Detection of *N,N'*-Diphenyl-*p*-phenylenediamine (DPPD) in Dehydrated Alfalfa and in Mixed Feeds

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N,N'-Diphenyl-*p*-phenylenediamine (DPPD), which is added to alfalfa to stabilize carotene and to mixed poultry feeds to protect chicks against encephalomalacia, interferes in the determination of carotene and of vitamin A. A test for DPPD in meals and feeds has been developed, which requires only a small sample and can be completed in a few minutes. The DPPD is extracted by a hexane-acetone mixture and interfering substances are removed by adsorption on magnesia. In the presence of hydrochloric acid solution and copper ion, a blue colored solution is formed when DPPD is present.

THE ANTIOXIDANT *N,N'*-diphenyl-*p*-phenylenediamine (DPPD) helps in the preservation of carotene in dehydrated alfalfa (*3*). When this chemical is put in poultry feeds at a level of 0.0125%, it protects chicks against encephalomalacia (*5*). DPPD is used commercially for both the foregoing purposes. However it interferes in the determination of carotene in alfalfa meal (*1*), and in determination of vitamin A in mixed feeds both by the spectrophotometric method at 328 mμ (*7*) and by the antimony trichloride method (*6*).

Mitchell and Silker (*4*) published a carotene method applicable to alfalfa containing DPPD. Procedures have been suggested for removing DPPD from extracts of mixed feeds (*2, 7, 8*), but these methods are still under investigation and have not been published. It is important to know when DPPD is present in samples of alfalfa meal and mixed feeds, so that appropriate methods of analysis may be chosen. A simple test for identifying DPPD in feeds and alfalfa is presented here. The test is a variation of one suggested for copper (*9*).

Test for *N,N'*-Diphenyl-*p*-phenylenediamine

Add 30 ml. of hexane-acetone (1 to 1) to 2 grams of alfalfa meal or 10 grams of mixed feed in a small beaker and stir thoroughly. Add 2 grams of activated magnesia, stir, and allow solids to settle. If the sample is alfalfa meal, decant the yellow supernatant liquid from the meal and filter directly into a test tube. If the sample is mixed feed, decant the supernatant liquid and to it again add 2 grams of activated magnesia and stir. Then decant and filter as with alfalfa extract. To either alfalfa or mixed feed extract add 5 ml. of hydrochloric acid solution (1 to 3) and 1 drop of 0.25% copper sulfate solution and shake. A clear blue to blue-green color in the acid-water layer is evidence of DPPD in the original sample. In some cases when DPPD is present, a light blue color is observed even if copper sulfate is not added, presumably from traces of copper in sample or reagents. In absence of magnesia treatment, a blue-green pigment extracted from the alfalfa, which is not due to DPPD, causes interference in this test.

This test is sufficiently sensitive to detect DPPD in a feed containing 5% alfalfa, in which the only source of the chemical is the ingredient alfalfa, containing 0.0125% DPPD. If the whole

Table I. Results of Blue Color Test on Feed and Alfalfa Meal Samples

Sample and Treatment	*N,N'*-Diphenyl-*p*-phenylenediamine Content, %	Blue Color
Alfalfa meal		
Sun-cured	None	−
Sun-cured + 16 lb. oil per ton	0.015	+
Dehydrated	None	−
Dehydrated, 80 lb. oil per ton	None	−
	0.015	+
Dehydrated, 80 lb. oil per ton, heated 100° C.	0.015	+
	None	−
Stored 60° C. 4 wk.	None	−
	0.015	+
Chick starter		
Commercial A	None	−
Mash	None	−
Layer mash, commercial	None	−
Chick starter		
Commercial B	0.0125[a]	+
Commercial C	0.0125[a]	+
Commercial C, stored 1 wk., 60° C.	0.0125[a]	+ (light)
Exptl.	None	−
	0.0075	+
Exptl., stored 4 wk., 60° C.	0.0075	+ (light)
Exptl.	0.0025	+
Exptl., stored 4 wk., 37° C.	0.0025	+ (light)
Exptl., stored 4 wk., 60° C.	0.0025	−
Exptl.	0.0125	+
Exptl., stored 2 wk., 60° C.	0.0125	+
Special	[b]	+
Exptl. + 5% alfalfa	[c]	+ (light)
Exptl. + 10% alfalfa	[c]	+ (light)
Exptl. + 5% alfalfa, stored 4 wk. room temp.	[c]	−
Exptl. + 10% alfalfa	None	−
Exptl. + 10% alfalfa, stored 4 wk., 60° C.	None	−

[a] Added by manufacturer, presumably at amt. shown.
[b] Special mix 2/3 feed containing no DPPD and 1/3 feed containing 0.0125% DPPD.
[c] Only alfalfa contained DPPD (0.015%).

feed contains 0.0125% DPPD (the level recommended for protection against encephalomalacia), only a 2-gram sample is needed for the test. Results obtained by use of this test are shown in Table I. Feeds stored as long as 4 weeks at 60° C. gave a test for DPPD if 0.0075% or more was originally added. Determination of carotene in alfalfa stored under the same conditions indicates that these accelerated storage conditions were equivalent to at least 6 months under normal conditions (25° C.).

Under specified conditions, the test appears specific for DPPD in feeds or alfalfa meal. Many materials were checked to determine whether they interfered in the test, including 16 amino acids, 11 amines and substituted amines, 9 vitamins, 8 drugs and 5 antibiotics used in feeds, 3 quinones, and miscellaneous feedstuffs.

A more sensitive test, but one not so specific for DPPD, is the following. Place 10 to 20 drops of the filtered hexane-acetone extract, as prepared in the foregoing test, in a small evaporating dish and evaporate nearly to dryness. Add 2 drops of concentrated nitric acid. The solution first turns blue and then red in presence of DPPD. A somewhat similar color is obtained in this test from 6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline (Santoquin), but the acid–copper sulfate solution will not give a blue color with this chemical.

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PROTEIN QUALITY AND SUPPLEMENTATION

Relative Nutritive Values of Proteins in Foods and Supplementary Value of Amino Acids in Pearled Barley and Peanut Flour

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A study was carried out on the relative nutritive values of the proteins in various foods at different levels of intake and during several periods of experimentation. The results are expressed as gains in body weight of the albino rat per gram of protein intake. Data are included on the influence of heat and duration of heating on the nutritive value of the proteins in dried nonfat milk solids (skim milk). Data are also recorded on the supplementary value of certain amino acids to the proteins in milled barley and peanut flour. Addition of 0.4% L-lysine, 0.5% DL-threonine, and 0.5% DL-methionine to the proteins in milled barley resulted in 151.1% increased growth and 224.7% increase in protein efficiency ratio. Supplementation of the proteins in peanut flour with 0.5% DL-methionine and 0.5% DL-threonine was followed by 60.6% gain in body weight and 61.5% increase in protein efficiency ratio.

THERE ARE ESSENTIALLY TWO METHODS of determining the nutritive value of proteins in foods. In 1909, Thomas (*20*) was the first to define the term biological value as the percentage of absorbed nitrogen which is retained by the body for the repair or synthesis of nitrogenous tissue. The measurement requires that the nitrogen intake, the nitrogen excretion, and the endogenous metabolic nitrogen be known. In 1924, Mitchell (*6*) applied this method to growing rats. Later, Mitchell and Beadles (*7*) emphasized the method of paired feeding and described the methods in detail. Osborne and Mendel (*8–10*) undertook an investigation of the methods of measuring quantitatively the comparative nutritive value of proteins, which they expressed as gains in body weight per gram of protein intake, defined as the protein efficiency ratio. Mitchell has critically reviewed the methods in use for determining the nutritive efficiency of proteins (*5*). Referring to the work of Osborne and Mendel, he says:

> As originally presented, the method involved a comparison of different proteins based on the maximum gains per gram of protein consumed. As ordinarily used by other investigators, however, no systematic attempt is made to find the maximum value. In some cases comparisons are made at one level of intake only, and in some cases comparisons of different proteins are made at different levels of intake, quite arbitrarily chosen. The values obtained with different proteins apparently stand in quite different ratios to one another, depending upon the level of protein intake (*6*).

In spite of such criticism, the comprehensive table by Block and Mitchell on the nutritive efficiency of the proteins in 41 foods (*1*) covers experimental periods of 4 to 8 weeks, and while they attempted to give data on protein contents of 10% such foods as rice, maize, and wheat flour were included. The maximum protein content in rations containing such foods could not be over 5, 7, and 8%, respectively; hence, the data submitted in this table do not represent accurate relative values of the protein