THE EFFECT OF VARIETY AND AMINO ACID SUPPLEMENTATION ON THE NUTRITIVE QUALITY OF OAT PROTEIN

By

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1952

Submitted to the Faculty of the Graduate School of
the Oklahoma Agricultural and Mechanical College
in partial fulfillment of the requirements
for the degree of
MASTER OF SCIENCE
July, 1954

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ACKNOWLEDGMENT

The author wishes to express his sincere thanks to Dr. Ruth Reder for enthusiastic assistance and guidance so generously given during the study of this problem.

Thanks are also due to Dr. A. M. Schlehuber who furnished the grain used in this study.

The author also wishes to acknowledge the financial assistance received as a graduate fellow in the Department of Chemistry and the facilities provided him by the Department of Agricultural Chemistry Research.

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INTRODUCTION

An investigation of factors affecting the protein quality of oats was initiated in this laboratory in 1950. Results of earlier studies of the effects of variety and of location and time of planting were reported by Thomas (34). The present report includes further studies of varietal differences as well as the effects of amino acid supplementation on the protein quality of certain oat varieties.

Biological value of the proteins studied was estimated by the protein efficiency quotient method. Eighteen oat varieties were compared in two experiments conducted in 1952 and 1953. Two of these varieties, Desoto and Forkedeer, were selected for use in the supplementation studies. Lysine, methionine, and leucine were the amino acids used in an attempt to enhance the biological value of the protein of these varieties.

LITERATURE REVIEW

The nutritional value of any protein is determined to a large extent by the relative amounts and nature of the amino acids which it supplies to the animal body. Evaluation of the relative protein quality of different food sources by animal assay, however, is affected not only by the amino acid composition of the food proteins but also by the digestibility of the proteins and the extent to which the end-products of digestion, largely amino acids, are absorbed from the alimentary canal and utilized by the animal. Mitchell (20) points out three avenues of approach to the evaluation of relative protein values: (a) the analytical determination of amino acid components of the food protein by either chemical or microbiological methods; (b) the measurement of the amount of nitrogen retained in the animal body; and (c) the measurement of the ratio of gain in weight to protein intake for growing animals.

Of the three assay methods mentioned, the one most commonly employed for protein evaluation involves the determination of the ratio, commonly known as the protein efficiency quotient, of gain in body weight to protein consumed, when growing albino rats are fed a complete diet in which protein is the only limiting factor. This is a modification of a method originally proposed 45 years ago by Osborne, Mendel, and Ferry (31). This method essentially consists of comparing the maximum ratio of gain in weight to protein consumed on an experimental diet over a period of from four to six weeks. Although this method enjoys extensive and widespread useage, it has several critical disadvantages which have been pointed out by Mitchell (20, 18, 19). In the use of this method most investigators have employed one arbitrarily selected protein level, usually around 10 percent, which is the value selected by Osborne, Mendel and Ferry (31). These authors point out, however, that when protein is fed at a single level or food intake is restricted arbitrarily, only relative values are obtainable, depending on the degree of retention of the protein in the diet. The ideal condition, as stated by these authors, would be if animals of the same starting size were to "eat the same amount of food in the same number of days and gain the same amount of weight, the protein factor being the only variable." Mitchell (19) has pointed out that it is necessary to control food intake if true differences are to be measured. Ad Libitum feeding may not only grossly exaggerate the difference in the nutrititive value of two protein mixtures, due to the fact that the better mixture will be consumed in greater amounts, but it may also have an unpredictable effect on the measurement arising from differences in the palatability of the test rations. Another source of error encountered in ad libitum feeding is that as the amount of dietary nitrogen consumed is raised from low to high levels, there is a level at which nitrogen equilibrium will be met. When this level is surpassed, the amino acids will be deaminized and the residue used for storage of fat and glycogen (19). Therefore, as the level of protein in the diet is increased, the efficiency utilization of food nitrogen as tissue nitrogen decreases.

Chemical and microbiological methods for assaying protein have certain disadvantages that are not necessarily applicable to the other methods used for measuring protein values. For example, they do not take into consideration the differential rates of availability of amino acids in digestion. The ultimate synthesis of body protein from animo acids made available through digestion and absorption is dependent on the simultaneous appearance in the blood stream of each of the essential amino acids in suitable proportions. Kuiken and Lyman (14) have found a wide range of availibility of different amino acids in various food stuffs. There is also the differential sparing effect of carbohydrate and fats in the diet on amino acids. Munro (26) found that nitrogen balance improved when carbohydrate was fed in the same diet with protein as compared to protein fed apart from carbohydrate. Fat seems to exert no statistically significant effect on nitrogen balance. Digestibility is a third factor of considerable importance in an assay of protein quality. Mitchell and Block (21) have noted that protein sources differ in their percentage digestibility. The protein of beef muscle, for example, is 100 percent digestible, while the protein of white rice is 78 percent digestible.

As Mitchell and Carman (22) point out, "the composition of the gains in weight made by growing rats varies considerably even though the rations consumed, the initial weight of the rats, and the length of the growth period may be the same. The gain in weight of an animal, no matter how carefully experimental conditions may be prescribed, is not a definite measure of the nutritive effect of the rations fed, but is subject to considerable variation due to the operation of unknown factors."

It is generally agreed among investigators that cereal grains when fed alone do not result in satisfactory nutrition (17, 11, 23, 2, 3). Osborne and Mendel (30) partially attribute this to the biological incompleteness of grain protein, resulting from an amino acid deficiency. Although oat protein is superior to other cereals such as rye, corn, wheat, and barley, (11, 30, 13) it does not approximate the biological value of whole egg protein or casein, even though the latter is deficient in the essential amino acid methionine (21). As early as 1914 Osborne and Mendel (28) supplemented corn with lysine and tryptophan and obtained normal growth. Similar results were obtained by Hogan (10) and Marais and Smuts (17). Mitchell and Smuts (25) demonstrated that oats and other cereals are deficient in lysine. They further showed that the supplementation of oats with lysine resulted in a slight increase in growth-promoting value. In experiments with corn and oats, Mitchell and Block (21) found that lysine supplementation caused a distinct though slight increase in the growth-promoting values of these cereals but that because of a secondary deficiency, tryptophan in the case of corn, and an unidentified factor in the case of oats, superior growth did not result. Casein is known to be lacking in the essential amino acid methionine and supplementation with this amino acid results in a better growth-promoting value.

Further instances of the complexities of amino acid suplementation have been cited by Rose et al (32). These investigators found that glutamic acid and possibly other amino acids, which are neither stimulatory nor essential at a low rate of growth, become stimulatory when added to rations which permit a higher growth rate. They also observed (36) that the addition of tyrosine to a diet which gave indications of a partial deficiency of the amino acid phenylalanine, caused an increased rate of growth; tyrosine greatly spared the requirement for phenylalanine to which it appeared to be a precursor. A similar relationship was noted between methionine and cystine; cystine stimulates growth when methionine is fed in suboptimal amounts (35).

As early as 1929 Mitchell and Hamilton (24) noted the poor nutritive quality of the alcohol-soluble proteins of cereal grains, especially corn, wheat, and oats. They attributed this to a deficiency in one or two amino acids, possibly lysine and tryptophan. Frey (7) recently demonstrated a positive correlation between the total nitrogen and the alcohol-soluble nitrogen content of oats. Application of this correlation to nineteen different oat varieties showed that "it is evident that the alcohol-soluble nitrogen remains a constant proportion of total nitrogen in the oat grain regardless of thetotal nitrogen percentage." If this relationship is true, then oats differ significantly in this respect from corn. Frey (6) has shown that as the percentage of total protein in corn increases, the percentage of zein increases. Since zein is low in tryptophan and lysine, the quality of the protein of high protein strains is consequently inferior to that of strains of lower protein content.

Frey (8) endeavored to correlate increased protein content to an increase in certain essential amino acids in oat varieties grown in 1947 and 1949. Data from fourteen oat varieties tested showed that the percentages of protein, leucine, and lysine in the earlier year were significantly correlated with those in 1949, exceeding the one percent level of significance, while the percentages of tryptophan and methionine were not significantly correlated at the one percent level in these two years. The average content of lysine, leucine, and methionine were, 47, 47, and 43 percent higher, respectively in 1949, than in 1947 but the average content of tryptophan was only 12 percent higher. Since all varieties employed did not change as much in their amino acid composition as did others, it seems probable that his previous suggestion for improving oats as a source of protein by selection of a high protein strain appears applicable for some varieties, but not for others.

The various proteins in oats have not been as well defined as those in corn. Classification of the various proteins in oats has been based largely on solubility. In the latter part of the nineteenth century

Osborne (27) isolated and separated oat proteins into three fractions. An oat sample containing 11.80 percent protein gave the following fractions: (a) a prolamin, gliadin, soluble in weak alcohol, amounting to 1.25 percent of the grain; (b) a globulin or salt soluble fraction, avenalin, amounting to 1.50 percent of the grain; and (c) an "insoluble fraction", a glutelin called avenin, which was determined by difference and constituted 9.00 percent of the grain. Luers and Siegert (16) isolated five proteins from oats; (a) an albumin, (b) a globulin, soluble in 10 percent cold salt solution, (c) a globulin soluble in 10 percent salt solution at 65° C., (d) a prolamin, and (e) a glutelin. Oat prolamin is known to be free of tryptophan and very low in lysine (5), and oat gliadin contains no tryptophan (12). Csonka (5) has drawn attention to the fact that rolled oats become richer in protein than the original grain and have a higher percentage of the amino acids, lysine, tryptophan, and histidine. This is accounted for by the elimination of oat shorts and middlings which are separated in the milling process. These by-products of rolled oats contain a small amount of lysine and very little, if any, tryptophan. Avenin has been reported by Lamour (15) to be high in arginine and basic nitrogen. Csonka (4) found only one glutelin in oat protein; it had an isoelectric point of about a pH of 6.45.

Practically all the oat protein is concentrated in the kernal, only a small amount being present in the husks (33). From the viewpoint of the oat grower it would hardly seem economical to remove the husks from whole oats before feeding, but it might be possible to supplement whole oats to relieve amino acid deficiencies and thus promote more normal growth. It is the purpose of this research to explore these possibilities.

EXPERIMENTAL PROCEDURE

Material and Methods

Protein Sources

Cereal grains used in this experiment were furnished by Dr. A. M. Schlehuber of the Agronomy department of Oklahoma A. and M. College. They were planted in special plots specifically for this purpose, and were harvested by hand to avoid mixing of the different varieties. Eighteen oat varieties and one wheat variety were used in the experiments. All varieties were grown at Perkins, Oklahoma.

Chemical Analysis

Each variety was thoroughly mixed and sampled at random. The sample was finely ground in a Wiley Mill, mixed, and analyzed for proximate constituents by the official methods of the Association of Official Agricultural Chemists (1). Since these methods are well known, they need only a brief description.

Moisture was determined by heating a two-gram sample at 105° C. for six hours. The percentage of moisture was calculated from the weight loss.

Ash was determined on the above moisture sample by heating the dried sample in a muffle furnace at 625° C. for two hours. The sample was cooled in a desiccator and the weight of the ash determined.

Ether extract was determined by placing a 2-gram sample in fat tubes and extracting with anhydrous diethyl ether for 16 hours. The sample was then dried and reweighed. The percentage of ether soluble material was estimated from the loss of weight during extraction.

Crude Fiber was determined by digesting the above ether-extracted residues first in dilute sulfuric acid for thirty minutes, then in dilute sodium hydroxide for an equal length of time. The residue remaining after filtration through a linen cloth was thoroughly washed and dried at a temperature of 105° C. for six hours. The crucibles were cooled, weighed, and the loss in weight on ashing calculated as the percentage of crude fiber.

Protein nitrogen analyses were made in triplicate. Two-gram samples were taken of each of the varieties of oats and wheat; a 0.5-gram sample of casein was used for analysis. The conventional Kjeldahl procedure was applied, using copper sulfate and sodium sulfate to elevate the boiling point. Nitrogen was determined and the percentage of protein calculated, using the factor 6.25.

Ration Composition and Preparation

In each experiment the level of protein was held constant in all diets and was determined by the protein content of the oat variety having the lowest percent of protein. Protein level was adjusted by the addition of corn starch. Fiber content was compensated in those rations which were low in this product by the addition of commercial cellulose. Mineral requirements were met by the inclusion of two-percent Hegsted salt mixture (9) in the diet. All known vitamins were provided by a vitamin mixture which was included in the diet and by the addition of fortified cod liver oil to the diet at each feeding. The quantity of vitamin per kilogram of ration is shown in Table 1. A mechanical feed mixer was used in preparing the rations. The dry ingredients were mixed first and after a homogeneous mixture was obtained, corn oil was added. In the last two experiments in which amino acids were included in the rations, hand mixing was employed to insure the proper distribution of the supplements.

Table I. Composition of vitamin mixture.

Vitamin	Amount (mg.) per kilogram of ration
Thiamin	4.0
Riboflavin	6.0
Pyridoxine·HCl	3.0
Nicotinic acid	20.0
Ca, Pantothenate	20.0
Inositol	20.0
Para-amino benzoic acid	20.0
Pteroylglutamic acid	20.0
Choline·Cl	100.0

The rations were stored in a deep freeze prior to and during each experiment to prevent deterioration.

Rat Assay

For use in the assays female albino rats weighing approximately 50 grams were obtained from Sprague-Dawley of Madison, Wisconsin. On arrival, they were randomly selected and placed in individual cages in a room in which the temperature was maintained around 75° F. The mean weight of the different groups in an experiment was adjusted so as not to vary more than two grams. Each rat was offered 70 grams of food each week; 20 grams on alternate days, three times a week, and 10 grams on the seventh day. Once each week the animals were weighed and non-consumed food collected and weighed. The total amount of food consumed during the period was calculated for each rat.

Table II. Percentage proximate composition of protein sources in experiment 1.

Protein source	Total protein	Ash	Ether extract	Crude fiber	Moisture
Oat Varieties — **Winter**					
Stanton Strain 1	10.25	3.28	7.05	7.05	8.45
Forkedeer	10.84	3.71	5.26	6.96	8.41
Tennex	11.06	3.84	5.55	9.08	6.54
Wintok	11.28	3.49	7.19	6.47	6.58
Traveler	11.72	3.27	5.47	8.19	6.16
Desoto	10.06	3.17	6.17	8.25	6.74
W. Fulghum Sel. (6570)	9.83	3.58	7.18	6.89	6.62
Wd. Comp. Sel. (4829)	10.39	4.08	5.30	8.47	6.80
" " " (5106)[1]	10.84	3.80	6.07	7.89	6.91
" " " (5106)[2]	11.22	3.53	5.86	5.95	6.38
Spring					
Cherokee	12.98	3.55	4.26	4.26	6.53
Neosho	13.40	3.54	4.65	8.67	6.41
Andrew	11.82	3.53	2.74	9.02	6.02
Clinton	12.19	3.57	3.74	11.13	6.46
Nemaha	13.30	3.35	3.78	10.88	6.34
O-200	11.55	3.58	3.94	10.80	6.29
Kanota	12.92	3.86	6.11	8.91	6.11
Wheat					
Comanche	11.84	1.37	1.05	1.09	7.17
Casein	82.50	3.20	0.45	----	8.10

[1] Spring seeded [2] Fall seeded

Table III. Composition of diets used in experiment 1.
Protein level 9.44%.

Protein source	Percentage constituent in diet Protein	Corn Starch	Corn Oil	Salt mixture
Oat Varieties Winter				
Stanton Strain 1	92.10	3.90	2.00	2.00
Forkedeer	87.09	9.91	2.00	2.00
Tennex	85.35	10.65	2.00	2.00
Wintok	83.69	12.31	2.00	2.00
Traveler	80.55	15.45	2.00	2.00
Desoto	93.84	2.16	2.00	2.00
W. Fulghum Sel. (6570)	96.00	0.00	2.00	2.00
Wd. Comp. Sel. (4829)	90.86	5.14	2.00	2.00
" " " (5106)[1]	87.09	8.91	2.00	2.00
" " " (5106)[2]	84.14	11.86	2.00	2.00
Spring				
Cherokee	72.73	23.27	2.00	2.00
Neosho	70.45	25.55	2.00	2.00
Andrew	79.86	16.14	2.00	2.00
Clinton	77.44	18.56	2.00	2.00
Nemaha	70.98	25.02	2.00	2.00
O-200	81.73	14.27	2.00	2.00
Kanota	73.07	22.93	2.00	2.00
Wheat				
Comanche	79.73	16.27	2.00	2.00
Casein[3]	10.21	83.79	2.00	2.00

[1] Spring seeded [2] Fall seeded [3] Agar added to the 2 percent level.
Each diet contained 2 gram vitamin mixture per kilogram.
Fortified cod liver oil added at each feeding.

Experiment 1

The seventeen oat varieties compared in the first experiment included both Spring and Winter types. Winter varieties were Stanton Strain 1, Forkedeer, Tennex, Wintok, Traveler, Desoto, Winter Fulghum Selection (6570), and Woodward Composite Selection nos. 4929 and 5106; the latter was both spring- and winter-seeded. Spring varieties were as follows; Neosho, Andrew, Clinton, Nemaha, 0-200, and Kanota.

The proximate composition of these varieties and of Comanche wheat and casein which served as protein sources in control diets is presented in Table II.

Quality of the protein from the 19 sources was compared in replicated assays; the first extended from January 10, to February 21, 1953, the second from April 9, to May 21, 1953. In the first replication there were 5 rats on each diet, in the second, there were six.

The protein level on all diets in the two assays was 9.44 percent. This was determined by the protein content of a diet containing 96 percent of the oat variety, Winter Fulghum Selection (6570) which had a lower protein content than any of the other varieties. Composition of the diets employed is given in Table III.

Experiment 2

Ten oat varieties of the Winter type were compared in experiment 2. These were Wintok, Forkedeer, Woodward Composite Selection (5106), Tennex, Stanton Strain 1, Woodward Composite Selection (4828), Winter Fulghum Selection (6570), Desoto, Woodward Composite Selection (4829), and Traveler. The proximate composition of each of these varieties and of the two control protein sources, Comanche wheat and casein, is shown in Table IV.

Table IV. Percentage proximate composition of protein sources in experiment 2.

Protein source		Total protein	Ash	Ether extract	Crude fiber	Moisture
Oat Varieties Winter						
Desoto		12.34	3.09	5.22	12.93	8.21
Forkedeer		15.97	2.96	5.75	9.01	9.04
Stanton Strain 1		13.47	2.97	7.10	10.00	7.92
Tennex		14.37	3.56	5.62	9.56	8.38
Traveler		13.62	2.70	6.09	9.51	8.24
Wintok		11.97	2.71	7.30	7.47	8.99
Wd. Comp. Sel.	(4828)	13.81	2.87	5.29	10.37	8.40
" " "	(4829)	14.81	3.13	6.10	11.17	8.49
" " "	(5106)	14.84	2.78	6.15	9.21	8.61
W. Fulghum Sel.	(6570)	15.59	3.10	6.36	11.01	8.02
Wheat						
Comanche		14.72	1.48	1.55	2.98	9.90
Casein		81.37	3.52	0.43	----	7.86

Table V. Composition of diets used in experiment 2.
Protein level 11.50%.

Protein source		Percentage constituent in diet Protein	Corn starch	Corn oil	Salt mixture
Oat varieties Winter					
Desoto		93.11	2.89	2.00	2.00
Forkedeer		71.95	24.04	2.00	2.00
Stanton Strain 1		85.30	10.70	2.00	2.00
Tennex		79.96	16.04	2.00	2.00
Traveler		84.36	11.64	2.00	2.00
Wintok		96.00	-----	2.00	2.00
Wd. Comp. Sel.	(4828)	84.36	11.64	2.00	2.00
" " "	(4829)	77.58	18.42	2.00	2.00
" " "	(5106)	77.43	18.57	2.00	2.00
W. Fulghum Sel.	(6570)	73.70	22.30	2.00	2.00
Wheat					
Comanche		78.06	12.45	2.00	2.00
Casein		14.14	74.05	2.00	2.00

Each diet contains 2 grams of vitamin mixture per kilogram.
Fortified cod liver oil added at each feeding.

The protein level for all diets in this experiment was determined in the same manner as in experiment 1. Wintok was the oat variety having the lowest protein content, 11.97 percent, and the level of protein for the experiment was 11.50 percent. Composition of the diets is given in Table V. The assay was conducted over a period of six weeks, from November 5, to December 17, 1953.

Experiment 3

The third experiment was conducted to determine whether the nutritive value of the protein of a variety having a low protein efficiency quotient could be increased by amino acid supplementation to equal that of a variety having a consistently high PEQ and, further, to determine whether the nutritive value of the better variety might also be increased in a similar manner. Desoto and Forkedeer were the two varieties selected for use in this experiment; the former having previously shown a relatively low, the latter a comparatively high, PEQ. Casein served as the protein source in a control diet. The proximate composition of the protein sources is given in Table VI. Protein level of the three basal rations was 12.42 percent and was determined by the protein content, 12.94 percent, of the Desoto variety. The basal diet was prepared in

Table VI. Percentage proximate composition of protein sources in experiment 3.

Constituent	Desoto	Forkedeer	Casein
Protein	12.94	14.41	81.25
Ash	2.96	2.86	3.32
Ether extract	6.71	7.17	0.47
Crude fiber	10.54	9.17	-----
Moisture	7.09	7.47	8.51

the same manner as in experiment 1. All necessary additives as vitamins, minerals, fats, and carbohydrates were supplied to make the diet biologically complete.

The experiment was conducted over a period of five weeks and included seven experimental groups, with seven animals in each group. Four groups were fed the Desoto basal diet; the first group received no amino acid supplement; the second group received lysine; the third, lysine and methionine; and the fourth group, lysine, methionine, and leucine. Two groups received Forkedeer basal ration. The first group received no supplements and the second was given lysine, methionine, and leucine during the first three weeks after which they received in addition, tryptophan and histidine. The casein diet was not supplemented. The level at which the amino acids were fed is presented in Table VII.

Experiment 4

The fourth experiment was carried out with one oat variety, Desoto, to determine the effect on nutritive quality of supplementing the protein of this variety with three amino acids, lysine, methionine, and leucine; alone and in all possible combinations. Casein served as the source of protein in a control diet. The level of protein in the three basal rations was 9.50 percent.

Proximate composition of the protein sources is given in Table VIII.

Table VIII. Percentage proximate composition of protein sources in experiment 4.

Constituent	Desoto	Casein
Protein	12.19	83.00
Ash	3.05	3.06
Ether extract	6.67	0.47
Crude fiber	10.00	------
Moisture	8.10	8.28

The basal diets were made up as in previous experiments with the required additives being supplied in the proportions that were needed to complete the diet.

Table VII. Percent amino acid supplementation in experiment 3.

Basal diet	L-lysine	DL-methionine	L-leucine	L-histidine	L-tryptophan
Desoto	--	--	--	--	--
"	0.58	--	--	--	--
"	0.58	0.41	--	--	--
"	0.58	0.41	0.92	--	--
Forkedeer	--	--	--	--	--
"	0.58	0.41	0.92	0.16*	0.16*
Casein	--	--	--	--	--

* Introduced at end of third week

Table IX. Percent amino acid supplementation in experiment 4.

Basal diet	L-leucine	DL-methionine	L-lysine
		First period	
Desoto	--	--	--
"	0.80	--	--
"	--	0.60	--
"	0.80	0.60	--
Casein	--	--	--
		Second period	
Desoto	--	--	--
"	0.80	--	--
"	--	0.60	--
"	--	--	1.00
"	0.80	0.60	--
"	0.80	--	1.00
"	--	0.60	1.00
"	0.80	0.60	1.00
Casein	--	--	--

The experiment was conducted with 50 animals over a period of eight weeks. During the first four weeks there were five experimental groups; four received the Desoto basal ration diet; the fifth, the casein control diet. The first group on the Desoto diet received no supplement; the second received leucine; the third, methionine; and the fourth, leucine and methionine.

At the end of four weeks each group except the casein control group was subdivided into two groups. Both subgroups continued to receive the same supplements as during the first half of the experiment, but one of the subgroups also received lysine in addition to the supplements originally fed.

The form and level of the amino acid supplements received by the different groups during the two four-week periods of the experiment are shown in Table IX.

RESULTS AND DISCUSSION

Experiment 1

Nutritive values of the 19 protein sources used in the first experiment may be compared in Table X. This table presents mean weights, food intake, and PEQ values for each protein source when included in a diet at a 9.44 percent level of protein. PEQ values obtained in the two replications of the experiment are shown graphically in Figures 1 and 2.

Overall means for weight gains and PEQ values for all groups and for the 17 groups which received oats as a sole source of protein are summarized below, together with significant effects of protein source on growth and the least significant difference of mean weight gains.

	All diets (19)		Oat diets (17)	
Replication	1	2	1	2
Mean gain (gm.)	54 14	58 15	54 9	58 7
Significance of effects of protein sources	**	**	*	*
L.S.D.	9.6	10.5	6.9	5.6
Mean PEQ	1.44	1.58	1.45	1.53

* Significant at 0.05 level
** Significant at 0.01 level

The biological value of the protein of wheat was inferior to that of any of the sources studied. Growth on wheat protein was significantly less than on any other protein source in each replication and the PEQ values for this protein, 1.07 and 1.12, in the two trials, respectively, were exceeded by those for all other sources. The low quality of wheat protein is attributed to a deficiency of two essential amino acids, ly-

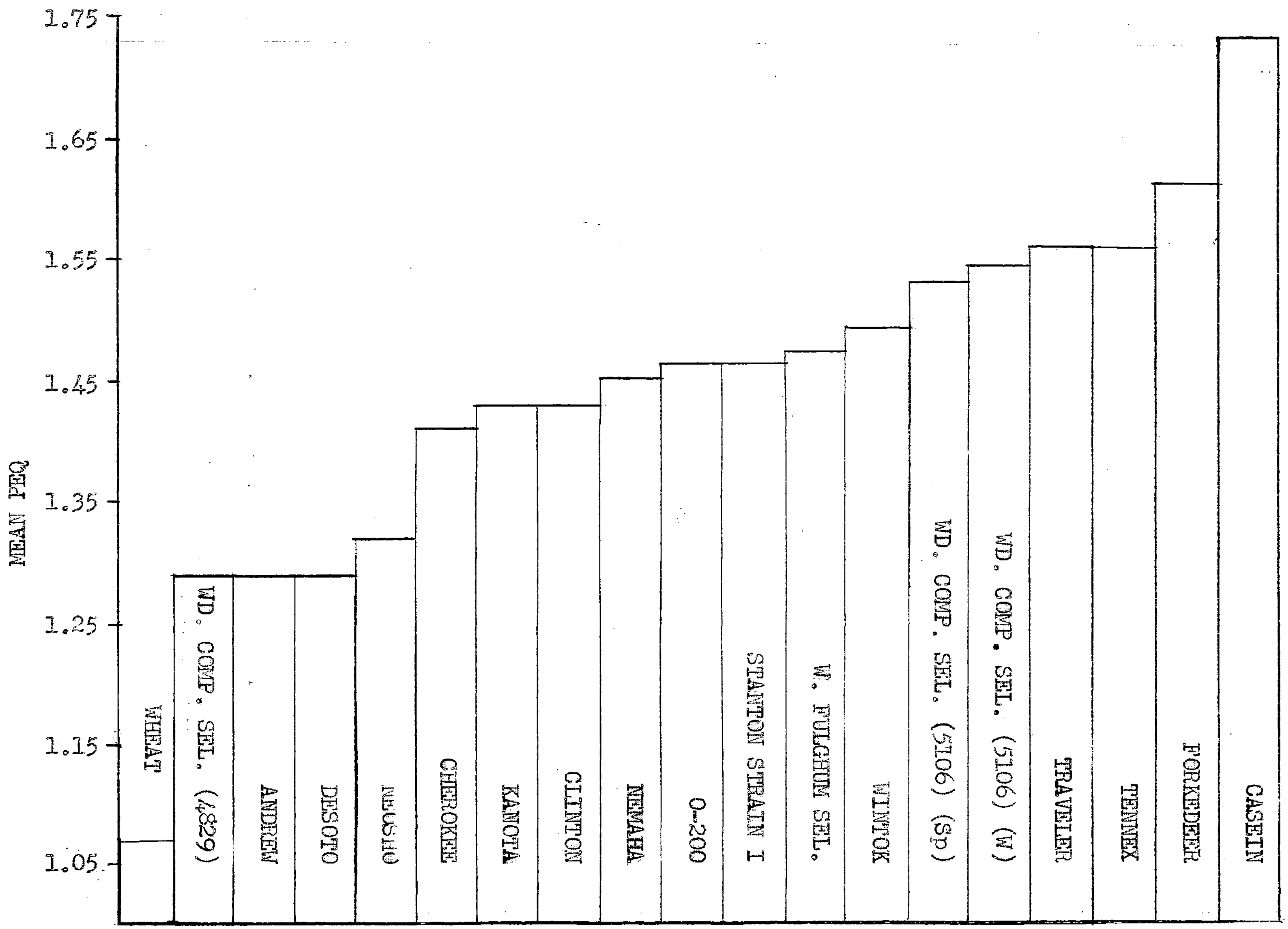

Figure 1. Protein efficiency quotients for 19 protein sources in **first replication of** experiment 1. Protein level 9.44 percent.

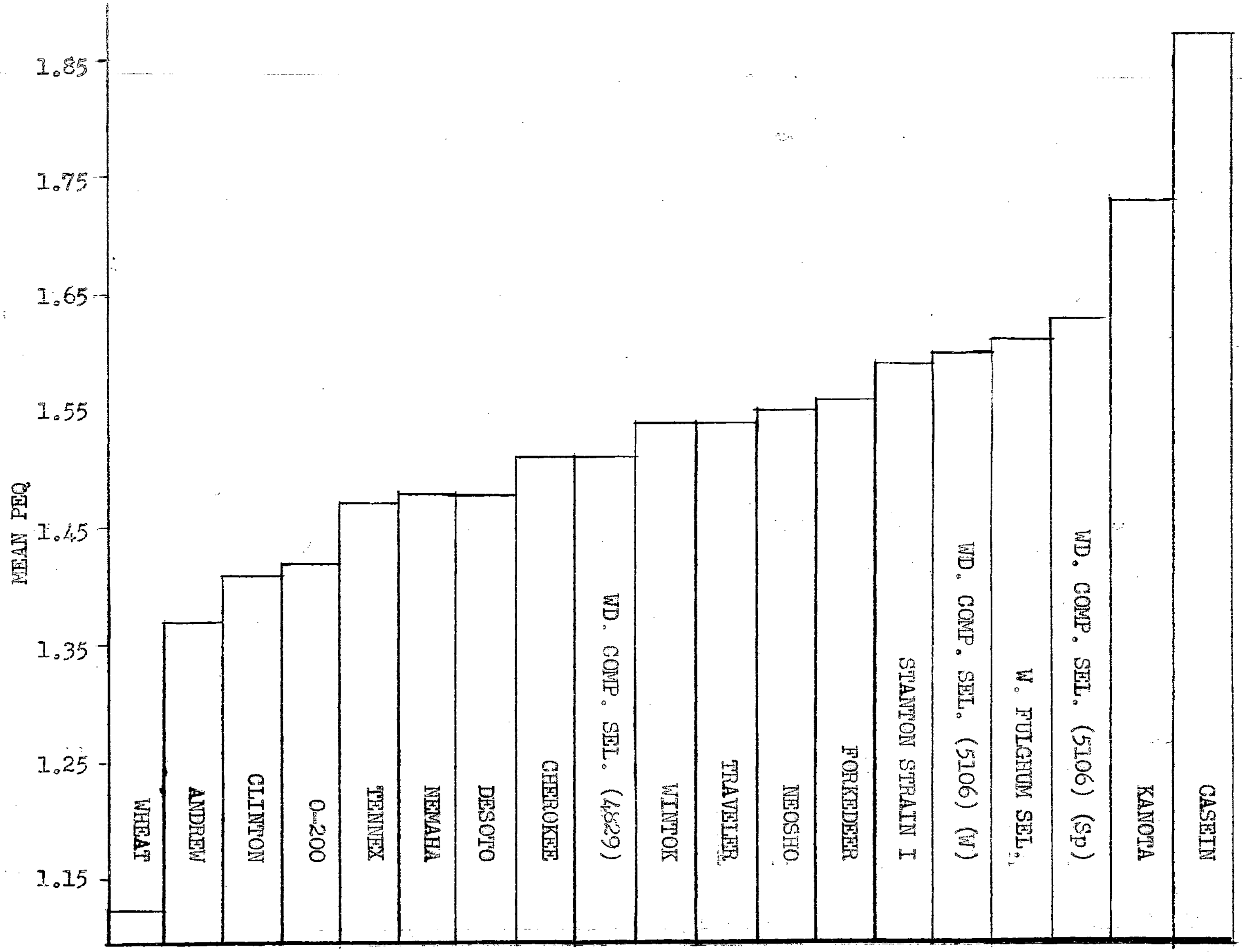

Figure 2. Protein efficiency quotients for 19 protein sources in second replication of experiment 1. Protein level 9.44 percent.

Table X. Mean weight gains, protein intake, and protein efficiency quotients in two replications of experiment 1. Protein level 9.44%.

Type of oat	Protein source	Mean total gain (grams)	Total Protein consumed (grams)	Protein efficiency quotient
		Replication 1		
	Wheat	35	33	1.07
W.	Wd. Comp. Sel. (4829)	45	35	1.29
W.	Andrew	48	38	1.29
W.	Desoto	45	35	1.29
Sp.	Neosho	50	38	1.32
Sp.	Cherokee	50	35	1.41
Sp.	Kanota	53	37	1.43
Sp.	Clinton	55	38	1.43
Sp.	Nemaha	57	39	1.45
Sp.	O-200	56	38	1.46
W.	Stanton Str.	55	38	1.46
W.	W. Fulghum Sel.	57	39	1.47
W.	Wintok	55	37	1.49
W.	Wd. Comp. Sel. (5106)[1]	59	39	1.53
W.	Wd. Comp. Sel. (5106)[2]	58	38	1.54
W.	Traveler	56	36	1.56
W.	Tennex	58	37	1.56
W.	Forkedeer	61	38	1.61
	Casein	61	36	1.73
		Replication 2		
	Wheat	38	34	1.12
W.	Andrew	52	38	1.37
Sp.	Clinton	54	38	1.41
Sp.	O-200	56	39	1.42
W.	Tennex	57	39	1.47
Sp.	Nemaha	57	38	1.48
W.	Desoto	57	39	1.48
Sp.	Cherokee	58	38	1.51
W.	Wd. Comp. Sel. (4829)	58	38	1.51
W.	Wintok	57	37	1.54
W.	Traveler	59	38	1.54
Sp.	Neosho	59	38	1.55
W.	Forkedeer	60	39	1.56
W.	Stanton Str.	61	38	1.59
W.	Wd. Comp. Sel. (5106)[2]	61	38	1.60
W.	W. Fulghum Sel.	61	38	1.61
W.	Wd. Comp. Sel. (5106)[1]	62	38	1.63
Sp.	Kanota	65	37	1.73
	Casein	69	37	1.87

[1] Spring seeded [2] Fall seeded

sine and tryptophan (29). Best growth and the highest PEQ values, 1.73 and 1.87, in the two trials, respectively, were obtained with casein as the protein source.

The nutritional quality of casein, however, was not superior to that of all the oat varieties studied. Growth on the casein diet in the first trial was significantly greater (0.05 level) than that of only five varieties, the first varieties listed in Table X. The mean PEQ for casein in this trial was 1.73, as compared to a mean of 1.32 for the five oat varieties. In the second trial, growth on the casein diet was significantly greater (0.05 level) than on nine oat varieties, the first listed for this trial. The mean PEQ for casein in this instance was 1.87, the mean for the nine varieties, 1.47. In both trials casein was a consistently and significantly better protein than the protein of four oat varieties: Andrew, Desoto, Cherokee, and Woodward Composite Selection (4829), all but the last of which are spring varieties.

Mean growth response to the different oat varieties varied 16 grams in the first and 13 grams in the second trial; PEQ values ranged from 1.29 to 1.61 in the first trial and from 1.37 to 1.73 in the second. Differences between replicates in growth response to the same variety were less than one gram in the case of five varieties, from 2 to 6 grams for seven varieties, and from 9 to 13 grams for five varieties. In both the latter groups growth was greater in the second trial that in the first. The greatest differences in growth between replicates were obtained with Neosho, Cherokee, Desoto, Kanota and Woodward Composite Selection (4829). The cause for these differences in response is not clear; it may be due to inherent differences in the animals. Results obtained by Thomas (34) in previous experiments with three of these varieties are in agreement with the lower values obtained in the first trial.

Differences in growth response and the PEQ values obtained with the oat varieties are presumably caused by difference in the relative amounts of the amino acids present in the grain. This, in turn, is probably the result in the shifting in the proportionality of the different proteins occurring in the oats. The results indicate that, in general, winter oats have a higher quality of protein than spring oats. Six of the ten winter types used, Stanton Strain 1, Winter Fulghum Selection, Wintok, Traveler, Forkedeer, and Woodward Composite Selection (5106) were the only varieties giving PEQ values which were above the average in both trials. The latter variety showed superior quality even when planted in the spring. Three of the winter varieties, however, Woodward Composite Selection (4829), Andrew, and Desoto were definitely inferior to the other winter types and resembled three spring varieties, Cherokee, Nemaha, and Clinton, in that they fell below the average PEQ in both trials. Three spring varieties, Neosho, Kanota and O-200, and one winter variety, Tennex, exceeded the average in one trial; these varieties appear to occupy an intermediate position between the better and poorer varieties. These findings are in essential agreement with those of Thomas (34).

Experiment 2

Of the ten winter oat varieties from the 1953 crop which were assayed in experiment 2, all but one, Woodward Composite Selection (4828), had been tested previously in experiment 1. Protein content of all varieties (Tables II and IV) was appreciably higher in the second year, when it varied from 11.97 to 15.97 percent, than in the first year, when it varied from 9.83 to 11.72 percent. Because of the generally increased protein content of the oats, protein level on the diets in experiment 2 was established at 11.50 percent, which was 21.9 percent higher than the level employed in the first experiment.

Mean weight gains, protein consumed, and PEQ values obtained for the 12 groups in experiment 2 are presented in Table XI and the PEQ values are shown graphically in Figure 3.

Differences in weight gains among all protein sources were highly significant (0.01 level) in this experiment as were differences among the oat varieties. Mean gain in weight was 70±24 grams for all diets and 70±16 grams for the oat diets; the L.S.D. between weight gain means was 24 grams for all diets and 5.54 for diets containing oats. The mean PEQ was 1.52 and 1.51 for all diets and oat diets, respectively.

As was observed in the preceding experiment, the lowest mean weight gain and the lowest mean PEQ value were obtained on wheat protein, the highest on casein. The mean weight gain on wheat was significantly lower than that on casein but was not significantly lower than the mean gain on any of the oat varieties. The mean gain on casein was significantly greater than that on all oat varieties.

Five oat varieties had a PEQ equal to or greater than the average; Wintok, Forkedeer, Woodward Composite Selection (5106), Winter Fulghum Selection, and Traveler. These varieties had been rated similarly in

Table XI Mean weight gains, protein intake, and protein efficiency quotients in experiment 2. Protein level 9.50 percent

Protein source	Mean total gain (grams)	Total protein consumed (grams)	Protein efficiency quotient
Wheat	59	46	1.28
Desoto	65	47	1.39
Stanton Strain 1	65	46	1.43
Tennex	64	44	1.46
Wd. Comp. Sel. (4829)	66	45	1.46
" " " (4828)	69	46	1.50
W. Fulghum Sel.	73	48	1.52
Traveler	72	46	1.55
Forkedeer	75	47	1.60
Wintok	77	46	1.65
Wd. Comp. Sel. (5106)	78	47	1.66
Casein	88	48	1.85

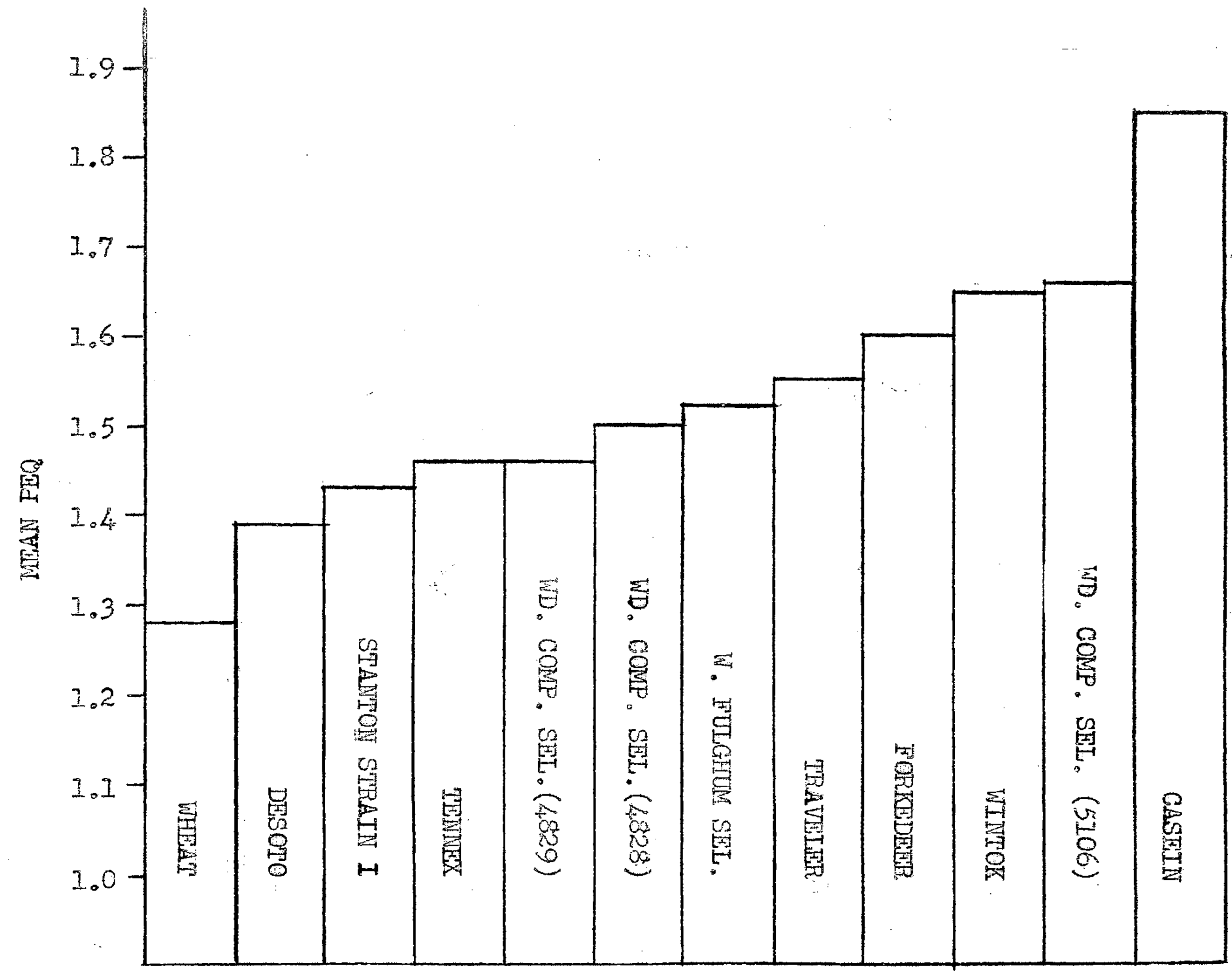

Figure 3. Mean protein efficiency quotient for 12 protein sources in experiment 2. Protein level 11.50 percent.

both trials in the first experiment. Four of the remaining varieties having a PEQ which fell below the average were similarily rated in either one or both replications of the first experiment. Thus when food intake was held constant, an increase in protein level of diets from 9.44 percent to 11.50 percent, and an increase in the protein content of the oats did not affect the PEQ values of the oat varieties in respect to each other.

The effect on weight gains and PEQ values of increasing protein level 21.9 percent while maintaining food intake at a practically constant level is shown in the following summary:

Protein source	Mean weight gain Protein level %				Mean PEQ Protein level %			
	A 9.44	B 11.50	B-A	(B-A)/A %	A 9.44	B 11.50	B-A	(B-A)/A %
Wheat	35	59	24	68.5	1.07	1.28	0.21	19.6
Desoto	45	65	20	44.4	1.29	1.39	0.10	7.8
Stanton Str.	55*	65	10	18.2	1.46	1.43	-0.03	-2.0
Tennex	58*	64	6	10.3	1.56	1.46	-0.10	6.4
Wd. Comp. Sel.[1]	45	66	21	46.7	1.29	1.46	0.17	13.1
W. Fulghum Sel.	57*	73*	16	28.1	1.47	1.52	0.05	3.4
Traveler	56*	72*	16	28.5	1.56	1.55	-0.01	-0.6
Forkedeer	61*	75*	14	22.9	1.61	1.60	-0.01	-0.6
Wintok	55*	77*	22	40.0	1.49	1.65	0.16	10.7
Wd. Comp. Sel.[2]	58*	78*	20	34.4	1.54	1.66	0.12	7.8
Casein	61	88	27	44.2	1.73	1.85	0.18	6.9

* Above average in trial [1] (4829) [2] (5106)

In no instance was the change in PEQ value occasioned by an augmented protein level commensurate with the increase in weight gains. The greatest change in PEQ, an increase of 19.6 percent, was obtained with wheat and was associated with a 68.5 percent increase in weight gain. The greatest increases in PEQ values for oat varieties, 13.1 and 10.7 percent, were obtained with Woodward Composite Selection (4829) and Wintok, respectively, and were associated with increases in weight gains amounting to 46.7 and 40.0 percent respectively. In the case of Desoto, Tennex, Winter Fulghum Selection, Woodward Composite Selection (5106) and casein, increases in PEQ were small, from 3.4 to 7.8 percent and were associated with increases in weight gains varying from 10.3 to 44.4 percent. Changes in PEQ values for Stanton Strain, Traveler, and Forkedeer were negative in sign and negligible in amount; increases in weight gains by these varieties were from 18.2 to 28.5 percent. These results suggest that an improvement in the protein quality of wheat and two oat varieties, Woodward Composite Selection (4829) and Wintok was effected by increasing the protein level of the diet.

Experiment 3

Results obtained in a study of the effects of amino acid supplementation on the nutritive quality of two oat varieties, Desoto and Forkedeer, are presented in Table XII and Figure IV.

The protein quality of Desoto was significantly lower than that of Forkedeer when the dietary protein level was held at 9.44 percent and 11.50 percent in experiment 1 and 2, respectively. However, weight gains and PEQ values obtained with these varieties did not differ significantly in experiment 3 when protein level in the diets was increased to 12.42 percent. Mean weight gains on the basal Desoto and Forkedeer diets over a period of five weeks were 58 and 63 grams respectively; the PEQ values 1.39 and 1.47. Incorporation of lysine alone, lysine and methionine, or lysine, methionine, and leucine in the basal Desoto diet did not significantly affect weight gains, nor was there an appreciable increase in PEQ value. Mean weight gains by all groups receiving the supplemented Desoto diet were significantly less than the mean gain on the casein diet, and the mean PEQ values were considerably below 1.85, the PEQ for casein.

Quality of the unsupplemented protein of Forkedeer was inferior to that of casein. Mean weight gain, 63 grams, and PEQ, 1.47, for this protein were significantly less than the mean weight gain, 79 grams, and PEQ, 1.85, for casein. On supplementation of the basal Forkedeer diet with lysine, methionine, and leucine mean weight gain was increased to 73 grams and PEQ to 1.74, which were not significantly different from the corresponding values for casein. Further supplementation of the Forkedeer diet with histidine and tryptophan during the last two weeks of the experiment did not significantly affect weight gain; during this period the mean gain by animals on the unsupplemented diet was 24 grams as compared to 27 grams for animals on the supplemented diet. Growth

Table XII. Mean weight gains, protein intake, and protein efficiency quotients in experiment 3. Protein level 12.42%.

Basal diet	Supplement	Mean total gain (grams)	Total protein consumed (grams)	Protein efficiency quotient
Desoto	None	58	42	1.39
"	Lysine	63	42	1.52
"	Lysine, Methionine	63	42	1.50
"	Lysine, Leucine, Methionine	58	41	1.40
Forkedeer	None	63	43	1.47
"	Lysine, Leucine, Methionine, Histidine, Tryptophan	73	42	1.74
Casein	None	79	43	1.85

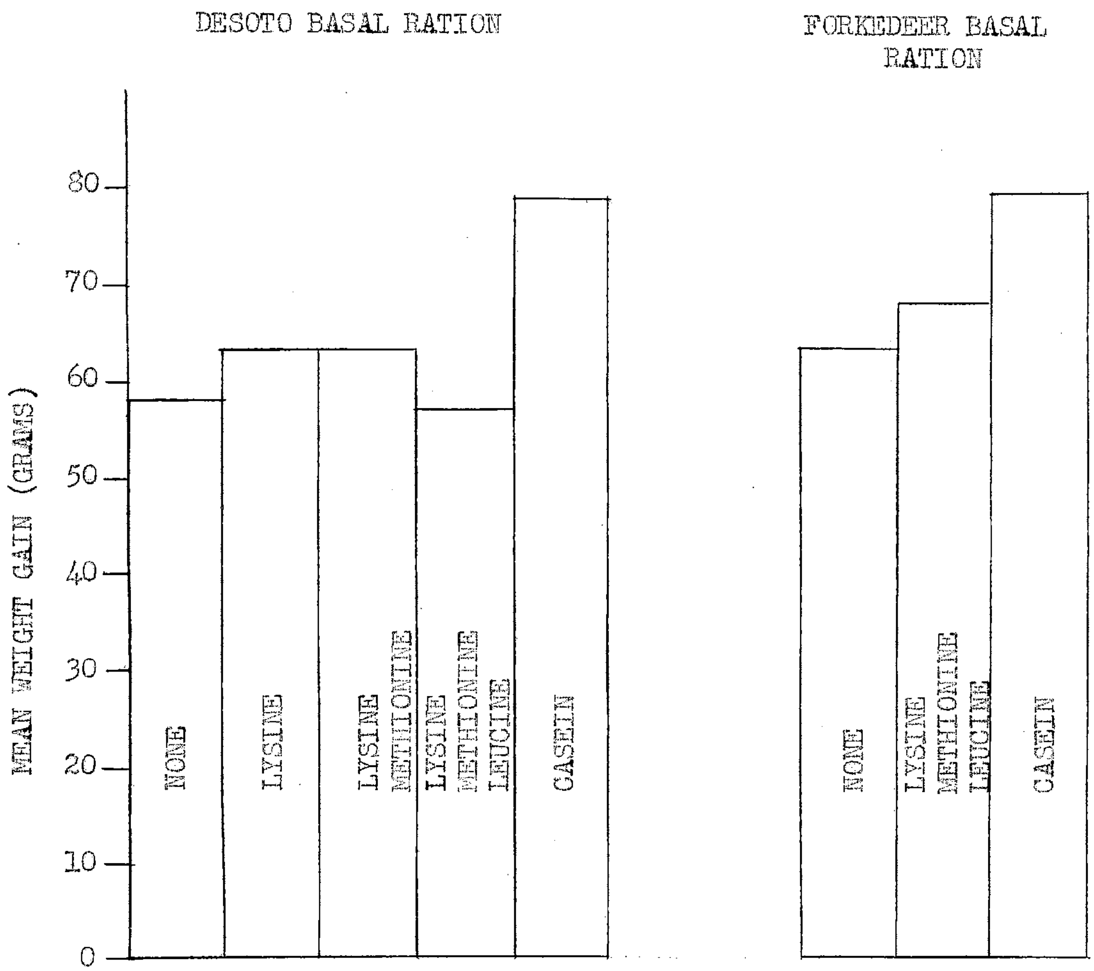

Figure 4. Mean weight gain (grams) over a period of five weeks by animals on three protein sources. Protein level 12.42 percent.

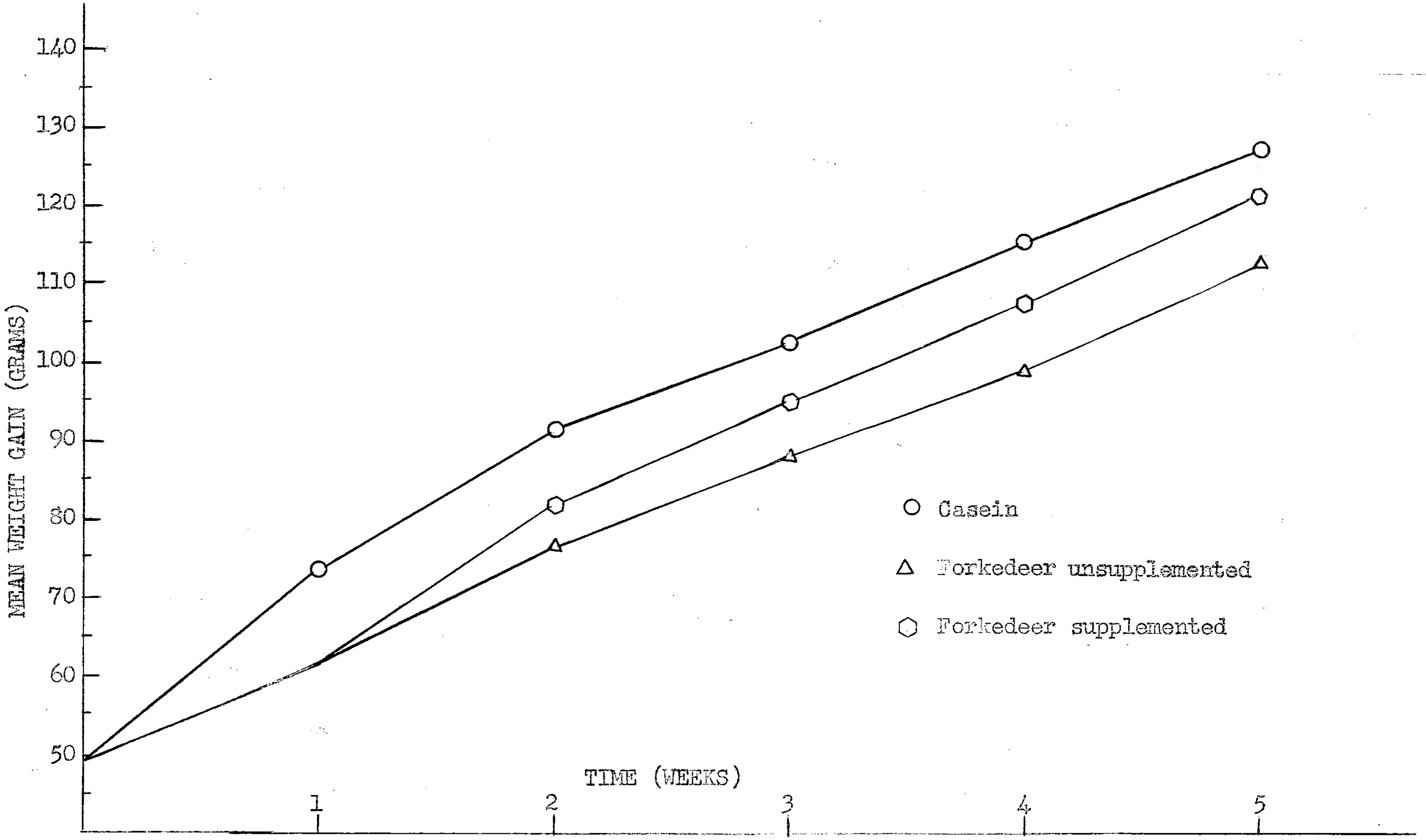

Figure 5. The effect of amino acid supplementation on growth of animals receiving a basal Forkedeer ration. Protein level 12.42 percent.

curves for animals on the Forkedeer diets and a control casein diet are presented in Figure 5.

Supplementation of the basal Desoto ration containing 12.42 percent protein with lysine, lysine and methionine, or lysine, leucine and methionine failed to improve the nutritive quality of the protein, but supplementation of the basal Forkedeer diet of equal protein content with these amino acids markedly improved the quality of the protein.

Experiment 4

Results obtained in a further study of the effect of amino acid supplementation on the nutritive value of the protein of Desoto are presented in Table XIII.

The mean gain in weight (47 grams) by ten animals receiving the basal Desoto diet during the first four weeks of the experiment was not significantly different from the mean gain (50 grams) by an equal number of rats on the casein diet, and the PEQ values for the two protein sources 1.88 and 2.06 respectively, are similar. Addition of either or both the amino acids leucine and methionine affected neither growth rate nor the PEQ obtained with similar groups of animals during this period; mean weight gain in these groups varied from 45 grams to 49 grams, the mean PEQ from 1.88 to 2.02.

Half of the animals on each of these diets except casein were continued on the same diets for an additional four-week period. Differences in mean weight gains by (Table XIII) the five groups during the eight week period were statistically significant (0.05 level). The mean weight gain for all groups was 87.3 ± 19.3 grams, the mean PEQ was 1.69, and the L.S.D. for group gains was 12. Growth was significantly greater on the casein diet than on any of the other diets but supplementation of the protein of Desoto with either leucine, methionine, or both, had no effect on weight gains by these groups. The mean PEQ for casein group was 1.98 as compared to 1.69 for the four groups on the Desoto diet.

When lysine was added to the diet of one-half of the animals of each of the four Desoto groups in the last half of the experiment, differences in growth response and PEQ values obtained for the various groups became apparent. Significant effects obtained in this portion of the experiment are indicated in the summary of the analysis of variance of weight gains in Table XIV. The method of calculation used to determine significant main effects and interactions is indicated in Table XV.

The difference between the mean gain in weight by animals on the Desoto diets and those on the casein diet were highly significant (0.01 level). The mean gain in weight by all Desoto groups was 36 grams, and that by animals on the casein diet was 47 grams; corresponding mean PEQ values were 1.42 and 1.80, respectively. Inclusion of either lysine or methionine in the basal diet had a significant effect on weight gain, the effect of methionine was significant at the 0.01 level, that of lysine was significant at the 0.05 level. The mean weight gain by the four groups receiving lysine was 38 grams as compared to a mean gain of 34 grams by the groups which did not receive this supplement; mean PEQ values for these groups were 1.51 and 1.32, respectively. Groups which received methionine made an average gain of 39 grams, those which received no methionine, 33 grams. Mean PEQ values for these groups were 1.53 and 1.31, respectively.

In addition to the effects of lysine and methionine, there was a highly significant (0.01 level) interaction of leucine x methionine. Inclusion of methionine in the basal Desoto diet effected a greater increase in weight in the presence than in the absence of leucine: the difference in the mean gain by the group receiving methionine in the presence of leucine was greater than the gain by the group receiving methionine in the absence of leucine, as follows;

	Methionine	-	No Methionine	=	Gain
In presence of leucine	38.2 grams	-	28.6 grams	=	9.6 grams
In absence of leucine	37.2 grams	-	33.3 grams	=	3.9 grams
In presence of leucine	1.44 (PEQ)	-	1.14 (PEQ)	=	0.30 (PEQ)
In absence of leucine	1.42 (PEQ)	-	1.28 (PEQ)	=	0.14 (PEQ)

Table XIII. Mean weight gains, protein intake, and protein efficiency quotients obtained in experiment 4 on a casein diet and a basal Desoto diet with and without amino acid supplementation. Protein level 9.50%.

Protein source Basal diet	Supplements	Mean total gain (grams)	Total protein consumed (grams)	Protein efficiency quotient
		First 4 weeks (10 animals on each diet)		
Desoto	None	47	25	1.88
"	Leucine	46	24	1.88
"	Methionine	49	25	2.02
"	Leucine, Methionine	45	23	1.95
Casein	None	50	24	2.06
		Eight weeks (5 animals on each diet)		
Desoto	None	84	51	1.63
"	Leucine	77	50	1.55
"	Methionine	85	51	1.68
"	Leucine, Methionine	80	49	1.61
Casein	None	99	50	1.98
		Second 4 weeks (5 animals on each diet)		
Desoto	None	33	26	1.28
"	Leucine	29	25	1.14
"	Methionine	37	26	1.42
"	Lysine	40	26	1.52
"	Leucine, Methionine	38	27	1.44
"	Leucine, Lysine	31	25	1.28
"	Methionine, Lysine	40	26	1.58
"	Leucine, Methionine, Lysine	42	25	1.66
Casein	None	47	26	1.80

Table XIV. Summary of analysis of variance of weight gains in the second half of experiment 4.

Source of variance	D.F.	Sum of squares	Mean sum squares	Standard deviation
Total	49	2,712.08		
Treatments	8	1,684.78	210.597	14.51
Within treatments	41	1,027.30	25.056	
				F[1] value
Treatments	8	1,684.78		
Casein vs. Desoto	1	928.77		37.07**
T_1 Lysine	1	133.23		5.316*
T_2 Leucine	1	50.63		N.S.
T_3 Lysine and Leucine	1	2.03		N.S.
T_4 Methionine	1	429.03		17.123**
T_5 Lysine and Methio.	1	0.63		N.S.
T_6 Leucine and Methio.	1	133.23		5.316*
T_7 Lysine x Leucine x Methionine	1	7.23		N.S.

[1] $F = \frac{\text{Treatment mean squares}}{\text{Within treatment mean square}}$

Table XV. Main effects and interactions in a 3- factor experiment (No. 4).

	Combination of treatments									
	None	Ly.	Leu.	Ly. Leu.	M.	M. Ly.	Leu. M.	All		
Sum[1]	166	192	143	156	186	201	191	210	Total	Sum of squares[2]
Effect of										
Lysine	-1	1	-1	1	-1	1	-1	1	73	133.25
Leucine	-1	-1	1	1	-1	-1	1	1	-45	50.625
Lysine x Leu.	1	-1	-1	1	1	-1	-1	1	- 9	2.025
Methionine	-1	-1	-1	-1	1	1	1	1	131	429.025
Lysine x Meth.	1	-1	1	-1	-1	-1	1	1	- 5	0.625
Leucine x Meth.	1	1	-1	-1	-1	-1	1	1	73	133.225
Ly. x Leu. x M.	-1	1	1	-1	1	-1	1	1	17	7.225

[1] Sum of weight gains by animals in group

[2] Sum of squares = $\frac{\text{Total square}}{\text{40 (No. of animals)}}$

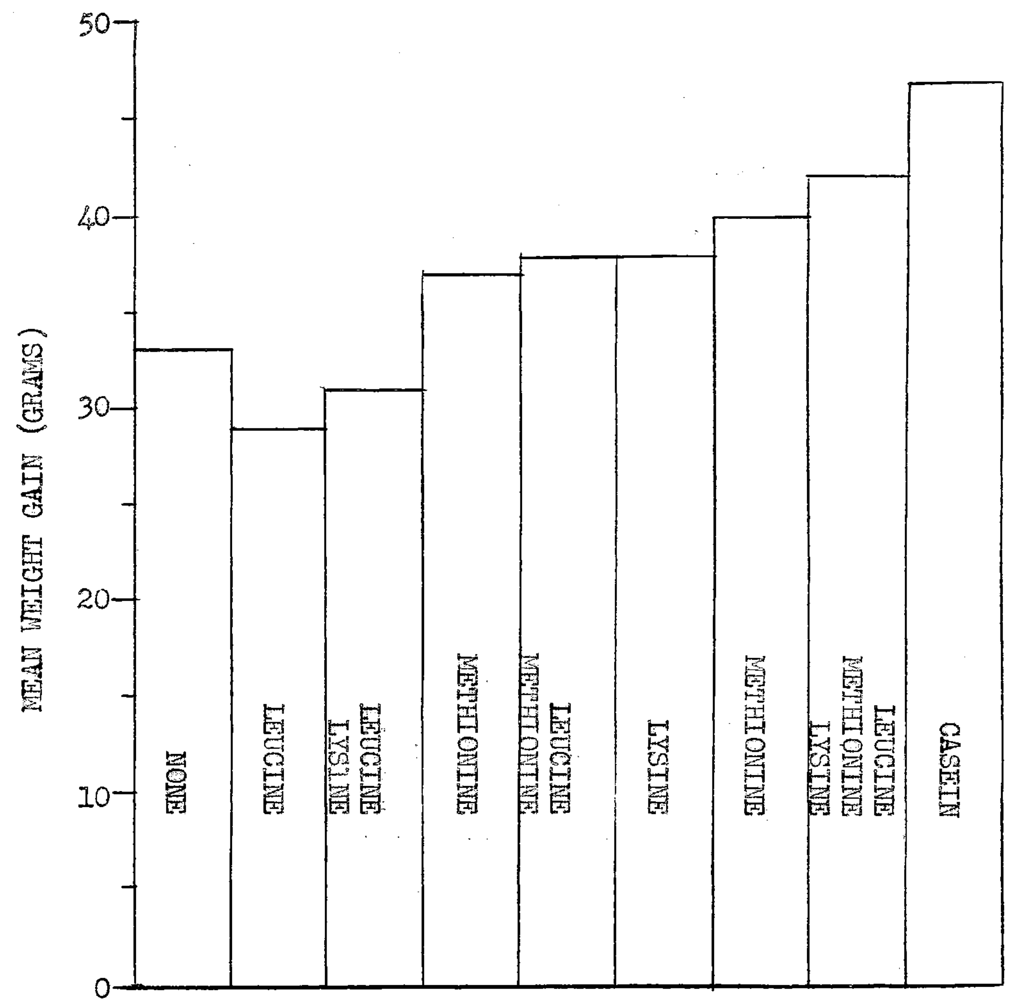

Figure 6. Mean weight gain (grams) over a period of four weeks by animals on Desoto basal diet with and without amino acid supplementation.

SUMMARY AND CONCLUSIONS

Experiments were conducted to study varietal differences in the nutritive quality of oat protein and to determine the effect of amino acid supplementation on the biological value of the protein of certain oat varieties.

Protein quality was evaluated by determination of weight gain and the ratio of gain in weight to the amount of protein consumed (PEQ) over a period of four to six weeks by young female rats on diets in which protein was the only limiting factor. In each experiment protein sources were incorporated in all diets at the same level of protein and food intake was restricted to 10 grams per day. Casein and wheat served as protein sources in control diets.

Seventeen oat varieties were used in two experiments. The first experiment included replicated trials with 16 varieties from the 1952 crop; six of the spring type, ten of the winter type, one of which was both winter and spring seeded. Protein level in this experiment was 9.44 percent. The second experiment was a single trial with ten winter varieties from the 1953 crop, nine of which had previously been tested with varieties from the 1952 crop. Protein level in this experiment was 11.50 percent.

Quality of oat protein, as evaluated by PEQ values, differed with variety. In general, the protein of winter oat varieties was of better nutritional quality than that of spring varieties. On the basis of protein nutritive quality the varieties studied may be grouped as follows:

Better than average	Intermediate	Below average
Stanton Strain 1 (W)	Neosho (Sp)	Woodward Comp. Sel. (4829) (W)
Winter Fulghum Sel. (W)	Kanota (Sp)	Andrew (W)
Wintok (W)	O-200 (Sp)	Desoto (W)
Traveler (W)	Tennex (W)	Cherokee (Sp)
Forkedeer (W)		Nemaha (Sp)
Woodward Composite Sel. (5106) (winter or spring seeded)		Clinton (Sp)
Woodward Composite Sel. (4828) (W)		

Results with nine varieities produced and tested in successive years suggest that a change in the protein content of a variety was not, in all probability, accompanied by a marked change in the percentage of the various essential amino acids present. The relationship of PEQ values for the different varieties was about the same in both years despite the fact that there was a general increase in protein content in the second year.

A difference in response to amino acid supplementation of the protein of two oat varieties incorporated in basal diets at the same level of protein suggests a varietal difference in amino acid compositions. Inclusion of lysine (0.58 percent), methionine (0.41 percent), and leucine (0.92 percent) in a basal diet in which Desoto supplied protein at a 12.42 percent level had no effect on the PEQ which was 1.39 on both unsupplemented and supplemented diets. On the other hand, addition of these amino acids to a similar basal diet in which Forkedeer was the protein source caused the PEQ to increase from 1.43 to 1.74.

Evidence of a deficiency of both lysine and methionine in the protein of Desoto was obtained in a factorial experiment in which lysine (1.0 percent), leucine (0.8 percent) and methionine (0.6 percent) were included, separately and together, in a basal Desoto diet having a 9.50 percent protein content. The effect of supplementation of the protein of Desoto with lysine or methionine was an increase in weight gain and

PEQ significant at the 0.05 level for lysine and at the 0.01 percent level for methionine. The effect of leucine was not significant but there was a significant interaction of methionine and leucine. The effect of methionine was greater in the presence than in the absence of leucine.

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THESIS TITLE: THE EFFECT OF VARIETY AND AMINO ACID SUPPLEMENTATION ON THE NUTRITIVE QUALITY OF OAT PROTEIN

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