(8) Moulton, F. R. (editor), *Pub. Am. Assoc. Advance. Sci.*, 1946.
(9) Nelson, E. A., M.S. thesis, Utah State University, Logan, Utah, 1953.
(10) Peirce, A. W., *Australia Council Sci. Ind. Research, Bull.* **121** (1938).
(11) Peirce, A. W., *Nutrition Abstr. & Revs.* **9,** 253 (1939).
(12) Phillips, P. H., Greenwood, D. A., Hobbs, C. J., Huffman, C. F., *Natl. Research Council Publ.* **381** (1955).
(13) Roholm, K. A. F., "Fluorine Intoxication," H. K. Lewis, London, 1937.
(14) Shrewsbury, C. L., Hatfield, J. D., Doyle, L. P., Andrews, F. N., *Ind. Agr. Expt. Sta., Bull.* **499** (1944).
(15) Slagsvold, L., *Norsk Vet.* **46,** 1 (1934).
(16) Snedecor, G. W., "Statistical Methods," 4th ed., Iowa State College Press, Ames, Iowa, 1946.
(17) Velu, H., *Acad. vet. France bull.* **4** (N.W.), 392 (1931).

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AMINO ACID SUPPLEMENTS

Lysine Supplementation of a Breakfast Cereal and Milk Combination

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Cereal proteins in such sources as wheat flour and bread have been improved considerably in protein quality with supplements of synthetic lysine. This investigation was made to determine whether a positive growth response would be obtained from the addition of 0.5% DL-lysine hydrochloride to a wheat flakes breakfast cereal, with and without the milk used in a customary serving. A 4-week rat feeding test indicated that the lysine supplement gave a growth response when wheat flakes were fed without milk—no significant increases in growth or protein efficiency were noted with milk.

WHEAT PROTEIN is deficient in lysine, which would be the limiting amino acid if wheat were the only source of protein in the diet. Rosenberg, Rohdenburg, and Baldini (*5*) found that the addition of 0.2% L-lysine to a diet containing 90% dried bread supplemented with salts, vitamins, and fat gave a significant increase in growth to weanling rats. Albanese *et al.* (*1*) showed that body weights and nitrogen balances of infants consuming milk were improved by a lysine supplement.

Many prepared breakfast cereals are composed of wheat, and whether supplementation of these foods with lysine would improve their nutritive value when fed with milk and sugar, as normally eaten, is of interest. This may be of special importance, as Baldwin, Lowry, and Thiessen (*3*) showed that much of the lysine content of proteins may be rendered unavailable to the body as a result of heat treatment of food, such as occurs in the manufacture of toasted cereals.

Materials and Methods

A mixture was made to give the ratio of 4 ounces of fresh whole milk and 8 grams of sugar (1 teaspoon) to 1 ounce of wheat flakes. Whole dry milk solids (Parlac) was used in place of fresh whole milk.

The wheat flakes cereal was pulverized in a Fitzpatrick mill before it was blended with the other dietary constituents. A similar mixture was made to which DL-lysine hydrochloride was added to give a concentration of 0.5% based on the cereal. These two mixtures, as well as the wheat flakes and the lysine-supplemented wheat flakes, were incorporated into diets for a 28-day rat feeding test.

A fifth diet was made with casein as the reference protein. Each kilogram of diet was supplemented with the following vitamins expressed as milligrams per kilogram of diet: choline chloride, 1000; *dl*-α-tocopherol diacetate, 100; pyridoxine hydrochloride, 20; calcium pantothenate, 50; riboflavin, 50; thiamine hydrochloride, 12; nicotinic acid, 90; biotin, 0.3; folic acid, 0.9; inositol, 200; *p*-aminobenzoic acid, 200; and 2-methyl-1,4-naphthoquinone diacetate, 2. This mix was diluted to 2 grams with cornstarch before blending into each diet. Corn oil was used to adjust the fat content of the wheat flakes diets to 6% and to give the casein diet a fat content equal to that of the cereal-milk diets (8%). Three drops of cod liver oil were given twice weekly to each rat to supply vitamins A and D. Salt mix, USPXIV, was added at a 4% level to all diets.

Each diet was fed to six weanling Wistar strain male albino rats obtained from Carworth Farms. The average weight of each group of animals was 59 to 60 grams at the beginning of the test. Food intakes were adjusted each day to equalize the protein intakes of the animals eating the two wheat flakes diets and those consuming the other three rations. Distilled water was given *ad libitum*. Each animal was housed in an individual raised-bottom cage in an air-conditioned room kept at 75° ± 2° F.

The protein content of the wheat flakes diet was 10% and that of the wheat flakes, milk, and sugar diet 12.5%. The casein diet contained 14% protein.

Table I. Four Weeks' Rat Growth from Lysine Supplementation of Wheat Flakes, with and without Milk and Sugar

Diet	Av. Body Weight Change, G.	Food[a] Efficiency	Protein[b] Efficiency
Wheat flakes	− 9 ± 0.9	Negative	Negative
Wheat flakes + 0.5% DL-lysine	− 1 ± 0.9	Negative	Negative
Wheat flakes, milk, and sugar	+87 ± 5.1	0.29	2.30 ± 0.07
Wheat flakes + 0.5% DL-lysine HCl, milk, and sugar	+83 ± 3.4	0.27	2.15 ± 0.06
Casein	+93 ± 3.5	0.33	2.37 ± 0.05

[a] Food efficiency = gram gain in body weight per gram food consumed.
[b] Protein efficiency = gram gain in body weight per gram protein consumed.

Results and Discussion

As shown in Table I, the supplement of lysine lessened the body weight loss obtained when the wheat flakes were consumed without the milk and sugar. However, the animals were still unable to grow, even with the lysine supplement.

When the milk and sugar were fed along with the wheat flakes, *t* tests showed that no significant differences (p = >0.05) in growth or protein efficiency resulted from the added lysine. The casein diet gave a significantly better (p = <0.05) protein efficiency than the lysine-supplemented milk-sugar-wheat flakes diet. No other significant differences were shown in protein efficiency or growth when the cereal and milk diets were compared to the casein diet (p = >0.05).

Under the conditions of this experiment, no positive response in growth or efficiency in protein utilization was shown when the milk-sugar-wheat flakes diet was supplemented with 0.5% DL-lysine hydrochloride. The lysine supplement would be equivalent to 0.2% L-lysine. The DL form was used because L-lysine was not available commercially at the time the experiment was performed. The lack of growth response from the lysine may be explained by the relatively high level of this amino acid in the milk alleviating the lysine deficiency of the wheat flakes protein. Amino acids other than lysine would probably be necessary to give a positive growth response.

Table II gives the amino acid contents of the basal wheat flakes and the milk-sugar-wheat flakes mixture. These data were calculated from microbiological analyses of wheat flakes and the basic mixture used in a different feeding test at this laboratory. The methionine, histidine, lysine, and phenylalanine contents of the basic mixture were below the levels which may be required for the rat (Table II). Therefore, low levels of various essential amino acids may have limited the effectiveness of the lysine. Growth patterns obtained from supplementation of cereal proteins with combinations of amino acids calculated from microbiological amino acid analyses in unpublished work at this laboratory show that such analyses as a basis for calculating amino acid deficiencies in various food proteins are not completely reliable.

Gessert and Phillips (*4*) showed that a lysine supplement to a low protein ration containing skim milk and cereal grains retarded growth in young dogs. In the range of protein levels of 10 to 15%, the amino acid balance is very critical. The positive effect from the supplementation of wheat flakes alone with lysine might be expected, as lysine is the limiting amino acid of wheat protein, and it is the amino acid which is considered to be most adversely affected by cereal processing.

Although the cereal component of the milk-sugar-wheat flakes mixture supplied about 40% of the protein of the diet, the body weight gain and protein efficiency were similar to those obtained from the casein diet.

Table II. Amino Acid Composition of Wheat Flakes, with and without Milk and Sugar

	Calculated Amino Acid Composition of Basal Test Diets		
Amino Acid	*In wheat flakes diet, %*[a]	*In wheat flakes, milk, and sugar diet, %*[a]	*Requirement for Growing Rat as of Diet*[b]
Arginine	0.26	0.53	0.2
Histidine	0.15	0.29	0.4
Isoleucine	0.42	0.50	0.5
Leucine	1.00	1.34	0.8
Lysine	0.13	0.76	1.0
Methionine	0.17	0.18	0.4
Phenylalanine	0.34	0.59	0.7
Threonine	0.30	0.51	0.5
Tryptophan	0.09	0.17	0.2
Valine	0.66	0.67	0.7

[a] Calculated from microbiological amino acid analyses of wheat flakes and a wheat flakes, milk, and sugar mixture without added lysine. Values adjusted to allow for supplements of corn oil, vitamins, and minerals.
[b] Requirements suggested by Almquist (*2*).

Literature Cited

(1) Albanese, A. A. Higgons, R. A., Hyde, G. M., Orto, L., *Am. J. Clin. Nutrition* **3,** 121–8 (1955).
(2) Almquist, H. J., in "Amino Acid Handbook. Methods and Results of Protein Analysis" (R. J. Block, ed.), p. 164, Charles C Thomas, Springfield, Ill., 1956.
(3) Baldwin, R. R., Lowry, J. R., Thiessen, Reinhardt, Jr., *Food Research* **16,** 107–17 (1951).
(4) Gessert, C. F., Phillips, P. H., *J. Nutrition* **58,** 423–32 (1956).
(5) Rosenberg, H. R., Rohdenburg, E. L., Baldini, J. T., *Arch. Biochem. Biophys.* **49,** 263–7 (1954).

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FORAGE NUTRIENTS

Free Reducing, Acid-Hydrolyzable, and Total Sugars and Total Available Carbohydrates in Ladino Clover, Nutritionally Significant Chemical Components of Forage Legumes

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Although legumes are a major forage crop in the United States, relatively little is known about their content of such biochemical constituents as carbohydrates, saponins, and organic acids.

The readily available carbohydrates of legumes are an important source of energy for ruminants, and as carbohydrates have been designated as one of the possible causative factors in bloat (*2, 3, 6*), the carbohydrate content of Ladino clover as affected by such factors as plant part, season, and time of day was determined.

Materials

Dormant Ladino clover stolons were obtained during the dormant seasons of 1947–48 and 1948–49 as described under plant parts.

Whole herbage and plant parts samples were taken in 1954 from five