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

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

INTRODUCTION

The concept of fortification of protein diets with certain amino acids has long been thought of as an answer to solve the protein deficiency situation which occurs among the highly populated areas of the world. With this hope in mind, numerous workers have studied the supplementation of amino acids to cereal grains. Our present knowledge, however, does not allow us to consider this idea on a practical scale. Further studies should either point to the basic problems, such as, the biological role and inter-relationships of amino acids, or to the practical considerations, such as the effect of amino acid supplements of various sources of proteins.

The purpose of the experiments reported in this paper were to study the nutritive value of various varieties of oats, either with or without the supplemental amino acids. The experiments were carried out with *ad libitum* feeding of rats. It is hoped that the results presented will be valuable for the further studies with the same objective.

LITERATURE REVIEW

Numerous studies on the biological value of cereal grains have led to the conclusion that they are rather poor in nutritive value when supplied as the only source of dietary protein (48, 20, 38, 26, 21). The nutritional value of oat protein, regardless of the confusion and confliction statements of the earlier workers (39), has been proven to be greater than that of other cereal grains (21). In nitrogen metabolism experiments with rats, Mitchell (31) demonstrated that oat protein had a higher nutritive value than corn protein at different levels of protein intake. Mitchell and Smuts (36) stated that the poor nutritive value of the protein of wheat, corn and oats is due to the deficiency of the amino acid lysine. Mitchell and Block (35) observed a superiority of the protein of rolled oats over that of other cereals. In a conclusive experiment, Jones (21) found that the protein of oats had a higher nutritive value than that of other cereal grains. The experiment was carried out with ad libitum feeding of rats and the superiority of oat protein was observed with protein levels ranging from 4.5 to 12 per cent.

It seems to be a logical trend that the finding of a deficiency of certain amino acids in cereal proteins led to the study of amino acid supplementation, with the hope to increase their biological values. It should be emphasized that the poor nutritive value of a protein is due to the imbalance of its essential amino acids (5, 15). The effect of the amino acid supplements is primarily to improve the balance of amino acids in the protein.

Mitchell and Smuts (36) in a paired-feeding experiment on rat showed that the addition of lysine to an oat diet resulted in a distinct though slight increase in the growth. On the other hand, Maris and Smuts reported that they received "no significant effect" of supplemented lysine on oats seed (25). Thomas and Reder (57) of this experiment station, observed a slight increase in the growth of rats when 0.5% of L-lysine was supplemented to an oat diet. The further addition of 0.5% of DL-threonine did not further increase the growth. Weber and Reder (59) found that an increase in PEQ (protein efficiency quotient) with the oat variety, Forkedeer, when lysine, methionine and leucine were supplemented. The similar supplementation of these three amino acids to DeSoto oat variety did not improve the PEQ. Tormo and Reder (58) supplemented lysine to a hulled oat diet and found a depression of growth in rats. The supplementation of lysine, threonine and methionine to an unhulled oat diet showed a distinct increase of PEQ.

The increase in biological value of wheat or wheat flour resulting from the supplementation of amino acids have been studied by many investigators. Osborne and Mendel demonstrated over 40 years ago that addition of lysine to wheat protein markedly increased its biological value (37). This finding has been confirmed by later studies (46, 5, 54). Light and Frey (24) showed that the protein of white and whole wheat breads can be further improved by supplementing the lysine with valine. Petitpierre and Volet (44) reported a 40 per cent increase in the growth rate of rats when 0.7 per cent of lysine and 0.3 per cent of methionine were supplemented to wheat flour. Sure (54) observed a marked growth response of rats to the addition of lysine, valine in the presence of lysine, and threonine in the presence of lysine as supplements to the whole-hard-wheat flour basal diet. Westerman et al. (60) in a recent experiment found that the supplemental lysine improved the growth of rats on a diet containing 44 per cent of wheat in the presence of milk and egg. The supplemental value was found to be equivalent to that of meat.

Pecora et al. (42) found that the supplementation of lysine alone did not improve the biological value of polished rice. In a later study, the same authors showed a three fold increase in biological value when threonine was supplemented to a polished rice diet in the presence of lysine (41). In confirmation of this observation Harper et al. (12) demonstrated a similar result in the growth response of rats. They also found that a supplementation of 0.4 per cent of L-lysine·HCl with either 0.24 or 0.5 per cent of DL-threonine to rice protein was adequate to prevent the accumulation of fat in the liver of rats.

Sure (53) studied the growth responses of rats fed amino acid supplements with corn diets and found that marked improvement of either growth or PEQ was obtained by adding 0.5 per cent threonine to the ration in the presence of lysine and tryptophan. In further studies the same author showed that the addition of 0.5 per cent DL-valine to whole rye flour produced a large response in the rat (54). He also demonstrated an increase of 224.7 per cent in protein efficiency ratio by adding 0.4% of L-lysine, 0.5% of DL-threonine and 0.5% of DL-methionine and 0.5% DL-threonine to peanut flour (55). Smuts and Marais (51) studied soybean meal and found that its nutritive value increased when 0.2% of cystine was supplemented.

It was not until recent years that a few workers have turned their interest to study the effect of enviroment of planting and variety on the nutritional value of cereal proteins. Frey (8) studied ninteen different oat varieties and found that the alcohol soluble nitrogen levels were essentially constant at 18 per cent of the total nitrogen, though the protein levels were quite different between varieties. In additional studies Frey (9) has reported the influence of varieties and environment on protein and amino acid levels of oats. It was found that the average protein content of 14 oat varieties was 25 per cent higher in 1949 than in 1947. Also significantly higher contents of lysine, leucine and methionine were observed in 1949. The tryptophan level was found to be more constant than those amino acids mentioned above with the change of environment. The differences in amino acid contents between the different varieties was found to be much less than those between the same variety planted in different years. Thomas and Reder of this experiment station (57) observed a higher biological value of a winter oat variety, Wintok, over that of a spring variety, Cherokee, in rat-feeding experiments. The DeSoto variety was found to have very poor nutritive value which was lower than most of the oat varieties. Weber and Reder (59) found in rat assay that most winter varieties of oats have higher biological values than those of spring varieties.

It has been suggested by several workers that lysine, when expressed as a percentage of protein, decreased as the total protein increased in wheat (47, 27, 45), barley and oats (27). Pence (43) found that the amino acid composition of the fluten isolated from 17 wheat flours, despite the wide range in type and source of the wheat and in the protein contents and baking behavior of the flours from which the glutens were obtained, was essentially constant. Likewise, Miller and co-workers (29) reported that no differences in lysine or glutamic acid with respect to environment were observed. They also demonstrated that no differences among wheat varieties were found for either cystine, lysine, methionine or glutamic acid. A significant difference was observed between the percentage of cystine in samples grown in 1946 and those grown in 1947. Gunthardt and McGinnis have recently studied the effect of nitrogen fertilization on amino acid contents of wheat (11). They found that the higher protein wheat samples contained significantly less lysine (expressed in percentage of crude protein) than did the low protein samples. They further stated that the lower lysine content of the crude protein resulted when nitrogen was applied to the soil. The lysine content appeared to vary with year or yield or both, while the levels of other amino acids remained rather constant.

In addition to amino acid imbalances, the amino acid inter-relationships also have an effect on the biological value of proteins. These inter-relationships, including the amino acid antagonism and toxicity, have further complicated the consideration of amino acid supplementation of proteins. Harper, Benton and Elvehjem (12) found that leucine, when present in excess in a diet, acted as an antagonist of isoleucine. A more complicated antagonism was found by Benton et al. (1). Leucine increased the rats requirement for valine as well as isoleucine while isoleucine, valine and phenylalanine each increased the rats requirement for leucine. Similar relationships were observed between the rats requirements for threonine and tryptophan and the level of phenylalanine in the diet (2).

As early as 1925, Lewis (23) observed a detrimental effect of adding cystine to an oat and cabbage diet fed to rabbits. Since then, excessive amounts of most of the essential and many of the non-essential amino acid have been found to cause toxic reactions in animals. Harper (14) in reviewing the toxicity of amino acids stated: "It is unlikely, however, that toxic effects of excessive amounts of amino acids will prove generally to be the result of amino acid antagonisms". The results of several studies (28, 13) also suggested that "a shift in the equilibrium of some metabolic reaction" is the basis for the toxicity of amino acids.

The term biological value was first defined by Thomas (56) in 1909 as the percentage of absorbed nitrogen which is retained by the body for the repair or synthesis of body protein. The determination requires that the nitrogen intake, the nitrogen excretion, and the endogeneous metabolic nitrogen be known. Mitchell (30) in 1924 applied this method to the growth of young rats. Mitchell and Beadles (32) developed the method of paired feeding and reported it in detail. Osborne, Mendel and Ferry (38) expressed the comparative nutritive value of proteins as gains in body weight per gram of protein intake, defined as protein efficiency ration. Later, Hegsted and Worcester (16) showed that comparison in terms of gains in body weight was as good as that of the conventional protein efficiency ratio.

Both paired feeding and <u>ad libitum</u> feeding have been criticized with respect to their suitabilities for comparing the growth-promoting properties of proteins as well as determining protein efficiencies, as indices of qualities of proteins (6, 40, 34). Most criticism of the <u>ad libitum</u> feeding method, as pointed out by Mitchell (33), is that it might excert an undesirable effect due to different quantities of diet consumed as well as that due to a shift in the nitrogen equilibrium resulted from different protein intake. In spite of such criticism, the <u>ad libitum</u> feeding method has been proved in a recent experiment conducted by Sherwood and Weldon (49) to have higher sensitivity then other feeding methods. It was found that much greater differences in response to different sources of protein were obtained by *ad libitum* feeding method. Moreover, the method is far more favored over others in its labor-saving procedures. The authors concluded that "there was no advantage in using protein efficiencies in place of gain in body weight as the metameter" of the biological assay experiments.

EXPERIMENTAL PROCEDURE

Protein Sources

Oat grains used in the experiments were furnished by Dr. A. M. Schlehuber of the Agronomy Department of Oklahoma State University.

Chemical Analysis of Protein Nitrogen

Total nitrogen content of each of the oat samples used in the experiments was determined by the Kjeldahl procedures. Two-gram samples of ground oats were used and the acid digestion was catalyzed by copper sulfate and sodium sulfate. All samples were run in duplicate. The percentage of protein was calculated from the percentage of nitrogen by multiplying by the factor 6.25.

The results of these determinations are given in Table I.

Preparation and Composition of Rations

The whole oat grains were ground in a Willy mill and well mixed before they were mixed with other components of the rations. The compositions of the rations used in the four experiments are given in Table IV, V and VI. The basal diet contained 85% of oats, 4% of Salt 4 (15, Table II), 5% of corn oil, 0.15% of choline chloride, 0.025% of vitamine mixture (Table III) and sucrose which was added to make up the ration mixture to 100 per cent. The supplemental amino acids replaced an equal amount of sucrose. The rations were stored in the refrigerator.

TABLE I

Percentage of Protein in Oats and Casein
Used in the Experiments

Experiment	Protein Source	Total Protein (%)
I	Oat (Winter Mix.)	15.29
III	Oat (Winter Mix.)	14.83
	Oat (DeSoto var.)	13.68
II & IV	Oat (Winter Mix.)	14.64
I to IV	Casein (Vitamine free)	88.30

TABLE II

Composition of Salt 4

Salt	Gram
$CaCO_3$	600.0
K_2HPO_4	645.0
$CaHPO_4$	118.0
$MgSO_4 \cdot 7H_2O$	204.0
NaCl	335.0
Fe(citrate)	55.0
KI	1.6
$MnSO_4 \cdot 4H_2O$	10.0
$ZnCl_2$	0.5
$CuSO_4 \cdot 5H_2O$	0.6

TABLE III

Composition of Vitamin Mixture

Vitamin	Gram	Vitamin	Gram
Thiamin-HCl	0.5	p-amino benzoic acid	2.0
Riboflavin	0.6	Folic acid	0.02
Pyridoxin-HCl	0.3	Biotin	0.01
Niacin	2.0	Vitamin B_{12}	0.002
Ca-pantothanate	2.0	2-methyl-1-4-naphthoquinone	0.05
Inositol	2.0		
Sucrose	240.0		

Rat Assay

Male albino rats were obtained from the Holtzman Company weighing from 45 to 55 grams and were divided, according to their body weight, into similar groups of six animals each. They were housed in individual cages with raised screen bottoms. In grouping animals, all rats were weighed, and were divided into six groups, according to their body weight. The final groups were formed by taking one animal from each of the six groups. The reason for using this grouping technique is that the variation between mean weight of groups can be minimized and each group contains the rats with similar distributions of body weight.

The animals were fed *ad libitum*, and weighed twice a week. Vitamin A and D were given to the rats orally once a week. The amount given to each rat was approximately 220 I.U. of vitamin A and 22 I.U. of vitamin D_3 daily. One group of rats in each feeding experiment was fed a diet containing 20% of casein.

Blood Amino Acids

At the end of the feeding period of experiment II, blood samples were taken from three rats in each group by heart puncture. Heparin was used to prevent cloting.

Protein-free Plasma --- The procedure of Hier and Bergerin (19) was followed in preparing the protein-free blood plasma. The blood cells were separated by using a centrifuge, sodium tungstate and acid were used to precipitate the plasma protein. The pH of the final protein-free plasma was adjusted to 6.8, using bromothymol blue as an outside indicator. The volume of the protein-free filtrates were mostly from five to ten ml.

Microbiological Assay -- The procedure of Henderson and Snell (17) was applied for determination of lysine, methionine and threonine in the protein-free filtrate. The standard curves were run for the three amino acids in a lower level and the standards were L-lysine·HCl 15 micro gram/ml, L-methionine 4 micro gram/ml and L-threonine 8 micro gram/ml. The pH of assay medium was adjusted to 6.8. The assay micro-organisms used were:

Lysine:	*Leuconostoc mesenteroides*
Methionine:	*Leuconostoc mesenteroides*
Threonine:	*Streptococus faecalis*

RESULTS AND DISCUSSION

Experiment I and II

In experiment I, nine groups of rats were fed an oat diet and supplements of lysine, methionine and threonine. The objective of this experiment was to study the supplemental effect of these amino acids with an *ad libitum* feeding method. Experiment II was a repeat of experiment I, without group 7. The levels of amino acid supplements and weight gains from these two experiments are shown in Table IV and Figure I.

The supplementation of 0.6% L-lysine to a basal diet caused a significant increase in the growth rate of rats. In experiment I (Table IV), the supplementation raised the mean weight gain from 19.3 to 23.4 grams per week. Similar results were observed in experiment II (Table IV) and experiment III (Table V). All these differences were found to be highly significant ($p<0.01$). These observations tend to support the finding of Mitchell and Smuts (31) that the oat protein is deficient in lysine. It was also noted that the growth promoting effect of lysine supplementation in these experiments was much larger than that observed by Mitchell and Smuts. Whether this was due to the fact that a lower level of lysine was used by those authors, or as has been mentioned previously in the literature review that the *ad libitum* feeding method has a much higher sensitivity to show the difference in the quality of proteins. By using this feeding method, we have been able to reproduce this difference in experiments I, II and III.

TABLE IV

The Effect of Amino Acid Supplementation

on the Growth of Rats Fed Oats

Group		Supplement	wt. gain* (gm. per week)	
Expt. I	Expt. II		Expt. I	Expt. II
1	1	None	19.3±0.7	25.5±6.7
2	2	0.6% L-Lysine	23.4±1.3	32.5±4.5
3	3	0.5% DL-Methion.	14.1±0.8	25.8±6.5
4	4	0.75% DL-Threon.	19.7±0.8	29.5±3.7
5	5	Lysine + Meth.	24.7±1.4	33.9±4.9
6	6	Lysine + Threon.	22.3±1.2	34.0±4.9
7	-	Meth. + Threon.	16.0±0.3	
8	7	Lys. + Meth. + Thre.	26.4±0.6	38.4±5.9
9	8	20% Casein	25.2±0.8	?**

*The value represent the mean ± standard error of the mean for six animals.

**This value is not presented since the rats failed to show a consistent growth.

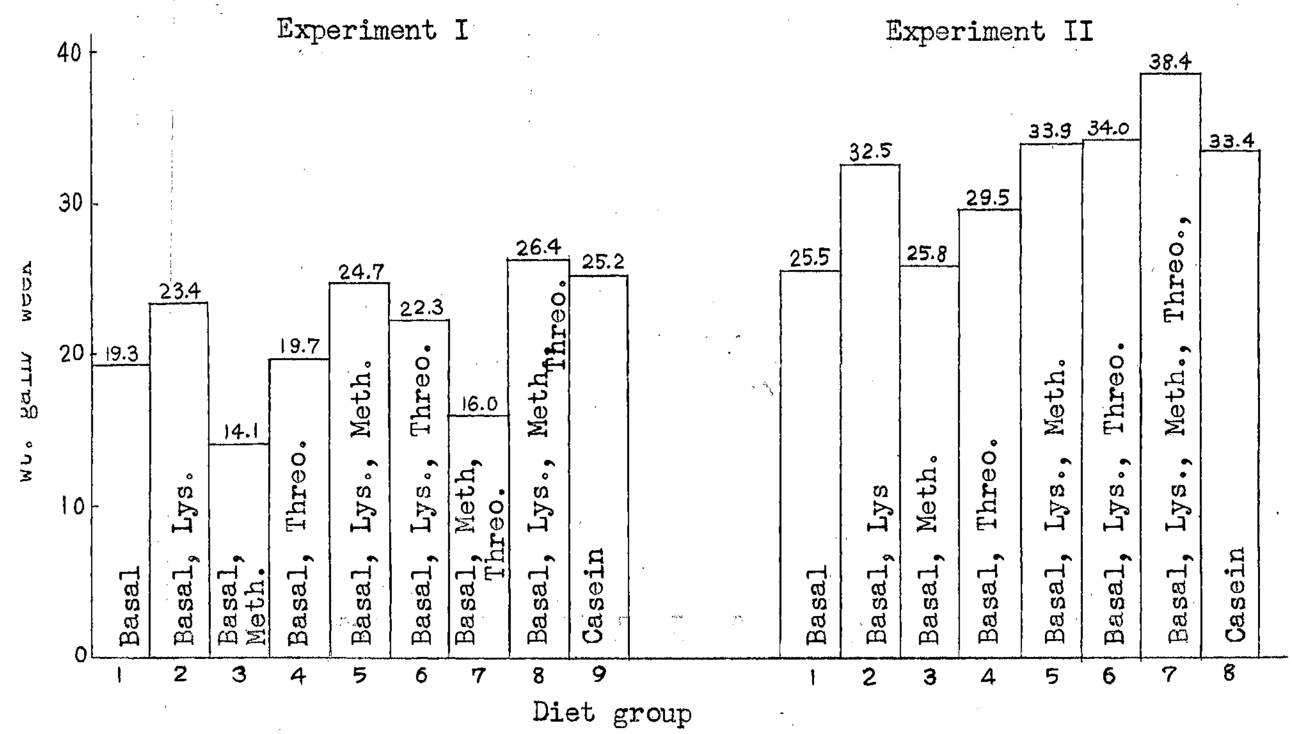

Figure I. Mean Weight Gain of Rats in Experiment I & II

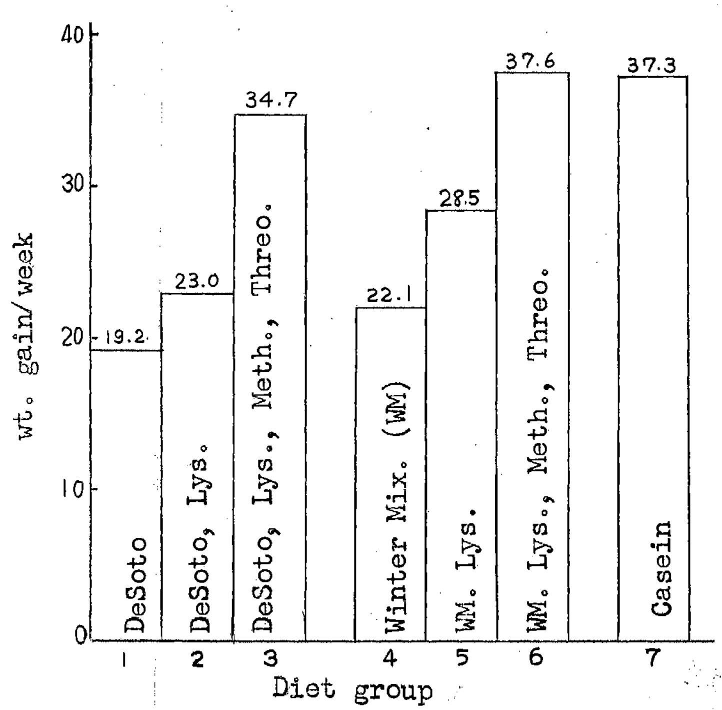

Figure II. Mean Weight of Rats in Experiment II:

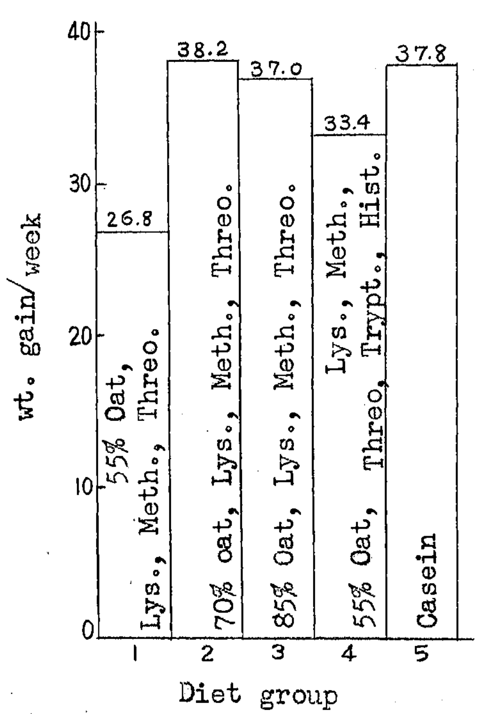

Figure III. Mean Weight gain in Experiment IV

It is of interest to note that the supplementation of 0.5% of DL-methionine to the basal diet resulted in a detrimental effect in the experiment I. The mean weight gain of the rats dropped from 19.3 (basal diet group) to 14.1 grams per week. This difference was found to be highly significant ($p<0.01$). The addition of 0.5% DL-methionine in the presence of 9.75% DL-threonine also caused a growth depression. It has been found in many studies that the supplementation of the second limiting amino acid to a diet caused a detrimental effect (14). These effects have been attributed to amino acid imbalances in the diet, and it has been shown that they can be prevented by the supplementation of the most limiting amino acid to the diet. Since the amount of methionine supplemented in this experiment would not cause a toxic effect in the presence of adequate protein, it seems that the growth depression caused by methionine supplementation was due to the amino acid imbalances. Although the amino acid imbalances caused by supplementation of methionine have been studied (10), more extensive study on the amino acid contents of different varieties of oats as well as the inter-relationship of amino acids would be necessary for a full explaination on this situation.

It was found in both experiments I and II that the supplementation of 0.75% DL-threonine alone did not increase the growth rate. The supplementation of either methionine or threonine in the presence of lysine did not further increase the nutritive value as compared to that of the diet supplemented with lysine alone. The supplementation of lysine, methionine and threonine to the basal diet resulted in significantly better growth over the group of rats fed the oat diet supplemented with lysine ($p<0.05$). There was essentially no difference between the growth response of this group and that of the group fed the 20% casein diet. The above observation indicates that lysine is the most limiting amino acid in the protein of oats. None of the amino acids other than lysine, when supplemented along to the oat diet, caused a growth response. It has already been shown that the supplementation of lysine alone, though markedly increasing the growth rate, does not increase the growth rate to that of the group fed 20% casein. Also the further supplementation of either methionine or threonine in the presence of lysine failed to show a significant growth response over the group receiving lysine. These observations suggested that both methionine and threonine are the second limiting amino acids in oats. The growth response to supplementation of lysine alone was limited by this second deficiency.

The addition of methionine to a diet containing lysine and threonine supplements caused a growth response in the experiment I. This addition significantly increased the weight gain of the rats ($p<0.01$). The same treatment in experiment II failed to show a significant difference. The growth depression which caused by the supplementation of methionine alone in experiment I, failed to show a significant difference in experiment II. These observations tend to suggest that the picture of amino acid compositions were different in the oats of experiment I and II.

When lysine, methionine and threonine together are supplemented to the oat diet the growth was identical to that of the rats fed the 20% casein diet, and this growth was significantly greater than that obtained from the oat diet supplemented with lysine alone. This indicates that the amino acid imbalance can be improved only by supplementing of lysine, methionine and threonine to the basal diet.

Experiment III

The objective of this experiment was to compare the difference in the nutritive value between oat varieties. Two different oat samples were used in this study. The DeSoto variety, which was found to have poor nutritive value (57), was compared to a mixture of winter varieties, which were known to have higher biological values (59). The basal diet of the DeSoto variety contained 85% of oats. The oat level in the basal diet of the mixture of winter varieties was decreased to 78.8% (according to the analytical data of protein levels in Table 1) to maintain the same protein level in the diets. Either lysine or lysine, methionine and threoine were added to the diet. The levels of amino acid supplements were the same as in experiments I and II. The results are presented in Table V and Figure II.

A significant difference between the biological values of the two oat samples was observed in this experiment. The growth rate of the rats that received the DeSoto oat basal diet was 19.2 grams per week while that of the rats fed the mixture of winter oat varieties was 22.1 ($p<0.05$). The results on the growth of rats fed two oat diets supplemented with either lysine or lysine, methionine and threonine, were similar to those observed in the experiment I. A difference between the growth responses was obtained with lysine supplementation to the diets containing the two different samples of oats. The mixture of winter oat varieties when supplemented with lysine caused a growth rate of 28.5 grams per week, which was significantly higher ($p<0.01$) than the growth rate caused by the DeSoto oat diet with the same level of lysine supplement (23.0 gram per week). The supplementation of all three amino acids to the winter mixture oat diet caused a growth rate which was essentially identical to

TABLE V

Effect of Varieties of Oats on
Growth Response to Amino Acid Supplements

Group	Protein Source	Supplement	Wt. Gain* (gm./wk.)
1	DeSoto	None	19.2 ± 1.2
2	DeSoto	Lysine	23.0 ± 1.1
3	DeSoto	Lys.+Meth.+Thre.	34.7 ± 0.6
4	Winter Mixture**	None	22.1 ± 0.5
5	Winter Mixture	Lysine	28.5 ± 0.7
6	Winter Mixture	Lys.+Meth.+Threo.	37.6 ± 0.7
7	20% Casein		37.3 ± 1.1

*The value represent the mean ± standard error of the mean of six animals.

**The percentage of oats in group 4, 5, and 6 were decreased to 78.8 per cent in order to have the same level of diet protein as that in diet of group 1, 2, and 3.

that of a group fed 20% casein diet. The same level of supplementation of three amino acids on the DeSoto basal diet showed a much lower nutritive value. The difference in growth response of these two diets was statistically significant ($p<0.02$).

Since the protein levels in the two oat basal diets were adjusted to a same level, it is reasonable to assume that the poorer nutritive value of the protein of DeSoto oat variety was due to a poorer balance of its essential amino acids. Laudick (22) analysized these two oat samples and found that there were no differences in the levels of lysine, methionine and threonine between the two samples. He also found that the digestibility of the mixture of winter oat varieties was poorer than that of the DeSoto oats. These observations further support the view that amino acid balance was poorer in the DeSoto oats.

It has been found by several workers that a high level of one amino acid in a low protein diet caused a higher requirement for an other amino acid in experimental animals (9, 1, 2). It seems possible that the same situation occurs in the DeSoto oat protein. However, no conclusion should be made with respect to whether or not an amino acid antagonism is a factor involving in the poor nutritive value of DeSoto oats.

Experiment IV

Observations were made on the growth of five groups of rats fed various levels of oats with amino acid supplements. The objective of this experiment was to study the minimum protein requirement for maximum growth of rats fed oats supplemented with lysine, methionine and threonine. Three levels of oats, 55, 70 and 85 per cent, were fed. The levels of supplemental amino acids were reduced in the diets containing lower levels of oats in order to maintain a similar balance of amino acids in each diet. Tryptophan and histidine were also supplemented to the 55% oat diet in the presence of supplemental lysine, methionine and threonine. The results are presented in Table VI and Figure III.

Similar weight gains were observed with the groups fed diets containing 85 and 70 per cent of oats. This growth rate was equal to that of the group fed the 20% casein diet. The further reduction of the oat level to 55% depressed the growth rate significantly. The supplementation of 0.13% DL-tryptophan and 0.24% L-histidine to this diet increased the growth rate from 26.8 to 33.4 gram per week, but it was not as rapid as that of the group fed the 20% casein diet (37.8 gram per week).

In Figure IV, the rats requirement for the five amino acids used in this experiment are compared with the amino acid levels in the diets of this experiment. The amino acid requirements of the rat were those cited by Block (3). The levels of amino acids in the oats were taken from the analytical data for oats found in the literature (4), and they are estimated as:

Lysine	3.6 (% of the crude protein)
Methionine	2.0
Threonine	3.6
Tryptophan	1.3
Histidine	2.3

TABLE VI

Growth of Rats Fed Various Levels of Oats
with Amino Acid Supplements

G'p	Oat (%)	L-lys. (%)	DL-meth. (%)	DL-threo. (%)	Rate of gain* (gm/wk.)
1	55	0.40	0.32	0.49	26.8 ± 1.2
2	70	0.50	0.41	0.62	38.2 ± 0.9
3	85	0.60	0.50	0.75	37.0 ± 0.5
4	Diet #1 + 0.13% DL-Tryp. + 0.24% L-hist.				33.4 ± 1.1
5	20% Casein				37.8 ± 2.1

* The value represent the mean ± standard error of the mean of six animals.

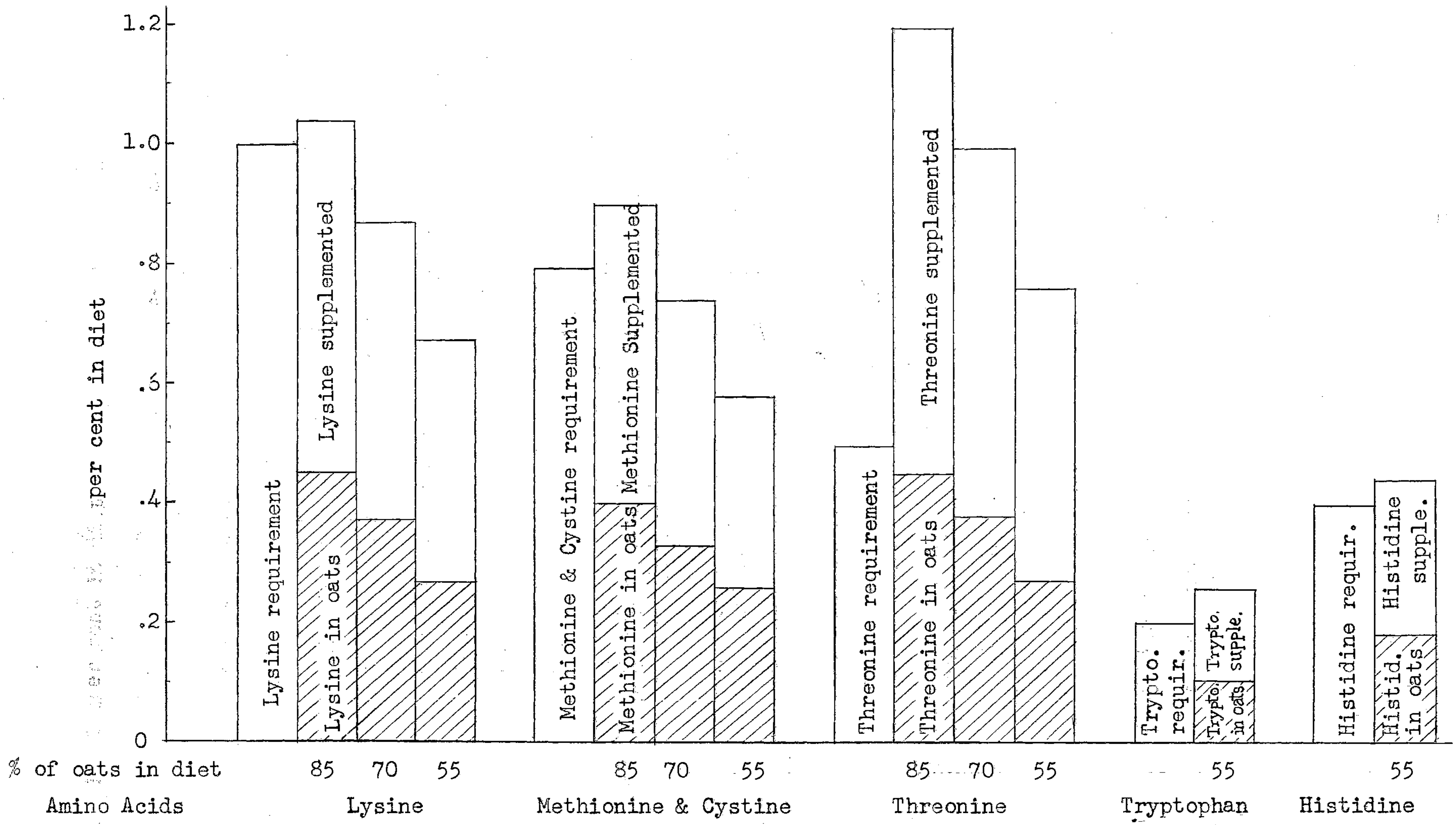

Figure IV. The comparison of amino acid requirements of rats and amino acid levels in diets of the Experiment III.

The lysine level in a 85% oats diet was only half of that required for growth by the young rat. The supplementation of 0.6% and 0.5% of L-lysine respectively to 85% and 70% of oats diet increased the level of lysine in the diet approximately to that of the rats requirement. The supplementation of 0.4% of L-lysine to a 55% oat diet produced a lysine level significantly lower than the rat's requirement. A similar situation occurs in the comparison of the rat's requirement to the dietary level of methionine (including cystine) (Figure IV). The level of threonine in a 85% oat diet seems close to the rat's requirement level. If this estimation is true, threonine would not be a primary deficience amino acid in a diet containing 85% of oats.

It has been shown that the supplementation of tryptophan and histidine to a diet containing 55% of oats supplemented with lysine, methionine and threonine caused an increase in the growth rate. This suggests that when the level of oats is decreased to 55% of the diet, either tryptophan or histidine become the limiting amino acid. The estimated levels of both tryptophan and histidine in the 55% oat diet are only approximately one-half of the levels required for growth of young rats. The supplementation of 0.13% DL-tryptophan and 0.24% L-histidine increased the levels of these two amino acids to that of the rat's requirement.

The Blood Amino Acids

At the end of experiment II, the levels of lysine, methionine and threonine in the blood plasma of the rats were determined by microbiological assay. The objective of this determination was to study the effect of amino acid supplement on the levels of these three amino acids mentioned. The results are presented as micro grams per ml. of blood plasma in the Table VII and Figure V.

It was found that the variations between individuals were rather high. This has been observed by the previous workers in the studies of blood amino acids (19). The levels of lysine, methionine and threonine in the blood from group 8 (20% casein group) were higher than that found by Henderson et al. (19). They fed the rats a diet containing 18% of casein and found that the level of lysine, methionine and threonine in the blood plasma were 58, 9.5 and 44 micro gram/ml respectively. Lysine was 88% higher, Methionine was 31 % higher and Threonine was 58% higher in this experiment than that observed by Henderson et al.

The plasma level of lysine in the rats of group 1, fed the basal oat diet, was found to be only 11.15 micro gram/ml while that of the group fed the 20% casein diet was 109.96 micro gram/ml. The evidences in the previous experiments have shown that lysine is the most limiting amino acid in oats. It seems possible that this difference in the blood level of lysine was the result of this deficiency. Henderson et al. found that the level of blood lysine increased from 58 to 72 micro gram/ml as a result of fasting (19). Wiss (55) observed that feeding a high protein diet (85% horse meat and 15% wheat) to rats resulted in a higher plasma lysine (188 micro gram/ml) than that of the fasting animals (88 micro gram/ml). The fasting group was found to have a higher level of

TABLE VII

Effect of Amino Acid Supplementation on the Plasma Levels of Lysine, Methionine and Threonine of Rats fed Oats

Group	Supplements	Lysine* (micro gram/ml plasma)	Methionine* (micro gram/ml plasma)	Threonine* (micro gram/ml plasma)
1	None	11.15 (5.80-14.66)	4.69 (2.47-6.83)	22.75 (16.33-30.68
2	0.6% L-lysine	111.28 (71.95-152.47)	6.07 (4.04-7.94	16.08 (11.05-20.65)
3	0.5% DL-methione	30.97 (13.23-51.75)	11.63 (4.38-19.26)	36.32 (36.19-36.45)**
4	0.75% DL-threonine	19.53 (11.84-25.54)	5.73 (4.10-7.61)	188.55 (126.9-228.9)
5	Lysine + Methionine	106.33 (104.2-108.5)	14.58 (5.93-26.55)	24.05 (20.25-27.84)
6	Lysine + Threonine	96.40 (92.70-99.18)**	3.51 (3.38-3.60)	143.37 (126.7-157.5)
7	Lys. + Meth. + Threo.	111.83 (79.62-142.11)	19.06 (17.5-21.3)	154.45 (105.7-220.5)
8	20% Casein	109.96 (101.89-119.2)	12.48 (12.15-13.17)	69.45 (65.9-71.4)

* The values represent the mean and the range of three data obtained from different rats.

** The mean were calculated from two values because too small volume of plasma sample was obtained.

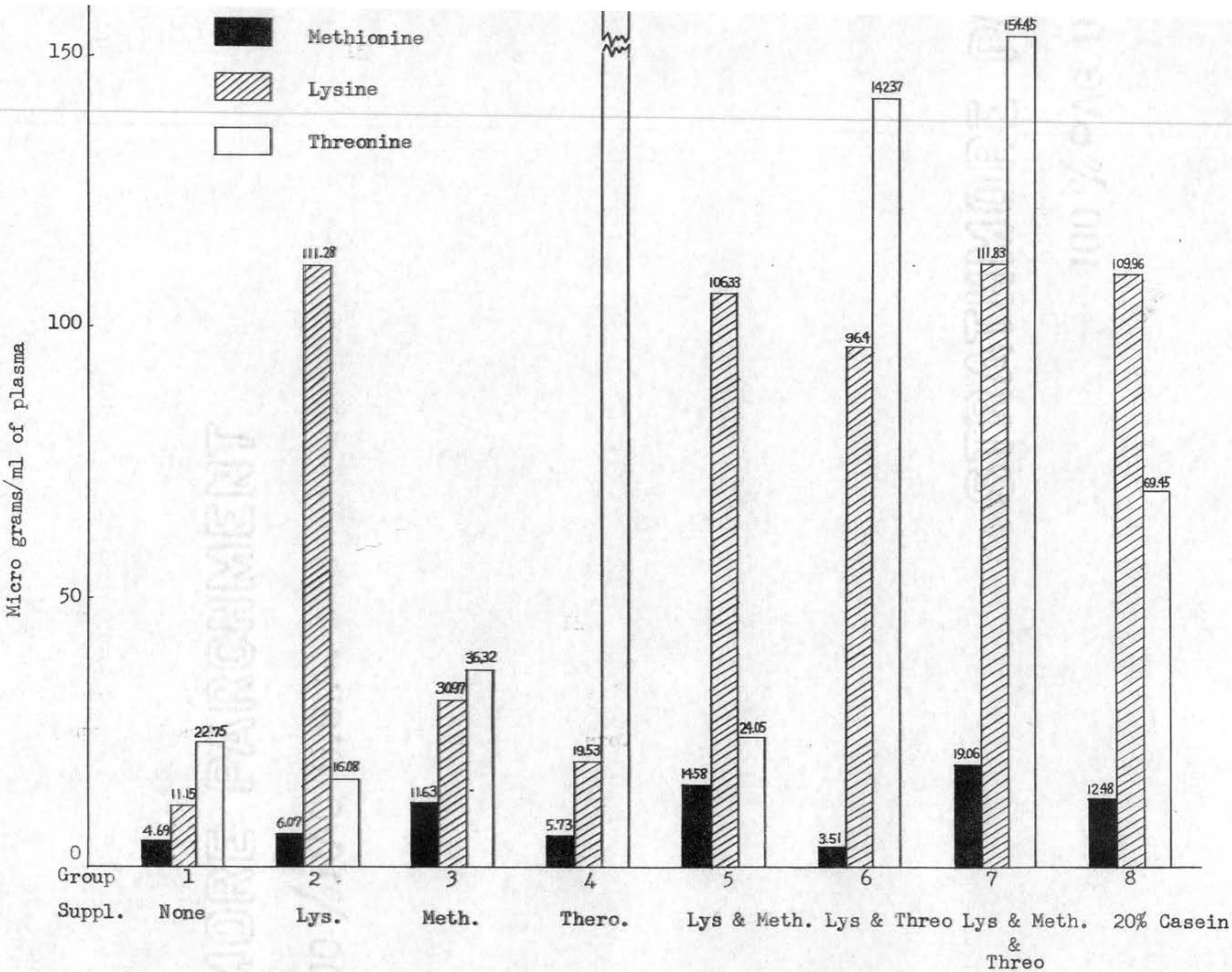

Figure V. The levels of Plasma Lysine, Methionine and Threonine in the Rats of Experiment II.

plasma lysine than those of groups fed either high fat or high carbohydrate diets. These workers demonstrated that the plasma level of lysine in rats can be increased either by fasting or by feeding the animals a high protein diet. Therefore, the plasma level of lysine is not always a good indication of the level of this amino acid in the diets. The supplementation of lysine, either with or without the other amino acids, increased the blood level of this amino acid approximately 10 fold. This was approximately the same level of lysine as was found in the plasma of the group fed the 20% of casein diet. The threonine supplement to the basal diet did not cause an increase in the plasma lysine level. The supplementation of methionine alone increased the level of lysine from 11.15 to 30.97 micro gram/ml., however, more information is required for a full explaination.

The level of blood methionine of the group fed the basal diet was 4.69 micro gram/ml while that of the group fed 20% casein was 12.48 micro gram/ml. The supplementation of methionine in the diet, either with or without other amino acid supplements, increased its level in the blood plasma 2 to 4 fold. Since the level of this amino acid was lowest of the three, smaller differences in the level of methionine resulted from the supplementation.

The plasma threonine level of the group fed the basal diet was 22.75 micro gram/ml, which was about one third of that of the group fed the 20% casein diet (69.49 micro gram/ml). The low value for both methionine and threonine in the basal diet tends to support the suggestion that they are both the second limiting amino acid in the oats. It is of interest that the supplementation of threonine to the diet increased the plasma threonine level to a much greater value than that of the animals fed the 20% casein diet while supplements of lysine or methionine produced levels of these amino acids approximately the same as those of the group fed the 20% casein diet. It has already been shown in the experiment IV that the dietary level of threonine was higher than the requirement of rats. The high level of plasma threonine caused by this supplement seems to be a result of this high level of threonine in the diet.

SUMMARY

The effect of amino acid supplementation on the nutritive value of different varieties of oats were observed in rat-feeding experiments. The animals were fed ad libitum, and the gains in body weight were noted. The following trends were noted from the data obtained:

1. To a diet containing 85% of oats, a supplement of 0.6% L-lysine increased the weight gained. A supplement of 0.5% DL-methionine alone decreased the weight gained and a supplement of 0.75% DL-threonine alone was without effect. Further addition of either methionine or threonine to lysine supplement caused no more weight gain than lysine alone. A supplement of lysine, methionine and threonine caused a greater growth response than obtained from other combinations of amino acid supplements. This last diet caused a weight gain which was the same as that of a group fed 20% casein. These observations suggest that lysine is the most limiting amino acid in the protein of oats, and both methionine and threonine are the second limiting amino acids. The amino acid imbalance of oats protein can be improved by the supplementation of all three amino acids.

2. Differences were observed on the weight gain when different oat varieties were fed to rats. The oats of poor nutritive values were of the DeSoto variety while the mixture of winter varieties used had a high nutritive value. A lysine supplement caused a greater growth response with the mixture of winter varieties of oats. A supplement of lysine, methionine and threonine to both oat samples resulted in significantly different

weight gains. Although growth responses were obtained in both cases. It was suggested that the amino acid balance is poorer in DeSoto oats than in the mixture of winter varieties of oats.

3. Similar weight gains were observed with the groups fed diets containing 85% and 70% of oats, each supplemented with lysine, methionine and threonine. This growth rate was equal to that obtained from 20% casein diet. A diet containing 55% of oats and three amino acid supplements caused a significantly lower growth rate. The further addition of tryptophan and histidine to this last diet significantly increased the growth rate, but was not as rapid as the growth obtained from 85% or 70% oat diet.

4. The levels of lysine, methionine and threonine in the plasma of rats were found being effected by the supplementation of these three amino acids in an oat diet. The levels of three amino acids in the plasma of the rats fed 85% oat diet was much lower than that of the rats fed 20% casein. A supplement of lysine or methionine, either with or without other amino acid supplements, resulted in an increase of plasma level of lysine or methionine approximately to that resulted from feeding rats with 20% casein. A supplement of threonine caused an increase in the plasma level of threonine which was much higher than that obtained from the 20% casein group.

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