AMINO ACID DEFICIENCIES OF GRAIN PROTEINS

by

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April 1950

THE UNIVERSITY OF ALBERTA

AMINO ACID DEFICIENCIES
OF GRAIN PROTEINS

A DISSERTATION

SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

FACULTY OF AGRICULTURE

by

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EDMONTON, ALBERTA,

April, 1950.

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The undersigned hereby certify that they have read and recommend to the School of Graduate Studies for acceptance, a thesis entitled "Amino Acid Deficiencies of Grain Proteins" submitted by Ruth Olive Ann Renner, B.Sc., in partial fulfilment of the requirements for the degree of Master of Science.

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April, 1950.

ACKNOWLEDGEMENTS

I am particularly indebted to Dr. L. W. McElroy, Professor of Animal Husbandry, who initiated and supervised the study herein reported and who, through helpful criticisms and suggestions, rendered much valuable and appreciated assistance in carrying out this research and in the preparation of this manuscript. Thanks and apprecaation are also extended to Dr. R. D. Sinclair, Chairman of the Department of Animal Science, for the use of the facilities of the Department, and to the Department of Biochemistry for providing the test animals and the space for conducting feeding trials. The very valuable advice provided by Dr. D. R. Clandinin, Associate Professor of Poultry Husbandry, regarding the conduction of microbiological assays is gratefully acknowledged.

Financial assistance received from Burns and Company, Limited, Calgary, through the Friends of the University Fund, and from a University of Alberta Research Scholarship is gratefully acknowledged.

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INTRODUCTION

Results of amino acid assays indicate that grains are lacking in certain amino acids and thus theoretically supplementation of grains with deficient amino acids should increase their nutritive value. However, results of rat feeding experiments conducted in the Department of Animal Science in 1947, to study the effect of supplementing farm grains with a number of amino acids were inconclusive. Rats fed a basal ration of low protein barley supplemented with the amino acid lysine made more rapid gains and required less feed per gram gain than did control rats fed the unsupplemented ration, but supplementation with tryptophan or methionine, threonine, valine and tryptophan in addition to lysine failed to bring about any further improvement in rate of growth.

Animal feeding trials were discontinued at that time until fundamental information regarding the amino acid composition of grains could be obtained. As detailed data with respect to nine essential amino acids in Alberta grains are now available from results of microbiological assays, the feeding trials reported herein were conducted to supplement the earlier experiments in which grains, fortified with pure amino acids, were fed to rats. The preliminary experiments, summarized in Part I of this report, were devoted to amino acid assays on the grains

selected for use in future feeding trials involving supplementation with pure amino acids and to repetition of a rat feeding experiment previously reported from this laboratory (5) in which rats were fed rations containing oats, barley or wheat as the sole source of protein.

PART I

AMINO ACID ASSAYS AND FEEDING EXPERIMENTS WITH GRAINS OF DIFFERENT SPECIES AND PROTEIN CONTENT

Literature Review

A number of early investigators, Sherman and Winters (28) and McCollum, Simmonds and Parsons (14) concluded that rye, wheat, corn, barley and oats varied little with respect to the nutritive value of their proteins. However, it has since been shown that differences do exist when a diet is fed that is nutritionally complete except for the protein. By means of nitrogen metabolism experiments with rats Mitchell (18) showed a distinct superiority of oat protein over corn protein at different levels of intake. In 1946 Mitchell and Block (19) concluded that the protein of rolled oats was definitely superior to that of other cereals tested. Recently Jones, Caldwell and Widness (12) in an experiment comparing cereal grains by rat growth studies showed that growth was proportional to protein level and at the 12% protein level, oats had a strikingly higher value than hard wheat. These results agree with those reported by McElroy, Lobay and Sinclair (17) who found that rate of growth was markedly in favor of high protein grains and that both barley and oats produced more rapid growth than wheat of similar protein content.

The results of rat growth experiments reported in the literature substantiate results recorded for the amino acid

composition of cereal proteins. In 1949 McElroy, Clandinin, Lobay and Pethybridge (16) from results of microbiological assays presented evidence indicating that the protein of Marquis wheat may contain significantly less of certain of the amino acids, notably lysine, than do the proteins of Victory oats and Newal barley. Thus, the lower lysine consumption of rats receiving wheat may partially explain the difference in growth promoting value of wheat, oats and barley. As McElroy et al. (17) did not record feed consumption, correlation coefficients to assess the relationship between lysine consumption and growth could not be calculated.

Therefore the following experiment was conducted to:

(1) determine if the grains available for the present experiments would give essentially the same results as were obtained in an earlier trial (17) in regard to the growth promoting value of high and low protein grains, and the relative growth promoting value of wheat, oats and barley.

(2) secure further information from feed consumption records.

Experiment 1

Grains Used and Methods of Analysis

The grains used, together with their protein content and amino acid composition, are listed in Table I. The methods of analyses employed were the same as those reported by Pethybridge (22).

TABLE I
Amino Acid Content of Grains
(g. per 16 g. nitrogen)

	% N	% Protein	Lysine	Leucine	Isoleucine	Phenylalanine	Valine	Methionine	Arginine	Histidine	Threonine	Total
H.P. Oats	2.99	18.69	3.34	6.28	4.40	4.47	4.97	1.15	6.02	1.45	2.91	34.99
L.P. Oats	1.90	11.88	3.75	6.74	5.08	4.38	5.18	1.33	6.44	1.63	3.01	37.54
H.P. Barley	2.73	17.06	3.10	6.33	4.58	4.96	4.85	1.26	4.72	1.58	3.05	34.43
L.P. Barley	1.73	10.81	3.31	6.57	4.94	4.19	5.04	1.36	5.50	1.69	3.30	35.90
H.P. Wheat	3.52	20.10	2.24	6.04	4.35	4.44	4.14	1.10	4.66	1.48	2.70	31.15
L.P. Wheat	1.84	10.51	2.68	6.13	4.81	4.07	4.34	1.39	5.06	1.82	2.95	33.25

Experimental Animals

Weanling white rats from the University of Alberta, Department of Biochemistry rat colony were used as the test animals. Forty-two male rats were divided into seven groups of six rats each, and were allotted as uniformly as possible with respect to breeding and weight. The rats were put on trial at 21 days of age and were maintained on the experimental ration for 6 weeks.

Test Groups

The test group numbers and the rations fed were as follows:

1. Low Protein Oats.
2. High Protein Oats.
3. Low Protein Barley.
4. High Protein Barley.
5. Low Protein Wheat.
6. High Protein Wheat.
7. Low Protein Barley + 8% Casein.

Rations

With the exception of the ration for group 7 the only source of dietary protein was grain. All rations were supplemented with fat, minerals and vitamins in amounts estimated to be adequate to support normal growth in rats, so that any difference between groups in growth response could be attributed primarily to the differences in the amount, or the amount and quality of protein in the grain used in the rations.

Details of the ration are shown below.

(a) Basal Ration

Basal Mixture for groups 1-6

Grain*	930 g.
Crisco	30 g.
Salt Mixture**	40 g.
Total	1000 g.

Basal Mixture for group 7

Grain*	850 g.
Crisco	30 g.
Vitamin-free Casein	80 g.
Salt Mixture**	40 g.
Total	1000 g.

*All grains were ground in a Wiley mill to pass through a 1 mm. screen mesh.

**McCollum's salt mixture (15)

Calcium lactate	$Ca.[CH_3CH(OH)COO]_2.5H_2O$	352 g.
Calcium phosphate	$Ca(H_2PO_4)_2.H_2O$	146 g.
Potassium acid phosphate	KH_2PO_4	258 g.
Sodium acid phosphate	$NaH_2PO_4.H_2O$	94 g.
Sodium chloride	NaCl	47 g.
Magnesium sulfate	$MgSO_4.7H_2O$	147 g.
Iron citrate		32 g.
		1076 g.

(b) Vitamin Supplements

The following vitamin supplements were added to each kilogram of basal mixture:

Fish oil (2400A - 400D)	4 g.
Thiamine	10 mg.
Riboflavin	20 mg.
Pyridoxine	10 mg.
Pantothenic acid	20 mg.
Nicotinic acid	10 mg.
Choline chloride	1 g.
Biotin	10 μg.

In addition each rat was dosed orally once a week with 2 mg. of alpha tocopherol in soybean oil and 2 drops of fish oil.

Housing, Feeding and Weighing

All rats were housed in individual wire screen cages. Food and water were supplied to each rat *ad libitum* daily. All animals were weighed once a week and daily records were kept of their feed consumption.

Results

The results of this experiment are summarized in Figure 1 and Table II.

TABLE II
Growth Response of Rats Fed Grains
of Different Species and Protein Content

Group	Av. Initial Wt., g.	Av. Final Wt., g.	Av. Daily Gain, g.	Av. Daily Feed Consumption, g.	Feed Consumed /g. gain, g.	Av. Daily N Consumption, g.	Grams N Consumed /gram gain
L.P. Oats	42.6	106.5	1.5	8.0	5.3	0.1226	0.0806
H.P. Oats	42.5	181.6	3.3	12.9	3.9	0.3176	0.0959
L.P. Barley	42.6	103.1	1.4	8.8	6.1	0.1221	0.0848
H.P. Barley	42.5	156.1	2.7	11.9	4.4	0.2690	0.0995
L.P. Wheat	42.5	76.1	0.8	6.7	8.4	0.0990	0.1238
H.P. Wheat	42.4	114.8	1.7	7.8	4.5	0.2233	0.1296
L.P. Barley + 8% casein	42.9	205.2	3.9	12.4	3.2	0.3205	0.0829

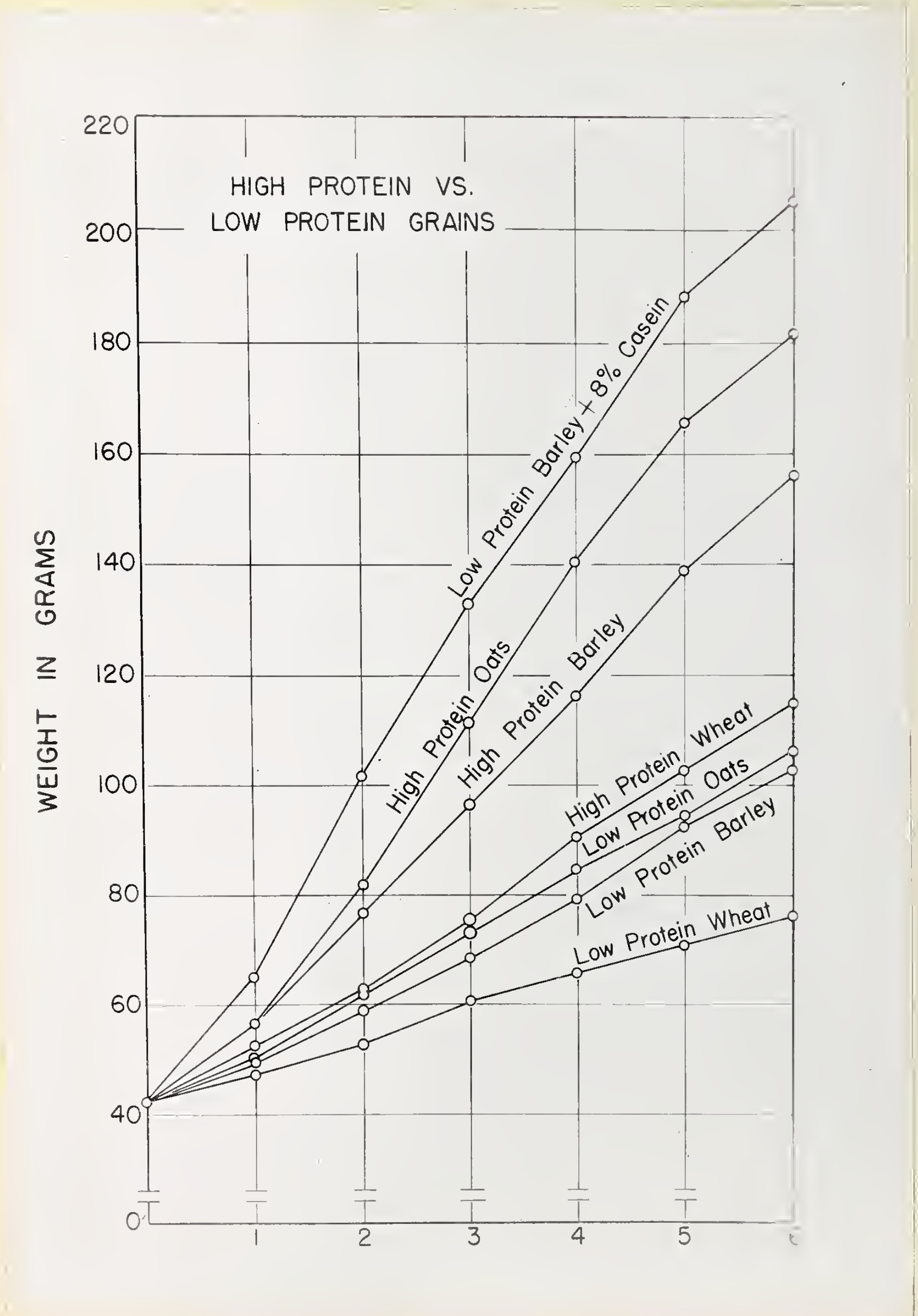

Fig.1. Growth curves of weanling rats fed grains of different species and protein content.

Discussion

The results of this experiment are essentially the same as those secured by McElroy *et al.* (17). As in their experiment, the rate of gain of rats was markedly in favor of the high protein grains. However, while in their experiment rats fed high protein barley made practically as rapid gains as those fed low protein barley + 8% casein, in this experiment rats fed high protein barley or high protein oats gained at a somewhat slower rate.

From an examination of the data in Table II it will be noted that rats fed high protein barley gained at a slower rate than rats fed high protein oats. It will also be noted that rats fed high protein oats consumed more feed daily than rats fed low protein barley + 8% casein or high protein barley. Thus when the rations are compared on the basis of grams nitrogen consumed per gram gain, it will be noted that rats fed low protein barley + 8% casein made the most efficient use of the nitrogen in their ration, while those fed high protein oats or high protein barley consumed approximately the same amount of nitrogen per gram gain. Therefore it would seem from results of this experiment that the ration of low protein barley + 8% casein contained protein of higher quality for the rat than the high protein oat or barley ration, while high protein oats and barley seem to contain protein of approximately the same quality. This is substantiated by the fact that if the quality of the protein as

indicated from results of microbiological assay given in Table I is taken as the criterion it will be noted that high protein oats and barley contain approximately the same total amount of the nine essential amino acids.

The data in Table II also indicate that, although rats fed the rations containing either low protein oats or barley made slower gains, they made more efficient use of the nitrogen in the ration than rats fed rations containing either high protein oats or barley. One explanation of this apparent increase in the efficiency of use of nitrogen may be that the rats fed rations containing low levels of protein used a higher proportion of the dietary protein for growth and a smaller proportion for energy than those fed high protein grains. It is shown in Table I and in the report of McElroy, Clandinin, Lobay and Pethybridge (16) that the quality of the protein in low protein grains may be slightly better than that of high protein grains as measured by lysine content or by content of nine essential amino acids. Better quality of the protein in low protein grains may have contributed to the more efficient utilization of nitrogen observed in the groups fed low protein grains in the present experiments. Conclusions regarding this possibility must await further experimentation.

Comparison of the figures in Table II for average daily gain and nitrogen intake shows that, as would be expected, the rate of gain of rats fed grains of the same species, but of different protein content, tended to be directly proportional

to the nitrogen intake. However, when the results for rats fed wheat are compared with those for rats fed oats or barley, the relationship between daily gains and nitrogen intake is found to be less direct. For example, rats fed high protein wheat consumed 0.2233/0.1226 or 1.82 times as much nitrogen per day as those fed low protein oats, but their rate of gain was only 1.7/1.5 or 1.13 times that of those fed the low protein oats.

In an earlier paper from this laboratory (6) it was reported that oat and barley protein contained 1.4 to 1.5 times as much lysine as wheat protein. It is shown in Table I that similar results were obtained for the grains used in the present experiments. The lysine values listed in Table III were therefore calculated to determine the relationship that existed between lysine intake and daily gains of rats fed wheat as compared to those fed oats or barley.

TABLE III
Lysine Consumption and Growth of Rats
on Grains of Different Species and Protein Content

Ration	Av. Total Lysine Consumption per rat	Daily Gains
	g.	g.
L.P. Oats	1.21	1.5
H.P. Oats	2.78	3.3
L.P. Barley	1.06	1.4
H.P. Barley	2.19	2.7
L.P. Wheat	0.70	0.8
H.P. Wheat	1.31	1.7
L.P. Barley + 8% casein	4.69	3.9

If the data for groups fed high protein wheat and low protein oats are compared on this basis it will be noted that rats fed high protein wheat consumed 1.31/1.21 or 1.08 times as much lysine and grew 1.13 times as fast as those fed low protein oats. These, and other factors that may be derived from the data in Tables II and III, indicate that in feeding trials involving comparisons of wheat with either oats or barley, lysine consumption is likely to give a better indication of growth to be expected than nitrogen consumption.

However, it will be noted from Table II that the rats fed wheat ate considerably less than those fed either oats or barley. These inter-species differences in feed consumption do not appear to be related to either the total nitrogen or total lysine content of the grains. Two possible reasons for the relatively low feed consumption of rats fed wheat are: 1. that wheat is less palatable to rats than either oats or barley, and 2. that the amino acids of wheat protein are less available than those of oats or barley. Since a number of workers have shown that the nutritive value of some proteins can be increased by heating for short periods, the experiments described in Part II were carried out to determine if heat treatment would improve the palatability or increase the nutritive value of wheat.

PART II

EFFECT OF HEAT ON THE NUTRITIVE VALUE OF GRAIN PROTEINS

Literature Review

From a brief review of the literature it was noted that in some cases heating for short periods of time increased the nutritive value of proteins. In 1947 Riesen, Clandinin, Elvehjem and Cravens (23) concluded that the nutritive value of soybeans was increased when they were heated in an autoclave for 4-15 min. at 15 lb. pressure. They concluded from results of microbiological assay that the increase in nutritive value following proper heat treatment was apparently due, not only to the destruction of the trypsin inhibitor but also to an alteration in the protein that made it more readily attacked by proteolytic enzymes.

The decrease in the nutritive value of soybeans by overheating was attributed by the above workers to the destruction of lysine, arginine and tryptophan by the combination of the free carbonyl groups with sugars. Clandinin (5) has also concluded from results of microbiological assays and chick growth experiments that herring meals dried by the flame method at a stack temperature of 220°F. are decidedly inferior in nutritive value to meals dried by the same method at 185°F. Overheating in this case results in decreased liberation of all essential amino acids by enzymatic hydrolysis and a decreased liberation

of lysine, arginine and probably threonine by acid hydrolysis.

Thus to avoid the possibility of decreasing the nutritive value of wheat by overheating it was decided to heat in the autoclave for 8 min. at 15 lb. pressure.

Experiment 2

Experimental Grains and Animals

The low and high protein wheats used were from the same source as those used in Experiment 1. To prepare the heated grain, the wheat was ground coarsely and mixed with 20% H_2O, heated in an autoclave for 8 min. at 15 lb. pressure, dried at 97°F. and reground in the Wiley mill to pass through a 1 mm. mesh screen. The rats used were from the high and low protein wheat groups in Experiment 1. Half the rats from the respective groups were placed on high and low protein heated wheat rations. The remaining rats were maintained as controls on the unheated high and low protein wheat rations. The animals were weighed daily.

Test Groups

Group No.	Ration
1.	High Protein Wheat.
2.	High Protein Wheat (heated).
3.	Low Protein Wheat.
4.	Low Protein Wheat (heated).

Rations

The rations were compounded from grain, Crisco, salt mixture and vitamin supplement as described in Experiment 1.

Results and Discussion

The results of this trial are summarized in Table IV.

From this experiment it would seem that heating the wheat for 8 mins. at 15 lb. pressure has the effect of not only increasing rate of gain but increasing the efficiency of feed utilization. Heating low protein wheat decreased the feed required per gram gain by 14.2%, while heating high protein wheat decreased the feed required per gram gain by 16%. It will also be noted that heating low protein wheat increased daily gain by 29%, while heating high protein wheat increased daily gain by 30%.

As this was merely a pilot experiment, another feeding trial was run to determine if these results were reproducible with weanling rats.

TABLE IV
Growth Response of Rats Fed Heated and Unheated Wheat
(18 day test)

Group	No. on Test	Av. Initial Weight	Av. Final Weight	Av. Daily Gain	Av. Daily Feed Consumption	Feed Consumed /g. gain	Av. Daily N Consumption	G. Nitrogen Consumed /gram gain
		g.	g.	g.	g.		g.	
L.P. Wheat	3	77.6	90.3	0.7	6.8	9.62	0.1005	0.1424
L.P. Wheat (heated)	3	78.8	94.6	0.9	7.1	8.25	0.1054	0.1186
H.P. Wheat	3	119.2	161.2	2.3	10.6	4.54	0.3042	0.1304
H.P. Wheat (heated)	3	116.8	171.4	3.0	11.5	3.80	0.3310	0.1091

Experiment 3

In the preceding experiment using nine week old rats it was noted that rats fed heated wheat made faster gains and required less feed per gram gain than those fed unheated wheat. Therefore the object of this experiment was to repeat Experiment 2 using weanling rats in order to determine if the same results could be obtained.

As a forerunner to future experiments in supplementing with pure amino acids a group was included to determine if the addition of 0.5% animal protein factor concentrate* to the high protein wheat ration would improve the growth of rats or their feed utilization.

Experimental Material

The grain used, the source, allotment, housing and feeding of rats were the same as in Experiments 1 and 2. Rats were weighed twice weekly.

Test Groups

Test groups were designated as follows and were made up of 4 male weanling rats each, allotted as uniformly as possible with respect to breeding and weight.

1. Low Protein Wheat
2. Low Protein Wheat (heated)
3. High Protein Wheat
4. High Protein Wheat (heated)
5. High Protein Wheat + 0.5% A.P.F.

*Furnished through the courtesy of Merck and Co. Ltd.] Montreal.

Results and Discussion

The results of this trial are summarized in Table V.

From the data given in Table V it will be noted that the differences between groups fed heated and unheated wheat were not as great as in Experiment 2. In the case of low protein wheat there were no marked differences between groups fed heated and unheated wheat as far as daily gains and feed consumption were concerned. It should be noted that in this experiment heating low protein wheat decreased the feed required per gram gain by only 6.9%, while in the preceding experiment it was decreased 14.2%. In this experiment heating increased the efficiency of use of nitrogen only 6.7% while in the preceding experiment nitrogen efficiency was increased by 16.7%. Thus it would appear that heating low protein wheat causes only a small increase in its nutritive value for weanling rats. An inadequate amount of protein in the low protein wheat ration is probably the first limiting deficiency.

TABLE V
Growth Response of Weanling Rats Fed Heated and Unheated Wheat
(28 day test)

Group	Av. Initial Weight	Av. Final Weight	Av. Daily Gain	Av. Daily Feed Consumption	Feed Consumed /g. gain	Av. Daily N Consumption	Grams N Consumed /gram gain
	g.	g.	g.	g.	g.	g.	
L.P. Wheat	43.5	71.0	1.0	7.1	7.28	0.1057	0.1076
L.P. Wheat (heated)	44.4	72.7	1.0	6.9	6.78	0.1014	0.1004
H.P. Wheat	45.0	115.4	2.5	10.3	4.11	0.2868	0.1180
H.P. Wheat*(heated)	47.6	134.8	3.1	11.5	3.69	0.3296	0.1058
H.P. Wheat + A.P.F.	44.2	116.9	2.6	10.7	4.11	0.3061	0.1179

*Four rats were included in the original group but one "runty" rat was discarded.

It will also be noted from Table V that, although the differences between the groups fed high protein wheat, heated and unheated, were not as great as in Experiment 2, heating high protein wheat increased daily gains by 24%, decreased feed required per gram gain by 10% and increased nitrogen efficiency by 10 per cent.

From comparison of results obtained in the groups fed high protein wheat and high protein wheat + A.P.F. it will be noted that the addition of A.P.F. to the ration did not bring about any real improvement in rate of gain or efficiency of utilization of feed or nitrogen. As female rats in the Biochemistry Department are maintained on Purina Fox Chow which is supplemented with A.P.F. and also contains fish and meat meal, the weanling rats might be expected to have a reserve store of the animal protein factor.

Experiment 4

From previous experiments it was noted that rats fed wheat as the only source of protein in an otherwise nutritionally complete diet made much slower gains than rats fed oats or barley of a comparable protein content. It was also noted that if rats were fed heated wheat not only was their rate of gain increased but they required less feed per gram gain.

Although in previous experiments unheated oats and barley gave good growth, the results obtained with heated

wheat suggested the desirability of studying the effect of heat treatment on coarse grains. If the nutritional quality of these grains could be shown to be improved by heating it would be necessary to consider the advisability of heating barley for subsequent experiments involving amino acid supplementation of low protein barley. As both rats and cage space were limited it was decided to use only high protein wheat, oats and barley, as the effect of heating might be expected to be more pronounced with a high protein grain than with a low protein grain.

Experimental Method

The grain used, the source, allotment, housing, weighing and feeding of rats were the same as in Experiment 3. The wheat, oats and barley were heated in the same manner as the wheat in Experiment 2.

Test Groups

Test groups were designated as follows and were made up of 6 male rats each allotted as uniformly as possible with respect to breeding and weight.

1. High Protein Wheat.
2. High Protein Wheat (heated).
3. High Protein Oats.
4. High Protein Oats (heated).
5. High Protein Barley.
6. High Protein Barley (heated).

Results and Discussion

Results of this experiment are summarized in Table VI.

TABLE VI
Growth Response of Rats Fed Heated and Unheated Wheat, Oats and Barley
(28 day test)

Group	Av. Initial Weight	Av. Final Weight	Av. Daily Gain	Av. Daily Feed Consumption	Feed Consumed /g. gain	Av. Daily N Consumption	Grams N Consumed /gram gain
	g.	g.	g.	g.	g.	g.	
H.P. Wheat	46.6	117.7	2.5	9.7	3.81	0.2774	0.1092
H.P. Wheat (heated)	46.5	143.6	3.5	12.0	3.45	0.3432	0.0990
H.P. Oats	46.2	132.6	3.1	12.1	3.91	0.2978	0.0965
H.P. Oats (heated)	46.1	142.7	3.5	13.6	3.93	0.3352	0.0972
H.P. Barley	46.4	135.8	3.2	12.7	3.98	0.2886	0.0904
H.P. Barley (heated)	46.2	130.7	3.0	12.2	4.04	0.2764	0.0916

From an examination of the data given in Table VI it will be noted that heating oats or barley did not increase rate of gain, efficiency of utilization of feed or nitrogen to any appreciable extent. This is in agreement with results obtained by Stewart, Hensley and Peters (29). They concluded from gain in weight of rats per gram protein consumed that ordinary household preparation of oatmeal, precooking of oat flour by drum dried process and toasting of extruded oat flour did not impair protein quality. Although their heat treatment was more severe than the method used in this experiment, still no damage was done.

However, it will be noted that, as in previous experiments, rats fed heated wheat grew faster and required somewhat less nitrogen per gram gain than did rats fed the unheated wheat. In order to determine if the difference in nitrogen utilization of rats was significant a statistical analysis was run according to Goulden (6), using the t test and pairing, as litter mates were being used. By this method of analysis the difference in nitrogen utilization of rats fed the heated and unheated wheat was significant at the 5% level. That is, the probability that such a difference would occur by chance alone is less than five times out of a hundred. Thus it can be concluded from results obtained in three experiments and summarized in Table VII, that high protein wheat heated in an autoclave for 8 min. at 15 lb. pressure with 20% water added has a higher nutritional value for the rat than unheated wheat.

TABLE VII
Summary of Effects of Heat on High Protein Wheat.

Experiment	% Increase in Daily Gain	% Decrease in grams N Consumed/g. gain
2	30	16.2
3	24	10.0
4	40	9.3

It will also be noted from data given in Table VI that rats fed unheated high protein wheat gained faster and made more efficient use of the nitrogen in the ration than did rats fed unheated high protein wheat in Experiment 1. In order to determine if the differences indicated by the results of Experiment 4 for the nutritional value of unheated wheat as compared to oats or barley were significant, a statistical analysis was run according to Goulden (6) using a complex randomized block type of experiment. Results of analysis, given in Table VIII, indicate that high protein wheat had a significantly lower nutritional value than oats or barley when compared on a basis of grams nitrogen consumed per gram gain and, as in Experiment 1, high protein oats and barley were of about the same nutritional value.

TABLE VIII
Complex Analysis of Variance
for Grams Nitrogen Consumed per Gram Gain
by Rats Fed Heated and Unheated
Wheat, Oats and Barley

Variance due to	D.F.	Sum Square	Mean Square	F
Species	2	0.0015403	0.0007702	4.37*
Treatment	1	0.0001928	0.0001928	0.055
Species x treatment	2	0.0005123	0.0002062	1.17
Replicates in general	10	0.0015856	0.0001586	-
Error	20	0.0035279	0.0001764	-
Total	35	0.0073589	-	-

*Significant at 5% level.

Minimum Significant Difference for Species is 0.0108 g. N per g. gain.

Results of this experiment indicate that when, as a result of heating the grain, consumption of wheat is increased to that of oats or barley, rate of gain and efficiency of nitrogen utilization of rats fed wheat is equal to that of rats fed oats or barley. An attempt to determine, by the use of a restricted feed intake technique, whether this increase in consumption was due entirely to increase in palatability, or whether heating caused some change in composition that improved protein quality, was the object of the next experiment.

Experiment 5

The objects of this experiment were:

1. To determine whether the apparent increase in the nutritive value of high protein wheat after heating for 8 min. at 15 lb. pressure is due to an increase in palatability, or whether it is due to some change in the protein.

2. To determine the effect of lysine supplementation under restricted feed intake conditions.

Experimental Method

The grain used and the heat treatment were the same as in preceding experiments. Inasmuch as the animals fed the diet containing unheated wheat consumed the least food, rats on the other diets were pair fed with respect to this group. In making up pairs special attention was given to weight, sex and breeding.

Lysine was added in an amount calculated to be equivalent to that supplied by 8% casein.

Test Groups

Test groups were designated as follows and were made up of 8 rats each.

1. High Protein Wheat.
2. High Protein Wheat (heated).
3. High Protein Wheat + Lysine.

Results and Discussion

The results of this feeding trial are set forth in Table IX.

TABLE IX
Growth Response of Rats Fed Heated and Unheated Wheat and Wheat Supplemented with Lysine
(21 day test)

Group	Av. Initial Weight	Av. Final Weight	Av. Daily Gain	Av. Daily Feed Consumption	Feed Consumed /g. gain	Av. Daily N Consumption	Grams N Consumed /gram gain
	g.	g.	g.	g.	g.	g.	
1. H.P. Wheat	43.6	103.4	2.9	10.7	3.75	0.3063	0.1076
2. H.P. Wheat (heated)	43.7	100.6	2.7	10.1	3.72	0.2890	0.1067
3. H.P. Wheat + lysine	43.6	116.0	3.5	10.2	2.95	0.2920	0.0847

From an examination of the data given in Table IX it will be noted that when the feed intake of rats fed heated wheat was restricted to that of rats fed unheated wheat, heating did not increase rate of gain or nitrogen utilization. Therefore it can be concluded that heating wheat in an autoclave at 15 lb. pressure for 8 min. simply causes some change which increases palatability and there is no change in the protein which makes it more nutritious. Thus the increase in utilization of nitrogen obtained by heating wheat in previous experiments must be the result of increased consumption.

It was noted that rations containing heated wheat were much easier to mix than rations containing unheated wheat, as the grain was more granular in texture and did not become gummy when the vitamin solution or choline solution was added. This may be one reason for the increase in palatability as heated wheat may be easier for the rat to eat than unheated wheat.

Results summarized in Table IX also indicate that addition of lysine to high protein wheat using a restricted food intake technique increased gain and nitrogen utilization. Thus it can be concluded that supplementation of wheat with lysine increases its nutritive value. Mitchell and Smuts (20) demonstrated as early as 1932 that supplementation of wheat with lysine gave a large increase in growth.

PART III

SUPPLEMENTATION OF GRAIN WITH AMINO ACIDS

Literature Review

From early experiments comparing the growth promoting value of grains it was noted that grains lacked certain amino acids and beneficial effects could be obtained by supplementing grain with these amino acids. This was first demonstrated in the classical experiments of Osborne and Mendel (21) who demonstrated that gliadin and zein are nutritionally inadequate for the rat but may be rendered satisfactory by the addition of missing amino acids, lysine in the case of gliadin, tryptophan and lysine in the case of zein. Since that time many more attempts have been made to supplement proteins with their missing amino acids. In 1932 Mitchell and Smuts (20) demonstrated that the proteins of lean beef and soybeans are deficient in cystine and that supplementation with cystine considerably improved their growth promoting value. Hoagland *et al.* (10) have shown that beef protein is deficient only in cystine and methionine, and when it is supplemented with 0.2-0.4% of either, the growth promoting value is equal to that of egg protein. Mitchell and Smuts (20) also demonstrated that supplementation of wheat with lysine gave a large increase in growth and that supplementation of corn and oats with lysine resulted in a small but distinct increase in growth. They concluded that

in these cases a second amino acid deficiency apparently develops after the addition of a minimum proportion of lysine. In the case of corn the second limiting amino acid is tryptophan. Jeppeson and Grau (11) have shown that a diet for chicks, in which all of the crude protein was provided by a concentrate of whole wheat protein, supplied adequate amounts of arginine, leucine, methionine and tryptophan, but that a lysine supplement was required to promote optimum growth.

Thus for many years it has been known that grains and other proteins lack certain amino acids, but it was not until recent years, after the development of the microbiological assay method, that the amino acid composition of many proteins was determined and could be used as a guide in supplementing proteins with the required amino acids.

Supplementation of proteins with amino acids was also hindered by the fact that the amino acid requirements of the rat, chick, dog and human were not known. However, since purified rations containing all the essentials necessary to support normal growth in experimental animals and humans have been developed, a great deal of research has been reported on the amino acid requirements of these animals - rat, Rose (24); chicken, Almquist (1), Hegsted (9); dog, Rose and Rice (27); moûse, Bauer and Berg (2) and man, Rose (25).

With information now available on dietary amino acid requirements and amino acid composition of food proteins, attempts are being made to supplement proteins with pure amino acids and in doing so a great deal of information is being

obtained on the metabolic interrelationships of amino acids. Hankes, Henderson, Brickson and Elvehjem (7), in supplementing a 9% casein ration with amino acids, have discovered that the addition of dl-threonine or dl-phenylalanine in amounts present in 2% acid hydrolyzed casein aggravates a niacin-tryptophan deficiency which is reversed by the addition of niacin or tryptophan. From later experiments (8) they concluded that a tryptophan deficiency accompanied by an adequate or generous intake of threonine and cystine is much more serious than when these amino acids are supplied at a low level. They concluded that whether similar results can be obtained with other proteins limiting in tryptophan and other amino acids remains to be seen.

Results of experiments conducted at the University of Alberta in 1946 and 1947 by Lobay (13) showed that rats fed low protein barley supplemented with lysine made more rapid gains and required less feed per gram gain than did control rats fed the unsupplemented basal ration, but supplementation with tryptophan or a mixture of methionine, threonine, valine and tryptophan in addition to lysine, failed to bring about any further improvement in rate of growth. Experiments were discontinued at that time until fundamental information regarding the amino acid composition of grains could be obtained. As these data are now available (Table I) the following experiments were conducted to reinvestigate the problem of supplementing grains with pure amino acids.

Experiment 6

The calculated amounts of ten essential amino acids supplied by a daily intake of 10 g. of barley are listed in Table X. Tryptophan values were calculated from the data of Baumgarten, Mather and Stone (3); values for the other nine amino acids were taken from Table I. Study of this table suggests that the amino acids most likely to be limiting in low protein barley are: methionine, histidine, tryptophan, lysine and phenylalanine. It will be noted, however, that arginine is the only amino acid supplied in an amount equivalent to that recommended by Rose (26).

TABLE X
Determination of the Degree to which Low Protein Barley fulfills the Requirement of Amino Acids for the Rat

	Barley mg./10 g.	Daily Requirement, Rose (26) mg.	% "Requirement" supplied by 10 g. barley
Lysine	31.9	120	26.6
Leucine	63.4	120	52.9
Isoleucine	47.7	80	59.6
Phenylalanine	40.4	120	33.7
Valine	48.6	100	48.6
Methionine	13.1	80	16.4
Arginine	53.3	40	113.3
Histidine	16.3	70	23.3
Threonine	31.8	70	45.4
Tryptophan	9.6	40	24.0

From Table X it would seem that one possible explanation

of why Lobay (13) failed to get any improvement in growth on addition of tryptophan or a mixture of methionine, threonine, tryptophan and valine, in addition to lysine, may be that some amino acid other than those added was limiting; e.g. histidine or phenylalanine. Thus the object of this experiment was to compare the growth response of rats fed low protein barley plus lysine plus either histidine or histidine and phenylalanine.

Experimental Material

The low protein barley used was from the same source as that used in Experiment 1. The source, allotment, housing and method of feeding of rats were the same as in previous experiments, and the rats were weighed twice weekly.

Test Groups

Test groups were designated as follows and were made up of 4 rats each allotted as uniformly as possible with respect to breeding and weight.

1. Low Protein Barley + 8% casein.
2. Low Protein Barley + lysine.
3. Low Protein Barley + lysine + histidine.
4. Low Protein Barley + lysine + histidine + phenylalanine.

Rations

The rations were compounded from grain, Crisco, salt mixture and vitamin supplement as described in Experiment 1. The values reported by Block and Mitchell (4) were used to estimate the amounts of each essential amino acid supplied by 8% casein. Rose (24), on investigating the nutritive value of the optical isomers of the essential amino acids, found that

only the naturally occurring forms of valine, leucine, isoleucine, lysine and threonine were utilized, whereas both isomers of methionine, tryptophan and phenylalanine were effective. The amino acid isomers used and the quantities added to the basal ration to make 1 kilogram are shown in Table XI. The amino acids were finely ground in a mortar and were mixed thoroughly with the basal ration.

TABLE XI
Isomeric Forms and Amounts of Amino Acids Added to the Basal Ration.

Amino Acid	Natural Isomer	Amt. Calculated equiv. 8% casein grams	Isomer Used	Amount added grams
Lysine	l(+)	6.32	l(+)***	7.90
Leucine	l(-)	7.92	l(-)	7.92
Isoleucine	l(-)	5.20	dl**	10.40
Phenylalanine	l(-)	4.48	dl*	4.48
Valine	l(+)	5.36	dl**	10.72
Methionine	l(-)	2.80	dl*	2.80
Arginine	l(+)	3.36	l(+)***	4.06
Histidine	l(-)	2.4	l(+)***	2.96
Threonine	d(-)	3.28	dl**	6.56
Tryptophan	l(-)	0.96	dl*	0.96

*Unnatural isomer can replace the natural form.
**Unnatural isomer cannot replace the natural form.
***Monohydrochloride.

Results and Discussion

The results of this trial are summarized in Table XII.

TABLE XII
Growth Response of Rats Fed the Basal Ration
Supplemented with Amino Acids
(14 day test)

Group and Supplement	Av. Initial Weight	Av. Final Weight	Av. Daily Gain	Av. Daily Consumption	Feed consumed /gram gain
	g.	g.	g.	g.	g.
1. casein	49.0	123.5	5.3	12.9	2.42
2. lysine	47.4	88.4	2.9	11.3	3.85
3. lysine + histidine	48.0	84.1	2.6	10.3	4.01
4. lysine + histidine + phenylalanine	48.8	83.1	2.5	10.4	4.25

From an examination of the data in Table XII it may be noted that supplementation of low protein barley with lysine and histidine or lysine, histidine and phenylalanine was of no greater value in promoting growth than supplementation with lysine alone. Similar results were obtained by Lobay (13) when he supplemented L.P.B. with tryptophan or tryptophan, methionine, threonine and valine in addition to lysine. He concluded that such supplementation of L.P.B. had a slight inhibitory effect but whether or not an actual growth inhibition occurred in either the present experiment or the earlier trial conducted by Lobay cannot be stated without repeating the experiments with larger numbers of rats.

As, at the end of two weeks, no differences in growth were being obtained, it was decided that a better approach would be to supplement barley with the amino acids in which it is most lacking and then attempt to determine which amino acids were giving the increase in growth response. Thus to the ration of half the rats on the low protein barley + lysine + histidine + phenylalanine, methionine and tryptophan were added to give a group receiving a ration in which the low protein barley was supplemented with the five amino acids in which it is calculated to be most lacking (Table X). Also, half the rats on the low protein barley + lysine + histidine ration were placed on a ration containing low protein barley supplemented with these five amino acids plus threonine and valine. From the data in Table X, these seven appear to be the amino acids most likely to be limiting in

barley.

Thus, the following 6 groups of rats were made up from the four original groups of Experiment 6.

1.	Low Protein Barley	+ casein				
2.	" " "	+ lysine				
3.	" " "	+ lysine	+ histidine			
4.	" " "	+ "	+ "	+ phenylalanine		
5.	" " "	+ "	+ "	+ "	+ methionine	+ tryptophan
6.	" " "	+ as 5	+ threonine	+ valine.		

Results and Discussion

The results of this trial are summarized in Table XIII.

From a comparison of the data in Tables XII and XIII it may be noted that supplementation with 5 amino acids failed to bring about any improvement in rate of growth or economy of feed utilization. However, the addition of the mixture containing 7 amino acids (group 6) appeared to promote more rapid growth and efficient use of feed.

TABLE XIII
Growth Response of Rats Fed the Basal Ration
Supplemented with Amino Acids
(7 day test)

Group and Supplement	No. of Rats	Av. Initial Weight	Av. Final Weight	Av. Daily Gain	Av. Daily Consumption	Feed consumed /gram gain
		g.	g.	g.	g.	g.
1. casein	4	123.5	157.2	4.8	17.8	3.71
2. lysine	4	88.4	106.0	2.5	14.0	5.56
3. lysine + histidine	2	80.6	97.8	2.5	13.7	5.57
4. lysine + histidine + phenylalanine	2	83.8	101.0	2.5	13.4	5.45
5. 5 E.A.A.*	2	82.3	97.4	2.2	12.6	5.83
6. 7 E.A.A.**	2	87.6	111.4	3.4	12.8	3.76

*lysine + histidine + phenylalanine + methionine + tryptophan
**lysine + histidine + phenylalanine + methionine + tryptophan + valine + threonine.

Experiment 7

Supplementation of low protein barley with lysine, histidine, phenylalanine, methionine, tryptophan, valine and threonine was found to increase the rate of gain and decrease feed consumed per gram gain in the preceding experiment. Experiment 7 was set up with the object of determining whether the growth stimulating effect of the above seven amino acids could be duplicated with weanling rats. In addition the experiment was designed to study the effect of adding (a) leucine, (b) isoleucine, and (c) leucine and isoleucine to the above mixture of seven amino acids.

Experimental Material

The grain used, the source, allotment, housing, feeding and weighing of rats were the same as in Experiment 6. Amino acids were added in an amount calculated to be equivalent to that supplied by 8% casein as set forth in Table XI.

Test Groups

Test groups were designated as follows and were made up of three male rats each.

1. Low Protein Barley + casein
2. " " " + lysine
3. " " " + 10 essential amino acids.
4. " " " + lysine + histidine + phenylalanine + methionine + tryptophan + valine threonine
5. As 4 + leucine
6. As 4 + isoleucine
7. As 4 + leucine + isoleucine
8. Low Protein Barley.

Results and Discussion

The results obtained over a 21-day period are summarized in Table XIV.

Supplementation of low protein barley with the ten essential amino acids was almost as effective as supplementation with 8% vitamin-free casein as measured by the growth rate and economy of feed utilization of the rats in groups 1 and 3. Similar results were reported by Lobay (13), who found that the growth of rats supplemented with 10 essential amino acids was 95% of that of control rats supplemented with 8% casein. The corresponding figure for the present experiment was 88 per cent. If the ration of low protein barley + casein is assigned a nutritional value of 100 the remaining rations could be evaluated as shown below.

Low Protein Barley	+ casein	100
" " "	+ 10 E.A.A.	88.4
" " "	+ 7 E.A.A. + leucine + isoleucine	86.3
" " "	+ 7 E.A.A.	68.2
" " "	+ 7 E.A.A. +isoleucine	55.6
" " "	+ lysine	48.2
" " "		38.1
" " "	+ 7 E.A.A. + leucine	26.6

Supplementation of low protein barley with lysine increased the gain significantly, but the increase was not as great as that reported by Lobay (13). In this experiment addition of lysine gave a 10 point increase over the basal diet while Lobay (13) reported a 17 point increase. It should also be noted that addition of lysine decreased feed required per gram gain by 13% as compared to 16% in Lobay's experiments.

TABLE XIV
Growth Response of Rats Fed the Basal Ration
Supplemented with Amino Acids
(21 day test)

Group and Supplement	Av. Initial Weight	Av. Final Weight	Av. Daily Gain	Av. Daily Consumption	Feed Consumed /gram gain
	g.	g.	g.	g.	g.
1. casein	41.6	138.1	4.6	14.2	3.08
2. lysine	41.3	87.8	2.2	10.9	4.91
3. 10 E.A.A.	41.6	126.9	4.1	12.8	3.15
4. 7 E.A.A.	41.5	107.3	3.1	11.4	3.63
5. as 4 + leucine	41.0	66.7	1.2	7.1	5.77
6. as 4 + isoleucine	41.9	95.6	2.6	9.9	3.88
7. as 4 + leucine + isoleucine	42.6	125.9	4.0	12.2	3.08
8. L.P.B.	42.4	79.2	1.8	9.9	5.63

Supplementation of low protein barley with 7 E.A.A. (group 4) increased gain and efficiency of food utilization significantly and it can therefore be concluded that the growth stimulating effect of these 7 E.A.A., observed in the previous experiment, was real. Although lysine accounts for 10 of the 30 point increase it will remain for future experiments to determine which of the other six contribute most to the remaining 20 point increase.

It should also be noted from Table XIV that the performance of rats supplemented with 9 amino acids (arginine omitted, group 7) was essentially the same as that of rats supplemented with 10 amino acids (group 3). This substantiates the results of microbiological assays (Table I and X) which show that barley is relatively rich in arginine.

The apparent inhibitory effect of leucine and isoleucine in the absence of one or the other (groups 5 and 6) is difficult to explain. It will be noted that the addition of leucine to the ration containing L.P.B. + 7 E.A.A. caused a decrease in growth of 41.6 points, while addition of isoleucine to the 7 E.A.A. depressed growth 12.6 points. However, the addition of both leucine and isoleucine to the 7 E.A.A. resulted in an increase in growth of 18.1 points (Group 7).

Experiment 7A

At the end of 21 days groups 2, 3 and 8 were taken off trial. The remaining groups were maintained for 7 days to determine if removal of leucine or isoleucine from the diet

would result in any change in rate of growth, and to determine if addition of leucine or isoleucine to the ration containing 7 E.A.A. would depress growth. The rations for groups 1, 4, 5, 6 and 7 were therefore changed as follows:

Experiment 7	Experiment 7A
1. L.P.B. + casein	1. L.P.B. + casein + leucine
4. L.P.B. + 7 E.A.A.	4. L.P.B. + 7 E.A.A. +leucine
5. L.P.B. + 7 E.A.A. + leucine	5. L.P.B. + 7 E.A.A.
6. L.P.B. + 7 E.A.A. + isoleucine	6. L.P.B. + 7 E.A.A.
7. L.P.B. + 7 E.A.A. + leucine + isoleucine	7. L.P.B. + 7 E.A.A. + isoleucine.

Results and Discussion

The results of this trial are summarized in Table XV.

From this experiment it can be concluded that the addition of leucine or isoleucine singly to the low protein barley + 7 E.A.A. has an inhibitory effect on growth which can be overcome by removing them from the ration. The effect of adding and removing leucine or isoleucine to and from the diet of the same rats is set forth in Table XVI and Figure 2.

TABLE XV
Growth Response of Rats After Addition and Removal of Leucine or Isoleucine from Diet of L.P.B. + 7 E.A.A.
(7 day test)

Group and Supplement	No. of Rats	Av. Initial Weight	Av. Final Weight	Av. Daily Gain	Av. Daily Consumption	Feed Consumed /gram gain
		g.	g.	g.	g.	g.
1. casein + leucine	3	138.1	175.3	5.3	18.6	3.51
4. 7 E.A.A.+ leucine	3	107.3	114.0	1.0	10.7	11.13
5. 7 E.A.A.	3	66.7	94.8	4.0	11.5	2.86
6. 7 E.A.A.	3	95.6	122.7	3.9	14.1	3.65
7. 7 E.A.A.+ isoleucine	3	125.9	146.0	2.9	15.0	5.23

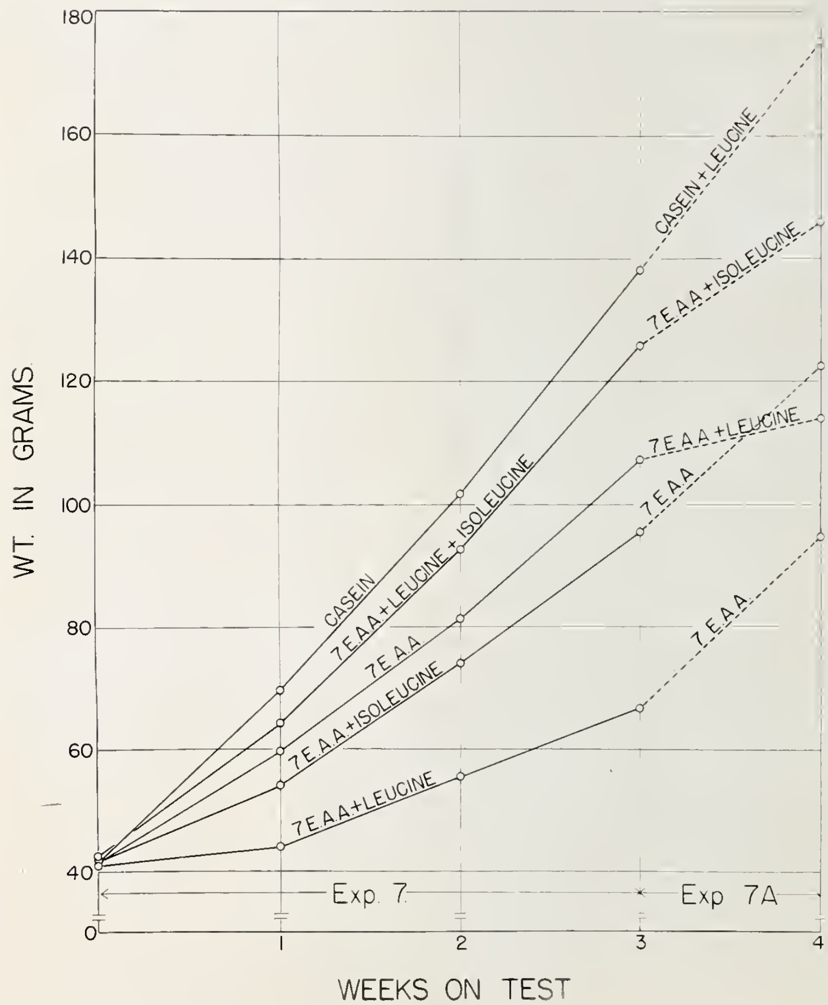

Fig.2. Growth curves of rats on addition and removal of leucine or isoleucine to or from the diet.

TABLE XVI
Effect of Addition and Removal
of Leucine or Isoleucine from Diet
of Low Protein Barley + 7 E.A.A.

Group and Supplement	Daily Gain	Daily Feed Consumption	Feed Consumed per gram gain
	g.	g.	g.
Effect of removing leucine:			
5. 7 E.A.A. + leucine Table XIV	1.2	7.1	5.77
5. 7 E.A.A. Table XV	4.0	11.5	2.86
Effect of adding leucine:			
4. 7 E.A.A. Table XIV	3.1	11.4	3.63
4. 7 E.A.A. + leucine Table XV	1.0	10.7	11.13
Effect of removing isoleucine:			
6. 7 E.A.A. + isoleucine Table XIV	2.6	9.9	3.87
6. 7 E.A.A. Table XV	3.9	14.1	3.65
Effect of adding isoleucine:			
7. 7 E.A.A. + leucine +isoleucine Table XIV	4.0	12.2	3.08
7. 7 E.A.A. + isoleucine Table XV	2.9	15.0	5.23

From the data given in Table XVI and Figure 2 it will be noted that when leucine was removed from the ration gains increased from 1.2 to 4.0 g. daily and feed required per gram gain decreased from 5.77 g. to 2.86 grams. Addition of leucine to the ration of rats previously receiving low protein barley + 7 E.A.A. decreased daily gain from 3.1 to 1.0 grams and increased feed required per gram gain from 3.63 to 11.13 grams.

Removal of isoleucine from the ration had a similar effect although not as marked. When isoleucine was removed

from the ration (Table XVI and Figure 2) daily gains increased from 2.6 to 3.9 grams. As daily feed consumption also increased the difference in economy of gain was not very large. Feed required per gram gain decreased from 3.87 to 3.65 grams. Similarly feeding a ration containing low protein barley + 7 E.A.A. + isoleucine decreased daily gains from 4.0 to 2.9 g. and increased feed required per gram gain from 3.08 to 5.23 grams.

Supplementation of low protein barley + 8% casein with leucine had no effect on gain or economy of utilization of feed. This was to be expected as the inhibitory effect of leucine was noted only when isoleucine was not added. As sufficient isoleucine to meet the requirements of growth was present in the casein supplement, addition of leucine had no effect.

Why the addition of leucine or isoleucine singly to the 7 E.A.A. should have an inhibitory effect on growth is difficult to explain. It seems that leucine and isoleucine should be added either together or not at all. Applying the theory of Hankes, Henderson and Elvehjem (8) it would seem that the addition of leucine to such a mixture causes a marked increase in the requirement for isoleucine with consequent growth retardation, while addition of isoleucine causes an increase in the requirement for leucine.

Experiment 8

As only a limited number of rats were available at this time, it was decided to duplicate Groups 4 and 5 of Experiments 7 and 7A to obtain additional data on the apparent inhibitory effect of supplementing a ration of low protein barley plus 7 E.A.A. with leucine.

Experimental Material

The grain used, amino acid supplementation, source, allotment, housing, feeding and weighing of rats were the same as in Experiments 7 and 7A.

Test Groups

Test groups for the two periods were designated as follows and were made up of 4 rats each.

First Period

1. Low Protein Barley + 7 E.A.A.*
2. " " " + " + leucine

Second Period

1. Low Protein Barley + 7 E.A.A. + leucine
2. " " " + "

*7 E.A.A. - lysine, histidine, phenylalanine, methionine, tryptophan, valine, threonine, in an amount equivalent to that supplied by 8% casein as set forth in Table XI.

Results

Results of this trial are summarized in Table XVII.

TABLE XVII
Growth Response of Rats on Addition or Removal of Leucine to Diet of L.P.B. + 7 E.A.A.

Group and Ration	Days on Trial	Av. Initial Weight g.	Av. Final Weight g.	Av. Daily Gain g.	Av. Daily Consumption g.	Feed Consumed /gram gain g.
1. L.P.B. + 7 E.A.A.	18	38.8	90.8	2.9	9.6	3.31
1. L.P.B. + 7 E.A.A. + leucine	6	90.8	94.2	0.6	9.8	17.3
2. L.P.B. + 7 E.A.A. + leucine	18	39.6	54.4	0.8	6.1	7.41
2. L.P.B. + 7 E.A.A.	7	54.4	84.2	4.3	11.5	2.7

From the data presented in Table XVII it will be noted that as in Experiment 7 and 7A addition of leucine to the supplement of 7 E.A.A. dedreased rate of growth of rats and increased feed required per gram gain, while removal of leucine increased rate of growth and efficiency of utilization of feed.

Hankes, Henderson, Brickson and Elvehjem (7) concluded that the addition of dl-phenylalanine or dl-threonine, in amounts equivalent to those present in 2% acid hydrolyzed casein, to a diet containing 9% casein and .2% l-cystine, aggravated a niacin-tryptophan deficiency. In a later paper from the same laboratory (8) it was concluded that l-threonine and d-phenylalanine are the isomers responsible for the growth inhibitory effect.

Although six out of ten of the amino acids used in the present study were fed in racemic form, good growth was obtained on supplementation of L.P.B. with 10 E.A.A. However, the possibility exists that unnatural forms may have an inhibitory effect when fed in incomplete mixtures. For example , Hankes, Henderson and Elvehjem (8) found that if adequate tryptophan or niacin was present dl-phenylalanine had no toxic effect. However, if adequate niacin or tryptophan was not present addition of d-phenylalanine caused growth inhibition, while addition of l-phenylalanine had no such inhibitory action. Thus, the relatively poor growth obtained with a number of the incomplete amino acid mixtures used in the present study (Expts. 6, 7, 7A and 8) may be attributable to inhibitory effects of unnatural isomers when fed in such combinations, or possibly,

simply to an increased requirement for a limiting amino acid when others are supplied in adequate amounts.

Experiment 9

Tuba, Cantor and Richards (30) concluded from experiments using weanling rats, that supplementation of L.P.B. with individual amino acids (lysine, histidine, phenylalanine, methionine, tryptophan, valine, threonine and leucine) produced no marked improvement in growth and in some cases, notably with phenylalanine and tryptophan, there seemed to be a retardation in growth. Lobay (13), using the same barley, obtained faster growth on his basal ration and a greater stimulation from lysine supplementation than did Tuba, Cantor and Richards. The differences may be attributable to the fact that Lobay used a basal ration containing considerably less fat than that employed by Tuba, Cantor and Richards.

The purpose of Experiment 9 was to determine the effect of supplementation of L.P.B. with individual amino acids using a diet containing 30 grams Crisco and 4 grams fish oil per kilogram.

Experimental Material

As for previous experiments.

Test Groups

Test groups were designated as follows and were made up of three rats each.

1. L.P.B.
2. " + lysine
3. " + histidine
4. " + phenylalanine
5. " + tryptophan
6. " + methionine
7. " + threonine
8. " + valine
9. " + leucine
10. " + isoleucine

Results and Discussion

Results of this trial are summarized in Table XVIII.

From the data given in Table XVIII it will be noted that supplementation with lysine increased growth and utilization of feed to the same extent as reported in Experiment 7. However, it will be noted that supplementation of L.P.B. with the other 8 amino acids singly did not increase the rate of growth. These results suggest that lysine is the first limiting amino acid in barley protein, so that in the absence of supplemental lysine, addition of other amino acids singly is not beneficial. Additional experiments would be required to show whether the decreased rate of growth observed in the groups supplemented with histidine and phenylalanine is significant.

It should also be noted that supplementation of the basal low protein barley ration simply with leucine or isoleucine did not cause any decrease in rate of growth. These results are in contrast to those obtained in Experiments 7, 7A and 8, which indicate that the addition of leucine or isoleucine to a supplement consisting of 7 essential amino acids inhibits growth.

TABLE XVIII
Growth Response of Rats Fed Low Protein Barley
Supplemented with Individual Amino Acids
(21 day test)

Ration	Av. Initial Weight g.	Av. Final Weight g.	Av. Daily Gain g.	Av. Daily Consumption g.	Feed Consumed / gram gain g.
L.P.B.	44.8	83.3	1.8	10.5	5.74
L.P.B. + lysine	41.3	88.1	2.2	10.3	4.60
L.P.B. + histidine	44.8	79.1	1.6	10.0	6.12
L.P.B. + phenylalanine	46.4	79.0	1.6	9.1	5.89
L.P.B. + tryptophan	45.2	83.1	1.8	10.4	5.78
L.P.B. + methionine	45.1	83.8	1.8	10.2	5.53
L.P.B. + threonine	44.7	81.5	1.8	9.4	5.38
L.P.B. + valine*	48.8	85.5	1.8	10.1	5.79
L.P.B. + leucine	44.6	82.9	1.8	9.8	5.35
L.P.B. + isoleucine	45.0	85.6	1.9	10.6	5.47

*Averages for 2 rats, the third rat in this group became sick and was discarded.

Experiment 10

It was observed in Experiments 7, 7A and 8 that a supplement made up of a mixture of seven amino acids was adequate to provide for reasonably good growth when added to the basal low protein barley ration. Previous experiments reported by Lobay (13), and in this paper, indicated that other mixtures, composed of from two to five amino acids that were estimated to be most limiting in barley protein, gave no greater growth than a supplement of lysine alone. The following combinations were found ineffective:

1) lysine and tryptophan,
2) lysine and histidine,
3) lysine, histidine and phenylalanine,
4) lysine, histidine, phenylalanine, methionine and tryptophan,
5) lysine, methionine, tryptophan, valine and threonine.

Thus, in summary, mixture I, outlined below, appeared to be effective, whereas mixtures II and III were not:

I-	lysine,	histidine,	phenylalanine,	methionine,	tryptophan,	threonine,	valine
II-	"	"	"	"	"	—	—
III-	"	—	—	"	"	threonine,	valine

The primary purpose of Experiment 10 was to determine the effect of mixtures of the six amino acids remaining after threonine, valine, histidine or phenylalanine were deleted from mixture I above.

Experimental Material

As for previous experiments, except that both male and female rats were used. In assigning the weanling rats to test groups an attempt was made to equate them for both sex and weight.

Test Groups

The following groups were placed on test, groups 1 - 5 being included as controls for comparative purposes.

1. Low Protein Barley.
2. Low Protein Barley + casein.
3. Low Protein Barley + 10 E.A.A.
4. Checkers.
5. Low Protein Barley + lysine.
6. Low Protein Barley + 7 E.A.A.
7. As 6 - threonine
8. As 6 - valine
9. As 6 - histidine.
10. As 6 - phenylalanine.

Results and Discussion

The results of this trial are summarized in Table XIX.

TABLE XIX
Growth Response of Rats Fed Basal Ration
Supplemented with Amino Acids
(21 day test)

Group and Ration	No. on Test	Av. Initial Weight g.	Av. Final Weight g.	Av. Daily Gain g.	Av. Daily Consumption g.	Feed Consumed / gram gain g.
1. L.P.B.	3	38.6	72.3	1.6	8.9	5.55
2. L.P.B. + casein	4	40.5	118.4	3.7	11.5	3.10
3. L.P.B. + 10 E.A.A.	4	41.2	106.8	3.1	9.9	3.18
4. checkers	4	40.9	130.7	4.3	13.1	3.06
5. L.P.B. + lysine	3	41.8	77.5	1.70	8.3	4.87
6. L.P.B. + 7 E.A.A.	4	40.3	78.7	1.8	7.6	4.14
7. As 6 - threonine	3	40.4	74.6	1.6	7.9	4.82
8. As 6 - valine	4	40.9	96.2	2.6	9.5	3.61
9. As 6 - histidine	4	41.0	91.8	2.4	9.3	3.83
10. As 6 - phenylalanine	4	40.4	99.6	2.8	10.1	3.59

From a comparison of the results listed in Table XIX for groups 3, 5 and 6 with those for groups fed similar rations in previous experiments, including those of Lobay (13), it is evident that the rats used in Experiment 10 did not respond normally. Rats in groups 5 and 6, supplemented with lysine and a mixture of 7 E.A.A. respectively, failed to make appreciably more rapid growth than unsupplemented rats in group 1. It is therefore not possible to draw any conclusions from this experiment.

SUMMARY AND DISCUSSION

1. Results of feeding experiments with grains of different species and protein contents are essentially the same as those reported previously from this laboratory (17) and by Jones, Caldwell and Widness (12). As in their experiments, rate of growth was markedly in favor of high protein grains, and unheated wheat was found to produce less rapid growth than either oats or barley.

2. Heating wheat in an autoclave for 8 minutes at a pressure of 15 lb. per sq. in. was found to increase the palatability of wheat for rats.

3. The average daily feed consumption of rats fed heated wheat was approximately the same as that of those fed oats or barley. Under these conditions the rate of growth and efficiency of gains for rats fed wheat, oats or barley were essentially the same.

4. It was found that the amino acid content of grains, as estimated from the results of microbiological assays for 9 essential amino acids, constituted a fairly reliable index of the relative growth promoting value of these grains for rats.

5. Comparison of the amounts of essential amino acids supplied daily by low protein barley, as estimated from results

of microbiological assays, with the daily requirements as listed by Rose (26), indicated that low protein barley was in varying degrees, deficient in all the essential amino acids other than arginine. Supplementation of low protein barley with single amino acids, other than lysine, or with mixtures of from two to five amino acids estimated to be most limiting in barley protein, did not result in any faster growth than supplementation with lysine alone.

6. It was found that a supplement of seven essential amino acids made up of lysine, histidine, phenylalanine, methionine, tryptophan, threonine and valine, was adequate to provide for reasonably good growth when added to the basal low protein barley ration.

7. It was observed that the addition of either leucine or isoleucine singly to the above mixture of 7 essential amino acids resulted in a decreased rate of growth, while the addition of both resulted in growth equivalent to that obtained with a supplement made up of all ten essential amino acids.

8. The similarity between these results and those reported by Hankes, Henderson and Elvehjem (8) is discussed.

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