all the experimental products were effective fertilizers. The crop response to phosphate was equal to or better than that obtained from the phosphate in either concentrated superphosphate or calcium metaphosphate fertilizers. Nitrification studies showed that the experimental products were nitrified more rapidly than ammonium sulfate.

Field tests of the steam-treated material produced in pilot plant operation are under way in 11 states as far west as Utah and as far north as New York.


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**NUTRITIVE QUALITY OF CROPS**

# Methionine Content of Soybeans as Influenced by Location and Season


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**Results obtained in this study indicate differences in methionine content of protein in soybean varieties. Strains from Dunfield crosses and the strain Clark were superior to other strains tested. The strain Clark was significantly higher in methionine content than either of its parents, indicating transgressive segregation within the Lincoln × Richland cross. It should be possible to develop varieties high in methionine content by plant breeding. There were significant seasonal variations only in Group IV, methionine being generally higher in 1952 than in the two previous years. The variation which could be attributed to location effect was not consistent enough to show significance at the 5% level except in Group III. These results indicate that soybean protein is produced in the seed with variable proportions of amino acids.**


SOYBEANS have become our most important source of vegetable protein and many feeding experiments have shown that soybean protein is deficient in methionine. The purposes of this investigation were to determine whether there are differences in the methionine content of different lots of soybean protein, the factors influencing methionine content, and the feasibility of breeding soybeans with higher methionine content. A 3-year study was made of samples of 14 soybean varieties, each grown at from 12 to 18 locations composited by variety and by location. In most cases, season and location were not consistent enough in their effect to be significant, but there were definite genetic differences indicating that it should be possible to increase methionine in soybean protein by breeding.

Our greatest source of protein for livestock feeding is soybean oil meal. Although there are surpluses of many agricultural commodities, there is a potential demand for about twice as much soybean protein as is now being produced (*6*). It would be possible to effect the equivalent of an increase in protein for animal feeding, without increasing the quantity produced, if certain amino acid deficiencies could be overcome.

The proportions of amino acids in soybean protein as reported by Block and Bolling (*3*) and Alderks (*1*) come remarkably close to matching requirements for rat growth as reported by Rose (*12*). However, the nutritional value of the protein is limited by a deficiency in the proportion of methionine and cystine present, as shown by Flodin (*4*) in his report on amino acid balance in protein. Cystine is included with methionine, because experiments have shown that, to a limited extent, methionine deficiency may be alleviated by supplying cystine (*14*). These data should be considered as only indicative and subject to revision, in view of the limited accuracy of existing information both on protein composition and on amino acid requirements of animals. Final proof is obtained in actual feeding trials, many of which have shown that soybean protein is deficient in methionine.

Soybean and corn proteins tend to supplement each other in a mixed feed, but corn protein is not high enough in methionine plus cystine to make up this deficiency in the soybean, and soybean protein is not high enough in lysine to make up this deficiency in the corn protein. As corn protein is very deficient in lysine, a mixed feed composed of corn and soybean meal is deficient in lysine; therefore higher lysine in soybean protein would be desirable. Synthetic lysine has recently become available commercially, but the high cost and high animal requirement make it an expensive supplement.

Many of the problems of availability of amino acids in soybean protein have been solved by nutritional research. Proper heat treatment greatly improves the availability of methionine by destroying the antigrowth factor, antitrypsin (*2*). Heat treatment also destroys the growth inhibitor soyin (*8*) and others (*9*). Growth response is also increased greatly by the addition of vitamin $B_{12}$ to the ration. Antibiotics sometimes give additional growth response, although their use in rations for ruminants is of questionable benefit because of their adverse effect on rumen microorganisms (*11*). The nutritive value of soybean oil meal as finally marketed is dependent on proper processing to obtain maximum availability of the essential amino acids in the seed.

Breeding soybean varieties with higher methionine content appears to be one method of increasing the supply of this amino acid without adding to the feed cost. The use of larger proportions of protein in the ration, the substitution of animal protein for vegetable protein, and the addition of synthetic methionine all add appreciably to the cost of the product.

Very little information is available concerning factors that affect the percentage of methionine in soybean protein. Alderks (*1*) reported amino acid analyses of the beans from 20 strains of soybeans and showed a range of 1.28 to 1.53% methionine in protein on composite samples from several locations.

Sheldon, Blue, and Albrecht (*13*) found that soybean plants grown in sand culture contained over twice as much methionine when the nutrient solution in which they were grown contained 96 p.p.m. of sulfur as when it contained 16 p.p.m. This would indicate that the amount of sulfur present may be a factor in determining the amount of methionine produced in the plant.

**Table I. Per Cent Methionine in Protein of Variety Composite Samples**

| Variety | Year Grown 1950 | 1951 | 1952 | 3-Year Average |
|---|---|---|---|---|
| | | Maturity Group II | | |
| Adams | 1.45 | 1.38 | 1.53 | 1.45 |
| Blackhawk | 1.30 | 1.35 | 1.24 | 1.30 |
| Earlyana | 1.32 | 1.27 | 1.33 | 1.31 |
| Hawkeye | 1.32 | 1.44 | 1.31 | 1.36 |
| Lincoln | 1.32 | 1.29 | 1.42 | 1.34 |
| Richland | 1.36 | 1.24 | 1.52 | 1.37 |
| Mean | 1.35 | 1.33 | 1.39 | |
| S.D. = 0.011[a] | | | | 0.047[b] |
| | | Maturity Group III | | |
| Adams | 1.45 | 1.54 | 1.50 | 1.50 |
| Chief | 1.44 | 1.42 | 1.43 | 1.43 |
| Dunfield | 1.55 | 1.47 | 1.62 | 1.55 |
| Illini | 1.43 | 1.26 | 1.59 | 1.43 |
| Lincoln | 1.29 | 1.44 | 1.42 | 1.38 |
| Clark | 1.55 | 1.54 | 1.64 | 1.58 |
| Mean | 1.45 | 1.45 | 1.53 | |
| S.D. = 0.009[a] | | | | 0.044[b] |
| | | Maturity Group IV | | |
| Chief | 1.32 | 1.46 | 1.56 | 1.45 |
| Perry | 1.45 | 1.48 | 1.64 | 1.52 |
| L6-1656 | 1.31 | 1.50 | 1.70 | 1.50 |
| Wabash | 1.48 | 1.58 | 1.67 | 1.58 |
| Mean | 1.39 | 1.51 | 1.64 | |
| S.D. = 0.016[a] | | | | 0.030[b] |

[a] Standard deviation of means based on variability within pairs of duplicate samples.
[b] Standard deviation of 3-year averages based on variety × year interaction.

## *Materials and Methods*

Samples of beans of 14 soybean varieties grown at from 12 to 18 locations in 1950, 1951, and 1952 were composited by variety and by location and analyzed for percentage of methionine in the protein.

The samples were dried to about 5% moisture in a low humidity room at about 70° F. and were ground in a Bauer mill. Two-gram samples were wrapped in filter paper and extracted with petroleum ether in Butt extraction apparatus for 8 hours to remove oil. The wrapped samples were then transferred to Soxhlet extraction apparatus and extracted 4 hours with 50% ethyl alcohol to remove sugars.

The wrapped samples were then removed from the extraction apparatus, allowed to dry about 1 hour, and then unwrapped. The meal was pulverized by forcing it through a wire gauze and transferred quantitatively to glass-stoppered Erlenmeyer flasks for hydrolysis. The samples were hydrolyzed by the method of Horn, Jones, and Blum (*5*), except that the hydrolyzate was acidified with 4 drops of sirupy phosphoric acid and then nitrogen gas was passed through the sample to remove hydrocyanic acid. Also, the hydrolyzate was clarified by centrifuging at about 25,000 times gravity before being made up to volume, using washings from the precipitate to make a final volume of 100 ml. Methionine was determined by an adaptation for soybean protein of Horn's colorimetric method (*5*). During the intervals between the addition of reagents the samples were shaken on a Ross Kershaw-type mechanical shaker. The samples were clarified by centrifuging at about 25,000 times gravity and transmittance was read at 530 mμ on a spectrophotometer. Standards were run at the beginning of each day for the range 0.75 to 1.50 mg. of methionine and a standard curve was plotted on semilog paper. Standards were also run with each set of samples and new curves plotted if necessary. The synthetic hydrolyzate (solution A) used with the standard to simulate soybean protein hydrolyzate was made as follows:

Dissolve in 500 ml. of solution 300 mg. of L-arginine, hydrochloride, 100 mg. of L-histidine, hydrochloride, 200 mg. of DL-isoleucine, 250 mg. of L-leucine, 250 mg. of L-lysine, hydrochloride, 225 mg. of DL-phenylalanine, 200 mg. of DL-threonine, 50 mg. of L-tryptophan, and 200 mg. of DL-valine.

The use of this synthetic hydrolyzate in establishing the standard curve helps to compensate for any effect of amino acids other than methionine on the color developed.

Duplicate samples were hydrolyzed and duplicate determinations of methionine were made in each of the hydrolyzates, so that each percentage reported is the average of four determinations.

The data for each maturity group were analyzed separately by analysis of variance for statistical significance. Standard deviations were also calculated as a measure of differences between duplicate determinations. Mean squares were calculated as a measure of variability due to variety and to location.

## *Results*

Data in Table I show percentages of methionine in the protein from composite samples of each variety grown at locations to which it is best adapted. The varieties were separated into three groups according to time of maturity. Earliest maturing varieties are in Group II (*10*) and are adapted to areas as far north as southern Wisconsin, while Group IV varieties are adapted to areas as far south as southern Illinois. Group III varieties are adapted best to the latitude of central and south-central Illinois.

The data presented in Figure 1 show differences in methionine content in the location composites. Each sample was a composite of all the Group III varieties grown at the particular location. Average percentages of methionine in protein for the 3 years ranged from 1.25% for

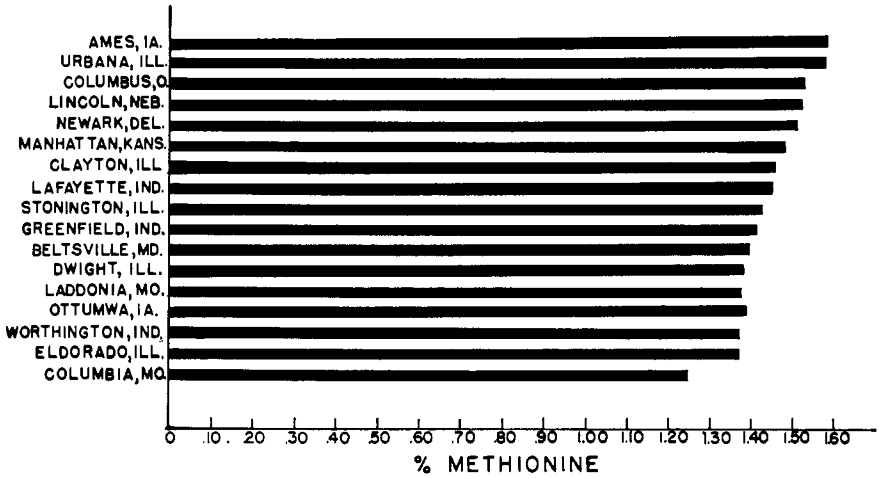


**Figure 1. Three-year average of methionine in protein of all Group III soybean varieties composited for each location**

Columbia, Mo., to 1.58% for Ames, Iowa.

### *Discussion*

An analysis of variance of data from location composite samples indicated that location differences were significant only in Group III. Much of this significance was due to one location, Columbia, Mo., but location differences were still significant if data from that location were omitted.

Standard deviations shown in Table I for each year's data are a measure of differences between duplicate samples and include sampling error and errors in chemical determinations. These indicate the significance of varietal differences for that particular year. Standard deviations shown for 3-year means are a measure of the effect of variety-year interaction and may be used to indicate the significance of consistent varietal differences during the 3-year period.

In many cases Dunfield or Dunfield related strains were high in methionine. Every year the Clark variety samples were also high. In order to determine the significance of these differences, an analysis of variance was made of the data.

The summary given in Table II indicates that in all three maturity groups, Dunfield and the varieties Adams and Wabash, from crosses with Dunfield as one parent, were significantly higher in methionine than the other varieties except Clark. In Group II, Adams was significantly higher than all other varieties at the 5% level of significance. In Group III, Adams and Dunfield were significantly higher than Chief, Illini, and Lincoln. Clark, a selection from the cross Lincoln × (Lincoln × Richland), was also significantly high and exceeded both parents, which indicates the possibility of increasing methionine by breeding. In Group IV, Wabash was significantly higher than the three other strains at the 5% level.

The range of values in the location composites was greater than in variety composites, which may indicate that a greater part of variation in methionine content is due to the location than to the variety. However, statistical analysis of the data shows about the same variability in the variety composites as in the location composites. The effect of location was not consistent enough during the 3 years tested to show significance at the 5% level, except in Group III, probably because of interaction of season and location. This might be interpreted to indicate that soil fertility was not always a controlling factor, but significance in Group III might indicate its possible importance along with climate in some locations. The fact that seed samples from Columbia, Mo., were consistently low each year in methionine may be related to sulfur nutrition, since Sheldon, Blue, and Albrecht (*13*) have reported an increase in methionine in hay when sulfur fertilizer was used. This would seem to indicate a sulfur deficiency in the soil. However, sulfur had been added as fertilizer to the soybean test plots at Columbia. Sulfur nutrition studies now in progress at this laboratory indicate that the use of sulfur as fertilizer causes an increase in the sulfur content of the plant, but in some cases a decrease in sulfur and methionine in the seed.

The use of composite samples made it possible to test a rather large amount of material, but obviously tends to minimize sample differences. Single samples from a given location might show a much greater range of methionine content but would be a less reliable measure of the general performance of the variety. For example, 10 single variety samples from Ames, Iowa, showed a range of 1.14 to 1.78% methionine in the protein. A few single samples of soybeans analyzed in this laboratory were almost as high in methionine as the rat requirements, but the highest variety composites were still deficient in methionine.

There seems to be a tendency toward higher methionine in material grown farther south—that is, Group IV varieties are generally higher than those of Groups II or III. This is in agreement with results obtained when Lincoln plants were grown in the greenhouse at two different day temperatures. Seed from plants grown in two replications at 90° F. contained 1.34 and 1.46% methionine in the protein, while in two replications seed grown at 70° F. day temperature the protein contained only 1.12 and 1.14% methionine.

It is interesting to note how closely results reported by Alderks (*7*) correspond to the average values obtained in

**Table II. Summary of Statistical Evaluation**

| | | *Df for Comparison* | *Df Error Term* | *F* | *Required for Significance* | |
|---|---|---|---|---|---|---|
| | Analysis of Variance | | | | | |
| Group II | Adams *vs.* remaining varieties | 1 | 10 | 5.30 | 0.05 | 4.96 |
| Group III | Adams, Dunfield, *vs.* Chief, Illini, and Lincoln | 1 | 8 | 5.61 | 0.05 | 5.32 |
| | Clark *vs.* remaining varieties | 1 | 10 | 5.97 | 0.05 | 4.96 |
| Group IV | Wabash *vs.* remaining varieties | 1 | 6 | 6.06 | 0.05 | 5.99 |
| Group IV | Variety composites: effect of season | 1 | 6 | 23.5 | 0.01 | 13.74 |
| Group III | Location composites: effect of location | 16 | 32 | 3.12 | 0.01 | 2.62 |

| | *Mean Square, 1950* | | *Mean Square, 1951* | | *Mean Square, 1952* | |
|---|---|---|---|---|---|---|
| | *Variety* | *Location* | *Variety* | *Location* | *Variety* | *Location* |
| | Variability among Variety Means and Location Means | | | | | |
| Group II | 0.088 | 0.124 | 0.124 | 0.080 | 0.204 | 0.132 |
| Group III | 0.156 | 0.108 | 0.176 | 0.092 | 0.156 | 0.116 |
| Group IV | 0.140 | 0.168 | 0.104 | 0.200 | 0.132 | 0.224 |
| Mean | 0.128 | 0.132 | 0.136 | 0.124 | 0.152 | 0.160 |

the present study for the same or closely related strains.

| Alderks | % | Present Study | % |
|---|---|---|---|
| A3-176 (Adams)[a] | 1.43 | Adams | 1.45 |
| A4-107-12 (Hawkeye)[b] | 1.34 | Hawkeye | 1.36 |
| Chief | 1.48 | Chief | 1.44 |
| Earlyana | 1.37 | Earlyana | 1.31 |
| Lincoln | 1.40 | Lincoln | 1.34 |
| Richland | 1.37 | Richland | 1.37 |

[a] Parent strain from which Adams was selected.
[b] One of component strains of Hawkeye.

The values reported by Alderks were determined by microbiological methods by Kuiken and Lyman (*7*), while the present authors' values were obtained by chemical methods. It is evident that the two methods give comparable results and that there are some consistent varietal differences. Most of the varieties which the authors found to be superior in methionine content in the present study had not yet been developed at the time of the Alderks report.

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**MINOR ELEMENTS IN NUTRITION**

# Effect of Dietary Cobalt on Growing Chicks and Rats

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Experiments were conducted to determine the effect of dietary cobalt on the growth of chicks and weanling rats fed diets with and without adequate choline and vitamin $B_{12}$. When cobalt was added at the rate of 12 mg. per kg. to a diet deficient in vitamin $B_{12}$ and low in choline, growth of chicks increased significantly when the diet contained only 5% fat; when the diet contained 20% fat, a significant depression in growth resulted. When folacin was omitted from the diet for rats, cobalt addition failed to produce a growth response; when folacin was added, an average of 18% increase in gain and an increase in the vitamin $B_{12}$ content of the liver resulted from addition of 12 mg. of cobalt per kg. of vitamin $B_{12}$–deficient diet containing 0.1% choline chloride. Cobalt did not improve growth when added to diets that contained adequate amounts of choline, methionine, or vitamin $B_{12}$. The results indicate that dietary cobalt is nutritionally important for nonruminants fed diets inadequate in choline and vitamin $B_{12}$.

Vitamin $B_{12}$ contains cobalt (*7, 9*) and rumen bacteria use cobalt in the synthesis of this vitamin (*1*). These facts elucidate the role of cobalt in ruminant nutrition, which was known to be important long before the discovery of vitamin $B_{12}$.

Many attempts to show a role for dietary cobalt in nonruminants have been unsuccessful. However, Klosterman and others (*5*) demonstrated a 13 to 18% increase in daily gains of swine on addition of approximately 2 mg. of cobalt per kg. of ration. Jaffe (*4*) observed a beneficial effect of cobalt when added to an all-vegetable ration for breeding rats at the rate of 9 mg. per kg. This ration was as good as a ration containing vitamin $B_{12}$ for breeding and lactation, but was inferior to a ration containing vitamin $B_{12}$ for young, growing rats. Davis and his associates (*3*) demonstrated an increase of 8 to 21% in the growth of female chicks when 2 to 10 mg. of cobalt was added per kg. of vitamin $B_{12}$–deficient ration containing penicillin.

The object of this work was to determine the effect of dietary cobalt on the growth of chicks and weanling rats fed diets with and without adequate amounts of choline and vitamin $B_{12}$ and to show a relationship of folacin to the utilization of cobalt. A preliminary report has been presented (*2*).

### Experimental

**Method for Chicks.** One-day-old New Hampshire chicks of mixed sex were distributed among various modifications of the basal diet according to weight. From 10 to 14 chicks were used per treatment and they were kept on experiment for 4 weeks. They were