conditioner chamber, where caked feed never dries and a continuous corrosion is taking place, aggravated by the high temperature as well as moisture.

It was difficult to follow a basal ration in the pellet mill, with one containing liquid acid and to maintain the same mill settings established by the previous run. The first obvious change needed was the reduction of steam intake to prevent choking up of the pellet die. With less steam going into the soft feed, the feeder setting had to be backed off to prevent the wall ammeter from reading beyond a safe amperage for the motor. If the same settings were used, constant attention would be necessary to prevent either a choke-up of the die or an overheating of the motor. When dry acid premix was used in the formulation, no change from the basal ration was made in the pellet mill operations and the only indication of the presence of acid was a slight increase in killowatt-hours per ton of feed produced.

The increase in pellet hardness in acid feeds was considered significant. The practical value of this was observed in a reduction of per cent fines brought back to be repelleted. The reduced amount of fines developed from pelleted, crumbled acid feeds did not entirely compensate for the lower feeder setting during the pellet operation. Because pellet hardness depends somewhat on moisture content, it is possible that the reduced moisture added to acid feeds resulted in a drier, harder pellet coming from the cooler. On the other hand, liquid (commonly water or steam) is used to bind soft feed material into a hard, compressed form as well as to lubricate the material and increase the pelleting rate. The total liquid added, acid, and steam, was larger in the acid feeds than in the basal ration, which may explain the hardness of the acid-containing pellets.

Undoubtedly, the use of a high-level acid premix, dry and free-flowing with completeness of acid-limestone reaction nearly 100%, would enable mixing, conveying, and pelleting operations to proceed as when using any other dry phosphate compound. A small continuous premixing line using stainless steel equipment could be used to feed phosphoric acid into rations, with adjusted formulas to allow for the composition of the premix.

Table VII. Production Data when Pelleting Basal, Liquid Acid, Dry Acid Mix Rations

	Basal Ration	*Liquid Acid*	*Dry Acid Mix*
Mixed, pelleted	5	5.8	2.25
Pelleting time, min./ton	33.2	34.6	33.3
Kw.-hr./ton	7.93	9.77	8.86
Steam, p.s.i.g.	48	60	48
Steam flow valve	2.8	1.8	2.8
Pellet feeder setting	35	32	35
Fines returned to mill, %	22	17	No data
Pellet hardness	10.0	10.5	9.9
pH soft feed, 1% slurry	6.2	5.6	5.9
Water added, as steam, %	5.2	4.5	5.2

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NIACIN DETERMINATION

Chemical Method for Estimation of Niacin in Poultry Feeds and Premixes

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The need for a chemical method for the routine determination of niacin in biological materials has been evident for some time. A suitable method involving initial purification of extracts followed by colorimetry is presented. Recoveries of niacin averaged 98.6%. Values found by this method agreed closely with those determined by microbiological assay. The procedure can be adapted to the routine determination of niacin in mixed feeds or premixes as part of a feed control program. With suitable modifications, it may also be used to determine niacin in a variety of feed ingredients.

A SIMPLE chemical method for the routine determination of niacin in manufactured feeds and premixes has been needed for some time. Among the numerous methods proposed for estimation of niacin in biological material (*1*, *5*, *6*, *9*, *10*), that of Chitre and Desai (*4*) appeared most adaptable for routine use. These authors used the potassium permanganate decolorization procedure of Krehl, Strong, and Elvehjem (*8*) to purify the extracts, and Koenig's reaction of α- or γ-unsubstituted pyridine derivatives with cyanogen bromide and an aromatic amine to give a color reaction (*7*). Aniline hydrochloride was the aromatic amine used rather than Swaminathan's aniline (*11*).

The method presented herein is a modification of the above procedure, and has proved very satisfactory for routine determination of niacin or niacinamide in manufactured feed and premixes.

Analytical Procedure and Results

Reagents. A 3% solution is prepared by dissolving cyanogen bromide (Eastman Kodak) crystals in distilled water, or by adding a 10% solution of potassium or sodium cyanide, dropwise, to a cold saturated solution of bromine until the solution is just decolorized. The filtered solution will keep for 4 to 5 months if stored in the cold, in an amber-colored bottle.

Aniline Hydrochloride. To 40 ml. of redistilled aniline in an ice bath, under a hood, 100 to 130 ml. of concentrated hydrochloric acid are added, a few milliliters at a time with constant stirring, until the creamy white precipitate is completely formed. The pre-

cipitate is collected in a Büchner funnel on a hardened filter paper and the excess acid is removed by washing with cold diethyl ether followed by suction. The precipitate is dried between folds of filter paper and dissolved in water to prepare a 10% solution. The filtered solution, stored in the cold in an amber-colored bottle, will keep for 4 to 5 months.

Niacin Stock Standard Solution (0.1 mg. per ml.). Pure niacin, 25 mg., dissolved in a 25% solution of 95% ethyl alcohol in water, made up to 250 ml. in a volumetric flask with the same solvent, and stored in the cold.

Niacin Working Solution (5.0 γ per ml.) is made up fresh daily by diluting 5 ml. of the stock solution to 100 ml. with distilled water. (Working Standard)

Preparation of Samples. The samples used were representative of commercial broiler mashes and premixes from a commercial feed mill. The samples were reground in a Wiley mill to 40-mesh, and used for subsequent analysis.

Extraction Procedure for Mashes. Two grams of a feed containing 20 to 45 mg. of niacin or the amide per pound are weighed into a 150-ml. Erlenmeyer flask; 40 ml. of 1*N* sulfuric acid are added, and the contents are autoclaved at 15 pounds' pressure for 30 minutes. To the cooled extracts are added 5 grams of anhydrous sodium sulfate or 8 grams of ammonium sulfate. They are set aside with occasional shaking for 15 minutes. The solution is then filtered and the residue on the filter paper is washed with two 5-ml. portions of distilled water. The filtrate and washings are collected in a 50-ml. volumetric flask and made up to volume. The extracts may be stored if necessary.

Decolorization. A 15-ml. aliquot is pipetted into a 150-ml. Erlenmeyer flask, to which a 1% potassium permanganate solution is added dropwise from a buret, until the rate of disappearance of the pink color of the permanganate is markedly reduced, and the color of the extract is considerably lightened. A few more drops may be added until the solution cannot be decolorized further and the pink color formed on addition of 1 to 2 drops of permanganate persists for a little over 15 seconds. The extract is neutralized to pH 6.5 to 6.8 with bromothymol blue as external indicator, transferred to a 50-ml. volumetric flask, made to volume, and filtered. An aliquot is used for colorimetry.

Extraction Procedure for Premixes. Two grams of a premix (containing approximately 1600 to 3300 mg. of niacin per pound) are weighed into an Erlenmeyer flask, 40 ml. of 1*N* sulfuric acid added, and the contents autoclaved for 30 minutes at 15 pounds' pressure. The cooled extracts are filtered and the residue is washed with two 25-ml. portions of distilled water. The filtrate and washings are collected in a volumetric flask and diluted to approximately 200 ml. The contents are neutralized to pH 6.5 to 6.8 and made to volume. A 10-ml. aliquot of the above solution is pipetted into a 250-ml. volumetric flask and made to volume, and an aliquot is used for colorimetry. The extract need not be decolorized, as little color remains after dilution.

These methods of extraction are designed to provide an optimal range of 3 to 6 γ of niacin in the final aliquot employed for colorimetry. Table I suggests suitable modifications in the weights of samples that might be employed to maintain the same dilutions as used in the procedure for mashes.

Colorimetry. The following procedure, set up for the Coleman Jr. spectrophotometer, employs a 10-ml. final volume. Test tubes, 18 × 150 mm. are set up as follows, the various components being pipetted in.

	Extract, Ml.	*Water, Ml.*	*Standard, Ml.*	*6N HCl, Drops*
Sample blank	5	1	...	1
Sample	5	1	...	...
Sample plus standard	5	...	1	...

The tubes, so prepared, are placed in a water bath maintained at 75 to 80° C. for 5 minutes. At the end of this period, 2 ml. of cyanogen bromide are added to each tube from a buret, and the tubes allowed to remain in the bath for an additional 5 minutes. They are then removed and cooled to room temperature in tap water. Aniline hydrochloride reagent (2 ml.) is added and the color developed is read after 1 to 1.5 minutes. The color produced reaches a maximum in 1 to 1.5 minutes, remaining stable thereafter for 3 minutes. It should consequently be read within this period. The per cent transmittance is read at a wave length of 420 mμ in 19 × 105 mm. Coleman round cuvettes, the instrument being set to 100% transmittance with distilled water. Per cent transmittance, T, is converted to absorbance A, by $2 - \log T$.

Some typical measurements of per cent transmittance employing this method are presented in Table II.

If tests are carried out as above, the following calculations are used for computing the amount of niacin in the sample.

Mashes

$$\frac{A(\text{sample}) - A(\text{sample blank})}{A(\text{sample plus standard}) - A(\text{sample})} \times 5 \times 50/5 \times 50/15 \times 454/2 \times 1/1000 = \text{mg. of niacin per pound of sample}$$

Premixes

$$\frac{A(\text{sample}) - A(\text{sample blank})}{A(\text{sample plus standard}) - A(\text{sample})} \times 5 \times 250/5 \times 500/10 \times 454/2 \times 1/1000 = \text{mg. of niacin per pound of sample}$$

Using the above method, niacin was determined in samples of feed and premixes obtained. Table III lists the recovery of niacin added to representative samples.

Discussion

As pointed out by Bandier (*3*), niacin, niacinamide, and nicotinuric acid, on an equimolar basis, yield colors of varying intensity. The use of 1*N* acid hydrolysis converts niacinamide to niacin. Nicotinuric acid under these conditions yields 30% of the color given by an equimolar amount of niacin; however, as nicotinuric acid is unlikely to be present in manufactured feeds to any large extent, 1*N* acid hydrolysis was employed prior to colorimetry. Sulfuric acid (1*N*) was preferred in this procedure, both to supply the requisite acidity, and to be available during the decolorization procedure with the permanganate solution.

The blank of Wang and Kodicek (*12*) was used rather than the more complicated one of Friedemann and Frazier (*5*), as identical results were obtained.

A pH of 6.5 to 6.8 in the extract for colorimetry was preferred to the usually recommended pH of 4.5, as a wider range of sensitivity was observed at pH 6.5 to 6.8. Potassium dihydrogen phosphate, recommended by Bandier (*3*) for the elimination of nonspecific reactions, did not appear necessary with the samples employed. Aside from depressing the range of sensitivity it gave values similar to those obtained without its use.

Table I. Variations in Sample Weight with Variation in Niacin Content

Sample Taken for Analysis, Grams	*Niacin Content, Mg. per Pound*
4.0	10 to 25
2.0	20 to 45
1.0	40 to 90
0.5	80 to 180

Table II. Typical Per Cent Transmittance Readings of Niacin Determination

	Sample Blank	*Sample*	*Sample plus Standard*
Mixed feeds	73.0	59.0	35.0
	74.0	58.5	34.0
Premixes	96.5	80.5	65 0
	97.0	79.0	63.5

The recoveries obtained were entirely satisfactory (Table III) and the values determined chemically on 10 premixes and 18 broiler mashes agreed very well with those found by microbiological assay (*2*). Correlation coefficients of 0.904 and 0.971 were obtained between the microbiological and the chemical method for the feed samples and premixes respectively. Average values of 28.4 mg. per pound (range 24 to 34.3 mg. of niacin per pound) in the mashes and 2061.0 mg. per pound (range 1613 to 2322 mg. of niacin per pound) in the premixes were observed.

The method described employs a smaller sample than that suggested in the AOAC procedure (1 ounce). The potassium permanganate decolorization procedure gives extracts of lighter color than those of untreated samples, even after precipitation of protein. While the superiority of the sample blank employed in this procedure over that employed in the AOAC procedure may be questionable, it is the authors' experience that cyanogen bromide does contribute toward the blank. They have consequently preferred to use all reagents rather than to omit the cyanogen bromide reagent for the sample blank measurement.

The smaller quantities of sample employed, the smaller dilutions, and the greater stability of reagents, make this method adaptable to routine use.

Table III. Recovery of Niacin Added to Representative Feed and Premix Samples

Sample	Niacin in 2-G. Sample, γ	Niacin Added to 2-G. Sample, γ	Niacin Determined, γ	Recovery, %
Mixed feed	126	50	180.0	108.0
		100	230.0	94.0
		150	275.0	99.3
	112	50	160.0	96.0
		100	210.0	98.0
		150	264.0	101.3
Premix	7061	1500	8500.0	95.9
		3000	10050.0	99.5
	8700	1500	10160.0	97.3
		3000	11600.0	96.6
			Av.	98.59

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SWEET POTATO PIGMENTS

Relationship of Tristimulus Colorimeter Readings to Carotenoid Pigments in Sweet Potatoes

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Changes in the carotenoid content have been found to be a sensitive index of the response of sweet potatoes to various growing and storage conditions. If this index is to be widely used, a rapid method of measuring these changes is needed. Because carotenoids are the pigments responsible for the color of the flesh it seemed likely that the Hunter color and color-difference meter might be used to measure these changes and thereby provide the fast method needed. Coefficients of correlation between total carotenoids of sweet potatoes, as determined chemically, and the values obtained with the Hunter meter are statistically highly significant, but are not sufficiently close to give assurance that even relatively large differences will be detected.

THE FLESH OF SWEET POTATOES may range in color from creamy white through yellow and orange to salmon-pink, depending on the concentration of carotenoid pigments. β-Carotene is the principal carotenoid in varieties having deeply colored flesh, and as the precursor of vitamin A it is the most important pigment in all varieties from the nutritional standpoint. Genetic factors are largely responsible for differences in carotenoid concentration between varieties, but other factors may readily and markedly affect the concentration of the pigments within a variety. Preharvest conditions may result in a lower or a higher concentration at harvest (*3*) or may influence the rate of accumulation after harvest (*6*). Environmental conditions after harvest may result in an increase, a decrease, or the maintenance of a rather uniform amount during storage (*4*).

Changes in the carotenoid concentration thus appear to be a sensitive indicator of the response of sweet potatoes to various environmental conditions, perhaps more sensitive than the subjective indicators commonly used. A major deterrent to its use for this purpose is the long, laborious, chemical procedure now used to measure these pigments and the changes they undergo. Edmond, Garrison, Wright, Woodward, Steinbauer, and Deonier (*2*) reported visual changes in the flesh color, but evalutions of such changes are difficult and only major changes or differences are likely to be detected. If a rapid, objective method