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Delayed Protein Complementation with Common Foods Used in the Middle East

LaDon J. Hilton

Abstract

DELAYED PROTEIN COMPLEMENTATION WITH COMMON FOODS USED IN THE MIDDLE EAST

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The purpose of this research was to determine whether whole wheat and lentils, food staples consumed in the Middle East, will exhibit delayed protein complementation when fed in alternating meals. Lentils are limiting in sulfur-containing amino acids, especially methionine, while whole wheat is limiting in lysine, and when these proteins are fed together they supplement one another.

Sixty male, Sprague-Dawley weanling rats were divided into six diet groups with ten in each group as follows: diets fed ad libitum; wheat, lentils, wheat and lentils combined; diets pair-fed; wheat and lentils combined, wheat alternating with lentils, lentils alternating with wheat. The design of the study was to feed lentils and wheat in alternating meals or in the same meal. The proportion of lentil to wheat protein was 1:1 and was given 1 hour 4 times a day with 4 hours between the meals. The diets included protein at 13.7 % in an otherwise complete diet. The rats were allowed water ad libitum. After a 2 week adjustment period data was collected for 3 additional weeks.

The rats fed ad libitum for three weeks the wheat diet showed significantly better growth than those on the lentil diet, with mean and standard deviations of 53.4 ± 5.62 g. and 23.5 ± 3.95 g. respectively ($p < 0.01$). Rats on the wheat-lentil diet grew better than those on the wheat or lentil diets alone with mean and standard deviation of 87.0 ± 8.42 g. ($p < 0.01$) There was a similar relationship between the PER values for lentils 1.08, wheat 1.54, and wheat-lentils 1.95. The growth data demonstrates excellent mutual supplementation between these two protein sources.

In the rats that were pair-fed there was no significant difference in weight gain in the control group with mean and standard deviations of 41.5 ± 3.27 g. that was fed lentils and wheat in the same meal as compared with the wheat alternating with lentil (WLWL) group 39.4 ± 3.31 g. or that were fed lentils then wheat (LWLW) in alternating meals 44.7 ± 5.38 g. There was also a similar relationship between the PER values with mean and standard deviations of the control 1.7 ± 0.14, WLWL 1.62 ± 0.15 and LWLW 1.82 ± 0.16.

The results from this study show that whole wheat and lentils complement each other whether in the same meal or in alternating meals with 4 hours separating the meals. Thus there was adequate delayed protein complementation in this study.

LOMA LINDA UNIVERSITY

Graduate School

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LaDon J. Hilton

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Each person whose signature appears below certifies that this thesis in his opinion is adequate, in scope and quality, as a thesis for the degree Master of Science.

U. D. Register, Professor, Chairman

Albert Sanchez, Professor of Nutrition

Kenneth Burke, Professor of Nutrition

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INTRODUCTION

Protein supplementation is a broad term that includes fortification and complementation. While, fortification refers to the addition of one or more amino acids to a protein limiting in certain amino acids, complementation is the appropriate combination of proteins that complement one another (1-3). These terms supplementation and complementation will be used interchangeably in this study.

The objectives of supplementation and complementation are to increase the quality of protein. One method of measuring the protein quality is protein efficiency ratio (PER). Protein efficiency ratio (PER) is the present official method for determining protein quality for the United States and Canada (4). Legumes are limiting in sulfur-containing amino acids but contain adequate amounts of lysine while cereal grains are limiting in lysine but contain adequate amounts of sulfur-containing amino acids (1,5-7). The appropriate combination of cereals and legumes or the addition of lysine to cereals or methionine or sulfur amino acids to legumes result in protein that is of higher quality than any of these components alone (1,2,8-15).

It has been generally thought that supplementation can occur only when all the essential amino acids are present in the same meal (16-20). More recent studies indicate that limiting proteins may be supplemented, but the time varies

depending on the specific amino acid in question (16,19,21-26).

A few studies have examined the effect of delayed complementation using a few combinations of limiting proteins (14,15,27). Mills and Canolty found an inverse relationship between complementary efficiency and time when using time intervals of 0, 1, 2, and 3 days (27). Sanchez in two separate studies found with 4 hours between the meals analogous growth promoting capacity was observed when using rice and mungbeans or pinto beans with either rice, wheat or corn (14,15).

Wheat and lentils are some of the main staples eaten in the Middle East specifically in the Syrian Arab Rep., of Turkey and Jordan (28,29). With the people of the lower socio-economic class in these countries the use of chick peas and wheat should be no problem for adequate protein quality since the PER for chickpeas is similar to that of eggs. Lentils are much lower in methionine than pinto beans or mungbeans (30). It is known that when lentils and wheat are fed together to rats excellent supplementation is observed (13); however, no studies have been conducted on the possible delayed supplementation when lentils and wheat are fed separately.

The purpose of this study is to determine whether the delayed feeding of wheat and lentil proteins in alternating meals will produce a protein efficiency ratio (PER) that is

significantly different from the results obtained feeding these identical proteins in the same meal. Considering there is an eight hour delay overnight (as compared to a four hour delay during the day time) we also want to see if the sequence of the feeding will have an effect on the protein efficiency.

REVIEW OF LITERATURE

Bressani and Elias (1) have stated that in general, maximum supplementary results occur at 1:1 protein ratio for legumes and cereals. The proper proportion is a subject of continuing discussion (31-34). Research at Loma Linda University shows that 25-33 % of the protein from the complementary protein sources provide a protein of good quality (11,13). Various factors may effect the proportions of the cereal/legume mixture needed including the following: the digestibility of the protein, the total energy and protein content of the diet, and the variation in the protein content or amino acid distribution in each plant species (35).

Delayed Time Supplementation with Amino Acids

Earlier studies on delayed amino acid supplementation indicate that a limiting amino acid should be given in the same meal or the body would not be able to synthesize proteins based on the concept that the body cannot store amino acids for use at a later time (17,18). It is true that all amino acids must be present in the cell at the time of translation for protein synthesis (36). This concept has led to the belief that all the essential or indispensable amino acids must be present in right proportions every meal or the limiting amino acid must be provided with the

deficient protein.

The above concept was challenged by Yang et al. (21,22) and Howe and Dooley (23), when using wheat protein limiting in lysine, showed that there is supplementation up to 16 hours after feeding wheat without adversely affecting growth. Delayed supplementation also allows effective growth when threonine is fed 6 hours apart from gluten in which lysine is supplemented in adequate levels but while the gluten diet is low in threonine (23).

Geiger (17) studied delayed methionine supplementation in 3 groups of 2 rats each fed casein treated with formic acid and hydrogen peroxide which destroyed methionine and tryptophan. Tryptophan and methionine were added to the control diet but only tryptophan was added to the methionine deficient diet. The methionine deficient diet was fed for 12 hours and then a protein free diet supplemented with methionine was fed for 12 hours. Another group of two rats were offered the methionine free diet and in a separate container from the methionine supplement. The control group was fed the tryptophan-fortified casein combination with the methionine supplement mixed in the diet. Geiger reported that with the 12 hour delayed supplementation both rats lost weight and with the rats fed the supplemented methionine in separate jars one rat grew but at a slower rate and the other lost weight. From this study it was concluded that methionine must be present in the meal for optimal protein

utilization. Obviously, a study with such a limited number of rats is inconclusive.

Switoniak et al. (24) determined that methionine supplementation must be given either 1 hour before or after the non-supplemented meal for adequate protein utilization. Methionine supplementation 2 hours after the meal produced a decreased growth as compared to non-delayed supplementation but the growth was significantly greater than the non-supplemented group. In a similar study design but with sulfur amino acid supplementation (methionine and cysteine) rather than methionine alone, the results were comparable (25). The difference was that the 1 and 2 hours supplementation after the meal was somewhat less effective than with the fortified meal. Thus, with sulfur amino acids or methionine alone delayed supplementation works best when the amino acids are furnished very close to the un-supplemented meal.

The present data available on tryptophan (16,19,26) indicates that effective delayed supplementation occurs only with short time intervals from the deficient meal.

Delayed Time Supplementation with Vegetable Proteins

Mills and Canolty, (27) tested the effect of delayed complementary effects of wheat germ with mungbeans as well as sesame seeds with black beans over 0, 1, 2, and 3 day period.

They found that there was an inverse relationship between complementary capacity and time. They stated in their introduction that there was "divergence of opinion" on the amount of time needed for time delayed complementation for limiting proteins. The conflict of time was between 4 - 6 hours, a few hours or no time lapse. We wondered why they did their test on days instead of hours since they did not state the rationale for this extended time period. If the amino acids do have a limited time span i.e. lysine of 16 hours (21-23) then to test for 1, 2, and 3 days would not be expected to produce delayed complementation.

Using plant proteins for delayed protein complementation Sanchez et al., in two separate studies (14,15), reported that rats fed vegetable proteins in alternating meals with 4 hours between the meals had the same growth promoting capacity and protein quality as when fed these foods together at the same meal. In the first study rice and mungbeans with a 1:1 protein ratio and 10 percent protein were fed for 28 days with 10 rats in each group. Both weight gain and PER were the same whether the proteins were fed in the same meal or in separate meals. The second study demonstrated that when rats were fed pinto beans alternately with either wheat, corn or rice with conditions similar to the above study, the results indicated there was no significant difference between those fed the protein in alternate meals or in the same meal except in the limiting

corn-bean combination for growth in the 4th week. This exception could possibly be explained due to differing food intake since the PER for week 4 for this group was not significantly different from the rats fed alternating proteins versus both proteins in the same meal.

Similar delayed time supplementation studies have not been performed on humans. In many parts of the world protein complementation, especially in lower economic populations, is used as an economic source of protein and most food combinations are similar to the ones reported in this and other papers (14,15). One question we have tried to answer in this research is if an occasional meal does not have all the essential amino acids in the proper proportions will it cause a decreased protein efficiency? Whether or not rats are an approximate model for determining PER for humans is not agreed upon (37-39).

Human studies performed on women subjects (40,41) investigated the relationship between the feeding of low quality protein at one meal and high quality protein at lunch or dinner. There was no difference in the quality of protein whether the proteins were fed together or separately indicating delayed protein complementation. This was observed when the calorie and protein intake were adequate. Similar studies are needed to test the effect of time delayed supplementation using vegetable proteins in humans.

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METHODOLOGY

Animals

Male, weanling Sprague-Dawley rats were housed in individual raised bottom stainless steel cages at the Loma Linda Medical Center Animal Care Facility in a room maintained at 70-74°F, at 40-50% humidity and lighted from 7 pm to 7 am. The rats were placed on a standard laboratory rat diet for a two day equilibration period upon arrival. Prior to feeding the test diet, the rats were divided into 6 groups of 10 animals each and weighed. Each group was adjusted so that the mean group initial weight was within 1 gram of every other group. The rats were fed 4 times daily and water was allowed ad libitum. Food was placed in conical cups that were placed inside glass jars. Food for the rats fed ad libitum was placed in the above containers and wire mesh was placed inside the conical cup to minimize spillage.

Diets

Whole wheat flour was purchased from the Loma Linda Market. Pre-cooked and ground lentils were prepared by an anonymous source which cooked the lentils in moist heat at 250° F and they were dehydrated at 150° F.

The protein content of wheat and lentils were determined using the Kjeldahl method described by the Association of Official Analytical Chemists (42).

The experimental diets consisted of the following in grams percent:

1. Protein	13.7
2. Corn oil	10
3. Vitamin mixture	1
4. Minerals mixture, XIV	4
5. Choline Chloride	0.1
6. Corn Starch	(To make 100)

Zinc chloride (55.0 mg per 100 gm mineral mixture) and choline chloride (0.1% level in the diet) were added to each of the diets at or above levels recommended for rats (43). American Institute of Nutrition (AIN) Vitamin Mixture 76, Choline Chloride, and Salt Mixture XIV were obtained from ICN Nutritional Biochemicals. Zinc Sulfate was obtained from Fisher Scientific Company. The composition of the mineral mixture and of the vitamin mixture are given in Appendix A and B, respectively.

All the diets were prepared in one day. Each diet was mixed in a Hobart mixer for 5-7 minutes. After preparation the diets were stored at -10° F until needed for use then they were stored at 40-50° F.

Experiments

The experiment was conducted to determine the complementation of wheat and lentils at 1:1 protein ratio when given separately at alternate meals throughout the day.

Six groups of rats were fed the following diets for 3 weeks with a 2 week adjustment period before the study

began. Fresh food was placed in the conical cups daily. For groups 1-3 the food consumption was determined by weighing the food containers (conical cups and salve jars) at the beginning and end of the weekly time period to measure food not consumed from containers. For these same groups wide mouth jars which contained the food for this group were weighed at the beginning and end of each time period to determine weekly food consumption.

Groups 5 and 6 received both whole wheat and lentil diets on alternate meals; group 5 was given the wheat diet first then the lentil diet while group 6 was given the lentil diet first then the whole wheat diet. The feeding schedule of the various diets are given in Table 1. The food intake every meal was recorded for groups 4-6 for each individual rat. The rats were individually weighed weekly.

Pair-feeding for groups 4-6 was determined each day by calculating the total food intake from the previous day taking this value and dividing it by 120 which gives the average food intake for one meal for the following day. This quantity of food is the amount of lentils-wheat combination which was given to group 4 each meal the following day. This same quantity is the amount of wheat that was given to groups 5 and 6 the following day when wheat was offered. Lentils were given ad libitum and the containers measured before and after the feedings. Thus the amount of lentils eaten would determine the amount of food

which was to be given the next day. The rats consumed virtually all of the wheat that was given each day. Using this method of pair-feeding the rats consumed 1:1 ratio of wheat to lentil proteins.

A two week period was required for the rats in groups 5 and 6 to become adjusted to the feeding schedule. With the conical cup in the salve jar the food spillage was measured weekly and/or daily weighing depending upon the groups. The rats were weighed weekly and the PER values were calculated.

Groups 1,2 and 3 were given the whole wheat diet, lentil diet and combined whole wheat-lentil diet, respectively. Fresh food was placed in conical cups daily. The total food intake was measured each week for groups 1-3.

The amount of growth in grams gained divided by the protein intake in grams is the method used to determine the PER of a protein.

Table 1 Experimental Design

Group	Diet	Feeding Sequence
1.	Whole Wheat (W)*	W ad libitum
2.	Lentils (L)*	L ad libitum
3.	Whole Wheat-Lentils*	WL ad libitum

		6 a.m.	11 a.m.	4 p.m.	9 p.m.
4.	Whole Wheat-Lentils †	WL	WL	WL	WL
5.	Whole Wheat or Lentils †	W	L	W	L
6.	Lentils or Whole Wheat †	L	W	L	W

* Diet is given ad lib as compared to pair-fed.

† 1:1 protein ratio pair-fed.

RESULTS

In Table 2 are the results obtained for group 1-3 for the 21 day period evaluated. Means only were calculated on food intake, protein intake, and PER.The means and standard deviations for weight gain were calculated. There were significant differences between groups 1 and 2, 1 and 3, 2 and 3 for growth (at $p < 0.01$) for the 21 day period. Although no statistical analysis was done on the PER the values appear to be significantly different.

TABLE 2.

Mean Food and Protein Intakes and Weight Gain and Protein Efficiency Ratio of Diets Fed ad libitum During 21 day period.

Group	Diet	Food Intake,g	Protein Intake,g	Grams Gain mean ± SD		PER
	Single Proteins					
1.	Wheat	88.4	11.56	53.4	5.62*	1.54
2.	Lentils	51.2	7.28	23.5	3.95*	1.08
	Combined Proteins					
3.	Wheat and Lentils	108.5	14.90	87.0	8.43*	1.95

* significance at $p < 0.01$.

Means and standard deviations for groups 4-6 were calculated on food intake, protein intake, weight gain, and PER and is summarized in Table 3 for the 21 day period. There were no significant differences between the control group 4 and either of the experimental groups 5 or 6. There was a significance between the experimental groups 5 and 6 for grams gained (significance at $p < 0.05$) and PER (significance at $p < 0.01$).

TABLE 3.

Mean Food and Protein Intakes and Weight Gain and Protein Efficiency Ratio of Diets Pair Fed During 21 day period.

Group Number	Diet	Food Intake,g mean ± SD		Protein Intake,g mean ± SD		Weight Gain,g mean ± SD		PER mean ± SD	
Combined Proteins									
4.	Wheat and Lentils	58.2	ND	7.94	ND	41.5	3.27	1.7	0.14
Alternating Proteins									
5.	WLWL	58.2	3.88	7.95	0.53	39.4	3.31	1.62	0.15
6.	LWLW	59.6	4.01	8.14	0.55	44.7	5.38	1.82	0.16

No significant differences were found between the control and the experimental groups for growth or PER.

Statistical analysis (44) using analysis of covariance using weight gain and PER as dependent variable, food intake as the covariant and group identification as the independent variable was done to determine if there was a significance between groups 4-6 which showed that for both weight gain and PER there were significant differences between the groups. Group t-tests were then done to determine where the

differences were and what was the implication of the difference. The group t-tests indicated that the differences were between groups 5 and 6 and not among the control and the experimental groups. The statistical analysis is given in Tables 4(a), 5(a), 6(a) and 7(a). In Tables 4,5,6 and 7 are the means and standard deviations for the variables being analyzed on groups 4-6. Table 4(a) answers the question, is there a significant difference in Protein Efficiency Ratio between the control group 4 and experimental groups 5 and 6 the results for week 3-2, 4-2, or 5-2? Table 4(a) indicates for week 3 that the rats who consumed the diet pattern WLWL obtained a PER significantly inferior to groups 4 and 6. For weeks 3 and 4 combined the rats with the diet pattern LWLW obtained a significantly elevated PER as compared to that of groups 4 and 5. The combination of weeks 3,4 and 5 showed that the rats on the LWLW diet pattern had a significantly elevated PER over that of the WLWL diet pattern but the latter diet pattern was not significantly different from that of the diet with both wheat and lentils given in the same meal. Thus only in week 3 was there any PER significantly lower than the control group.

TABLE 4.

Mean Protein Efficiency Ratio difference from week 2 to the end of 3rd, 4th and 5th weeks for 3 diet groups. Weight differences at the end of 3,4,5 week.

	WEEKS 3		WEEKS 3+4		WEEKS 3+4+5	
GROUP ID	MEAN	SD	MEAN	SD	MEAN	SD
4. BOTH PAIRED	1.52	0.23	1.54	0.23	1.71	0.14
5. WLWL PAIRED	1.16	0.25	1.53	0.21	1.62	0.15
6. LWLW PAIRED	1.64	0.20	1.85	0.29	1.82	0.16

TABLE 4(a).

Group t-test F-values of mean Protein Efficiency Ratio differences (Accumulative Protein Efficiency Ratio over three weeks) comparing diet group of TABLE 4.

GROUPS COMPARED	WEEK 3	WEEKS 3+4	WEEKS 3+4+5
4-5	3.37 **	0.16	1.52
4-6	-1.36	-2.60 *	-1.59
5-6	-4.85 **	-2.85 **	-2.94 **

* significance at $p < 0.05$.

** significance at $p < 0.01$.

In Table 5 and 5(a) for week 3 again as observed in tables 4 and 4(a) group 5 has a PER that is significantly

lower than groups 4 and 6. For week 4 group 6 has a PER that is significantly higher than group 4 the control group but not than that of group 5. For week 5 although group 4, the control group, was observed to be higher than groups 5 and 6 it was not significantly higher. Thus again only in week 3 was their any PER significantly lower than the control group.

TABLE 5.

Weekly mean protein efficiency ratio differences for the three diet groups.

	WEEK3		WEEK4		WEEK5	
GROUP ID	MEAN	SD	MEAN	SD	MEAN	SD
4. BOTH PAIRED	1.52	0.23	1.57	0.45	2.04	0.30
5. WLWL PAIRED	1.16	0.25	1.90	0.34	1.79	0.30
6. LWLW PAIRED	1.64	0.20	2.06	0.52	1.76	0.36

TABLE 5(a).

Group t-test F-values of differences between diets of mean Protein Efficiency Ratio differences in TABLE 5.

GROUPS COMPARED	WEEK3	WEEK4	WEEK5
4-5	3.37 **	-1.82	1.89
4-6	-1.36	-2.23 *	1.96
5-6	-4.85 **	-0.80	0.26

* significance at $p < 0.05$.

** significance at $p < 0.01$.

Table 6(a) answers the question, is there a significant difference in weight between the 3 diet groups for weeks 3-2, 4-2 or 5-2? The accumulative weight for week 3-2 shows a significantly lower value for group 5 at the 0.01 level as compared to groups 4 and 6. For weeks 4-2 group 6 was significantly at the 0.05 level higher than group 4 and 5. Week 5-2 accumulative weight in table 6(a) showed that group 6 was significantly higher at the 0.05 level than only group 5. Thus only in group 5 week 3-2 was there any value that was significantly lower than the control group (group 4).

TABLE 6.

Mean weight difference from week 2 to the end of 3rd, 4th and 5th weeks for 3 diet groups. Weight differences at the end of 3,4,5 week.

	WEEKS 3-2		WEEKS 4-2		WEEKS 5-2	
GROUP ID	MEAN	SD	MEAN	SD	MEAN	SD
4. BOTH PAIRED	10.1	1.5	22.6	3.6	41.5	3.3
5. WLWL PAIRED	7.5	1.7	23.0	3.0	39.4	3.3
6. LWLW PAIRED	10.9	1.6	28.0	5.2	44.7	5.4

TABLE 6(a).

Group t-test F-values of mean weight differences (weight for the week minus the weight at the end of week 2) comparing diet group of TABLE 6.

GROUPS COMPARED	WEEK 3	WEEK 4	WEEK 5
4-5	3.58 **	-0.27	1.43
4-6	-1.15	-2.68 *	-1.61
5-6	-4.59 **	-2.61 *	-2.66 *

* significance at $p < 0.05$.

** significance at $p < 0.01$.

Table 7(a) answers the question, is there a significant difference in weekly growth (as measured by weight gain) between the diet groups for weeks 3-2, 4-3, 5-4? In week 3-

2 like in table 6(a) group 5 was significantly lower than both group 4 and 6 at the 0.01 level. In week 4-3 the control group 4 is significantly lower than both group 5 and 6 at the 0.05 level. In week 5-4 group 4 is significantly higher than group 5 at the 0.05 level but not significantly higher than group 6. So for week 3-2 and 5-4 the experimental group 5 accumulative mean weight difference was significantly lower than the control group 4.

TABLE 7.

Mean weight differences (grams) from previous week for three diet groups.

	WEEKS 3-2		WEEKS 4-3		WEEKS 5-4	
GROUP ID	MEAN	SD	MEAN	SD	MEAN	SD
4. BOTH PAIRED	10.1	1.5	12.5	3.6	18.9	2.8
5. WLWL PAIRED	7.5	1.7	15.5	2.5	16.4	2.7
6. LWLW PAIRED	10.9	1.6	17.1	4.8	16.7	3.7

TABLE 7(a).

Group t-test F-values of differences between diets of mean weight differences in TABLE 7.

GROUPS COMPARED	WEEKS 3-2	WEEKS 4-3	WEEKS 5-4
4-5	3.6 **	-2.2 *	2.0 *
4-6	-1.2	-2.4 *	1.5
5-6	-4.6 **	-0.9	-0.2

* significance at $p < 0.05$.

** significance at $p < 0.01$.

DISCUSSION

The results from Table 2 for the PER for wheat is 1.54 and is similar to the PER of 1.55 obtained at 10% protein by Lakusta. (15). The PER of 1.02 for lentils at 18% protein is also similar to our results of a PER of 1.08 (13). In a study using 12 % gluten (the main protein in wheat) and 6% lentil protein with a total of 18% total protein using ad libitum feeding Sanchez (13) obtained a PER of 1.78 which was relatively close to the value we obtained of a PER of 1.95.

The PER value obtained from group 3 where the food intake was ad libitum, wheat and lentil protein quality is most likely to be similar to that observed with people where food intake is not restricted by famine or economic factors that severely restrict food purchases. Since both wheat and lentils are relatively inexpensive, adequate supplies of these products should be accessible.

On the ad libitum diet the lentils only diet (group 1) produced the lowest PER. The wheat only diet (group 2) was significantly better, but the combination of wheat and lentils 1:1 produced the largest value. Groups 1-3 were part of this study to show the non-delayed complementary effects of wheat and lentils on an ad libitum diet.

Food intake was affected significantly by the source of protein (Table 2). This observation has also been noticed

by Sanchez et al. and Rogers and Leung (14,45). It is generally known that wheat is limiting in lysine and that lentils are very low in methionine or sulfur amino acids (1,5-7). The rats which ate only lentils or wheat ad libitum consumed less than those which had lentils and wheat ad libitum.

Depressed food intakes and plasma amino acid changes have been associated with animals fed amino acid deficient diets. It has been suggested that the food intake depression is a normal homeostatic response to prevent drastic changes in plasma amino acid concentrations due to disproportions of amino acids (46-50). Although more recent studies have shown that amino acids play a part in the appetite-regulating center of the brain (51,52). The greater food intake of rats on the wheat diet and mixed diet suggest that these food and combinations result in an amino acid combination capable of stimulating the appetite and promoting better growth.

In Table 3, which is a basically a summary of the 21 day period as mentioned in the results, there is no significant difference in either the weight gain or PER for this study between the control group 4 and the experimental groups 5 or 6. These results agree with those of Sanchez et al. who observed delayed protein complementation with other legume and cereal combinations (14,15)

In table 3 it is also observed that there is a

significant difference in weight gain and PER between groups 5 and 6. This difference is most likely due to week 3 growth and PER values being so low (see Tables 5 and 7).

In Table 4 it is observed that in week 3 group 5 had both a reduced PER and weight gain as compared to the control. The above may have been an adjustment in the rats metabolism, and, if this were the case, then why did group 6 not have a similar adjustment period? In week 4 for both weight gain and PER with group 5 there seemed to be a rebound as compared to the control group, but not with group 6. In week 4 group 6 continued to showed excellent growth and PER value even significantly above that of the control group 4 for accumulative and weekly values. In week 5 the PER value and weight gain for the control group increased so that its accumulative score for PER was not significantly different from that of group 5 and 6, and the weight gain for week 5 was significantly greater than group 5 but not the accumulative weight gain. The accumulative weight gain and accumulative PER for group 6 were significantly greater than group 5 basically due to the initial adjustment period where growth and PER were significantly lower ($p > 0.01$). Yet even with this slower initial growth the group 5 accumulative weight gain and PER were not significantly lower than the control group.

This study was to determine whether a protein severely limiting in sulfur amino acids especially methionine could

complement a protein limiting in lysine with a 4 hour span between the protein administration. The most recent data indicates that proteins can complement from one meal to the next (14,15,53), providing evidence in favor of delayed protein complementation. PER and growth data from this study (Table 2) also confirm previous work (22,23,54) which support the adequacy of delayed amino acid supplementation with vegetable proteins.

Our study and those of the above investigators disagree with the conclusion of Geiger et al. (17-19). Although using proteins that are totally deficient in a specific amino acid (only gelatin) may require close to immediate supplementation of meal containing a single amino acid yet proteins as normally found in nature do not appear to need such immediate supplementation.

One reason why the above may be true is that one study using human subjects showed that among the essential amino acids methionine was absorbed at the most rapid rate while leucine and lysine were found at the highest concentration in the upper jejunum in the fasting state(58). In this same study (55) when multiple amino acids were given in the same meal the absorption of methionine was much slower than when given alone. Also, in one study dogs were fed either egg, zein or a completely protein free diet. After an hour and a half a tube was placed in the stomach and the contents were obtained and examined for amino acids. Because of the

similarities in the quantities of the essential amino acids, it could not be determined from the content of the stomach which food the dogs had eaten (56). For adult humans it is estimated that 50 to 100 grams of endogenous protein or amino acids are delivered to the digestive tract every day. Only about 10 to 15 grams are lost into the stool. Accordingly for the temporary irregularities in the dietary protein supply there is a homeostatic mechanism that serves to regulate protein metabolism in Nasset's opinion (56). Yet the administration of single amino acids may easily unbalance the amino acid pattern in the cell and extracellular fluid (57).

SUGGESTIONS FOR FUTURE RESEARCH

More studies in rats are needed to examine the delayed complementation of similar vegetable protein combinations used in this and other studies, but over longer time intervals such as practiced in some cultures and/or religious practices of eating only two meals a day. A delayed protein supplementation study should be done using a protein deficient in methionine with the alternating meal containing methionine with non-essential amino acids to make both meals to contain the same percent of protein. Other food combinations should be tried that are similar to foods eaten in different vegetarian or low socio-economic cultures, but with similar time span as done in this study.

Finally studies should be designed to determine if the results of this and previous studies can be applied to humans subjects.

SUMMARY AND CONCLUSION

This research was to study the delayed time supplementation of two vegetable proteins that are the main staples in the Middle East each limiting in different essential amino acids. Lentils are limiting in sulfur-containing amino acids but contain adequate amounts of lysine while whole wheat is limiting in lysine but contains adequate amounts of sulfur-containing amino acids. The design of the study was to feed lentils and wheat in alternating meals or in the same meal. The proportion of lentils to wheat was 1:1 and was given 1 hour 4 times a day with 4 hours between the meals. The diets included protein at 13.7 % in an otherwise complete diet.

Sixty male, Sprague-Dawley weanling rats were divided into six diet groups with ten in each group as follows: diets fed ad libitum; wheat, lentils, wheat and lentils combined, diets pair-fed; wheat and lentils combined, wheat alternating with lentils, lentils alternating with wheat. In the group that was pair-fed, wheat was controlled by the average amount of total food eaten by the two groups pair-fed alternating diets of lentils and wheat from the previous days calculations and lentils were fed ad libitum since this was found to be the best method of securing a 1:1 ratio of food intake. Water was allowed ad libitum.

After a two week adjustment period the study lasted

three weeks. There was no significant difference in the weight gains and PER in the rats fed wheat-lentils separately (group 5) or lentils-wheat separately (group 6) (the experimental groups) as compared to wheat-lentils fed together (group 4) (the control group).

These results show that whole wheat and lentils complement each other whether in the same meal or in alternating meals with 4 hours separating the meals. This data supports the theory of a homeostatic control mechanism for amino acid complementation from one meal to the next.

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APPENDIX A

AIN VITAMIN MIXTURE 76[1]

COMPOSITION:	per kg of Mixture
Thiamine Hydrochloride	600.0 mg
Riboflavin	600.0 mg
Pyridoxine Hydrochloride	700.0 mg
Nicotinic Acid	3.0 gm
D-Calcium Pantothenate	1.6 gm
Folic Acid	200.0 mg
D-Biotin	20.0 mg
Cyanocobalamin(Vitamin B-12)	1.0 mg
Retinyl Palmitate(Vitamin A)	
Pre-mix (250,000 IU/gm)	1.6 gm
DL-alpha-Tocopherol Acetate(Vit.E)	
Pre-mix (250 IU/gm)	20.0 gm
Cholecalciferol (Vitamin D_3)(400,000 IU/gm)	250.0 mg
Menaquinone (Vitamin K)	5.0 mg
Sucrose, finely powdered	972.9 gm

[1]ICN Biomedicals, Inc., Costa Mesa, California

APPENDIX B

U.S.P. XIV SALT MIXTURE[1]

As required in the various biological test diets listed U.S.P. XIV (1950).

COMPOSITION:

Calcium Carbonate.....6.86000%

Calcium Citrate.....30.83000%

Calcium Phosphate Monobasic.....11.28000%

Manganese Carbonate.....3.52000%

Magnesium Sulfate·$7H_2O$.....3.83000%

Potassium Chloride.....12.47000%

Dipotassium Phosphate.....21.88000%

Salt(Sodium Chloride).....7.71000%

Copper Sulfate·$5H_2O$.....0.00777%

Ferric Citrate(16-17%Fe).....1.52815%

Manganous Sulfate·H_2O.....0.02008%

Potassium Aluminum Sulfate.....0.00923%

Potassium Iodide.....0.00405%

Sodium Fluoride.....0.05070%

[1]ICN Biomedicals, Inc., Costa Mesa, California