Nonprotein Nitrogen in Soybeans

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There is an increasing interest in the development of high-protein soybeans. Since the protein content is usually measured by determining Kjeldahl nitrogen and multiplying by 6.25, the method is accurate only if the proportion of nonprotein nitrogen is small. Over 30% of the nitrogen in very immature soybeans was nonprotein nitrogen compared to 4 or 5% in mature seed. Mature soybeans were higher in nonprotein nitrogen when grown under adverse conditions. High-protein soybeans were not necessarily high in nonprotein nitrogen. Strains high in Kjeldahl nitrogen were high in actual protein.

Within the past 20 years, soybean production in the United States has increased from a crop of little commercial importance to one of the greatest sources of vegetable protein. The good balance of amino acids in soybean protein makes it an excellent constituent of animal rations. Soybean production has increased from 5,000,000 to over 500,000,000 bushels in 25 years. However, demand for high-protein materials has increased so rapidly that there is a demand for still more soybean protein. Although surpluses of many farm commodities have created a problem, there is need for about 30% more protein to feed livestock most efficiently (*7*). Demand for fats and oils, however, has not kept pace with soybean production and there is, therefore, a surplus of soybean oil.

Although protein is by far the major component of soybean seed percentagewise, it is seldom separated as such for animal rations. The percentage of protein reported for commercial feed is obtained by determining Kjeldahl nitrogen and multiplying by 6.25. This in effect assumes that protein is the only nitrogen compound present.

One possible solution to the problem of a demand for more protein, but less oil, would be to breed for high nitrogen in soybeans. Since most soybean meal is used in rations for monogastric animals, it is important that the nitrogen compounds can be utilized without the aid of rumen organisms.

When a protein-precipitating reagent is added to an aqueous extract of soybean meal, 3 to 10% or more of the total nitrogen remains in solution. If the meal is from immature seed, the percentage of nonprotein nitrogen may be over 30% of the total nitrogen. Muller and Armbrust (*6*) reported that protein-free extract of mature soybeans contained the following nitrogen compounds: adenine, arginine, choline, glycine, betaine, trigonelline, guanidine, tryptophan, and probably canavanine or a similar compound. The actual composition of the nonprotein fraction would be expected to vary, depending on whether the soybeans are mature or the protein has been damaged by weather or in storage. In a previous investigation (*5*), it was found that weather-damaged soybean seed was higher in nonprotein nitrogen than undamaged seed. Since the nonprotein nitrogen fraction contains amino acids, the extent to which nonruminants could utilize it would depend on amino acid balance.

During the past decade, many investigations on utilization of nonprotein nitrogen by ruminants were reported. Hale (*4*) reported that many nonprotein nitrogen compounds can be utilized by rumen organisms. Fifty per cent or more of a natural protein may be degraded or altered by rumen microorganisms. Too high a proportion of nonprotein nitrogen compounds, which are broken down more rapidly than protein, may be degraded more rapidly than they can be utilized. This condition may result in feed loss or even in toxicity.

Gallup (*3*) reported the utilization of nonprotein nitrogen by beef cattle in a series of nitrogen-balance tests. He found that beef cattle could utilize urea up to 50% of the total nitrogen supply in fattening rations and up to 25% in wintering rations. Carbohydrate feeds were essential for efficient utilization, and methionine supplements brought further improvement.

The present study is an investigation of the factors which affect the percentage of nonprotein nitrogen in soybeans.

Materials and Methods

To study the effect of maturity on nonprotein nitrogen, samples were harvested and analyzed beginning at a stage when the soybeans weighed less than 5 mg. per seed. Four replicate samples of two varieties, Clark and Hawkeye, each planted at two different dates, were harvested daily until maturity, when the seeds weighed about 150 mg.

To study the effect of the percentage of protein in the seed on the proportion of nonprotein nitrogen, 381 samples of mature soybeans with a wide range of protein contents were analyzed. Protein in samples ranged from 25.5 to 58.9%. In order to secure samples of seed with such a wide range of protein content, it was necessary to use seed from a wide variety of growing conditions and of many varieties of soybeans. The extreme protein percentages were sometimes due to unusual environmental conditions as well as to genetic differences.

To investigate further the effect of interactions between season, location, and variety, composite samples of mature soybeans were prepared and analyzed. Samples of soybeans of 16 to 23 varieties and strains grown at 16 to 24 midwestern locations were composited and analyzed each year from 1951 to 1957.

Weather conditions were reported by collaborators at each of the state experiment stations where soybean test plots were located.

Protein was determined by the Kjeldahl-Wilfarth-Gunning method (*1*). Nonprotein nitrogen was determined by the method of Becker, Milner, and Nagel (*2*). Net protein was calculated as the difference between total nitrogen and nonprotein nitrogen multiplied by the factor 6.25.

Results and Discussion

Effect of Maturity. In the maturity study, the percentage of total or crude protein changed only slightly during the growth of the seed (Figure 1). In the earliest samples, almost one third of the nitrogen was nonprotein nitrogen, but the percentage decreased steadily as the seed matured. At the same time, net protein increased so that the sum was

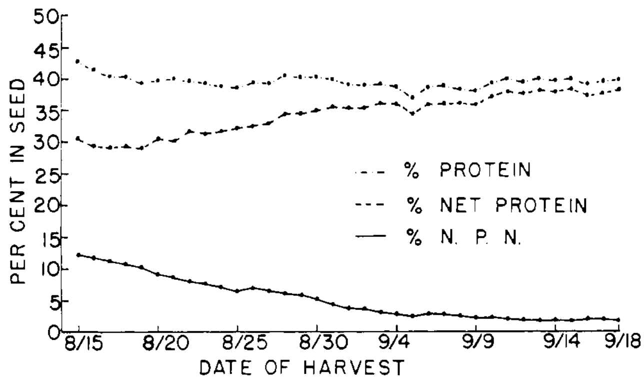

Figure 1. Protein fractions in maturing soybean seed as per cent N × 6.25

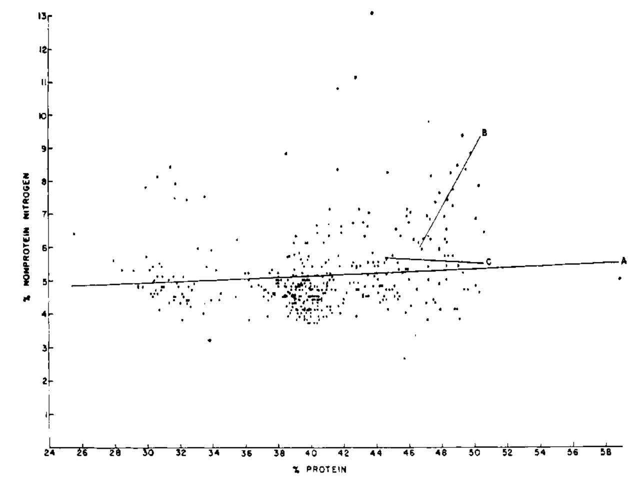

Figure 2. Relationship between percentages of protein and nonprotein nitrogen in soybean seed

Regression lines. *A* all 381 samples, *B* high-protein seed grown under dry conditions, *C* high-protein seed grown under favorable conditions

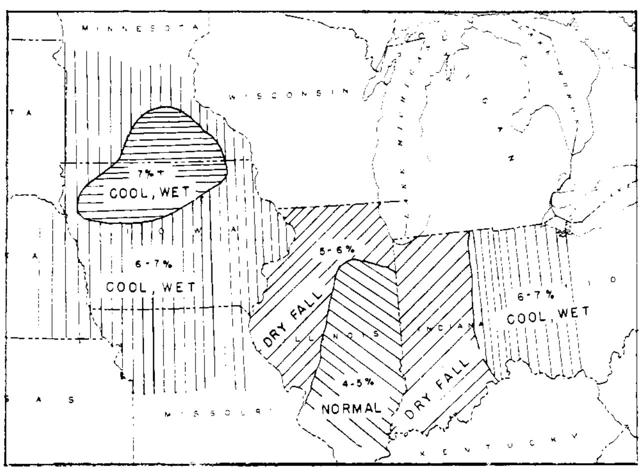

Figure 3. Effect of cool, wet season on per cent of nonprotein nitrogen in soybeans (1951)

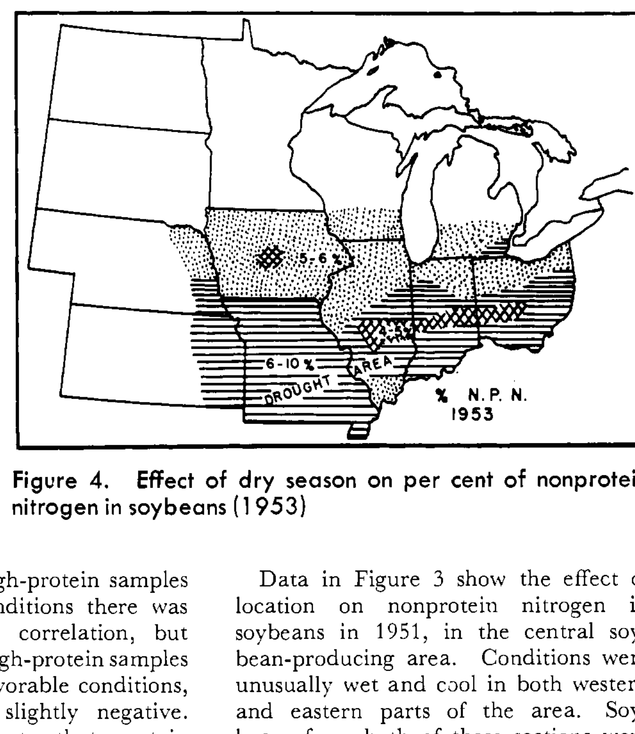

Figure 4. Effect of dry season on per cent of nonprotein nitrogen in soybeans (1953)

almost constant. Data shown are averages for Hawkeye first planting. The other three samplings of both Hawkeye and Clark varieties gave similar results. In terms of milligrams per seed, both total protein and nonprotein nitrogen increased rapidly during the early part of the growing period. However, as maturity was approached, nonprotein nitrogen decreased.

Effect of Protein Content of Seed. Over the complete range of protein, from 25.5 to 58.9% in 381 samples, the correlation between nonprotein nitrogen and protein content (calculated from total nitrogen) was only 0.073 (Figure 2). The slight tendency for nonprotein nitrogen to increase with increasing protein was too small to be of significance.

In the restricted range, from 39 to 44% protein in 174 samples, the correlation between nonprotein nitrogen and protein was also not statistically significant. This is of importance because practically all the soybeans grown commercially fall within this range.

In one group of high-protein samples grown under dry conditions there was a significant positive correlation, but in a similar group of high-protein samples grown under more favorable conditions, the correlation was slightly negative.

These results indicate that protein level is not a determining factor in nonprotein nitrogen content and that protein can be increased without a significant increase in nonprotein nitrogen.

Since soybean meal is used in animal rations mainly as a source of protein, an increase in protein nitrogen means an increase in nutritional value, assuming that amino acid balance is maintained.

Effect of Season, Location, and Variety. Most of the data from the variety-location study showed little varietal effect. Varieties adapted to the southwestern part of the area averaged higher in nonprotein nitrogen, but other data seem to indicate that higher nonprotein nitrogen was probably due to drought conditions in that area.

Data in Figure 3 show the effect of location on nonprotein nitrogen in soybeans in 1951, in the central soybean-producing area. Conditions were unusually wet and cool in both western and eastern parts of the area. Soybeans from both of these sections were unusually high in nonprotein nitrogen, with 6.0 to 7.6% of the total nitrogen. Adjacent to these areas were areas in which weather conditions were cool and wet in the spring, but dry in the fall. In this area nonprotein nitrogen was above normal, but lower than in the first areas. In the central part of the central soybean-producing area, conditions were about normal and nonprotein nitrogen was 4 to 5%. In 1953, weather conditions were droughty with most of the southern half of the region receiving insufficient rainfall. Soybeans from this area were above normal in nonprotein nitrogen (Figure 4). Soybeans from the drought area contained 6 to 10% of nonprotein nitrogen as compared to about 4 to 5% for normal mature soybeans. The effect of drought on nonprotein nitrogen was noticeable in each of the three succeeding years as well as in 1952, particularly in the southwestern part of the region, but drought areas were smaller than in 1953.

In 1957, weather conditions were more uniform, with no areas producing soybeans very high in nonprotein nitrogen and, in general, soybeans from northern areas tended to be slightly higher in nonprotein nitrogen, especially in areas where soybeans were slightly damaged by frost before they were mature. Unfavorable growing conditions, whether too cool and wet or too hot and dry, were associated with a high percentage of nonprotein nitrogen. Under drought conditions, growth may have been arrested before the seed was mature. Under these conditions, nitrogenous materials which would have normally developed into protein may have remained as nonprotein nitrogen in the harvested seed. Also, cool, wet weather appeared to have the same effect, that of increased nonprotein nitrogen in the seed, possibly because of slow and incomplete development. Wet weather continuing through the harvest season increased the probability of weather damage to the seed because of delayed harvesting and high moisture content. Under these conditions, weather damage may have caused breakdown of some protein, already formed, into nonprotein nitrogen compounds. Weather-damaged soybeans were often higher in nonprotein nitrogen than undamaged seed (*5*).

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NUTRITIVE VALUE OF CHICKEN MEAT

Nutritive Value of Chicken Meat and Its Value in Supplementing Rice Protein

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A study was made of the nutritive value of light and dark chicken meat and its effect on the improvement of whole and milled rice. The albino rat was used as the experimental animal. The protein efficiency of light and dark chicken meat has been determined. Data are presented on the value of the proteins of light and dark chicken meat in supplementing those of whole and milled rice and on the amino acid (essential and nonessential) content of light and dark chicken meat. The high nutritive value of chicken meat merits its continued promotion in its use for human foods and its recommendation for expansion of the broiler industry, where it is economically feasible.

HEPBURN, Sohn, and Devlin (*2*) found chicken meat to be less efficient than casein as a source of dietary protein and Millares and Fellers (*6*) reported that light chicken meat had higher protein content than dark chicken meat and that the biological value of proteins in chicken meat is equivalent to that of beef, pork, lamb, and veal. Since the latter investigators used yeast and liver extracts as a supplement to synthetic vitamins, it is possible that such extracts furnished supplementary nitrogen to the proteins of the various meats studied which might have influenced their results. Furthermore, little is known concerning the effect of chicken meat on the nutritional value of cereal grains, and although some information has been obtained on the content of essential amino acids, none is available on that of nonessential amino acids. It thus appears that information on the nutritive value of proteins in chicken meats is needed and is a new field for investigation.

This paper reports results of growth and metabolism experiments with young rats fed diets composed of light and dark chicken meat as the only source of protein. Included is a study on the effect of replacing rice proteins with those of chicken meat; 1, 3, and 5% of these solids replaced equivalent amounts of protein in whole brown and milled white rice rations which were fed to albino rats for 70 days. Another series of experiments was made on the effect of adding small amounts of these solids to rations containing whole brown and milled white rice as the only source of protein. Results are also presented of a study on the content of amino acids (including nonessentials) in light and dark chicken meat.

Experimental Procedure and Materials

In this study, 12 albino rats—sexes equally divided—were used for each group. The animals were about 28 days old when the experiments were started and weighed 50 to 54 grams each. Chicken meat and rice furnished the only sources of protein in the rations. The duration of the experiments was 10 weeks. The rations contained 4% of Sure's salt mixture No. 1 (*11*); 3% of hydrogenated vegetable shortening; 2% of cod liver oil; 1% of wheat germ oil; 2% of cellu flour; and the balance, percentagewise, was glucose (cerelose).