THE EFFECT OF AMINO ACID SUPPLEMENTATION ON THE NUTRITIVE VALUE OF THE PROTEIN IN OATS

By

PHYLLIS L. PORTER TORMO

Bachelor of Science

Central State College

Edmond, Oklahoma

1954

Submitted to the faculty of the Graduate School of the
Oklahoma State University in partial fulfillment of
the requirements for the degree of
MASTER OF SCIENCE
1957

THE EFFECT OF AMINO ACID SUPPLEMENTATION ON THE NUTRITIVE VALUE OF THE PROTEIN IN OATS

Thesis Approved:

Duane G. Benton
Thesis Adviser

Charles L. Crane

Laban MacLean
Dean of the Graduate School

ACKNOWLEDGMENT

This thesis is a tribute to the late Dr. Ruth Reder who so enthusiastically assisted and guided the author so generously during the study of this problem.

Thanks are also due to Mr. George Odell and Dr. D. A. Benton for their advice in completion of the work and to Dr. A. M. Schlehuber who furnished the grain used in this study.

The author also wishes to acknowledge the financial help received as a graduate fellow and the facilities provided her by the Department of Agricultural Chemistry of the Oklahoma State University.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Progress in the various fields of chemistry and nutrition enables us to view individual components thereof as opposed to the necessity during early development to consider groups of compounds as a whole. Thus we now think in terms of individual amino acids and their interactions rather than protein as a single dietary component.

An investigation of the nutritive value of the protein in oats as effected by variety and location and time of planting (38) as well as limited amino acid supplementation (39) was initiated in this laboratory with the hope of improving oats as a source of protein while retaining its economy as a feed.

The author feels that an even greater value of this study is in its touching on the problem of amino acid imbalance.

CHAPTER II

LITERATURE REVIEW

Osborne and Mendel (29) demonstrated that because of the biological incompleteness of grain proteins and deficiency of certain mineral salts and fat soluble vitamins none of the cereal grains alone afforded satisfactory animal nutrition.

Mc. Collum and coworkers (30) found that gelatin when combined with oat protein formed a much better protein mixture for growth than did casein and oat protein. Casein, although a protein of high quality (3,29) is relatively deficient in cystine and methionine.

It has been reported by numerous investigators (18,19,34) that the addition of an excess of individual amino acids to diets that may or may not be particularly deficient may result in deleterious effects in laboratory animals.

When the most limiting amino acid in a diet generally poor in protein is increased, a deficiency of the next most limiting amino acid may occur. It was noted by Harper et. al. (21) that a supplement of methionine to the low-protein diet of rats precipitated a threonine deficiency which was observed as fatty infiltration of the liver.

The same workers (20) similarly showed that the converse is also true. Addition of an excess of leucine to a diet already high in leucine but not deficient in isoleucine was capable of inducing a depression in growth which could be overcome only by increasing the

amount of isoleucine in the diet.

Similarly, supplementation with an amino acid that is not primarily limiting may increase the severity of the deficiency of the most limiting amino acid. Demonstrative of this is evidence presented by Winje and her collaborators (41) of a growth depression precipitated by lysine supplementation but corrected by the addition of histidine to a diet low in that amino acid. Sure (37) in investigating the nutritive values of whole wheat and rye protein found that these cereal diets, deficient in threonine, failed to support growth when valine was added.

The imbalance of certain proteins with regard to adequacy of the essential amino acids is heightened in some cases by the preponderance of certain amino acids which are actually inhibitory at high levels in the diet. Hier and others (24) observed that a supplement of gelatin caused a growth depression in rats fed a casein diet and they found this was due to the high level of glycine in the gelatin.

Sirny (27,36) in his work with Leuc. Mesenteroides noted that this bacteria in order to utilize limiting amounts of arginine required high levels of proline and vice-versa. This interdependance seemed demonstrative of a different peptide synthesis and the utilization of the peptide bound amino acid in preference to the free form.

The influence of the time of ingestion of the indispensable amino acids upon utilization in tissue synthesis has been noted by numerous workers (6,10,13,28,33). All agree that for optimum utilization of food proteins, all essential amino acids must be present in adequate amounts, at the same time and in proper relationship to one another. Melnick (28) further states that all must be liberated during digestion

in vivo at rates permitting mutual supplementation, thus accounting for differences in the biological values of proteins by the rate of release of individual amino acids during enzymatic digestion.

It was found in studies (33) with protein hydrolysates that the omission of a single essential amino acid from an otherwise adequate mixture resulted in poor utilization of the readily absorbed amino acids. Nitrogen retention was not improved by ingestion of the missing amino acid eight hours later. Similarly, Elman (13) found that injection of tryptophan six hours after the injection of an incomplete mixture of amino acids lacking only tryptophan failed to induce nitrogen balance, whereas injection of all amino acids simultaneously succeeded in doing so.

Cox and Mueller (10) called attention to the different rates of absorption of various amino acids from the gastro-intestinal tract. Also they noted in their work on plasma albumin regeneration. Also they noted that amino acids were liberated to different extents from resistant protein linkages.

Experimental evidence was presented by Christensen and his co-workers (8,9) that the cells actually reject the imbalanced amino acid mixtures. The studies show that the amino acid pattern of the cells can be upset by imbalanced amino acid levels in the extra-cellular fluid, leading to a loss of amino acids from the interior of the cells. This provides an explanation as to why certain acute amino acid imbalances work to the nutritional disadvantage of the animal.

> "Amino acids appear to show competitive inhibition among themselves for the means by which the cells concentrate the amino acids presented to them by extracellular fluid,

> indicating that these means do not operate independently for each amino acid. An identical mechanism could scarcely concentrate each of the large number of amino acids and yet maintain characteristic distributions for each in the face of the various amino acid mixtures presented."

Whatever the cause of an amino acid imbalance its results are soon apparent. A low lysine diet produced cessation of growth and hypoproteinaemia in young rats. Radiological examination showed a considerable decrease of subcutaneous fat, waste of muscle, and reduction of calcification in the bones. Certain organs, such as the eye and the kidney, continued to grow at the expense of others (22). In rats of initial weight 110-140 grams, lysine deficiency produced a marked weight loss, loss of protein from the liver and hypoproteinemia while body protein content remained seemingly unchanged (15).

A lysine deficient diet brought on nausea, dizziness and hypersensitivity to metallic sounds in man. These sensations were apparently associated with the phenomenon of high urinary excretion of non-ketone organic acids (26). Male subjects went into negative nitrogen balance. In their single female subject, Albanese and Holt (1) noted negative nitrogen balance on withdrawal of lysine except on onset of menstruation. The menstrual period was also characterized by an unusually scanty flow and by the complete absence of symptoms of the subject's usual premenstrual distress.

Rats on a diet deficient in sulfur-containing amino acids began to decline in six weeks. Symptoms, including failure to grow, general appearance associated with malnutrition and lifeless appearance of the hair with an actual loss of hair in many cases, were cured in the early stages by methionine or cystine. Later deficiency symptoms responded only to cystine (40).

Necrosis of the liver produced by a protein deficient diet was prevented by Glynn (16,17) upon administration of 20 mg. of methionine per day. Livers affected by acute dietary necrosis show, as compared to normal rat livers, increased amounts of water and protein and an absence of glycogen. The alterations appear suddenly with the onset of cellular damage (25).

Daft, Sebrell and Lillia (11) found that methionine, a choline precursor, and choline have a preventive action on the development of hepatic cirrhosis and that cystine and methionine have a preventive action on the development of the hepatic hemorrhage and necrosis. The importance here of the latter two would seem not to be in their action as sulfur-containing amino acids but in their suggested rolls in transmethylation (12) and the relation of the methyl group to liver damage (4).

CHAPTER III

EXPERIMENTAL PROCEDURE

Protein Sources

Cereal grains, oats of Desoto and Tennex varieties, used in this experiment were furnished by Dr. A. M. Schlehuber of the Agronomy Department of Oklahoma State University.

Chemical Analysis

Each variety was thoroughly mixed and sampled at random. In Experiment I the hulls were removed by vacuum cleaner and the hulls separated from the oats by hand. 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 (2). Since these methods are well known, they need only a brief description here.

Moisture: A two-gram sample was heated at 105°C for six hours, then weighed. The percentage of moisture was calculated from the weight loss.

Ash: The residues from the moisture determination were ashed in an electric muffle furnace maintained at 625°C for two hours. The sample was cooled in a dessicator and the weight of the ash determined.

Ether extract: Two-gram samples were extracted in fat tubes with anhydrous diethyl ether for 16 hours and afterwards dried and reweighed. The percentage of ether soluble material was estimated

from the loss of weight during extraction.

Crude Fiber: The ether extracted residues were digested first with dilute sulfuric acid for thirty minutes, then with dilute sodium hydroxide for an equal length of time and filtered through linen after each digestion. The remaining residue was dried, weighed and ashed, the loss in weight recorded as crude fiber.

Protein Nitrogen: Two-gram samples of grain or half-gram samples of casein were analyzed for total nitrogen using the conventional Kjeldahl procedure, with copper sulfate-sodium sulfate as a catalyst. The percentage of nitrogen obtained was converted to percentage of protein using the factor 6.25. All samples for nitrogen were run in triplicate.

Proximate composition of each protein source used in the experiments is given in Table I.

Preparation and Composition of Rations

In each experiment the level of protein was held constant in all diets at a 9.5% level and was adjusted by the addition of cornstarch. Fiber content with the exception of the diet to which hulls were added was equalized by the addition of commercial cellulose to the rations. Mineral requirements were met by the introduction of 2% Hegsted salt mixture (23) and an adequate supply of vitamins was assured by the addition of a vitamin mixture at the level per kilogram of ration shown in Table II plus fortified cod liver oil at each feeding. Amino acids were supplied at the desired supplementary levels (Table IV). Dietary oat supply was adjusted as amino acids were added, thus holding nitrogen level constant.

TABLE I

Percentage proximate composition of protein sources

Experiment	Protein source	Total protein	Ash	Ether extract	Crude fiber	Moisture
I	Desoto (hulled)	15.07	1.87	5.94	2.05	7.46
	Oat hulls	2.31	6.91	4.82	32.78	5.23
	Casein	81.90	--	--	--	7.83
II	Tennex A (whole ground)	12.20	3.41	3.98		7.66
	Tennex B	11.72	3.23	1.05		7.29
	Casein	91.89	--	--	--	6.25
III	Tennex	10.88		0.39	7.31	
	Casein	87.94	--	--	--	--

TABLE II

Composition of vitamin mixture

Vitamin	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	0.5
Choline·Cl	100.0

TABLE III

Composition of diets used in experiments

Protein level 9.50%

Diet No.	Protein Source	Percentage constituent in diet: Protein Source	Fiber from Oats	Corn Starch	Ground Hulls	Solkaflok
Experiment I						
1	Casein	11.60	---	83.13	0	1.27
2	Desoto (1)	63.04	1.39	32.96	0	0
3	Desoto (1)	61.45	1.26	23.94	10.61	0
4	Desoto (1)	57.66	1.18	37.60	0	0
5	Desoto (1)	61.45	1.26	30.73	0	0
6	Desoto (1)	56.07	1.15	38.89	0	0
Experiment II						
1	Casein	10.34		83.66		2.0
2	Tennex B (2)	81.06		14.94		0
3	Tennex B (2)	74.15		20.95		0
4	Tennex B (2)	73.04		21.70		0
5	Tennex B (2)	70.73		23.55		0
6	Tennex B (2)	72.70		21.96		0
7	Tennex B (2)	70.39		23.81		0
Experiment III						
1	Tennex (2)	83.79		11.79		0
2	Tennex (2)	81.84		13.38		0
3	Tennex (2)	77.19		17.79		0
4	Tennex (2)	81.76		13.52		0
5	Tennex (2)	75.24		19.38		0
6	Tennex (2)	79.84		15.08		0
7	Tennex (2)	75.16		19.52		0
8	Tennex (2)	73.21		21.11		0
9	Tennex (2)	87.32		8.68		0
10	Casein	10.80		83.20		2.0

(1) Hulls removed, kernels ground.
(2) Whole, ground.

Each diet contained 2 grams of vitamin mixture per kilogram, 2.0% of corn oil, and 2.0% of salt mixture.

Fortified cod liver oil was added at each feeding.

TABLE IV

Percentage of Amino Acid Supplementation Used in Experiments

Diet No.	L-lysine	DL-threonine	DL-methionine	DL-tryptophan
Experiment I				
1	--	--	--	--
2	--	--	--	--
3	--	--	--	--
4	0.68	--	--	--
5	--	0.36	--	--
6	0.68	0.36	--	--
Experiment II				
1	--	--	--	--
2	--	--	--	--
3	0.68	--	--	--
4	0.68	0.36	--	--
5	0.68	0.36	0.45	--
6	0.68	0.36	--	0.08
7	0.68	0.36	0.45	0.08
Experiment III				
1	0.20	0.10	0.12	--
2	0.20	0.10	0.48	--
3	0.80	0.10	0.12	--
4	0.20	0.40	0.12	--
5	0.80	0.10	0.48	--
6	0.20	0.40	0.48	--
7	0.80	0.40	0.12	--
8	0.80	0.40	0.48	--
9	--	--	--	--
10	--	--	--	--

In preparing the rations the weighed dry ingredients were first thoroughly mixed. The corn oil was added and the whole further mixed by hand to ensure the proper distribution of the supplements. The rations were stored in a refrigerator prior to and during each experiment to prevent deterioration.

Rat Assay

For use in the assays female albino rats weighing from 40 to 60 grams were obtained from Sprague-Dawley Co., Madison, Wisconsin. They were selected for the different diets and placed in individual cages in a room in which the temperature was maintained at 75°F.

Experiment I

Ten rats per diet were selected at random. Then the rats were fed ad libitum until a weight of 65 grams was reached. Food intake was restricted to ten grams a day. The animals were fed and weighed daily in order to take advantage of what Carpenter and Porter (7) termed the optimum growth period, the 65-85 grams range. In this and further experiments to secure an accurate measure of total food consumption, all refused or spilled food was recovered and weighed. The total amount of food consumed by each rat was calculated for each twenty gram period after the rat attained a weight of 65 grams.

Experiment II

Nine randomly selected rats were placed on each of seven diets and caged accordingly. As before, the animals were fed ad libitum up to their attainment of the weight of 65 grams. Food, limited to ten grams daily, was then given until the rats reached a weight of 85 grams. Feed consumption and protein quality were determined for that period. During the remainder of the experiment, the animals were

fed four times weekly, necessitating the feeding of 20 grams on three of these feeding days. Animals were weighed weekly with no attempt being made to stay within weight bounds. Instead, food consumption and growth were measured for the entire thirty-nine day period.

Experiment III

Selection of six rats for each of ten diets was less randomized than in previous experiments with rats being grouped in six blocks of similar weight ranges and one rat selected at random for each group. Feeding, weighing and determination of growth and food consumption were conducted for six weeks as in the latter part of Experiment II.

Supplementation

Protein efficiency quotient or P.E.Q., originally defined by Osborne, Mendel and Ferry (32), is the ratio 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. These experiments were conducted to determine whether the nutritive value of a protein of an oat variety having a low protein efficiency quotient could be increased by amino acid supplementation to equal that of casein, a protein of high quality although slightly deficient in cystine and methionine. Therefore, casein and a basal diet of oats alone were used as controls in each experiment.

In experiment I lysine and threonine, singly and together, were used to supplement hulled oats, while another diet of basal plus hulls was fed to check the effect of the hulls. In the latter stages of the trial, tryptophan and methionine were added to determine

if further growth responses could be obtained. The favorable results in the second part of experiment I led to feeding lysine, threonine, tryptophan, and methionine alone and in combination as supplements to whole oats in experiment II. Whole oats plus methionine, lysine and threonine at all possible combinations of two levels of supplementation formed the diets of rats in experiment III.

CHAPTER IV

RESULTS AND DISCUSSION

Experiment I

The growth rate and **P.E.Q.** of rats fed Desoto variety oat diets supplemented with lysine and threonine are presented in Figure I and Table V. Supplementation of the basal ration with 0.68% L-lysine resulted in gross retardation of growth. One-half of the animals on that diet failed to reach 65 grams. The mean protein efficiency quotient of the five rats which did reach that weight was equal to but no greater than the **P.E.Q.** of the basal group for the 65-85 gram range. The tabulated **P.E.Q.** can give no true indication of the detrimental effect of the basal supplemented with lysine as the rats which showed the greatest growth retardation failed to reach experimental range. Growth was slightly retarded by addition of 0.36% DL-threonine while lysine and threonine together raised the **P.E.Q.** a slight but insignificant amount, 0.06, over that of the basal diet. The protein efficiency quotient of the rats fed basal hulled oats plus hulls was 0.08 less than that of the animals fed the basal diet alone although the growth rate of the animals on the former diet was greater. This is probably due to the high level of fiber in the diet containing the hulls.

The fact that lysine and threonine together slightly raised the **P.E.Q.** suggests that an amino acid imbalance caused by addition

TABLE V

Mean Protein Intake and Mean Protein Efficiency Quotients in Experiment I. Protein Level 9.50%.

		Supplementation			Wt. range 65-85 gr.		Wt. range 85-105 gr.	
Diet No.	Protein Source	L-lysine	DL-threonine	Hulls	Prot. Int.	P.E.Q.	Prot. Int.	P.E.Q.
1	Casein	-	-	-	7.45	2.88	8.18	2.53
2	Desoto	-	-	-	10.60	1.94	9.93	1.66
3	Desoto	-	-	10.61	11.45	1.86	10.79	1.86
4	Desoto	0.68	-	-	10.40	1.95	-	-
5	Desoto	-	0.36	-	10.86	1.86	-	-
6	Desoto	0.68	0.36	-	9.03	2.00	-	-

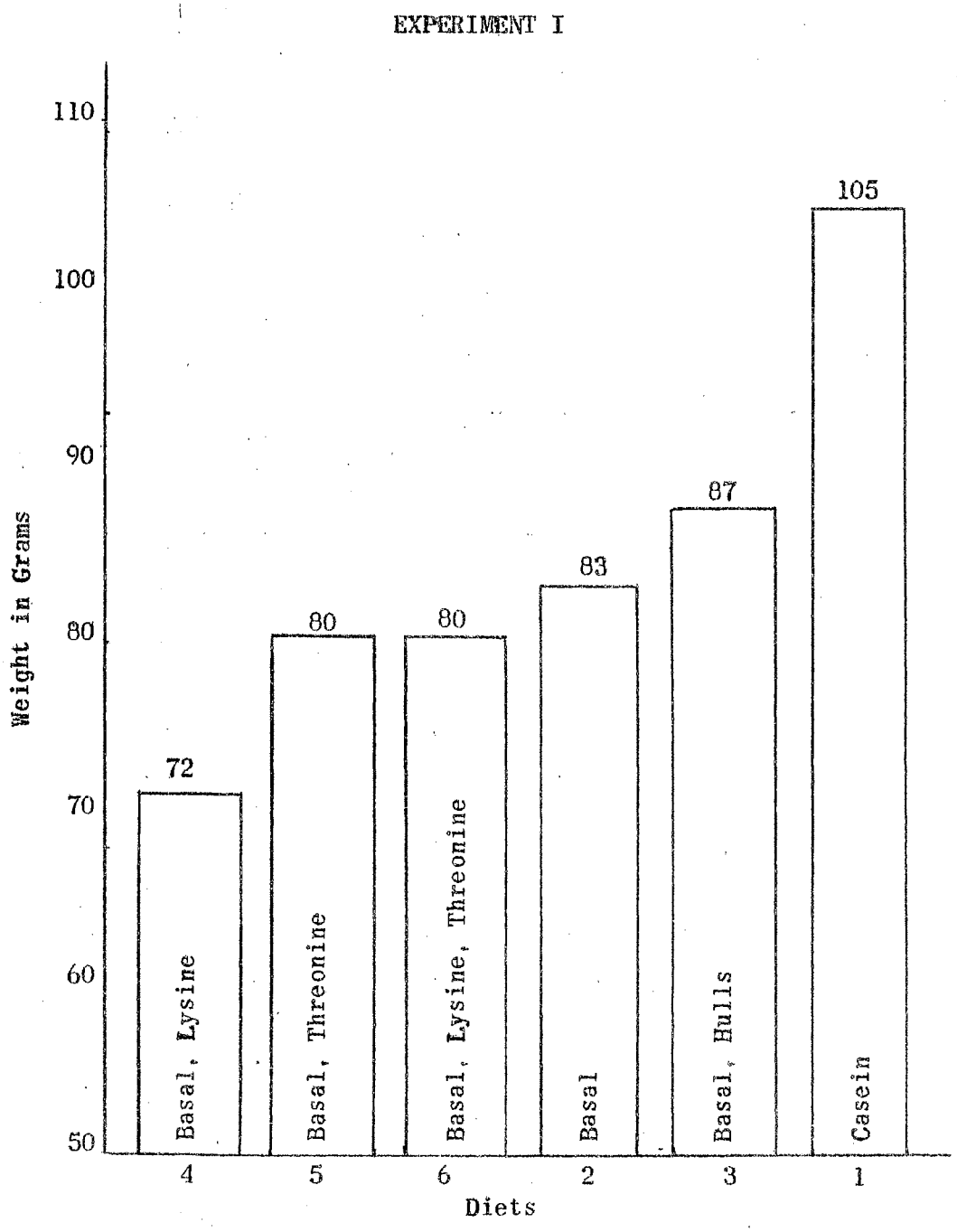

Figure I
Weight Gain of Rats in Experiment I
During a 28-Day Period.

TABLE VI

Effects of Supplementation Changes on Diets of Experiment I

Diet No.	Rat No.	Contained	Added	P.E.Q. Before	P.E.Q. After	Improvement
7	A	Threo.	Lys.+Meth.+Trypt.	1.94*	3.15*	62*%
7	B	Lys.+Threo.	Meth.+Trypt.	2.25*	2.78*	24*%
8	41	Threo.	Lys.+Meth.	2.03	3.51	73%
8	56	Lys.+Threo.	Meth.	1.33	4.11	209%
9	44	Threo.	Meth.+Trypt.	1.69	1.34	-21%
9	48	Threo.	Meth.+Trypt.	1.68	1.88	12%
10	13	Basal	Lys.+Meth.+Trypt.	2.69	1.73	-26%
10	15	Basal	Lys.+Meth.+Trypt.	1.95	2.94	51%
11	55	Lys.+Threo.	Trypt.	1.38	1.66	20%
11	57	Lys.+Threo.	Trypt.	2.15	2.38	11%
12	24	Basal+Hulls	Lys.+Trypt.	1.36	3.08	126%
12	25	Basal+Hulls	Lys.+Trypt.	1.36	2.04	50%

*Mean of five rats.

of either alone was offset. Furthermore, the possibility that the removal of the hulls contributed to an amino acid imbalance which was not noted until the diet was supplemented with lysine should not be overlooked. This factor alone could explain why supplementation with lysine in the experiments of Mitchell (29) and Weber (39) failed to have the same deterrent effect on growth. Both of these workers found that the addition of lysine to a diet of whole oats resulted in an increase in growth promoting value. They also realized that there was some other limiting factor which was unknown to them.

Lagging growth rate and loss of hair in several of the animals pointed up the lack of a sulfur-containing amino acid. Therefore methionine as well as tryptophan was added to the ration of the rats listed in Table VI in the latter stages of this experiment to produce diets 7-12. Immediate response was indicative of offsetting the deficiency created by the previous amino acid imbalance. Increase in P.E.Q. was as great as 20% for the rats on lysine-threonine supplement when methionine was added. Animals on a diet which lacked lysine in each case responded quite favorably to the addition of that amino acid. Animals also responded to the addition of lysine and methionine. Other differences probably were of no significance. The great degree of improvement in individual rats brought about by correction of an existing deficiency did not give a true indication of the worth of the diets tested, but was helpful in planning experiment II. Changes in diets with consequent changes in P.E.Q. are presented in Table VI.

Experiment II

Results obtained in a study of the effects of supplementation of whole ground oats of the Tennex variety with lysine, threonine,

methionine and tryptophan in combinations as indicated to be suitable in the latter stages of experiment I are presented in Table VII and Figure 2.

Mean **P.E.Q.**'s and grams gained are tabulated for the 39-day duration of the experiment after the rats reached a weight of 65 grams with special emphasis of the **P.E.Q.**'s for the 65-85 gram weight range.

The basal Tennex diet was not significantly improved by lysine and lysine and threonine supplementation contrary to results with Desoto in Experiment I in which a distinct growth depression occurred. Ration 5, basal oats plus lysine, threonine and methionine, and Ration 7 which contained tryptophan in addition produced **P.E.Q.**'s significantly greater than those affected by casein (Table VII , Figure 2). Tryptophan raised the **P.E.Q.** of the mixture only 0.04, failing to cause a significant growth response in the 65-85 gram weight range. Therefore, this amino acid was omitted from diet supplements in Experiment III.

After the fixed range of growth, 65-85 grams, useful in eliminating variations (7), was passed, diet 7 continued to outrank casein in nutritive value, while diet 5 fell behind. The overall **P.E.Q.**'s presented in Table VII point out that Tennex oats supplemented with lysine, threonine, tryptophan and methionine (**P.E.Q.** 1.82) is nutritionally equal to casein (**P.E.Q.** 1.80).

The effect of the addition of lysine to whole Tennex oats does not differ greatly from the outcome of the inclusion of that amino acid in a diet of hulled Desoto oats. No growth depression was noted in the second experiment but there was also no improvement

TABLE VII

Mean Protein Intake, Weight Gain and Protein Efficiency Quotients in Experiment II. Protein Level 9.50%

Diet No.	Prot. Source	Supplementation				Wt. Range 65-85 gm. P.E.Q.	Wt. After 65 gm.		
		L-lysine	DL-threonine	DL-methionine	DL-tryptophan		Prot. Int. in Grams	Wt. Gain in Grams	P.E.Q.
1	Casein	-	-	-	-	2.12	16.66	29	1.80
2	Tennex	-	-	-	-	1.45	26.57	34	1.25
3	Tennex	0.68	-	-	-	1.64	22.12	24	1.17
4	Tennex	0.68	0.36	-	-	1.92	19.77	29	1.43
5	Tennex	0.68	0.36	0.45	-	2.54	26.70	43	1.60
6	Tennex	0.68	0.36	-	0.08	2.10	22.03	33	1.48
7	Tennex	0.68	0.36	0.45	0.08	2.58	25.48	46	1.82

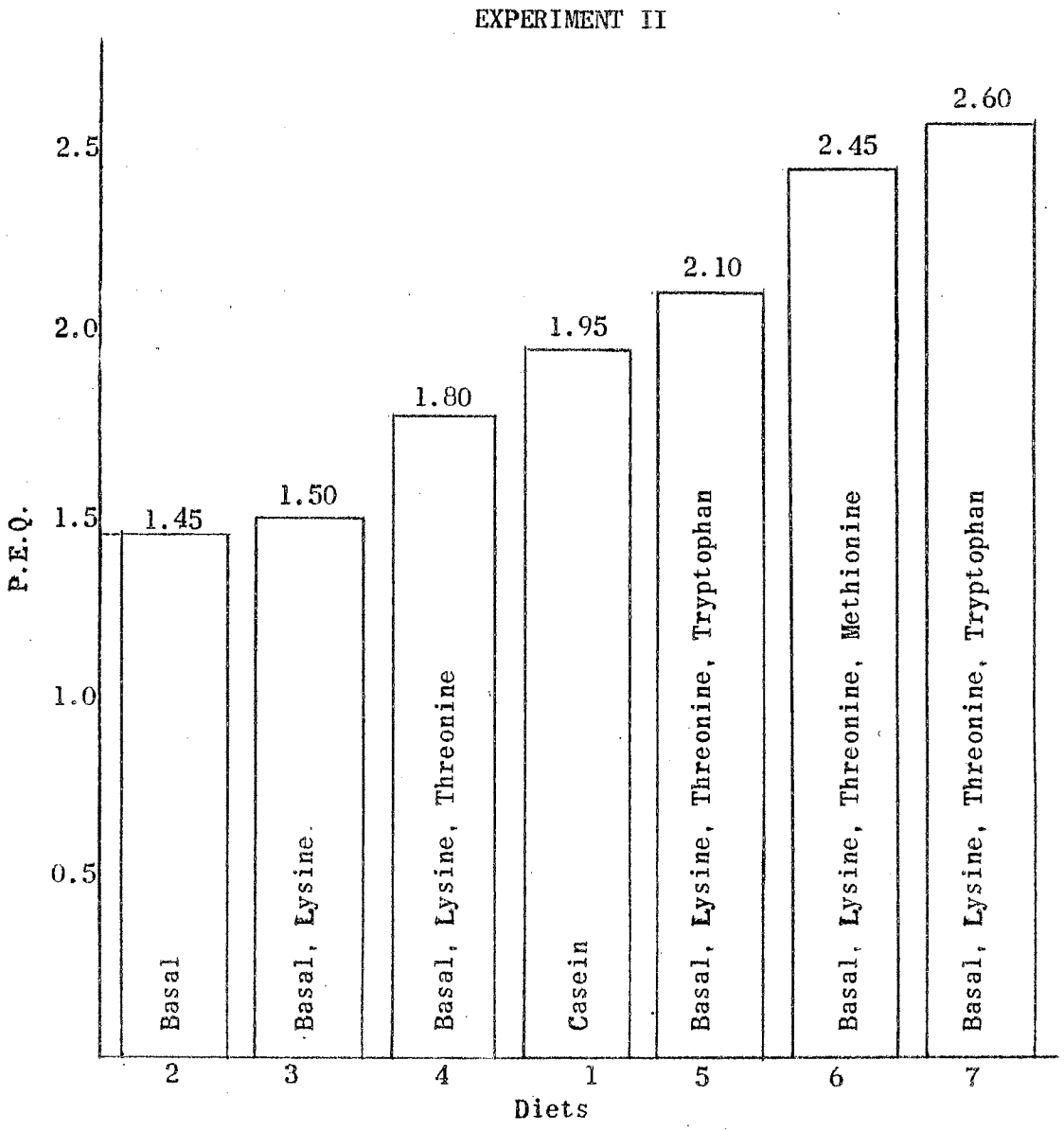

Figure II
Protein Efficiency Quotients of Rats of Experiment II
Within the 65-85 Gram Weight Range.

in biological value. These results tend to confirm conclusions drawn in Experiment I that the removal of the hulls from the oats of Desoto variety was responsible for deleterious effects noted in supplemented diets.

The results with lysine alone are not in agreement with those of Thomas (38) who achieved an increase in **P.E.Q.** of 0.27 when he supplemented Tennex with 0.5% DL-lysine. Weber (39) found that at the 11.50% protein level, incorporation of lysine in a diet of Desoto variety oats raised the **P.E.Q.** 0.13 while at 9.50% protein level, **P.E.Q.** rose from 1.28 for the basal Desoto to 1.52 for Desoto plus lysine. Failure of the results of this most recent experiment to concur with results previously reported in this laboratory may be associated with changes in proportions of certain amino acids in the oat protein due to changes of season and planting area. Evidence of this phenomena was first presented by Frey (14).

Weber also worked with methionine supplementation, but alone or in combination with leucine so that its effect could not be compared with the effect of methionine in this experiment.

Experiment III

Supplementation of whole Tennex variety oats with lysine, threonine and methionine at all possible combinations of two levels resulted in weight gains and **P.E.Q.**'s indicated in Table VIII. Supplementation with low (0.20%) L-lysine, low (0.10%) DL-threonine and low (0.12%) methionine resulted in a **P.E.Q.** of 1.88, only a 0.32 increase over the **P.E.Q.** of basal Tennex. Addition of high (0.48%) methionine and low levels of lysine and threonine to the basal diet (Ration 4) resulted in a **P.E.Q.** of 2.03 as did supplementation with

high (0.80%) lysine and low levels of threonine and methionine. However, only rats on low lysine, high threonine and low methionine equalled those on casein in mean weight gain. The mean protein efficiency quotient, 2.18, of the animals on Ration 4 exceeded that of those on casein by 0.02, while Diet 6, low lysine, high threonine and high methionine, succeeded in producing a P.E.Q. of 2.17. Only the basal oat diet was of significantly less nutritive value than casein (Figure 3), thus confirming the results of Experiment II. It is postulated that more significant increases over casein would have been achieved by determination of values within specific weight limits.

Results indicate that supplementation with all three amino acids at their lower levels does not improve the biological value of the basal oat diet. Low level lysine addition produces a greater growth response than inclusion of higher level lysine in the diet, while high level threonine supplementation seems preferable. The level of methionine supplementation seems to have little effect on P.E.Q. and growth rate. Differences observed in the effect on P.E.Q. and growth of the various levels tested have no great significance. The results serve in confirming conclusions reached in Experiment II but determination of the optimum level of supplementation requires further experimentation.

TABLE VIII

Mean Total Weight Gain, Protein Intake and Protein Efficiency Quotients in Experiment III. Protein Level 9.50% (Over 6 Weeks Period)

Diet No.	Protein Source	% Supplementation			Weight Gain	Protein Intake	P.E.Q.
		L-lysine	DL-threonine	DL-methionine			
1	Tennex	0.20	0.10	0.12	72	38.42	1.88
2	Tennex	0.20	0.10	0.48	78	38.75	2.03
3	Tennex	0.80	0.10	0.12	76	38.12	2.03
4	Tennex	0.20	0.40	0.12	83	38.47	2.18
5	Tennex	0.80	0.10	0.48	77	38.32	2.02
6	Tennex	0.20	0.40	0.48	81	37.50	2.17
7	Tennex	0.80	0.40	0.12	72	37.39	1.93
8	Tennex	0.80	0.40	0.48	73	37.28	1.97
9	Tennex	-	-	-	59	37.68	1.56
10	Casein	-	-	-	84	37.75	2.16

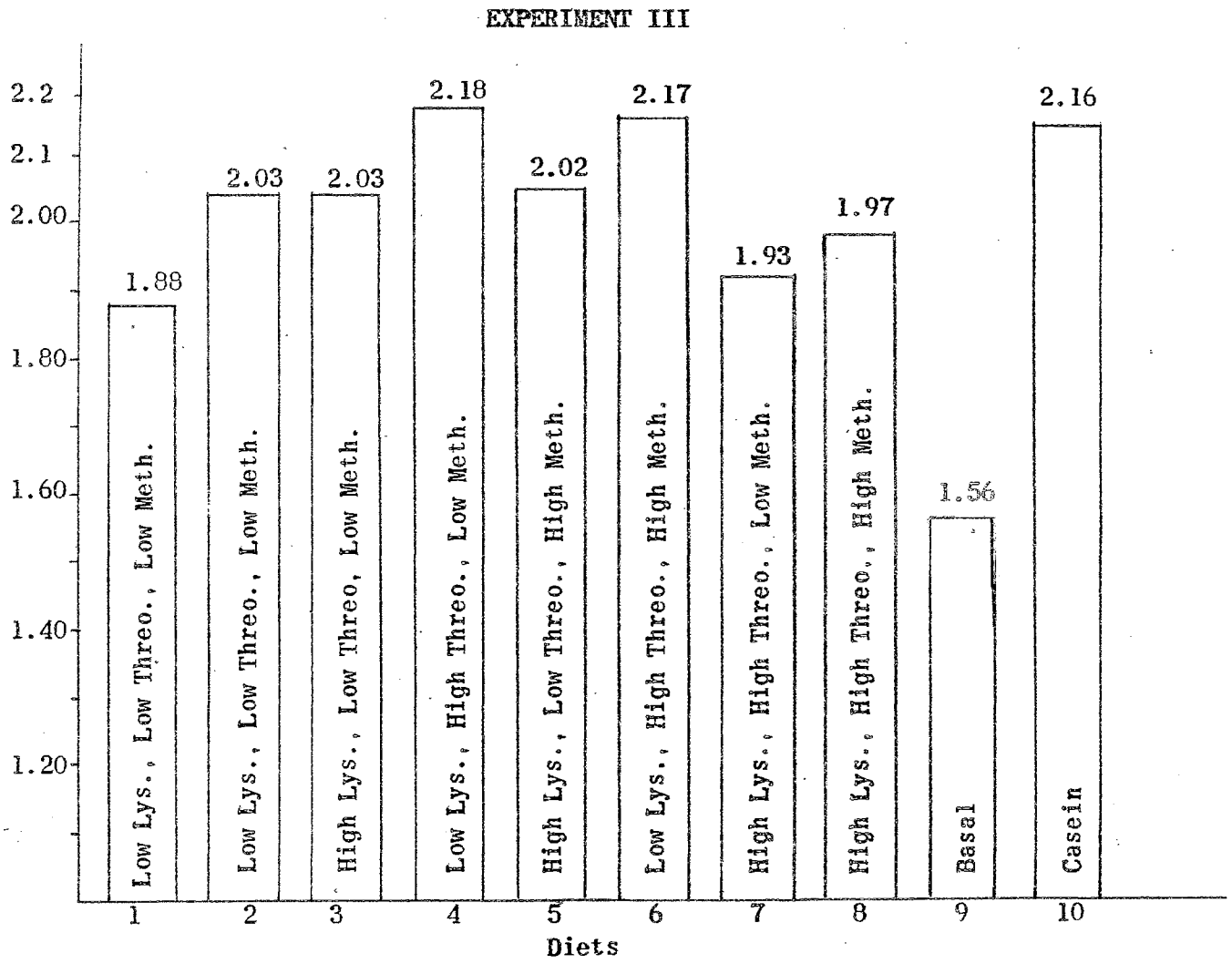

Figure III
Protein Efficiency Quotients of Rats of Experiment III over a Six Week Period.

CHAPTER V

SUMMARY AND CONCLUSIONS

Experiments were conducted 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 (P.E.Q.) 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 served as the protein source in control diets.

Desoto, a variety of oats demonstrated to be of below average nutritive value (39) was chosen for the first experiment but Tennex, a variety of intermediate quality (39) was incorporated in Experiments II and III because of its greater availability. Protein level in all experiments was held at 9.5 per cent.

Hulls were removed from the oats of Desoto variety and results in comparison with those of previous experiments (39) suggest the upset of the amino acid balance. Inclusion of lysine (0.68%) in the basal diet produced a severe retardation of growth, threonine (0.36%) retarded growth slightly while lysine and threonine together raised the P.E.Q. an insignificant amount.

Rats on a diet of unhulled Tennex oats demonstrated no growth response to lysine (0.68%) supplementation but a slight growth response to lysine plus threonine (0.36%). Addition of lysine, threonine and methionine (0.45%) produced a greater P.E.Q. than that achieved with casein. Inclusion of tryptophan (0.08%) in the above mixture had no significant effect. A difference in response to amino acid supplementation of the protein of the two oat varieties incorporated in basal diets at the same level of protein suggests a varietal difference in amino acid composition or a nutritional deficiency due to removal of hulls.

A third experiment to determine the optimum level of supplementation demonstrated no significant differences in the effect on growth and P.E.Q. of the various levels tested, serving only to confirm the results of Experiment II.

BIBLIOGRAPHY

1. Albanese, A. A., Holt, L. E., Jr., Brumback, J. E., Jr., Hayes, M., Kajdi, C., and Wangerin, D. M., Proc. Soc. Exptl. Biol. Med., 48, 728 (1941).

2. Association of Official Agricultural Chemists. Official and Tentative Methods of Analysis. 6th Ed. 1945.

3. Beadles, J. R., et. al., J. Biol. Chem., 88, 623 (1930).

4. Bennett, M. A., Gastroenterology, 5, 491 (1945).

5. Cannon, P. R., Protein and Amino Acid Deficiencies. Charles C. Thomas, Springfield, Ill. 1948.

6. Cannon, P. R., Steffee, C. H., Frazier, L. E., Rowley, D. A., and Stepto, R. C., Fed. Proc. Am. Soc. Exper. Biol., 6, 390 (1947).

7. Carpenter, K. J. and Porter, G., British J. Nutr., 6, No. 4 (1952).

8. Christensen, H. N., Cushing, M. K., and Streicher, J. A., Arch. Biochem. 23, 96 (1949).

9. Christensen, H. N., Streicher, J. A., and Elbinger, R. L., J. Biol. Chem. 172 515 (1948).

10. Cox, W. M., Jr. and Mueller, A. J., J. Clin. Investigation, 28, 875 (1944).

11. Daft, F. S., Sebrell, W. A., and Lillie, R. D., Proc. Soc. Exptl. Biol. Med., 50, 1 (1942).

12. Du Vigneud, V., Chandler, J. P., Moyer, A. W., and Keppel, D. M., J. Biol. Chem., 131, 57 (1939).

13. Elman, R., Proc. Soc. Exptl. Biol. Med. 40, 484 (1939).

14. Frey, K. J., Cereal Chemistry, 29, 77 (1952).

15. Gillespie, M., Neuberger, A., and Webster, T. A., Biochem. J., 39, 203 (1945).

16. Glynn, L. E., and Himsworth, H. P., J. Path. Bact., 56, 297 (1944).

17. Glynn, L. E., Himsworth, H. P., and Neuberger, A., Brit. J. Exper. Path., 26, 326 (1945).

18. Graham, C. E., et. al., J. Biol. Chem. 185, 97 (1950).

19. Groschke, A. C., Anderson, J. A., Combs, G. F., and Griggs, G. M., J. Nutrition, 45, 345 (1951).

20. Harper, A. E., Benton, D. A., Winje, M. E., and Elvehjem, C. A., Arch. Biochem. 51, 523 (1954).

21. Harper, A. E., Benton, D. A., Winje, M. E., and Elvehjem, C. A., J. Biol. Chem., 209, 159 (1954).

22. Harris, H. A., Neuberger, A., Sanger, F., Biochem. J., 37, 508 (1943).

23. Hegsted, D. M., et. al., J. Biol. Chem., 138, 459 (1941).

24. Hier, S. W., Graham, C. E., and Klein, D., Proc. Soc. Exptl. Biol. Med., 56, 187 (1944).

25. Himsworth, H. P., and Glynn, L. E., Biochem. J., 39, 267 (1945).

26. Holt, L. E., Jr., et. al., Proc. Soc. Exptl. Biol. Med., 52, 209 (1943).

27. Klungsoyr, M., Sirny, R. J., and Elvehjem, C. A., J. Biol. Chem., 189, 557 (1951).

28. Melnick, D., Oser, B. L., and Weiss, S., Science, 103, 326 (1946).

29. Mitchell, H. H., and Smuts, D. B., J. Biol. Chem., 95, 263 (1932).

30. Mc. Collum, E. V., Am. Med. Assn., 68, 1397 (1917).

31. Osborne, T. B., and Mendel, L. B., J. Biol. Chem., 41, 275 (1920).

32. Osborne, T. B., Mendel, L. B., and Ferry, E. L., J. Biol. Chem., 37, 223 (1919).

33. Plotz, H., Wertman, K., and Bennett, B. L., Withheld from Publication. As cited in 28.

34. Russell, W. C., Taylor, M. W., and Hogan, J. M., Arch. Biochem., 39, 249 (1952).

35. Salmon, W. D., Arch. Biochem., 51, 30 (1954).

36. Sirny, R. J., Cheng, L. T., and Elvehjem, C. A., J. Biol. Chem., 190, 547 (1951).

37. Sure, B., J. Agri. Food Chem., 2, 1108 (1954).

38. Thomas, J. P., Master's Thesis, Oklahoma A. and M. College, 1953.

39. Weber, E., Master's Thesis, Oklahoma A. and M. College, 1954.

40. Weichselbaum, T. E., Quart. J. Exper. Physiol., 25, 363 (1935).

41. Winje, M. E., J. Nutrition, 54, 155 (1954).

VITA

Phyllis L. Porter Tormo

Candidate for the Degree of

Master of Science

Thesis: THE EFFECT OF AMINO ACID SUPPLEMENTATION ON THE NUTRITIVE VALUE OF THE PROTEIN IN OATS

Major Field: Biochemistry

Biographical:

Personal data: Born: February 19, 1934, Oklahoma City, Oklahoma.

Education: Undergraduate study: Central State College, Edmond, Oklahoma, 1951-1954. Graduate study: Oklahoma State University, Stillwater, Oklahoma, 1954-1957.

Experience: Research Assistant, Agricultural Chemistry, Oklahoma State University. Instructor, Chemistry Department, Oklahoma State University.

Member of Phi Sigma.