINOACETOPHENONE.

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So far as we have been able to discover, very few azo dyes derived from *p*-aminoacetophenone have been described. Klingel[1] prepared *p*-acetophenoneazo β-naphthol, but states that it is soluble in dilute alkalies, while we have found it to be entirely insoluble in aqueous alkalies, as would be expected from analogy with benzeneazo β-naphthol. Fr. Baeyer & Co.,[2] under D. R. P. 81152, patented the preparation of azo dyes by coupling $\alpha_1\alpha_4$dihydroxynaphthalene mono- and disulphonic acids with diazotized aromatic amino ketones, or the diazo compounds of the esters, amides, and anilides of aromatic amino carboxylic acids. Pröscher[3] prepared a colored compound by the action of bilirubin on diazotized *p*-aminoacetophenone.

We have prepared both hydroxyazo and aminoazo compounds and oximes of some of these bodies. The aminoazo compounds in general turn from yellow to red on the addition of acids. The sensitiveness of the phenyl-, dimethyl-, and diethylamino derivatives and the oxime of the latter toward hydrogen ions was determined by a method similar to that described by Salm,[4] which was called to our attention by Dr. L. J. Henderson of this Laboratory and the Harvard Medical School, who had used a modification of Salm's method in studying the ionization constants of β-hydroxybutyric acid and acetoacetic acid.[5] To 5 cc. of a 0.27 *N* sodium acetate solution containing a few drops of an alcoholic solution of the dye, 0.851 *N* hydrochloric acid was added until a pure pink color was obtained. The hydrogen-ion concentration at which this change in color took place was calculated from the following approximately accurate formula:

$$(H)^+ = 2 \times 10^{-5} \frac{HCl}{CH_3COONa—HCl}$$

and was found to be for *p*-acetophenoneazodiethylaniline about 5×10^{-5}. The other three are less sensitive, the *p*-acetophenoneazophenylaniline being the least sensitive of all.

In the preparation of *p*-acetophenoneazophenylaniline a modification

[1] *Ber.*, **18**, 2695.

[2] *Ibid.*, **28**, R. 701.

[3] *Z. physiol. Chem.*, **29**, 411.

[4] *Z. physik. Chem.*, **57**, 471.

[5] Henderson and Spiro, *Biochemische Zeit.*, **15**, 105.