# EFFECTS OF NITROGEN FERTILIZATION ON LYSINE, THREONINE, AND METHIONINE OF HULLED AND HULL-LESS BARLEY CULTIVARS[1]

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**ABSTRACT**

Three hulled and two hull-less barley cultivars were grown in five environments (which included triplicate field plots from two locations) with zero, optimal, and twice the optimal amount of N-fertilizer. There was an overall linear relation between per cent crude protein of the grains and fertilizer level, but per cent protein showed a significant fertilizer-environment interaction. There was a significant fertilizer-cultivar interaction; increase in per cent protein was much larger in the low-protein hulled cultivars than in the high-protein hull-less barleys. The effect of fertilizer on lysine or the sum of lysine, threonine, and methionine (as per cent of protein) was not the same for all cultivars. No fertilizer-cultivar interaction was detected, however, for the amount of lysine or the sum of lysine, threonine, and methionine in the sample.

Any improvement through plant breeding is a long-range program and its contribution can be augmented by cultural practices. Application of water and nitrogen (N) can be manipulated to increase both yield and protein content of the grain. Late N application increases grain protein content, but this protein is of low biological value. Whitehouse (1) concluded that the best cultural solution to

[1]Cooperative investigations of the Barley and Malt Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Madison and the Wisconsin Agricultural Experiment Station, Madison.

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the protein problem would be to grow crops in a way that would produce maximum total yield and, therefore, the maximum yield of protein per unit-area of land. This practice would reduce the protein content of the grain, but the reduction may be offset (in part at least) by the higher biological value of the protein.

The effects of N-fertilization on the protein content and quality in wheat have been studied more extensively than in barley. The storage proteins (gliadin and glutenin) in wheat contribute to breadmaking quality, but in barley, an increase in storage proteins may impair performance in malting (2). The potential of several recent barley cultivars for the development of high-protein, high-lysine feed barleys with acceptable yield stimulated interest in the effects of N-fertilization on malting quality, protein content, and amino acid composition of barley cultivars.

Rhodes and Mathers (3) studied the amino acid composition of developing grains of the barley cultivars Hiproly and Maris Mink. Hiproly accumulated about 50% more protein per seed than Maris Mink, but maintained a balance of amino acids which was typical of a low-N barley. Hiproly and seven commercial cultivars were also grown with various levels of N-fertilization in a greenhouse. In the commercial cultivars the proportions of glutamic acid, proline, and phenylalanine in the protein were positively correlated with protein content, but the levels of the essential amino acids, particularly of lysine, showed a strong negative correlation with protein content. Hiproly, however, deviated significantly from this negative correlation of the essential amino acids with

**TABLE I**
**Means of Per Cent Protein and Some Amino Acids**

| Environment, Cultivar, or Fertilizer | Protein % | Lysine in Protein % | Sum Lysine, Threonine, Methionine in Protein % | Lysine in Sample % | Sum Lysine, Threonine, Methionine in Sample % |
|---|---|---|---|---|---|
| Environment | | | | | |
| South Dakota 1972 | 18.62 | 3.28 | 8.73 | 0.61 | 1.63 |
| Montana 1972 | 15.43 | 3.60 | 9.19 | 0.56 | 1.41 |
| Idaho 1972 | 15.35 | 3.55 | 9.21 | 0.55 | 1.41 |
| Idaho 1973 | 17.37 | 3.63 | 9.09 | 0.63 | 1.58 |
| Idaho 1974 | 16.09 | 3.77 | 9.25 | 0.61 | 1.49 |
| Cultivar | | | | | |
| Conquest | 14.25 | 3.67 | 9.51 | 0.52 | 1.35 |
| CI 4362 | 18.27 | 3.11 | 8.16 | 0.57 | 1.49 |
| Firlbecks III | 14.83 | 3.49 | 8.89 | 0.51 | 1.31 |
| Larker | 14.24 | 3.58 | 9.20 | 0.51 | 1.30 |
| Hiproly | 19.47 | 4.25 | 9.98 | 0.83 | 1.94 |
| Fertilizer | | | | | |
| None | 15.36 | 3.70 | 9.38 | 0.57 | 1.43 |
| Optimal | 16.23 | 3.62 | 9.10 | 0.59 | 1.48 |
| Double Optimal | 17.05 | 3.55 | 8.97 | 0.61 | 1.53 |

protein content. Subsequently, Rhodes and Jenkins (4) compared the amino acid composition of the barley cultivars Risø 1508, Hiproly, and Maris Mink grown in the greenhouse with three N treatments. Protein content of the grains averaged 12.1 to 14.6% and the lysine content of the protein averaged 4.88 to 6.30%, substantially above levels previously reported by Rhodes and Mathers (3). Also the amino acid recovery in the study of Rhodes and Jenkins (4) varied widely (72.5% for Hiproly, 83.5% for Maris Mink, and 95.9% for Risø 1508). The results of the greenhouse investigations indicated that in amino acid composition, Risø mutant 1508 differed fundamentally from the other cultivars. Risø 1508 had a high lysine content even when the protein content was increased by N-fertilization; the lysine content in the protein decreased from 7.1 to only 6.0% when grain protein greatly increased from 7.7 to 22.9%.

We previously reported results of studies on effects of N-fertilization on malting quality of two hull-less and three hulled barley cultivars (5). The present study was conducted to determine the effects of N-fertilization on protein content and amino acid composition of proteins in those five cultivars. Specifically, the following questions are hopefully answered: 1) How does N-fertilization affect protein content and does the fertilizer effect on protein content vary with cultivar? 2) How does N-fertilization affect protein quality as reflected in concentration of lysine (the first-limiting amino acid) or the sum of the three

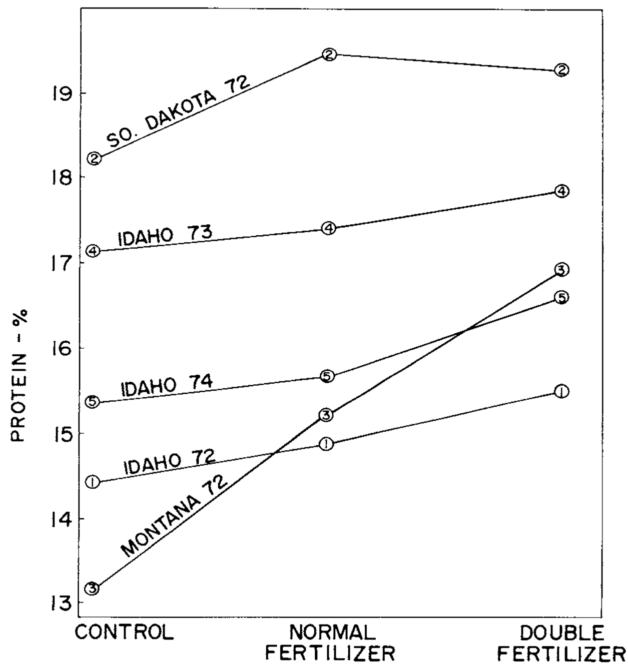


Fig. 1. Effects of interaction between fertilizer level and environment on barley protein content.

limiting amino acids—lysine, methionine, and threonine (SLTM)—in the protein, and does that fertilizer effect depend upon either cultivar or environment? 3) How does N-fertilization affect lysine and SLTM contents of the sample (rather than that of the protein)?

## MATERIALS AND METHODS

### Barley Cultivars

Five spring barley cultivars grown in each environment were Larker, Conquest, Firlbecks III, Hiproly, and CI 4362. Larker and Conquest are six-rowed, hulled malting barleys. Larker has a white aleurone and Conquest has a blue aleurone. Firlbecks III is a two-rowed, hulled, white-aleurone, malting barley. Hiproly and CI 4362 are two-rowed, hull-less, white-aleurone barleys that are not acceptable for malting. They are morphologically similar and often considered as an isogenic pair. Both are rich in protein, but Hiproly has shriveled seed, lower yield, and substantially more lysine in the protein than CI 4362 (6). Neither Hiproly nor CI 4362 is grown commercially, but Larker, Conquest, and Firlbecks III are important cultivars in the U.S. and Canada.

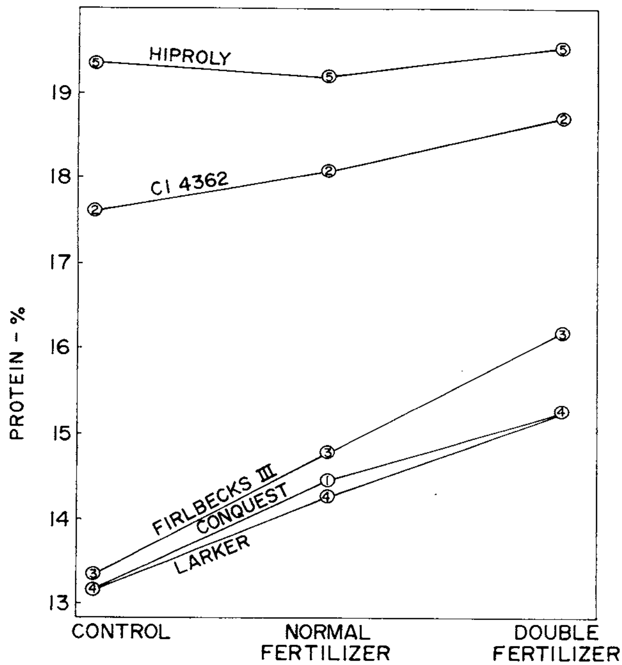


Fig. 2. Effects of interaction between fertilizer level and cultivar on barley protein content.

**Locations**

Barleys were harvested from five environments, which were designated as:

| *Environment* | *Location* | *Year* | *No. of Replicates* |
|---|---|---|---|
| 1 | Aberdeen, ID | 1972 | 1 |
| 2 | Brookings, SD | 1972 | 1 |
| 3 | Ft. Ellis, MT | 1972 | 3 |
| 4 | Aberdeen, ID | 1973 | 1 |
| 5 | Aberdeen, ID | 1974 | 3 |

**Fertilizer Treatment**

The N-fertilizer levels used were: none, the optimum amount recommended on the basis of soil analysis (25, 45, or 60 lb/acre, depending on location), and twice the optimum amount (5). The combination of environment (two having three replicates each) and fertilizer treatment yielded 27 samples for each cultivar and a total of 135 samples.

**Analytical Methods**

Crude protein (Kjeldahl) and amino acid analyses of acid hydrolyzates (on a Beckman 121 automatic analyzer) were determined in duplicate as described

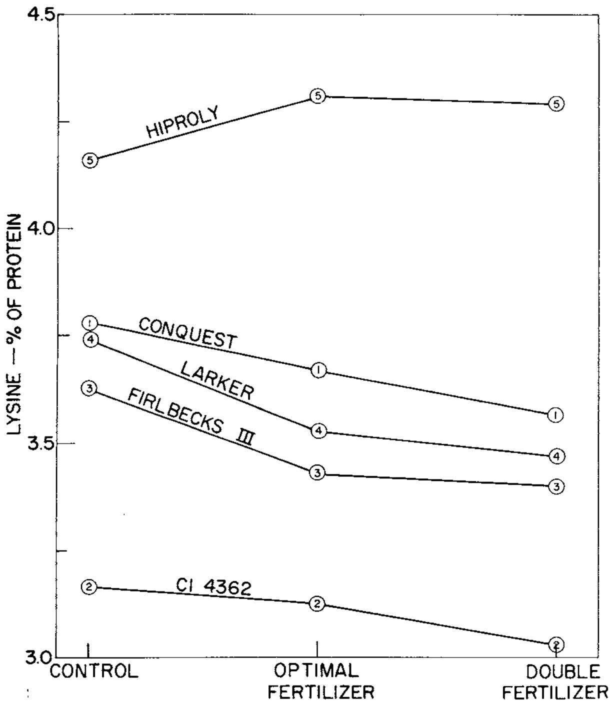


Fig. 3. Effects of interaction between fertilizer level and cultivar on per cent of lysine in the protein.

elsewhere (7). Values were obtained in grams amino acid per 100 g recovered, and are reported here as both per cent amino acid in protein and as per cent amino acid in the sample. Crude protein was estimated by the product of N × 6.25 and is reported on a moisture-free basis.

## RESULTS AND DISCUSSION

Data for per cent protein (PCP), per cent lysine in protein (LYS), the sum of lysine, threonine and methionine as a per cent of protein (SLTM), per cent lysine in the sample (SLYS), and the sum of lysine, threonine, and methionine as a per cent of the sample (SSLTM) were treated by analysis of variance. Some means of interest are presented in Table I.

Per cent protein had an interaction ($P < 0.01$) between fertilizer level and environment. The means, which illustrate the nature of this interaction, are presented graphically in Fig. 1. For the three Idaho environments, per cent protein increased gradually and consistently with fertilizer level; in Montana, the consistent increase was much sharper. In South Dakota, the recommended fertilizer level increased per cent protein, but doubling that level gave no additional increase. The benefit of fertilization was not the same for all

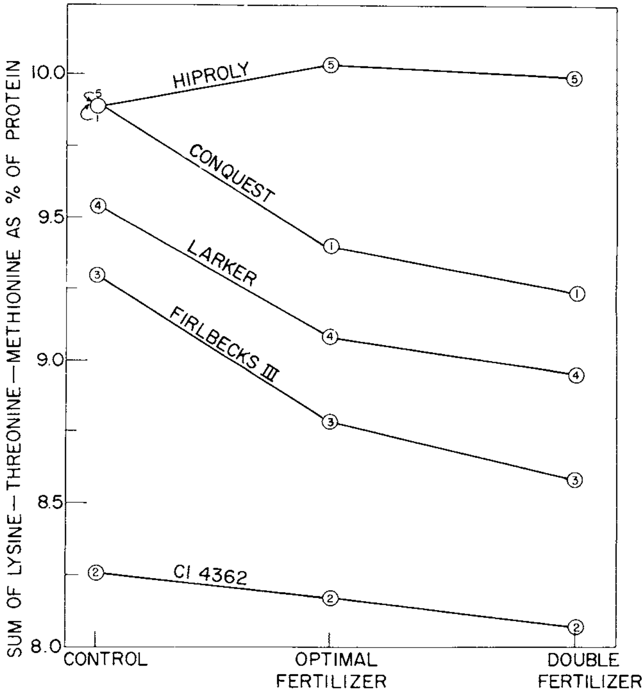


Fig. 4. Effects of interaction between fertilizer level and cultivar on per cent of lysine + threonine + methionine in the protein.

environments. The protein response to fertilizer application probably depends on the initial content and availability of soil N. This is borne out by our findings that the South Dakota barleys contained substantially more protein, especially when grown in unfertilized soil, than the Idaho and Montana samples; and that the protein response was greatest in the low-protein Montana barleys from 1972.

For per cent protein, there was an interaction ($P < 0.01$) with fertilizer level (Fig. 2). However, the presence of interactions between cultivars and fertilizer level and between environments and fertilizer level indicates that the fertilizer effect is not the same for all cultivars nor for all environments.

In considering the effect of N-fertilization on protein quality, it is necessary to again consider the dependence on cultivar. For both LYS and SLTM there was an interaction ($P < 0.05$) between fertilizer level and cultivar. This means that the fertilizer effect was not the same for all cultivars. Examination of Figs. 3 and 4 reveals a decrease in both LYS and SLTM as the fertilizer level is increased. This relation appears consistent for all cultivars except Hiproly, which actually increased in LYS and SLTM when fertilized as recommended but had no further increase when the fertilizer level was doubled. The failure of Hiproly to respond the same as the other four cultivars appears as the interaction between fertilizer level and cultivar. It should be recalled that both LYS and SLTM are reported as

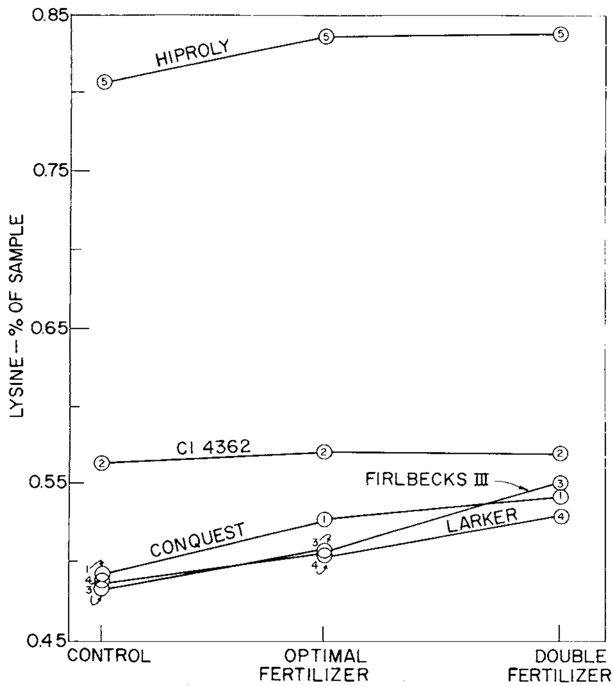


Fig. 5. Effects of interaction between fertilizer level and cultivar on per cent of lysine in the sample.

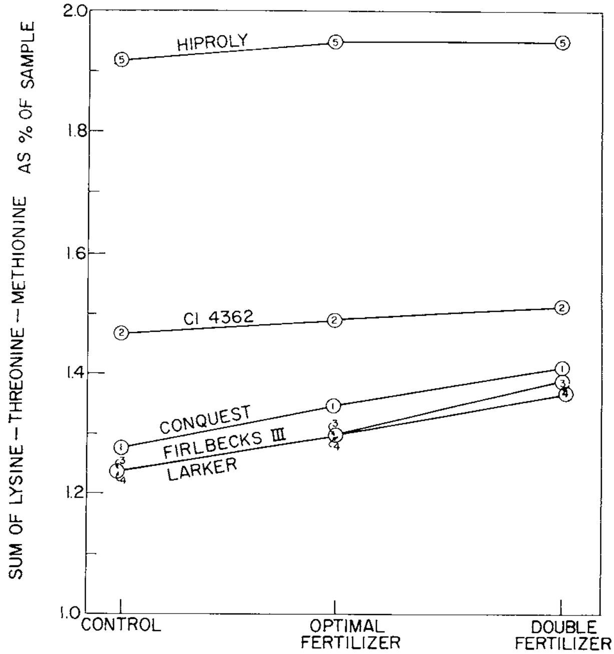


Fig. 6. Effects of interaction between fertilizer level and cultivar on per cent of lysine + threonine + methionine in the sample.

per cent amino acid in protein. This means that, with the exception of Hiproly cultivar, an increase in level of N-fertilization was accompanied by a decrease in the percentage of these amino acids in protein.

The amino acid values can also be expressed as a per cent of the total sample, rather than as per cent of protein. These values are denoted by SLYS and SSLTM. From Figs. 5 and 6, it is apparent that, although the percentage of these amino acids in protein may decrease with the application of N-fertilizer, SLYS and SSLTM either increase or remain constant. In fact, the effect of fertilization on these values is sufficiently consistent for all cultivars that no interaction can be detected between fertilizer level and cultivar. The overall effect of increasing fertilizer level on SLYS and SSLTM is a linear ($P < 0.01$) increase, with no detectable nonlinear component.

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