Effects of kind and rate of fertilizer and application time on lysinein winter wheat
by Mohammad Akmal

A thesis submitted in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in Crop and Soil Science
Montana State University

Abstract:
Wheat, a basic food for many people, is nutritionally lacking in lysine. This study was conducted to determine the effect on lysine level of wheat grain by additions of various fertilizers at several growth stages.

Winter wheat at 25 locations was topdressed with nitrogen, phosphorus or potassium singly or in combination at various growth stages in 1972, 1973, and 1974. At harvest, grain yield and protein were measured and the grain was bioassayed for lysine concentration.

The data were analyzed and means separated following common methods. All possible simple correlations for lysine, protein and grain yield means as influenced by various treatments were calculated for each location and cultivar. Correlations between soil temperature and lysine were also evaluated. Stepwise multiple regression methods were used to determine the relative importance of several factors in variation of lysine, protein and grain yield. Some treatment comparisons nested in location-years were evaluated separately.

Generally 80 lb and 20 lb N/acre produced higher levels of lysine than the control. The 30, 40, or 60 lb rate of nitrogen topdressing or foliar application did not generally change the lysine levels. Increasing rate of nitrogen application increased the protein percentages in the grain.

At one location, 30 lb dry N increased lysine more than the 60 lb liquid application. Inadequate information on the environmental factors that prevailed during the growing season precluded determining the exact reasons. Topdressing with phosphorus or potassium as late as FS 10.5-10.5.2 in one case increased lysine levels provided no nitrogen application was made at FS 6-7. Soil moisture and precipitation appeared to be responsible for over 90% of the variation in lysine levels at that location. Potassium, therefore, seemed to play an important role in improving water relations, while phosphorus probably was important in enhancing those chemical reactions leading to lysine synthesis. However, nitrogen applications at FS 6-7 might have limited the available soil moisture at FS 10.5-10.5.2 when P and K were applied.

Higher lysine levels were generally obtained from fertilizer applications at FS 3-6, 6-8 and 10.1-10.4 than at later stages. The same was also true of protein levels. In addition to the longer period of improved soil fertility, better environmental conditions such as soil temperature of 19°C, as in GR and RB during 1972, might enhance the lysine levels even at a growth stage as late as FS 11.1-11.2.

Location affected lysine levels of Winalta all three years. Similar response was obtained for Cheyenne in 1972 and 1973. During 1972, the interactions Location x Fertilizer and Location x Growth Stages were significant in Cheyenne and Winalta.

The environmental factors soil moisture, precipitation, soil temperature, soil NO3-N, and pan evaporation appeared to influence variation in the lysine levels.

In order to identify more specifically the effect of growth stage at time of fertilizer application on eventual lysine status of the grain, sampling procedures must be developed that will account for environmental effects.

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Approved:

Charles F. McGuire
Chairperson, Graduate Committee

Kurt C Feltner
Head, Major Department

Henry L Parsons
Graduate Dean

MONTANA STATE UNIVERSITY
Bozeman, Montana

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ABSTRACT

Wheat, a basic food for many people, is nutritionally lacking in lysine. This study was conducted to determine the effect on lysine level of wheat grain by additions of various fertilizers at several growth stages.

Winter wheat at 25 locations was topdressed with nitrogen, phosphorus or potassium singly or in combination at various growth stages in 1972, 1973, and 1974. At harvest, grain yield and protein were measured and the grain was bioassayed for lysine concentration.

The data were analyzed and means separated following common methods. All possible simple correlations for lysine, protein and grain yield means as influenced by various treatments were calculated for each location and cultivar. Correlations between soil temperature and lysine were also evaluated. Stepwise multiple regression methods were used to determine the relative importance of several factors in variation of lysine, protein and grain yield. Some treatment comparisons nested in location-years were evaluated separately.

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INTRODUCTION

The nutritional problem of the world's population is characterized mainly by a shortage of protein (Liebenow, C.R., 1966). The protein supplied to many people, especially in developing countries, comes from the cereals, including wheat. Since wheat is a basic food for many people, its nutritional adequacy is of paramount importance. As is the case with most cereal grains, wheat protein is nutritionally lacking in lysine, one of the essential amino acids.

Soil applications of fertilizer sometimes increases the protein content of the wheat grain (Sims and Jackson, 1974). The biological or nutritional value is not, however, proportional to this increase (Lawrence, et al., 1958). The biological value depends on the most limiting amino acid - lysine (Aykroyd and Doughty, 1970; FAO, 1965).

While literature on the effect of fertilizers on the grain protein percentage of cereals is voluminous, only a few efforts have been made to improve protein quality by fertilization at different growth stages of wheat. A major objective of this study was to determine the effect on lysine level of wheat grain by nitrogen, phosphorus, and potassium applied singly and in combinations to the soil at several growth stages in winter wheat.

LITERATURE REVIEW

It is now well established that the quantity of protein in a cereal is genetically controlled (Lonnquist, 1969). However, the fertility of the soil and the environments in which the cereal is grown are important factors influencing whether or not the plant achieves its full genetic potential for protein production. In addition to the influence of environment and fertility, other factors such as the time of fertilizer application as related to the growth stage of the plant can materially influence the protein content and its quality (Gunthardt and McGinnis, 1957). Nitrogen fertilization often increases the protein content of the wheat grain (Evald and Wenzel, 1967; Lawrence, et al., 1958; Stickler, et al., 1964). The amino acid composition of wheat grain protein varies when protein level changes as a result of N fertilization (Lawrence, et al., 1958).

Gunthardt and McGinnis (1957) compared the amino acid composition of four low (10.3%) and four high (16.5%) protein samples of Idaed wheat. The high protein samples were produced by a combination of nitrogen at 120 lb/acre and water application at the boot, flowering or milk stages of growth. The low protein samples received from 0 to 40 lb nitrogen at seeding time only. The high protein samples contained significantly less lysine expressed as lysine percent of crude protein than did the low protein samples. The lower lysine resulted when the nitrogen applications were as late as the boot, flowering or

milk stages of growth. The lysine content appeared to vary with the year or yield or both.

Sosulski, et al. (1963) found that reduced water supply, nitrogen fertilization, and higher air temperatures increased the protein content of Thatcher wheat grown under controlled environmental conditions in growth chambers. At higher temperatures, the soil moisture conditions exerted the greater effect on protein content while the largest responses to nitrogen fertilization were obtained at the medium moisture level. The relative distribution of nine amino acids in wheat was significantly correlated with changes in grain protein content. The correlation of lysine with protein was negative. The inverse relation between protein and lysine contents was also found by Hepburn and Bradley (1965), Chopra and Sidhu (1967), and Price (1950). According to the findings of Lawrence, et al. (1958), Mattern, et al. (1968), and Robinson and Sageman (1968) the relationship appears to be true only up to a certain limit. Lawrence, et al. (1958) reported that lysine decreased significantly with increasing protein contents up to 13.5% of protein.

Larsen and Nielsen (1966) determined nitrogen (micro-Kjeldahl) and amino acid content of Koga wheat grown in pots in a mixture of one part clay soil and two parts sand. They fertilized with a basal dressing and nitrogen as ammonium sulphate added at levels ranging from .025 gN/pot to 5.0 gN/pot. Increasing application of nitrogen to

wheat caused a decrease in the lysine from 3.6 to 2.6 in grams amino acid nitrogen per 100 g total nitrogen. Their results were in agreement with those of Campbell and Pickett (1968) and of Larsen (1968).

Srivastava, et al. (1971) studied the effect of nitrogen applied as urea at levels of 0, 20, 40, 60, and 80 kg/ha on the protein content and lysine in the protein of S_{227} wheat grown under rainfall conditions in India. In soil treatments the whole dose was applied before seeding. In foliar treatments, half was applied to the soil before seeding and the rest sprayed as a 3% urea solution. In each spray 10 kg/ha was applied; the first spray was at the post-tillering stage and then at 7-day intervals depending on treatments. The highest lysine content, 3.22% of the protein, was obtained from 40 kgN/ha applied to the soil. The highest protein percentage (10.43%) was obtained from soil plus foliar treatment at 80 kgN/ha from a sample of S_{227} wheat. The lowest protein (7.23%) was obtained from 0 kgN/ha. On comparison of these samples, lysine percent in the protein was found inversely correlated with total protein in the wheat.

Mamchenkov and Platonov (1971) found that as the protein content (Nx5.7) of Mironovskaya 808 winter wheat increased through the use of nitrogen, phosphorus, and potassium fertilizers combined with crop rotation, the percent lysine in the protein decreased.

Elonen, et al. (1972) studied the effects on spring wheat of irrigation and nitrogen fertilization, separately and together, in

five field trials in Finland in the years 1967 through 1970. The varieties were Svenno in trials 1 and 2, Norrona in trial 3 and Ruso in trials 4 and 5. Four of the trials were in successive years on the same farm in the neighborhood of Helsinki, the fifth one about 100 Km farther to the west. The soil was a silty clay with an average pH of 5.6, containing about 4% of organic carbon in the topsoil. The subsoil was heavy clay. The basal fertilizer dressing on the N_1 plots averaged 68 kgN, 53 kgP, and 81 kgK per hectare and was placed in rows at a depth of 8 cm. Additional nitrogen on the N_2 plots averaged 76 kg/ha. It was applied as a surface dressing for the shoots as calcium nitrate in trials 1 and 2 and as urea in trial 3. Nitrogen as urea or ammonium nitrate + limestone was drilled with the seed in trials 4 and 5.

Irrigation increased average yields by 1200 kg/ha. Grain protein percentage decreased with irrigation but this decrease could be offset by additional nitrogen fertilization. Irrigation significantly increased the proportion of lysine as percent of protein, but the decrease in total protein content gave rise to a net decrease in lysine content. Lysine declined with nitrogen fertilization. By increasing fertilizer nitrogen from 68 to 144 kg/ha and by irrigating twice in June, grain yields increased by 65% without any noticeable changes in flour protein level or lysine content.

Woodham, et al. (1972) experimented with 14 cultivars of barley under various conditions of N fertilization in different locations in the United Kingdom and examined their N content. Topdressing with nitrogeneous fertilizers during growth produced higher grain nitrogen content as well as increased overall yield of nitrogen/hectare. The locality where these cultivars were grown caused greater changes in the nitrogen content of the grain than did the N application.

Lysine content and total lysine per ton of grain were inversely proportional to crude protein content for barley containing between 8 and 11% crude protein (Woodham, 1972).

Khera, et al. (1972) examined the relationship between the phosphorus level in the soil and the protein quality of Kalyan Sona wheat grown in pots. To each 4.5 kg of soil in pots was added 89 ppm N, 37 ppm K and adequate amounts of trace elements. It was found that protein quality as measured by protein percent in the grain and lysine as a percent of grain protein was low when the available phosphorus levels were below 12.5 ppm. Below this level of phosphatic fertilizer kernel weight and protein quality suffered.

At Pahalvi University, Shiraz, Iran, Hojjati and Maleki (1972) studied the effect of potassium and nitrogen applied to the soil on the lysine, methionine, and total protein of Roushan bread wheat. The soil was calcareous silty loam high in potassium. Additional K was

added at the rate of 0, 25, 50, and 100 kgK/ha in the form of potassium sulphate. Four levels of nitrogen, 0, 50, 100, and 200 kgN/ha, were added as urea. All the potassium and half the nitrogen were applied in April 1968. The field was irrigated before seeding and during the growing season.

Increasing nitrogen fertilizer treatments consistently increased the protein content of the grain, from about 10% to 14%. An approximate increase of one percentage point of protein was recorded with each additional 50 kgN/ha. Lysine as a percent of the protein was negatively correlated with the protein content and with the N applied, but positively correlated with added K.

Smika and Greb (1973) collected data from several locations in Nebraska to identify major soil and climatic factors and their interrelations influencing protein content of hard red winter wheat grain. Protein content was negatively correlated with total precipitation more than 1.25 cm for a 15-day period 40 to 55 days before maturity ($r = -0.70^{**}$) or with available soil water at seeding to a depth of 1.2 or 1.8 m ($r = -0.79^{**}$). Grain protein levels were shown to be positively correlated by linear regression with total soil NO_3-N to depths of 1.2 or 1.8 m ($r = 0.82^{**}$) and by curvilinear regression with maximum air temperature for a 5-day period 15-20 days before maturity ($r = 0.74^{**}$). With all four factors combined, $r^2 = 0.96$. With

available soil water at seeding plus NO_3-N in the soil profile at seeding and protein content of wheat grain, $r^2 = 0.86$.

Labanauskas, et al. (1974) evaluated the effects of soil temperature and aeration treatments on the protein and free amino acids in the milk and mature stages of wheat grain. Soil temperature treatments of 5°, 15°, and 25°C were applied. Protein levels increased significantly from 28.69 micro mole/g dry grain in grain from the 5° treatment to 40.13 at the 25°C treatment. Lysine levels were not affected by soil temperature.

Fredrick (1975) cultivated 5 cultivars of common spring wheat under controlled conditions of five temperatures from 12 to 24°C. From the time of heading three day-length treatments were applied at each temperature; 10 hour, 24 hour and natural day length. Nitrogen content of the grain increased by 46 to 124% of the check with increasing temperatures. The relative amounts of lysine, valine and threonine decreased with increasing temperature and extension of the photoperiod. At the highest temperature this trend was hardly apparent. Protein fractionation showed that the decrease in lysine was correlated with an increase in the ratio of alkali-soluble to salt-soluble proteins.

METHODS AND MATERIALS

Plot Location and Design

The winter wheat fertility plots for this research were located throughout the winter wheat producing area of Montana. The research data were collected from 8, 10 and 7 locations, respectively, for the years 1972, 1973 and 1974. The field design for 1972 plots was randomized complete block and for 1973 and 1974, split plot. The individual plots were organized in randomized complete blocks with individual plots running across the rows. Individual plot sizes were uniform at a location but ranged from 200 ft^2 to 450 ft^2.

Criteria Adopted for Site Selection Location

A) A field should have:

1. received phorphorus fertilizer with the seed or have phosphorus applied and worked into the soil prior to seeding;
2. good stand of the crop;
3. limited weed problem, particularly cheatgrass, wild oats, and wild buckwheat;
4. recommended variety of winter wheat.
5. only the recommended cultivar if grown by the cooperator was included for this research;
6. only one cultivar was grown on each location.

B) Actual plot size should be:

1. Uniform in all visual aspects such as the soil type, and color, crop color and stage of growth;
2. No less than 20 ft from the side of the strip;
3. No less than 150 ft from the end of the strip.

C) Soil should be:

Montana bench mark soil or a representative soil of an extensive dryland grain acreage for the particular area.

Treatment Details

For simplification, results of grain yield, grain protein, or lysine at harvest as influenced by fertilizer applications at specific growth stages are referred to as "growth stage" results. The Feekes Scale (Appendix Table I) is used and abbreviated FS (Large, 1954). The treatments are detailed as under:

Year 1972. Four fertilizer levels 0, 20, 40, and 80 lb of N/acre were top-dressed at each plant growth stage. The growth stage at which N was applied at Amsterdam (AB), Broadus (DB), Brady (FB), Joliet (GR) and Coffee Creek (RB) were, respectively, FS 3-6, 6-8, 10.1-10.4, 10.5-10.5.4 and 11.1-11.2, at Cut Bank (JR) 3, 6, 10.1, 10.5, 10.5.4 and 11.1, at Bootlegger (BH) 5-6, 8, 10.5.2 and 11.1, at Broadview (LE) 4, 9, 10.4 and 11.1. Cultivars grown were Winalta at AB, DB, FB, RB and JR; Cheyenne at BH and LE, and Warrior at GR.

Year 1973. Five fertilizer rates, 0, 30 dry, 60 dry, 30 liquid, and 60 lb of liquid N/acre were applied at FS 3-6, 9-10.5.3 and 11.1-11.2 on all the locations. Winalta was grown at Amsterdam (AB), Brady (FB), Chinook (JJ), Cheyenne at Bootlegger (BH), Fort Benton (EL) and Broadview (LE); Winoka at Broadus (DB), Froid at Cut Bank (DV), Warrior at Joliet (GR) and Itana at Coffee Creek (RB). Winoka and Winalta cultivars have been treated alike for purposes of statistical analysis since Winoka is a stem rust resistant selection from Winalta. Aqueous solution of ammonium nitrate + urea was used for foliar applications.

Year 1974. Nitrogen, P_2O_5 and K_2O were applied singly or in combination at FS 5-7 and 10.1-10.5.2 at all locations. Winalta was grown at Broadus (DB), Brady (FB) and Chinook (JJ); Cheyenne at Bootlegger (BH) and Broadview (LE); Warrior at Joliet (GR); and Itana at Coffee Creek (RB).

Treatment details are attached as Appendix Tables II and III. The data used for regression analysis for each location are also detailed in Appendix Tables IV, V and VI, respectively, for the years 1974, 1973, and 1972.

Soil types and classification information on the locations are enclosed as Appendix Table VII. In order to detect Location x Treatment interactions in each year, the lysine data were pooled across locations when the same cultivars were grown.

Climatic Measurements

Open pan evaporation and rainfall were measured according to methods described by Sims and Jackson (1974). Pan evaporation was measured in this study to integrate humidity, wind velocity and air temperature variables.

Soil temperature was measured at a soil depth of 50 cm during the growing period of 1972 and 1974. Indoor-outdoor thermometers were employed in 1973 by placing the outdoor senser at a depth of 50 cm in a hole made with an oakfield tube and backfilling the hole with the soil cores. Soil depth of 50 cm was used since this depth is a standard for soil classification purposes. Diurnal effects on temperature are not great at this depth. Climatic measurements were recorded at 7- to 14-day intervals throughout the growing season (May 1 - August 15).

Soil Analysis

Soil water was determined by conventional gravimetric analysis using a forced draft oven at 60-65°C for 48 hours. Available soil water was estimated by the method described by Cole and Mathews (1954). Basically, this method uses soil water content at harvest as the limit of available water rather than soil water at 15-bar tension. Soil nitrate nitrogen (NO_3-N) was estimated by the phenoldisulfonic acid procedure as described by Bremner (1965).

Soil organic matter was measured by the colorimetric method published by Sims and Haby (1971).

Grain Harvest

The grain was harvested from a minimum of 7.44 m^2 near the center of each plot. A standard plot-sized sickle mower and thresher were utilized for cutting and threshing 1972 plots. A plot combine was employed on all 1973 and 1974 plots.

Bioassay

Various research workers as cited in the literature review used different methods for the amino acid analysis such as autoanalyzer, microbiological assay, Technicon autoanalyzer NC-1, Technicon autoanalyzer with technicon chromobeads, enzymatic decarboxylase method, paper chromatography, short-column chromatography, column chromatography, ion exchange chromatography and ninhydrin analysis. The method used for this research was modified microbiological assay developed by Waters (1976). This technique is inexpensive and efficient as compared to other techniques and differentiates lysine levels quantitatively. A trained technician using this bioassay can process at least 400-500 cereal samples weekly at an estimated cost of less than $0.50 per sample. This compares favorably with approximately 15-20 samples per week and $33.00 per sample for the amino acid analyzer.

A. Wheat sample preparation and hydrolysis. The wheat samples were prepared by grinding whole wheat grain in a UDY cyclone mill. Two grams of each ground grain sample were weighed out and placed in a 250 ml Erlenmeyer hydrolysis flask to which was added 25 ml of analytical grade 6N HCl. Each flask was fitted with a refluxing column approximately one meter in length. The samples in the flasks were hydrolyzed for 16 hours on a hot plate at 150°C ± 10°C. In order to get uniform heating and minimize variations in plate temperature, aluminum plates 1.27 cm thick were used on each hot plate. After hydrolysis, the samples were allowed to cool to room temperature.

B. Titration and volumization of hydrolyzate samples. Before titration, 20 ml of 6N NaOH was added to each sample hydrolyzate, cooled and then titrated with 2N NaOH and then it was brought to 100 ml with distilled water and a pH of 6.5 ± .03. Copenhagen Radiometer automatic titration was used. The samples were autoclaved cand stored for bioassay.

C. Assay medium preparation. Lysine assay medium (Difco) was used for the lysine determinations. The medium contained all the components required for growth of the assay organism except lysine and was prepared according to manufacturer's recommendations. To rehydrate the media, 10.5 grams were suspended in 100 ml of distilled water.

The media were prepared the same day the microbiological assay was performed.

D. Inoculum preparation

1. The bacterium used for the microbiological assay of lysine was Leuconostoc mesenteroides (ATCC 8042). Stock cultures were obtained from Difco Laboratories and stored at 0-3°C.

2. Preparation of brain-heart infusion medium. Bacto-brain heart infusion (Difco) was used as a liquid infusion medium for cultivation of the Leuconostoc mesenteroides. To rehydrate the medium 37 grams of the Bacto-brain heart infusion were dissolved in 1000 ml distilled water and sterilized in the autoclave for 15 minutes at 15 pounds pressure (121°C).

3. Bacteria were transferred from the stock culture to brain-heart infusion medium at room temperature. The brain-heart infusion cultures were then incubated for 72-96 hours at 37°C. After cooling to room temperature, a loopful of this culture was used to inoculate previously prepared depletion tubes. The depletion tubes are meant to metabolically exhaust available lysine by the bacteria. The tubes were incubated for 72-96 hours at 37°C.

4. The depletion tubes were prepared by adding 5 ml of distilled water to 5 ml of assay medium and autoclaved for 10 minutes at 121°C and 15 pounds pressure. The depletion tubes are free from

L-lysine but contain all the growth factors and amino acids necessary for the growth of Leuconostoc mesenteroides. Growth of bacteria in depletion tubes, therefore, depletes bacteria metabolically of any residual lysine. To ensure this, bacteria were depleted twice.

5. The depletion tubes were visually checked for the bacterial growth after the incubation period.

E. Hydrolyzate sample tube preparation. The following were combined in an incubation tube: 300 λ of hydrolyzate (by use of an Eppendorf pipet), 4.7 ml of distilled water, and 5 ml of lysine assay medium. The Eppendorf pipet tips were changed regularly and were rinsed thoroughly in distilled water between samples. Fresh tips were moistened in the sample being transferred to ensure uniform volume.

F. Standard tubes. Standard tubes were prepared which combined the following:

1. Tube No.	1	2	3	4	5	6	7	8	9
2. Lysine (μg)	0	10	20	30	40	50	60	70	80
3. Deionized water (ml)	5	4.9	4.8	4.7	4.6	4.5	4.4	4.3	4.2
4. Lysine assay medium (ml)	5	5	5	5	5	5	5	5	5
Total volume (ml)	10	10	10	10	10	10	10	10	10

Bacterial growth resulted in lactic acid production. The amount of lactic acid and hence the change in pH are proportional to the concentration of lysine. A standard curve can thus be developed relating pH and the concentration of L-lysine.

G. Autoclaving and pH determination. After all the samples and standard tubes were filled, they were autoclaved for 10 minutes at 15 pounds pressure, cooled and incubated. After 72 hours incubation at 37°C, the pH was determined on each tube by a combination electrode coupled to a pH meter (Beckman Expandomatic SS-2).

Computation Formula

To relate the L-lysine standard curve to % lysine, the equations used were as follows:

$$\mu g \text{ lysine} = a + b_1(pH) + b_2 \log(pH).$$

$$\% \text{ lysine in grain} = \frac{\mu g \times .000001 \times \frac{100}{\text{aliquot size (0.3) ml}} \times 100}{\text{wt. of sample in grams}}$$

$$\% \text{ lysine in protein} = \frac{\% \text{ lysine in grain}}{\% \text{ protein}} \times 100$$

Statistical Procedures

The data were analyzed and the F values were tested at approximately .05 and .01 levels of significance. Duncan's Multiple Range

test was used to detect differences among means wherever significant treatment effects were found.

All possible simple correlations for lysine, protein and grain yield means as influenced by fertility rates and growth stage applications were calculated for each location and each cultivar. These calculations were done on 1972 and 1973 data. Correlations between soil temperature and lysine were also estimated.

Some treatment comparisons within each location were made on 1973 and 1974 lysine data. The same comparisons were also completed for each cultivar; Cheyenne and Winalta grown on different locations.

Stepwise multiple regression methods using the MSU computer facilities were used to determine the relative importance of several factors in variation of percent lysine, percent protein and grain yield. The program was included in the Biomedical Computer program for the health sciences computing facility, Department of Biomathematics School of Medicine, University of Los Angeles available through the MSU Statistics Laboratory.

RESULTS AND DISCUSSION

The "Feekes Scale" (FS) always implies topdressing of fertilizer at that development stage of wheat growth and estimation of lysine, grain protein or grain yield after the harvest. There is only one exception. During 1974 at FS 10.5.4-11.2 soil temperature at 50 cm was recorded and has been included in Tables 8 and 9 and Appendix Table III. "Soil temperature" would always mean as recorded at 50 cm depth. "Lysine" (Lys) and "Protein" (PN) would mean, respectively, lysine as percent of protein and grain protein as percent by weight of protein in the kernel. The word yield (Yd) used in tables means grain yield. Treatments have been referred to by their numbers which are detailed in Appendix Tables II and III for the years 1974, 1973 and 1972.

Year 1974

Lysine data from locations where the same cultivar was grown were pooled and analyzed (Table 1). The mean squares for FB, DB and JJ locations where Winalta was grown produced a highly significant F test. Effects of location on the level of lysine in Winalta were thus of importance. Treatment x Location interaction was non-significant. The results are in agreement with Woodham, et al. (1972) who found that growth locality caused greater changes in the nitrogen content of the grain than did the N application. DB produced the highest lysine percent of 3.05, FB 1.92 and JJ 1.70. The differences were statistically highly significant. The higher lysine was associated with

higher soil moisture status at FS 5-7, rainfall and higher soil temperature at heading, flowering, and ripening stage.

Table 1. F values of lysine (% of PN) for cultivars grown at different locations during 1974

Source of variation	Cheyenne LE + BH	Degrees of freedom Denom.	Numer.	Winalta FB + DB + JJ	Degrees of freedom Denom.	Numer.
Blocks/ locations	1.83 NS	35	4	1.25 NS	54	6
Fertilizers	0.40 NS	35	9	0.62 NS	54	9
Locations	0.63 NS	35	1	183.12**	54	2
Fert x Loc	0.15 NS	35	9	0.87 NS	54	18

** Statistically significant at $P \leq .01$
NS Non-significant

The data were further analyzed for the following treatments within a location and pooled over locations for each cultivar, Cheyenne and Winalta.

Table 2. Fertilizer treatment comparisons

1 vs 2	5 vs 8
1 vs 5	
2 vs 3	2 + 5 vs 3 + 8
2 vs 4	
2 vs 6	
2 vs 9	

The results were, however, non-significant except for GR data where Treatment 9 significantly increased the percent lysine over Treatment 2. Although higher protein was obtained in Treatment 2 and higher yields in Treatment 9, they are statistically non-significant. No differences in test-weight were detected. Based on the regression analysis results it appears that P in conjunction with precipitation after heading did increase lysine. A possible explanation is that the treatment did, in some way, increase Mo availability (Stout, et al., 1951) which is essential for nitrate reductase thereby increasing the reaction rate essential for lysine metabolism.

The analysis of variance of lysine data for each 1974 location indicated significant differences for the RB means, but variation among treatment means for the rest of the sites were non-significant (Table 4).

In the case of RB, the highest percent lysine of protein was produced by Treatment 7. It was non-significant from Treatments 3, 4, 5, 8, and 10 but was statistically different from Treatments 1, 2, 6, and 9 (Table 4). Treatments 8 and 10 were non-significant from 1, 2, 3, 4, 5, 7, and 9 but outyielded Treatments 6 and 9 in lysine as a percent of protein.

These results indicate that top dressing with phosphorus at FS 10.5 (Treatment 10) enhanced lysine compared to Treatment 9 when nitrogen and phosphorus were respectively applied at FS 6-7 and 10.5.

Table 3. Mean values of lysine (% of PN) percent grain protein and yield and their significance for Joliet, 1974

Treatment No.	Fertilizer treatment Tillering	Heading	Lys (NS)	PN** (.01)	Yield**(kg/h) (.01)
1	0-0-0	0-0-0	2.19	9.8 ab	1113 a
2	40-0-0	0-0-0	1.88	11.1 cde	1700 b
3	0-0-0	40-0-0	1.97	11.97 ef	1156 a
4	40-0-0	40-0-0	2.05	12.57 f	1783 b
5	40-0-40	0-0-0	2.03	10.3 abc	1963 b
6	40-0-0	0-0-40	2.17	10.53 abcd	1937 b
7	0-0-0	0-0-40	2.15	9.83 ab	1119 a
8	0-0-0	40-0-40	2.06	11.43 de	1202 a
9	40-0-0	0-40-0	2.31	10.77 bcd	1808 b
10	0-0-0	0-40-0	2.03	9.60 a	1091 a

** Means followed by different letters within rows differ statistically according to Duncan's Multiple Range test at $P \leq .01$
* Tested against 18 d.f. as denominator and 9 d.f. as numerator
NS Statistically nonsignificant

Similar results were obtained with potassium in Treatment 7 versus 6. Treatment 7 also outyielded the control. Potassium and nitrogen top-dressed at FS 10.5 (Treatment 8) brought about significant increase in lysine over Treatment 6, when nitrogen was applied at FS 6-7 and potassium at FS 10.5. The results are supported by the findings of Khera, et al. (1972) and Hojjati and Maleki (1972). The former found that lysine as percent of protein was low when the available phosphorus levels were below 12.5 ppm. The latter found that lysine as percent of protein was positively correlated with added potassium.

Table 4. Fertilizer "treatment wise" mean values of lysine (% of PN) and their significance for 1974 locations or 3 cultivars

	Fertilizer treatments									
At FS 5-7	0-0-0	40-0-0	0-0-0	40-0-0	40-0-40	40-0-0	0-0-0	0-0-0	40-0-0	0-0-0
At FS 10.1-10.5.2	0-0-0	0-0-0	40-0-0	40-0-0	0-0-0	0-0-40	0-0-40	40-0-40	0-40-0	0-40-0
	1	2	3	4	5	6	7	8	9	10
Locations										
1. Bootlegger (BH)	2.11	1.73	1.80	1.69	1.87	2.20	1.92	2.13	1.90	1.93
2. Brady (FB)	1.89	1.83	1.9	1.82	1.88	2.01	2.21	1.95	1.81	1.94
3. Broadus (DB)	3.09	2.95	2.87	3.1	3.37	2.79	2.99	2.96	2.95	3.46
4. Broadview (LE)	2.08	2.00	1.86	1.90	2.11	2.15	2.07	1.92	2.05	2.02
5. Chinook (JJ)	1.87	1.76	1.71	1.67	1.61	1.64	1.76	1.77	1.61	1.59
6. Coffee Creek* (RB)	1.93 ab	1.95 ab	2.11 abc	2.03 abc	2.07 abc	1.84 a	2.26 c	2.2 bc	1.87 a	2.21 bc
7. Joliet (GR)	2.19	1.88	1.97	2.05	2.03	2.17	2.15	2.06	2.31	2.03

* Means within a row followed by different letters differ statistically according to Duncan's Multiple Range test at $P \leq .05$.

Non-significant differences in lysine were obtained between the control and where nitrogen was applied at FS 6-7 (Treatments 2,5,6, and 9) singly or in combination with phosphorus or potassium at FS 6-7 or FS 10.5. When nitrogen applied at FS 6-7 was also accompanied with nitrogen at FS 10.5 or where nitrogen was applied at FS 10.5 only, lysine levels were not affected (Treatments 3,4).

On the other hand, highly significant increases in protein were obtained by Treatments 2, 4, 5, 6, and 9 compared to the control. Where only nitrogen, phosphorus, or potassium was top dressed at FS 10.5, Treatments 3, 10, and 7, grain protein remained statistically unchanged (Table 5). Grain yields were not affected by the fertilizer treatment. It appears that nitrogen application at FS 6-7 increased the levels of lysine poor protein, thereby decreasing lysine as a percent of protein in such cases.

Protein and grain yield from DB and JJ were positively correlated (Table 6). Correlations of average values of lysine, protein and grain yield were computed with soil temperatures at 50 cm as existed at FS 5-7, 10.1-10.5.2 and 10.5.4-11.2 (Table 7). Non-significant correlation coefficients were obtained. Soil temperature as existed at FS 5-7 and 10.5.4-11.2, however, produced positive correlation trends with lysine and yield and negative with protein.

Average lysine over locations was positively correlated with soil moisture at FS 5-7 (Table 8a). In the case of Winalta, soil

Table 5. Fertilizer "treatment wise" values of lysine (% of PN), percent grain protein and grain yield for Coffee Creek and their significance

Treatment No.	Fertilizer treatments		Lys*	PN**
	Tillering	Heading		
1	0-0-0	0-0-0	1.93 ab	10.97 ab
2	40-0-0	0-0-0	1.95 ab	12.47 cde
3	0-0-0	40-0-0	2.11 abc	11.67 abcd
4	40-0-0	40-0-0	2.03 abc	13.23 e
5	40-0-40	0-0-0	2.07 abc	12.88 e
6	40-0-0	0-0-40	1.84 a	13.07 e
7	0-0-0	0-0-40	2.26 c	10.73 a
8	0-0-0	40-0-40	2.2 bc	12.03 bcde
9	40-0-0	0-40-0	1.87 a	12.70 de
10	0-0-0	0-40-0	2.21 bc	10.93 ab

Means followed by different letters within rows differ statistically according to Duncan's Multiple Range test
*, ** Significant at $P \leq .05$ and $P \leq .01$, respectively

temperature at FS 5-7 had a negative influence on average lysine level in locations. The grain yield in locations was positively correlated with test weight. NO_3-N level at FS 5-7 was, however, negatively correlated with test weight in Winalta as well as over locations. Soil temperature at FS 10.1-10.5.2 was negatively correlated with total precipitation. Soil temperature at FS 10.5.4-11.2 and soil temperature at FS 10.1-10.5.2 tended to be negatively associated. In Winalta the soil temperature at FS 10.5.4-11.2 had a positive correlation with precipitation after heading.

Table 6. Correlations among lysine (% of PN), percent grain protein and grain yield (b/A) for 1974 locations

Locations	Lys vs PN	Lys vs Yd	PN vs Yd
1. Bootlegger (BH)	-0.41 NS	-0.08 NS	-0.43 NS
2. Brady (FB)	-0.51 NS	0.16 NS	-0.54 NS
3. Broadus (DB)	-0.36 NS	-0.05 NS	0.69*
4. Broadview (LE)	-0.60 NS	0.32 NS	0.49 NS
5. Chinook (JJ)	-0.15 NS	-0.25 NS	0.66*
6. Coffee Creek (RB)	-0.59 NS	0.13 NS	0.23 NS
7. Joliet (GR)	-0.35 NS	0.08 NS	0.34 NS

* Statistically significant at $P \leq .05$
NS Statisticially non-signficant
Tested against 8 d.f. as denominator

Stepwise regression analysis of lysine, protein and grain yield and environmental factors was computed (Table 8b). Soil moisture accounts for 60.9% of the variability in lysine. Precipitation after heading plus soil moisture at FS 5-7 accounted for 90.1% of lysine variation. Soil temperatures at FS 10.1-10.5.2, and 10.5.4 to 11.2 and total precipitation accounted for the other 9.8% of lysine variation. Regarding grain protein, soil moisture at FS 5-7 accounted for 47.7% of the variability. This together with soil temperature at FS 10.1-10.5.2 contributed 90.3% variation. Eight point four percent variation was on account of soil temperatures at FS 5-7 and 10.5.4-11.2.

Table 7. Correlation of lysine (% of PN), percent grain protein and grain yield (b/acre) with soil temperature at three different Feekes Scales over locations during 1974

Locations	Average values			Soil temp °C at FS		
	Lys	PN	grain yd.	5-7	10.1-10.5.2	10.5.4-11.2
1. Bootlegger (BH)	1.93	15.73	27.11	6.67	17.78	17.78
2. Brady (FB)	1.92	14.97	20.42	5.56	15.56	17.22
3. Broadus (DB)	3.05	15.35	36.15	10.0	12.22	20.0
4. Broadview (LE)	2.02	11.31	32.39	10.0	12.22	20.0
5. Chinook (JJ)	1.70	11.53	43.26	7.22	17.78	18.89
6. Coffee Creek (RB)	2.05	12.05	25.2	10.0	16.5	17.78
7. Joliet (GR)	2.08	10.79	27.51	9.5	10.56	20.0
			Lysine	+ .52 NS	-0.53 NS	+0.47 NS
			Protein	-0.47 NS	+0.30 NS	-0.44 NS
			grain yield	+0.23 NS	+0.03 NS	+0.56 NS

NS Non-significant
Tested against 5 d.f. denominator

Table 8a. Simple correlations among agronomic traits and environmental factors for 4 cultivars of winter wheat over 1974 locations and for one cultivar Winalta grown on DB, FB, and JJ

Over Locations	Avg. PN	Avg. test wt.	Soil moisture at FS 5-7	Total ppt.	NO_3-N at FS 5-7	Soil temp at FS 5-7	Soil temp at FS 10.1-10.5.2	Soil temp at FS 10.5.4-11.2
Location	-.88 *	.36	-.52	.65	-.43	.09	-.37	.42
Avg. Lys	.40	-.24	.78 *	.52	.31	-.51	-.53	.47
Avg. PN	1.00	-.45	.69	-.40	.66	-.11	.30	-.44
Avg. Yd.		.85 *	-.35	-.13	-.74	-.05	.03	.56
Avg. test wt.		1.00	-.63	-.27	-.82 *	.22	.23	.27
Total precipitation				1.00	.04	-.18	-.84 *	.62
2 over Winalta								
Avg. Lys	.69	-.47	.80	.99 *	.51	-.99 *	-.97	.70
Avg. test wt.		1.00	-.91	-.51	-.99 *	.53	.68	.30
ppt. after heading					-.31	-.61	-.45	.99 *

Table 8b. Multiple correlation coefficients for association between lysine or protein or yield and environmental and agronomic factors for 1974 locations and Winalta grown on DB, FB, and JJ

Locations

Lys		PN		Yd	
Variable	R_2	Variable	R_2	Variable	R_2
1. Soil moisture at FS 5-7	60.9	Soil moisture FS 5-7	47.7	Average test wt.	71.7
2. Precipitation after heading	90.1	Soil temp at FS 10.1-10.5.2	90.3	Precipitation after heading	86.9
3. Soil temp at FS 10.1-10.5.2	99.2	Soil temp at FS 5-7	95.8	Nitrate-N at FS 5-7	96.6
4. Soil temp at FS 10.5.4-11.2	99.9	Soil temp at FS 10.5.4-11.2	98.6	Pan evaporation	98.9
5. Total Precipitation	100.0	Average yield	100.0	Total Precipitation	99.5
Winalta					
1. Total precip.	99.7	Soil moisture at FS 5-7	97.7	Organic matter NO_3-N	91.5

Grain yield varied 71.7% due to average test weight. Precipitation after heading, NO_3-N status at FS 5-7, pan evaporation and total precipitation contributed 15.2%, 9.7%, 2.3%, and 0.6% variation, respectively (Table 8b).

In Winalta (Table 8b) 99.7% variation in lysine was due to total precipitation, 97.7% of the variation in grain protein due to soil moisture at FS 5-7 and 91.5% of the variation in grain yield due to organic matter NO_3-N.

It is concluded that Winalta produced significant F test for the lysine level which appeared to be associated with soil moisture at FS 5-7, rainfall and soil temperature at heading, flowering and ripening stages. It is also concluded that nitrogen, phosphorus and potassium applied singly or in combination at FS 5-7 and 10.1-10.5.2 do not change the lysine levels. Only in case of RB, application of potassium at FS 10.5 increased lysine levels over the control without affecting any change in the protein. As soil moisture and precipitation contributed over 90% (based on R^2) of the variation in lysine levels, one logical reason appears that the potassium contribution may be important in stomatal adjustment of improving water relations in addition to enhancing certain enzymatic actions for nitrogen metabolism. These results were partly in agreement with Hojjati and Maleki (1972).

Topdressing with phosphorus or potassium or potassium + nitrogen at FS 10.5-10.5.2 increased lysine levels over phosphorus or potassium

topdressed at FS 10.5-10.5.2 but accompanied by nitrogen at FS 6-7. Phosphorus appeared to increase the number of chemical reactions essential for lysine metabolism in the plant which might not have been possible in later case due to soil moisture limitations. Protein levels increased where nitrogen was applied at tillering over the control.

Protein and grain yield were positively correlated in DB and JJ which shows the effect of fertilizers in increasing the protein percentage. Average lysine for all the locations was positively correlated with soil moisture at FS 5-7 and average grain yield with average test weight. In case of Winalta average lysine was positively correlated with total precipitation and negatively with soil temperature at FS 10.1-10.5.2.

Soil moisture at FS 5-7, precipitation after heading, soil temperature at FS 10.1-10.5.2 and 10.5.4-11.2 and total precipitation were the factors which contributed variation in lysine levels. In Winalta total precipitation contributed the major variation. In case of protein, the factors which contributed variation in decreasing effect were soil moisture at FS 5-7, soil temperature at FS 10.1-10.5.2, 5-7, and 10.5.4-11.2 and average yield. Soil moisture at FS 5-7 was the only source affecting protein level in Winalta. Important factors affecting grain yield were average test weight, precipitation after heading, NO_3-N at FS 5-7, evaporation and total precipitation. Organic matter

NO_3-N was the only major factor contributing variation in grain yield of Winalta.