THE VITAMIN B_1 AND C CONTENT OF MUNG BEANS AND MUNG BEAN SPROUTS

Thesis for the Degree of M. S.
MICHIGAN STATE COLLEGE

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1939

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By

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A THESIS

Submitted to the Graduate School of Michigan State College of Agriculture and Applied Science in partial fulfilment of the requirements for the degree of

MASTER OF SCIENCE

Department of Nutrition

Division of Home Economics

1939

THESIS

ACKNOWLEDGMENTS

The writer wishes to express her sincere appreciation to Miss Flora M. Hanning of the Home Economics Division of Michigan State College, under whose supervision this study was conducted, for her interest and helpful criticism, and to Dr. Marie Dye, Dean of the Home Economics Division of Michigan State College for her helpful suggestions.

Acknowledgment is also given to the La Choy Food Product Company, Detroit, Michigan for generously donating the bean sprouts used in this study.

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THE VITAMIN B_1 AND C CONTENT OF MUNG BEANS AND MUNG BEAN SPROUTS

Introduction

Green mung beans (Phaseolus aureus), sometimes called green gram beans, are very commonly used in China either in the form of dry beans or sprouts, but more often as sprouts. Mung bean sprouts take the place of vegetables in the diet. They are available all through the year and are quite cheap. In some parts of the country the sprouts are sold with the cotyledonal part cut off, probably for better palatability and appearance. Mung bean sprouts are undoubtedly an important item in the Chinese dietary.

There are many kinds of mung beans. Those commonly used in China are more or less round in shape, green in color, and about 2.5 mm. by 2.5 mm. by 3 mm. in size. This kind of beans was also used in this experiment.

The method often used in cooking the dry beans is boiling in a large quantity of water for hours until very tender. The details of the procedure of sprouting the beans differs slightly in different sections of the country; but the general principles are almost the same. They are:

1. Washed clean.
2. Soaked for about 5 to 10 hours.
3. Sprouted in a perforated bottom container.
4. Sprinkled with water many times a day to insure dampness.

5. Kept in a perfectly dark and warm condition.

The sprouts are usually 7 to 9 cm. in length from root tip to the expanded cotyledons, of which about 5 cm. is white, fleshy, and thick. The cotyledons are pale yellow in color and have expanded. Often the plumule has emerged from between the cotyledons. The plumule is usually yellow in color and ½ to 1 cm. in length.

Though some investigations have been made of the value of mung beans and their sprouts as sources of vitamins, the information is far from complete. This work was undertaken in the hope of increasing our knowledge of this important Chinese food. The object was to investigate the three points mentioned below:

1. The vitamin B_1 and C content of mung bean sprouts.
2. The difference of the content of these vitamins between dry and sprouted beans.
3. The distribution of these two vitamins in cotyledonal and non-cotyledonal parts.

Literature Review

Vitamin B_1

Most of the work on the vitamin value of mung beans and mung bean sprouts was done in India and China and published in those countries. Miller (11) reported that Embrey (5) had found that dry mung means, when fed as 45% of the weight of the diet, contained enough vitamin B_1 for the growth of mice. The bean sprouts also were fed in dried form, and at a 25% level, contained enough vitamin B_1 for the growth of mice. About 10 mice were used in each group. Embrey suggested as a result of her experiments that vitamin B_1 increased when the beans were sprouted.

Santos (12) also stated that the cooked sprouted beans showed greater vitamin B_1 potency than cooked beans. He used recovery of weight of animal as a measure of the vitamin B of the food tested. The age of the rats was not stated, but most of them weighed considerably over one hundred grams when placed on the experimental diet. Both the beans and the sprouts were cooked, then dried and fed as a vitamin B_1 supplement. Seven rats were fed on the beans, 4 receiving 1 gm., and 3 receiving ½ gm. daily supplements; and 6 rats on the sprouts, 3 receiving 1 gm., and 3 receiving ½ gm. supplements daily.

Miller (11) reported the vitamin value of mung bean sprouts, supplied by the local market in Honolulu. Sherman's method was followed in her research for the comparison of vitamin B_1 value between raw and cooked sprouts (steamed for 5 minutes). Nine rats were put on

3 gram raw sprouts level and 1 on 4 gm. level. For cooked sprouts 10 rats were put on 2.7 gm. level and 2 on 3.6 gm. level. The rats were fed daily except Sundays for a period of 8 weeks. She concluded that the amount of raw sprouts for maintaining net weight in standard rats for a period of 8 weeks was between 2.5 gm. and 3.0 gm., and between 2.2 and 2.7 gm. for the cooked sprouts (equivalent to 2.5 and 3.0 gm. of raw). According to Sherman's unit basis, raw bean sprouts contained 150 to 180 units per pound and the cooked sprouts 150 to 170 units per pound of which correction had been made for the difference in water content.

Jansen (9) stated that about 30% of katjang idjoe (mung beans) was enough to cure polyneuritis in pigeons. Donath (4) found that katjang idjoe were quite rich in vitamin B_1.

Van Veen (14) reported one International unit of vitamin B_1 per gm. of dry black mung beans (Phaseolus radiatus). Spruyt (13) found that black mung beans had the same amount of vitamin B_1 as green mung beans.

The work of Wilson (16) showed that the green mung beans contained 150 to 160 units of vitamin B_1 per 100 gm. of dry beans. The unit of vitamin B_1 was defined as the quantity which, when given to a rat each day would produce an increase of weight of 10 gm. per week for at least 3 weeks. They claimed that this unit was equal to an International unit and to 4 micrograms of the Jansen and Donath's crystals. The rats were put on vitamin B_1 free diet when they weighed about 30 gm. At least 6 rats were used for each test.

Vitamin C

Donath (4) found that dry katjang idjoe (mung beans) contained no vitamin C. Ghosh (7) found by titration method that dry beans contained 4.0 mg. of vitamin C per 100 gm. and sprouted beans contained 31.0 mg. per 100 gm. Ahmad (7) found that the vitamin C content of dry mung beans was 3.0 mg. per 100 gm. and of sprouted beans was 23.0 mg. per 100 gm. Guha (8) found that ascorbic acid in Phaseolus Mungo was increased 7 to 8 times by germination.

Miller (11) reported that 2.5 to 3.0 gm. of raw sprouts seemed to protect guinea pigs from scurvy, while 2.7 gm. of cooked sprouts (equivalent to 3.0 gm. raw) did not. However, according to her experimental data, she thought that the amount needed to protect against scurvy probably lay between 3 to 4 gm. Nine guinea pigs distributed on 4 different levels of raw sprouts, and 8 guinea pigs on 4 different levels of cooked sprouts were used in this experiment. The results obtained indicated that there was considerable destruction of vitamin C even in the 5 minutes of cooking. She concluded that, on the basis of Sherman's unit, raw mung bean sprouts contained 180 units of vitamin C per pound; and in the cooked state, the bean sprouts probably had approximately 150 units per pound or possibly a little less. She claimed that in the raw state mung bean sprouts have a vitamin C content equal to that of lemon, orange, and tomato juice; and when cooked 5 minutes, a slightly lower value.

Wats (15) reported that 3 gm. of sprouts of each of the varieties

of beans tested was enough to protect guinea pigs from scurvy. His result checked very closely with Miller's (11) findings.

Chi (3) found that mung bean sprouts contained 0.022 mg. per gm. determined by the Harris method, 0.15 mg. per gm. by the iodometric method, and [+(?)] by biological assays.

Determination of Moisture and the Proportion of the Cotyledonal and Non-cotyledonal Parts

Experimental Procedure

I. Moisture:

Since the method and condition for sprouting the beans is slightly different in different places, the moisture content is expected to be different. It would be of interest if the vitamin value could be compared on the dry basis so these determinations were made.

About 20 gm. of fresh or frozen bean sprouts, and about 10 gm. of dry beans were used in each determination. Two or four determinations were made on each sample. They were weighed on an analytical balance to one tenth of a mg. The samples were first dried at a temperature below 60°C. in an oven which had an electric motor to circulate air. Then, the samples were transferred into another ordinary drying oven at 65°C., and dried to constant weight. The moisture content of fresh sprouts from Detroit was determined at 60°C., but it was found there were not enough changes in weight to cause a decrease of 0.1% when put into the oven at 65°C.

II. Proportion of the two parts --- cotyledonal and non-cotyledonal:

In each determination 100 gm. of bean sprouts were used. The sprouts were cut at the point right next to the cotyledonal part. After all of them were cut, the two parts were weighed again. The weighings were made on a torsion balance. All of the processes were completed as quickly as possible and with minimum

handling so as to prevent loss of moisture.

Results and Discussion

I. Moisture:

Moisture content is given in Table I. According to the moisture content of sprouts from Lansing, 100 gm. of dry beans as purchased would give 1125 gm. of bean sprouts. According to the moisture content of bean sprouts from Detroit, 100 gm. of dry beans as purchased would give 1506 gm. of sprouts, assuming that the addition of water would account for the total change in weight.

Table I. Moisture Content

Material	% of Moisture
Dry beans	6.6
Powdered dry beans	6.5
Fresh bean sprouts from Lansing	91.7
Fresh bean sprouts from Detroit	93.8
Frozen bean sprouts from Detroit	93.4

II. Proportion of two parts --- cotyledonal and non-cotyledonal parts:

There were 19 determinations of 5 different samples made. The percentage of cotyledonal part ranged from 10.6 to 12.8% with an average of 11.4% by weight. The percentage of non-cotyledonal part ranged from 86.4 to 89.0% with an average of 87.8%. The sum of the percentage of these two parts was 99.2%. The weight lost due to evaporation was 0.8%. Assuming that the rate of evaporation

was the same on these parts, the percentage of the cotyledonal and non-cotyledonal parts would be 11.5 and 88.5%. This was termed as corrected proportion of the two parts. The results are given in Table 2.

Table 2. Proportion of cotyledonal part and non-cotyledonal part of the bean sprouts.

No. of determinations	Cotyledonal part	Non-cotyledonal part
1	10.8%	87.5%
3	11.0	89.0
2	12.8	86.4
4	12.0	87.0
9	10.6	88.8
Average	11.4%	87.8%
Corrected average	11.5%	88.5%

Vitamin B_1

Experimental Procedure

I. Preparation of supplements:

1. The bean sprouts.

The bean sprouts for the vitamin B_1 assay were kindly supplied by the La Choy Food Products Company in Detroit. During the period of transportation the sprouts were kept in dry ice. They looked perfectly fresh upon arrival. Immediately after the bean sprouts arrived, a small portion of them was cut in the same manner as was done in the determination of the proportion of the cotyledonal and non-cotyledonal parts of the sprouts. After this was completed (about 3-4 hours, during which they were kept cold) both the whole sprouts and the non-cotyledonal parts were put into wax paper cups of one pint capacity, placed in the freezing room in the Horticulture Building, and kept frozen.

2. The dry beans.

The dry beans were purchased from Lansing. These beans and the beans used by the La Choy Food Products Company for sprouting were shipped from Manchuria. The beans were washed with water, dried in air, and ground to fine powder.

3. The standard solution.

The standard vitamin B_1 solution was prepared by dissolving exactly 10 mg. of thiamin chloride hydrochloride in

100 c.c. of 20% alcohol solution. This solution was called stock solution. Two c.c. of the stock solution was diluted to 250 c.c. with distilled water. Five c.c. of the final solution equaled 4 gamma of thiamin chloride hydrochloride.

II. Preparation of basal ration:

Chase and Sherman's (2) vitamin B_1 deficient diet was used. The composition of the ration is as follows:

Casein free from vitamin B_1	18%
Salt mixture (McCollum and Steenbock)	4
Butter fat	8
Cod liver oil	2
Autoclaved yeast	15
Starch	53

1. Purification of casein.

The casein was purified by extracting with 60% cold alcohol (by weight). With 1.5 liter of 60% alcohol, 300 gm. of casein was treated and the whole was shaken for ½ hour and then allowed to stand for 5½ hours. The mixture was filtered with suction and thoroughly washed with 750 c.c. of 60% alcohol. The casein was shaken with another 1-liter portion of 60% alcohol for one half hour. After standing 19 hours, it was filtered, washed with 750 c.c. of 60% alcohol, and finally with one half liter of 95% alcohol and then dried in air.

2. Autoclaving yeast.

Dried powdered baker's yeast was mixed with 0.1 NaOH to make a smooth paste in a proportion of 100 gm. yeast to

125 c.c. NaOH. The mixture was then heated in a pressure cooker at 15 pounds pressure for 6 hours. The resulting yeast was neutralized with standard HCl. It was dried in an oven at 60° C. and then ground.

3. Salt mixture.

Salts were weighed on a torsion balance. They were ground separately in a mortor, mixed and ground. The final mixture was passed through a 40 mesh seive.

Composition of salt mixture #2 --- U.S.P.X.
(Modification of McCollum and Steenboch #40)

Sodium chloride (NaCl)	0.173
Anhydrous magnesium sulfate ($MgSO_4$)	0.266
Sodium phosphate ($NaH_2PO_4 \cdot H_2O$)	0.347
Potassium phosphate (K_2HPO_4)	0.954
Calcium acid phosphate $CaH_4(PO_4)_2 \cdot H_2O$	0.540
Ferrie citrate ($1\frac{1}{2}H_2O$)	0.118
Calcium lactate	1.300

4. Butter fat. --Commercial butter was melted, decanted, and filtered.

5. Cod liver oil. --Patches' cod liver oil was used.

6. Corn starch. --Argo's corn starch was used.

III. Selection and care of animals.

Rats were used as experimental animals. They were of known heredity. When the rats were about 4 weeks old and weighed about 47-55 gm., they were put on vitamin B_1 deficient diet for about

2 weeks. At the end of the first week harnesses were put on so as to prevent coprophagy. After the first week, rats were weighed every other day, and some times each day at the end of the second week. Growth curves were made. The weight of the rats usually went down after harnesses were put on but it would usually be up again on the fourth day. When the weight of a rat went down the second time, that rat was considered to have been depleted in vitamin B_1. The average weight of the whole group at the end of the depletion period was about 70 gm.

Rats were distributed into different groups according to sex and litter. About 4-5 rats were put on each supplement at the beginning of the experiment. Later on, more rats were put on the level where the growth curve was considered to be nearer to the positive control group. Table III shows the distribution.

After rats were put on supplement, they were weighed weekly and the food consumption was also recorded. At the time of weighing, harnesses were taken off and were not put back until late in the afternoon. During this period the rats could clean themselves.

IV. Feeding supplements.

1. Experimental period.

A 4 weeks growth test period was used. It has been found in this laboratory that the 4 weeks period has approximately the same significance as longer periods.

2. Technique in feeding.

All supplements were fed each day including Sunday. Supplements were limited, but basal diet was fed ad. libitum. The feeding was usually done at about 11 o'clock each morning.

Table III. Distribution of Animals in the Feeding Experiment

Supplement rats	Positive control 4 thiamin rat no.	*wt.	Negative control rat no.	wt.	Whole sprout 10 gm. rat no.	wt.	Whole sprout 8 gm. rat no.	wt.	Whole sprout 6 gm. rat no.	wt.	Non-cotyledonal part 10 gm. rat no.	wt.	Non-cotyledonal part 6 gm. rat no.	wt.	Dry beans 1 gm. rat no.	wt.
	3937♀ -	55	3939♂ -	76	3945♀L	65	3944♀ -	73	3939♂RR	70	3944♀RR	68	3967♀ -	68	3937♂L	70
	3941♂R	69	3937♂R	68	3937♂ -	78	3937♀L	68	3937♀R	76	3941♂B	74	3967♂ -	62	3939♂L	73
	3944♂R	81	3944♂L	78	3944♀B	59	3941♂L	75	3941♀B	61	3945♀R	64	3968♀ -	64	3944♀L	69
	3945♀ -	61	3941♀L	62	3941♀R	68	3945♀B	61	3945♂R	75	3939♂LL	74	3970♂ -	63	3941♀ -	67
	3937♀B	75	3945♂L	78	3945♂ -	71	3939♂R	77	3944♀R	65					3945♂B	72
	3944♂ -	78			3941♂ -	74			3967♂L	68					3937♂B	80
	3967♀L	69							3967♂R	70					3967♀R	60
	3967♂B	75							3968♂L	68					3968♀	58
	3968♀L	67							3968♀R	70					3968♂ -	68
	3970♂L	64							3970♀RR	60					3970♀L	70

*Initial weight of the experimental period.

Food cups were taken out so that the rats ate the supplement. In case of positive controls water bottles were taken instead of food cups. Any spilled food was picked up at one o'clock or at three in the afternoon, according to the schedule of the day. If all the supplement was eaten, food cup or water bottle was put back at that time. At night spilled food was again picked up, and all the water bottles and food cups were put back. If too much of the sprouts was left, the watter bottle was taken out and the food cup was put in. In case of dry beans, about 3-5 gm. more of basal ration was put in with the supplement. The reason for doing this was to be sure that they ate all the supplement and at the same time had enough food. Water and food were available all of the time except for special reasons as mentioned above.

3. Bean sprouts.

The approximate amount of sprouts needed daily was taken out each day from the freezing room. The amount left was put into an evaporating dish, wrapped with wax paper, and kept in the freezing unit of an electric refrigerator. The sprouts were weighed on a small sensitive torsion balance immediately after they were taken out from the freezing room. Care was taken to keep them from thawing. Weighing while they were cold was, of course, not the most accurate way, but, if time were taken to let them come up to room temperature, the bean sprouts would thaw and water would come out from the tissue. Since vitamin B_1 is water soluble, the loss of water this way probably induced higher error than weighing while they were cold.

4. Dry beans.

The dry beans were kept in a glass jar at room temperature. They were weighed also on the same torsion balance on which the sprouts were weighed. Each gm. of beans was mixed with about 1-2 gm. of basal ration and then fed to the rats. During the first week of the experiment beans were moistened with water; but later it was found that after drying, the beans became a hard mass, and the rats did not like it. Hence, the beans were mixed with food instead of moistened with water.

5. Thiamin chloride hydrochloride solution.

It was pipetted out with a 5 c.c. pipette into a small cup.

Vitamin B_1

Results and Discussion

The records of individual rats are given in Tables 9 to 24 in the appendix, and the summary of data may be found in Table 4. The curve of the growth of rats and the histogram of the food consumption of the rats are shown in Fig. 1.

The vitamin B_1 value, expressed in terms of micrograms of thiamin chloride hydrochloride and International units (equivalent to 3 micrograms of thiamin chloride hydrochloride), was calculated on the basis of gain in weight with the positive control group as a standard of comparison. The results from levels of 6 gm. of both the non-cotyledonal part of sprouts and the whole sprouts were used in calculating the vitamin B_1 value. The results are tabulated as follows:

Table 5. Vitamin B_1 Content of Mung Beans and Mung Bean Sprouts

Food	Vitamin B_1 Value of 100 gm. on raw weight: Microgram of thiamin per 100 gm.	on raw weight: International unit per 100 gms.	on dry weight: Microgram of thiamin per 100 gm.	on dry weight: International unit per 100 gm.
Dry beans	618	206	662	221
Whole bean sprouts	85	28	1288	429
Non-cotyledonal part of bean sprouts	66	22	----	----

Table 4. Summary of Data of Growth and Food Consumption of the Rats in Vitamin B_1 Assay.

	No. of Animal	Average initial weight	Weekly gain in gm.					Weekly Food Consumption in gms.				
			1st wk.	2nd wk.	3rd wk.	4th wk.	Av.	1st wk	2nd wk.	3rd wk.	4 th wk.	Av.
4 micrograms of thiamin hydrochloride	10	69.4	9.7	4.9	6.2	6.5	6.8	36.4	36.2	35.2	38.2	36.5
Dry beans	10	68.7	11.4	9.5	12.0	9.2	10.5	34.2	34.8	38.5	38.9	36.6
6 gm. non-cotyledonal part of sprouts	4	64.3	7.3	5.3	10.0	4.0	6.7	30.3	30.0	30.3	33.3	31.0
10 gm. non-cotyledonal part of sprouts	4	70.0	8.8	9.0	10.3	11.5	9.9	35.5	39.5	42.0	44.0	40.3
6 gm. whole sprouts	10	68.3	8.3	7.1	10.6	8.7	8.7	32.6	33.0	35.8	38.6	35.0
8 gm. whole sprouts	5	71.0	12.6	7.8	13.4	10.2	11.0	37.6	39.6	40.6	44.4	40.6
10 gm. whole sprouts	6	69.2	10.8	7.3	13.8	15.0	11.7	35.7	38.0	42.3	45.5	40.5
Negative control	7	67.9	-5.0	-7.5	-5.8	-11.5	-7.3	24.4	20.0	18.2	14.5	19.2

Average Weekly Food Consumption*

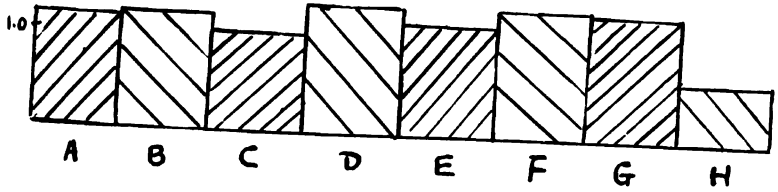
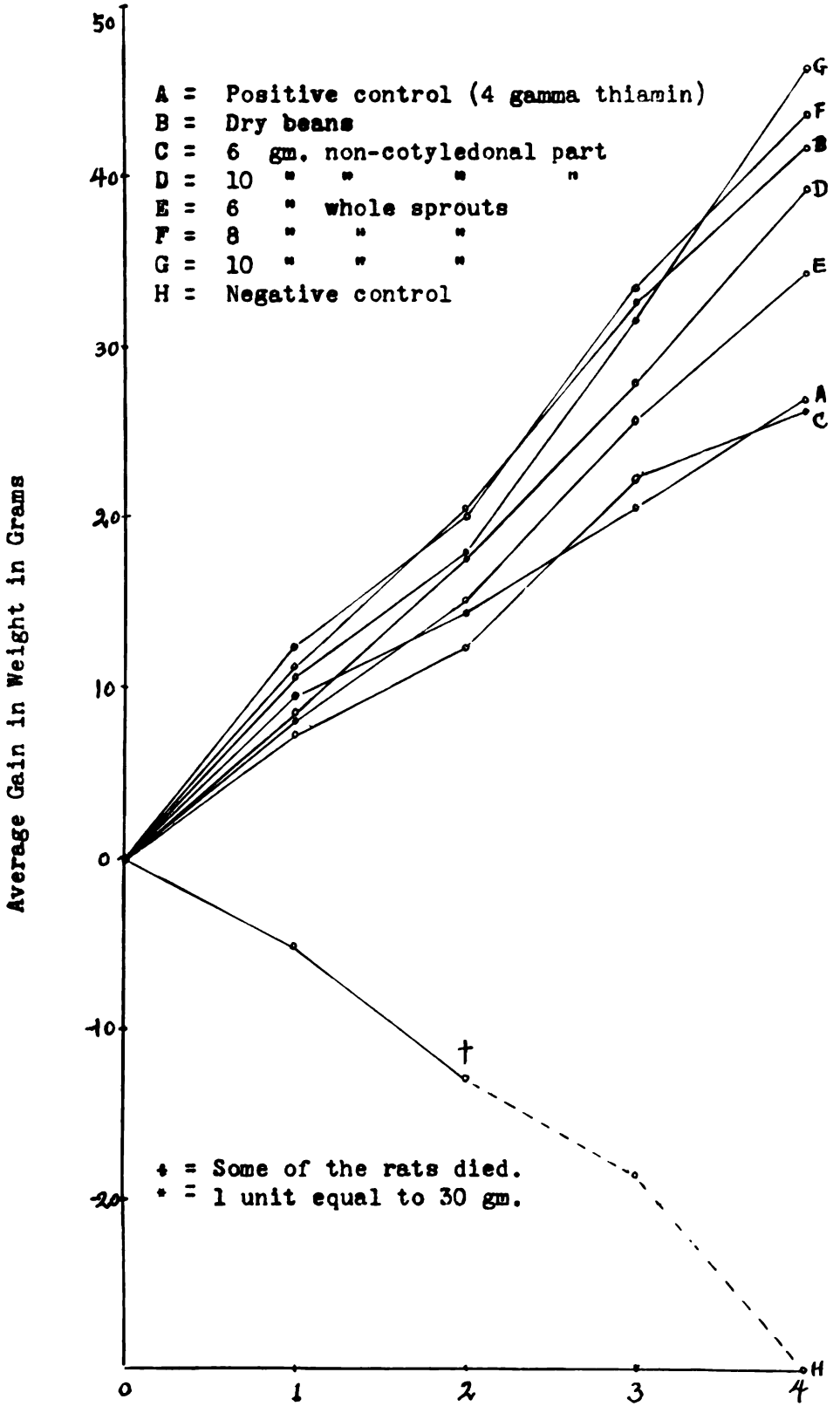


Fig. 1. Gain in Weight Curve of the Rats



The results showed that dry beans contained 618 micrograms of thiamin per 100 gm. raw weight, and 662 micrograms of thiamin per 100 gm. dry weight; and sprouts contained 85 micrograms of thiamin per 100 gm. raw weight and 1288 micrograms of thiamin per 100 gm. dry weight. Apparently the vitamin B_1 increased about 2 times as much as that of the original dry beans after sprouting.

The growth rate per week in the dry beans group was considerably higher than the positive control group. If the supplement level were decreased about one fourth of the amount fed in this experiment, the vitamin B_1 value might be a little higher than the present figure; but this would not effect the result very much as shown by the fact that the value calculated from the 8 gm. level sprouts gave almost the same value as from the 6 gm. level of sprouts. When the results from the 8 gm. level sprouts, in which the growth rate of the rats was even higher than that of the rats supplemented with 1 gm. of dry beans, was used in calculating, the vitamin B_1 content of bean sprouts was 81 micrograms of thiamin chloride per 100 gm. raw weight. The vitamin B_1 value was only 4 micrograms thiamin chloride less than that calculated from the results of the 6 gm. level of sprouts.

When the vitamin B_1 value was calculated from the results of the 10 gm. level of bean sprouts, the vitamin B_1 content per 100 gm. raw weight dropped to 69 micrograms of thiamin chloride. This might indicate that the amount of vitamin B_1 in 8 gm. of bean sprouts was almost enough for the maximum growth of rats. When the vitamin B_1 intake was increased beyond what the body needed, it might be simply stored in the body, probably excreted, or it might be utilized less efficiently by the body. When the amount of sprouts in the supplement

was increased from 6 to 8 gm., the gain in weight increased accordingly; but when it was increased from the 8 to the 10 gm. level, the gain in weight of the rats was considerably less per unit weight of the supplement. Therefore, the vitamin B_1 value calculated from the 10 gm. level of sprouts would not represent the true value of the sprouts. Furthermore, the number of animals used in the 8 gm. and the 10 gm. levels was less than that in the 6 gm. level; the average value from the 8 and 10 gm. levels would not be so significant as that from the 6 gm. level.

The vitamin B_1 value of non-cotyledonal part of the mung bean sprouts was decidedly lower than that of the whole sprouts. There were only 66 micrograms of thiamin chloride in 100 gm. of raw non-cotyledonal part of sprouts, while there were 85 micrograms in 100 gm. of raw whole bean sprouts. In other words, the vitamin B_1 was not evenly distributed in the whole sprouts but more concentrated in the cotyledonal part.

When the vitamin value was calculated from the results of the 10 gm. level, the vitamin B_1 content of the non-cotyledonal part of the sprouts was 58 micrograms of thiamin chloride per 100 gm. raw weight. This value was also lower than that calculated from the results of 6 gm. level. There were only 4 rats on each of these levels, which is rather a small group of rats in view of the individual variations of the rats.

This test has found that dry mung beans are a good source of vitamin B_1 (206 International unit per 100 gm. raw weight); whole bean sprouts are a fairly good source (28 International unit per 100 gm. raw weight); and the non-cotyledonal part of bean sprouts is a fair

source of vitamin B_1 (22 International unit per 100 gm. raw weight). After sprouting, the vitamin B_1 content of the beans increased to approximately two times the original value.

Vitamin C

Experimental Procedure

The beans used for this part of the experiment were purchased in two Chinese restaurants in Lansing. In both of the restaurants, the beans were generally sprouted for seven days. The length of the sprouts was about 7-9 cm., which was almost the same as those from the La Choy Food Products Company, used in the vitamin B_1 test. The sprouts were stored in a tightly covered glass jar and kept in an ice box before analysis. They were analyzed as they were, without being washed.

The vitamin C content was determined by the microchemical titration method. The details of Tillman's method modified by Bessey and King (1) were followed with few modifications. Bessey and King (1) found that the dye titration method checked with the biological assays. We have assumed that to be true of mung bean sprouts, and in this study, the reducing value of the materials tested is reported as vitamin C.

I. Standardization of 2-6 dichlorophenolindophenol solution.

Meander and Guerrant's method (10) was used. They found that the curve obtained for the titrations against sodium thiosulfate was comparable with that in which ascorbic acid was used. In successive portions of hot water, 0.1 gm. of dye was dissolved and filtered into a 200 c.c. volumetric flask. When all the dye was dissolved, the filter was washed with a small amount of hot water until the washings were nearly colorless.

Fifteen c.c. of the dye solution was pipetted into a 100 c.c. Erlenmeyer flask; 0.5 to 1.0 gm. potassium iodide and 0.5 to 1.0 c.c. of diluted sulfuric acid (1 to 4) were added. After shaking to facilitate the oxidation of the potassium iodide, the liberated iodine was titrated with 0.01 sodium thiosulfate, using 1 c.c. of 1% soluble starch as an indicator. The sodium thiosulfate was standardized with standard potassium dichromate solution. The potassium dichromate was of good quality, dried, and had been kept in a dessicator. Fales' procedure (6) of standardization was followed. One c.c. of 0.01 N sodium thiosulfate was equivalent to 0.88 mg. of ascorbic acid.

II. Extraction and titration.

About 5 gm. of dry beans or bean sprouts were weighed and ground with 2-3 gm. of acid washed white sand and a small amount of 2% meta-phosphoric acid until a paste was formed. The mixture was washed with some more 2% meta-phosphoric acid and transferred to a 50 c.c. centrifuge tube. The mixture was centrifuged then for about 5 minutes. The clear solution was filtered through a quick filter (Whatman No. 41) into a 100 c.c. Erlenmeyer flask. Another 10 c.c. or 15 c.c. of meta-phosphoric acid were used to wash the mortar and stirred into the solids, which were again centrifuged. The washing and rinsing were repeated twice more. The total volume of about 50 c.c. was titrated with standardized dye. A persistence of a pink color for about 15 seconds was taken as the end point of the titrations. The titration was carried out within two minutes. The time between weighing and titration was kept as short as possible -- usually within two hours.

During this period, direct sunlight was kept out of the laboratory as much as possible.

Distilled water was used instead of redistilled water as recommended by Bessey and King(1). The use of distilled water in the manipulations was checked with redistilled water twice. Lemon juice was used as a source of vitamin C. It was found that even when the distilled water was concentrated 5 times, the difference of the results between distilled and redistilled water was less than 1%, which is within the experimental error of the method. Therefore, distilled water was used all through the experiment.

The method of standardization was checked many times. The value of dye solution standardized by Meander's (10) method was practically the same as the value standardized against pure ascorbic acid. A difference of 1-2$ was within the experimental error.

During the period of acquiring technique, at least four determinations were run on each sample. Afterwards only duplicates or triplicates were run. Only two determinations were carried on at one time so as to lessen the atmospheric oxidation. The vitamin C content of dry beans, of whole sprouts, and of the non-cotyledonal part of bean sprouts was determined. The cotyledonal part was calculated by difference.

III. Recovery tests:

1. Test for atmospheric oxidation.

0.5 mg. portions of pure ascorbic acid solution dissolved in 2% metaphoric acid were pipetted out into 100 c.c. Erlenmeyer flasks and were let stand without covering for

the same amount of time as the grinding process would require. Then, these solutions were diluted to about 50 c.c. with more 2% metaphosphoric acid and let stand for the same amount of time as the actual determination would require. The results indicated that the destruction of vitamin C by atmospheric oxidation was less than 2% in cool weather, and the percentage of destruction was higher in hot summer weather. It went up to about 10% destruction on a very hot day.

2. Test for completeness of extraction:

0.5 gm portions of pure ascorbic acid solution were ground, washed, and filtered in the same manner as the actual determination. The results were compared with the same amount of ascorbic acid which was exposed to air for the same amount of time. The results showed that the extraction was complete.

3. Oxidation in presence of oxidase:

0.5 gm. portions of ascorbic acid solution were added to weighed samples of sprouts and the amount of vitamin C found in excess of the average amount of the vitamin C of the same sample was taken as the amount of recovery. In this case, it was very difficult to get a very uniform sampling of sprouts, but the average of the recovery might give some idea of the action of oxidase under the particular situation. Recovery tests were done on two samples of sprouts and one of dry beans. The average percentage of recovery of two determinations on one of the samples of sprouts was 101%, and the average of three determinations on the other sample was 99.7% (97.0%, 98.9%, 103.2%). The average percentage of recovery of two determinations on dry beans was 96% (94.0%, 98.0%).

Results and Discussion

The results of the vitamin C determination are given in Table 6. The average vitamin C content of dry beans was 6.7 mg. per 100 gm. raw weight, and 7.2 mg. per 100 gm. of dried weight (dried at 65°C.). The average vitamin C content of the whole bean sprouts was 9.4 mg. per 100 gm. fresh weight and 113.3 mg. per 100 gm. dried weight. The average vitamin C content of the non-cotyledonal part was also 9.4 mg. per 100 gm. fresh weight. Apparently the vitamin C distributed uniformly in these two parts, and the proportion would be the same as the proportion of fresh weight.

Wu (17) reported that the moisture content of whole bean sprouts was 91.7% and of the non-cotyledonal parts was 93.2%. If the vitamin C content of the non-cotyledonal parts was calculated on this basis, it would become 138.2 mg. per 100 gm., indicating a slightly higher content of vitamin C in the non-cotyledonal part on the dried weight basis.

One hundred gm. of raw dry beans contained 6.7 mg. of vitamin C, and the amount of sprouts (1125 gm.) from 100 gm. of beans would have 105.8 mg. of vitamin C. The vitamin C value of mung beans was increased 15.8 times when the beans were sprouted. A summary of the data is given in Table 7.

The reported value of viatmin C in green mung bean sprouts ranged from 2.2 mg. to 31.0 mg. (7) per 100 gm. of fresh sprouts. Miller (11) used biological assays and reported more than two times as much vitamin C as reported in this study. The methods of sprouting, the time of sprouting, the kind of beans, and the freshness of the sprouts may account for these differences.

Table 7. Summary of Vitamin C Content of Mung Beans and Mung Bean Sprouts.

	Percentage of moisture	Vitamin C content mg. per 100 gm Fresh basis	Dried basis
Bean sprouts	91.7	9.4	113.3
Non-cotyledonal parts of sprouts	93.2	9.4	138.2*
Dry beans	6.6	6.7	7.2

* Moisture content was not determined in this laboratory.

Table 6. Vitamin C Content of Mung Beans and Their Sprouts

Sample no.	Food	No. of determinations	Vitamin C value in mg/g of fresh weight of each titration	Av.	Remarks
1	Dry beans	2	0.062 0.066	0.064	
2	"	4	0.062 0.065 0.064 0.067	0.065	2 % metaphosphoric acid was used.
3	"	2	0.068 0.070	0.069	
4	"	4	0.063 0.070 0.070 0.072	0.069	5% trichloroacetic acid was used.
Av.	"			0.067	
5	Bean sprout	2	0.099 0.104	0.102	The sprouts were fresh.
6	"	3	0.096 0.100 0.103	0.100	Determinations were made 1 to 3 hrs. after
7	"	1	0.084	0.084	delivery.
8	"	1	0.093	0.093	
9.	"	2	0.090 0.091	0.091	
10	"	2	0.110 0.111	0.111	
11.	"	3	0.067 0.082 0.081	0.077	
Av.	"			0.094	
12.	"	2	0.089 0.092	0.091	Fresh determinations were made after stored over night.
13	"	3	0.086 0.088 0.098	0.091	
14	Non-cotyledonal part of bean sprout.	3	0.075 0.085 0.085	0.082	
15	"	2	0.088 0.105	0.097	They were fresh. Determinations
16	"	2	0.099 0.100	0.100	were made 1 to 4 hrs. after delivery.
17	"	2	0.095 0.099	0.097	
Av.	"			0.094	
18	"	2	0.090 0.092	0.091	Fresh, stored over night.

When the bean sprouts did not look very fresh, slightly dry, or light brownish in color at the tip of the sprouts, the vitamin C content was usually about one half of the fresh sprouts. The average vitamin C contents of two samples (21 and 23, not fresh) were 5.3 mg. per 100 gm. and 5.9 mg. per 100 gm. of the sprouts. Sample 20, though stored over one night, looked fresher than sample 21. The vitamin C content of sample 20 was accordingly higher. The vitamin C value of samples 19 and 22 varied so much in different determinations, that the results were not averaged. When the bean sprouts looked quite brownish all over or in part, and slightly soft, the vitamin C value according to the titration method, dropped to almost none. This effect of the condition of the samples is tabulated on Table 8.

If, however, the fresh sample was stored over night in a refrigerator and appeared in good condition, it gave results comparable to those determined with the shorter time before testing, as shown in samples 12, 13, and 18 of Table 7.

This test has found that dry mung beans are a poor source of vitamin C (6.7 mg. per 100 gm. raw weight); but the vitamin C content increases 15.8 times after sprouting. Both whole bean sprouts and the non-cotyledonal part of the sprouts are fairly good sources of vitamin C (9.4 mg. per 100 gm. fresh weight).

Table 8. Vitamin C Content of Bean Sprouts (not fresh).

Sample no.	Food	No. of determinations	Vitamin C value in mg/gm. of fresh weight of each titration					Av.	Remarks
19	Bean sprouts	3	0.066	0.080	0.162				5 days old, stored over night in ice box, leaves turned green and red.
20	"	5	0.075	0.081	0.083	0.087	0.097	0.085	6 days old, stored over night in ice box.
21	"	2	0.047	0.058				0.053	6 days old, stored over night in ice box in small quantity, looked dry.
22	"	2	0.044	0.100					6 days old, stored over night in ice box in large quantity.
23	"	3	0.057	0.059	0.060			0.059	Did not look fresh. Report from restaurant stated it had not been good for days.

Summary

I. Dry mung beans, mung bean sprouts, and the non-cotyledonal part of the sprouts were analyzed for their vitamin B_1 and C content.

II. The average vitamin B_1 content (microgram thiamin chloride hydrochloride per 100 gm. raw weight) of (a) dry mung beans was 618 micrograms, (b) bean sprouts was 85 micrograms, (c) non-cotyledonal part was 66 micrograms.

III. The average vitamin C content (miligrams per 100 gm. raw weight) of (a) dry mung beans was 6.7 miligrams, (b) bean sprouts was 9.4 miligrams, (c) non-cotyledonal part of the sprouts was 9.4 miligrams.

IV. The vitamin C content of mung beans was increased 15.8 times after the beans were sprouted, and the vitamin B_1 content of mung beans was increased about 2 times after the beans were sprouted.

V. The vitamin C of the bean sprouts was evenly distributed in the whole sprout, while vitamin B_1 probably was more concentrated in the cotyledonal part of the sprouts.

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Appendix

Table 9. Growth of Positive Control Rats

Rat number		3937♀	3944♂	3944♂R	3941♂R	3945♀	3937♀B	3967♀L	3967♂B	3968♀L	3970♂	Gain of the whole group Total	Average
Initial weight		55	78	81	69	61	75	69	75	67	64	694	69.4
Weekly gain	1st wk.	9	12	7	11	5	11	8	11	13	10	97	9.7
	2nd wk.	3	12	4	0	3	4	3	12	5	3	49	4.9
	3rd wk.	6	8	10	13	9	6	-5	-1	10	6	62	6.2
	4th wk.	3	10	2	12	8	13	2	12	0	3	65	6.5
Total gain		21	42	23	36	25	34	8	34	28	22	273	27.3
Average weekly gain		5.3	10.5	5.8	9.0	6.3	8.5	2.0	8.5	7.0	5.5	68.3	6.8

Table 10. Growth of Rats Supplemented with 1 gm. Dry Mung Beans Per Day

Rat number		3937♂B	3937♂L	3939♂L	3944♀L	3941♀	3945♂B	3967♀R	3968♀B	3968♂	3970♀L	Gain of the whole group Total	Average
Initial weight		80	70	73	69	67	72	60	38	68	70	687	68.7
Weekly gain	1st wk.	13	8	18	0	12	15	4	15	18	11	114	11.4
	2nd wk.	25	12	3	11	5	-1	10	10	12	8	95	9.5
	3rd wk.	15	15	20	7	16	20	12	8	6	1	120	12.0
	4th wk.	10	10	10	13	6	9	8	10	9	7	92	9.2
Total gain		63	45	51	31	39	43	34	43	45	27	421	42.1
Average weekly gain		15.8	11.3	12.8	7.8	9.8	10.8	8.5	10.8	11.3	6.8	105.3	10.5

Table 11. Growth of Rats Supplemented with 6 gm Non-cotyledonal Part of Mung Bean Sprouts Per Day.

Rat number		3967♀-	3967♂	3970♂	3968♀-	Gain of the whole group Total	Average
Initial weight		68	62	63	64	257	64.3
Weekly gain	1st wk.	8	8	7	6	29	7.3
	2nd wk.	7	4	8	2	21	5.3
	3rd wk.	16	4	10	10	40	10.0
	4th wk.	0	5	3	8	16	4.0
Total gain		31	21	28	26	106	26.6
Average weekly gain		7.8	5.3	7.0	6.5	26.6	6.7

Table 12. Growth of Rats Supplemented with 10 gm. Non-cotyledonal Part of Mung Bean Sprouts Per Day.

Rat number		3944♀RR	3941♂B	3945♀R	3939♂LL	Gain of the whole group Total	Average
Initial weight		68	74	64	74	280	70
Weekly gain	1st wk.	3	12	8	12	35	8.8
	2nd wk.	7	12	7	10	36	9.0
	3rd wk.	8	12	7	14	41	10.3
	4th wk.	10	12	8	16	46	11.5
Total gain		28	48	30	52	158	39.6
Average weekly gain		7.0	12.0	7.5	13.0	39.5	9.9

Table 13. Growth of Rats Supplemented with 6 gm. of Whole Mung Bean Sprouts Per Day

Rat number		3944♀R	3939♂RR	3945♂R	3941♀B	3937♀R	3967♂L	3967♂R	3968♀R	3968♂L	3970♀RR	Gain of the whole group Total	Average
Initial Weight		65	70	75	61	76	68	70	70	68	60	683	68.3
Weekly gain	1st wk.	4	14	10	7	7	16	8	3	8	6	83	8.3
	2nd wk.	8	0	5	6	7	10	3	12	16	4	71	7.1
	3rd wk.	6	10	14	16	9	12	11	7	11	10	106	10.6
	4th wk.	9	15	6	0	10	6	8	13	11	9	87	8.7
Total gain		27	39	35	29	33	44	30	35	46	29	347	34.7
Average weekly gain		6.8	9.8	8.8	7.3	8.3	11.0	7.5	8.8	11.5	7.3	86.8	8.7

Table 14. Growth of Rats Supplemented with 8 gm. of Whole Mung Bean Sprouts Per Day.

Rat number		3939♂R	3944♀	3945♀B	3941♂L	3937♀L	Gain of the whole group Total	Average
Initial weight		77	73	61	75	68	354	71
Weekly gain	1st wk.	16	13	7	19	8	63	12.6
	2nd wk.	18	0	1	10	10	39	7.8
	3rd wk.	20	12	9	17	9	67	13.4
	4th wk.	16	6	4	14	11	51	10.2
Total gain		70	31	21	60	38	220	44.0
Average weekly gain		17.5	7.8	5.3	15.0	9.5	55	11.0

Table 15. Growth of Rats Supplemented with 10 gm. of Whole Mung Bean Sprouts Per Day.

Rat number		3941♂	3945♀L	3945♂	3941♀R	3944♀B	3937♂	Gain of whole grp. Total	Av.
Initial weight		74	65	71	68	59	78	415	69.2
Weekly gain	1st wk.	17	8	11	14	11	4	65	10.8
	2nd wk.	4	8	10	1	3	18	44	7.3
	3rd wk.	21	9	18	8	9	18	83	13.8
	4th wk.	17	14	16	18	6	19	90	15.0
Total gain		59	39	55	41	29	59	282	46.9
Average weekly gain		14.8	9.8	13.8	10.3	7.3	14.8	70.5	11.7

Table 16. Growth of Negative Control Rats

Rat number		3939♂	3937♂R	3944♂L	3941♀L	3945♂L	3970♀B	3970♀-	Gain of the whole group Total	Average
Initial weight		76	68	78	62	78	56	57	475	67.9
Weekly gain	1st wk.	1	-7	-2	-2	-5	-10	-10	-35	-5.0
	2nd wk.	-2	-11	-13	-13	-7	- 4	- 2	-52	-7.5
	3rd wk.	-7	- 7	- 3	- 9	-12 died	- 6 died	-3	-29	-5.8
	4th wk.	-12	- 3 died	-11	- 4 died			-4 died	-23	-11.5
Total gain		-22	-28	-29	-28	-24	-20	-19	-170	-24.3
Average weekly gain		-5.5	-8.5	-7.3	-7.6	-8.8	- 7.4	-6.1	51.2	-7.3

Table 17. Food Consumption of Positive Control Rats

Rat number		3937♀-	3944♂-	3944♂R	3941♂R	3945♀-	3937♀B	3967♀L	3967♂B	3970♂	3968♀L	Consumption of the whole grp. Total	Average
Weekly consumption	1st wk.	35	40	40	34	33	34	37	40	36	35	364	36.4
	2nd wk.	30	46	41	32	32	44	33	38	33	33	362	36.2
	3rd wk.	35	38	41	38	34	41	27	32	35	31	352	35.2
	4th wk.	29	44	40	40	38	43	29	43	38	38	382	38.2
Total consumption		129	168	162	144	137	162	126	153	142	137	1460	146.0
Av. weekly consumption		32.3	42.0	40.5	36	34.3	40.5	31.5	38.3	35.5	34.3	365	36.5

Table 18. Food Consumption of Rats supplemented with 1 gm. of Dry Mung Beans Per Day

Rat number		3937♂B	3937♂L	3939♂L	3944♀L	3941♀	3945♂B	3967♀R	3968♀B	3968♂	3970♀L	Consumption of the whole group Total	Average
Weekly consumption	1st wk.	42	27	40	24	39	39	29	34	34	34	342	34.2
	2nd wk.	52	35	32	36	34	30	31	29	31	38	348	34.8
	3rd wk.	48	42	45	34	40	46	33	32	35	30	385	38.5
	4th wk.	42	38	42	43	37	40	35	37	35	40	389	38.9
Total consumption		184	142	159	137	150	155	128	132	135	142	1464	146.4
Av. weekly consumption		46.0	35.5	39.8	34.3	37.5	38.8	32	33	33.8	35.5	366	36.6

Table 19. Food Consumption of Rats Supplemented with 6 gm. of Non-cotyledonal Part of Mung Bean Sprouts Per Day.

Rat number		3967♀-	3967♂	3970♂	3968♀-	Consumption of the whole group Total	Average
Weekly consumption	1st wk.	31	31	30	29	121	30.3
	2nd wk.	31	29	31	27	118	30.0
	3rd wk.	34	27	32	28	121	30.3
	4th wk.	35	32	35	31	133	33.3
Total consumption		131	119	128	115	493	123.9
Av. weekly consumption		32.8	29.8	32	28.8	123.3	31.0

Table 20. Food Consumption of Rats Supplemented with 10 gm. of Non-cotyledonal Part of Mung Bean Sprouts Per Day.

Rat number		3944♀RR	3941♂B	3945♀R	3939♂LL	Consumption of the whole group Total	Average
Weekly consumption	1st wk.	35	37	32	38	142	35.5
	2nd wk.	33	43	39	43	158	39.5
	3rd wk.	41	44	36	47	168	42.0
	4th wk.	41	48	38	49	176	44.0
Total consumption		150	172	145	177	644	161.0
Av. weekly consumption		37.5	43.	36.3	44.3	161	40.3

Table 21. Food Consumption of Rats Supplemented with 6 gm of Whole Mung Bean Sprouts Per Day

	Rat number	3944♀R	3939♂RR	3945♂R	3941♀B	3967♀R	3967♂L	3968♂R	3968♀R	3968♂L	3970♀RR	Consumption of whole grp. Total	Average
Weekly Consumption	1st wk.	29	37	29	29	35	42	32	31	31	31	326	32.6
	2nd wk.	33	30	29	35	41	38	28	29	36	31	330	33.0
	3rd wk.	33	34	42	38	39	43	32	30	32	35	358	35.8
	4th wk.	33	40	41	36	41	34	48	38	38	37	386	38.6
	Total consumption	128	141	141	138	154	157	140	128	137	134	1398	139.8
	Av. weekly consumption	32.0	35.3	35.3	34.5	38.5	39.3	35.0	32.0	34.3	33.5	349.5	35.0

Table 22. Food Consumption of Rats Supplemented with 8 gm. of Whole Mung Bean Sprouts Per Day.

Rat number		3939♂R	3944♀	3945♀B	3941♂L	3937♀L	Consumption of the whole group Total	Average
Weekly consumption	1st wk.	40	37	32	42	37	188	37.6
	2nd wk.	50	36	27	45	40	198	39.6
	3rd wk.	44	40	35	47	37	203	40.6
	4th wk.	60	38	31	53	40	222	44.4
Total consumption		194	151	125	187	154	811	162.2
Av. weekly consumption		48.5	37.8	31.3	46.8	38.5	20.3	40.6

Table 23. Food Consumption of Rats Supplemented with 10 gm. of While Mung Bean Sprouts Per Day.

Rat number		3941♂	3945♀L	3945♂	3941♀R	3944♀B	3937♂	Consumption of whole grp. Total	Average
Weekly consumption	1st wk.	40	33	43	34	31	33	214	35.7
	2nd wk.	40	37	41	31	34	45	228	38.0
	3rd wk.	50	41	50	38	33	42	254	42.3
	4th wk.	52	47	49	41	33	51	273	45.5
Total consumption		182	158	183	144	131	171	969	16.2
Av. weekly consumption		45.5	39.5	45.8	36.	32.8	42.8	242.3	40.5

Table 24. Food Consumption of Negative Control Rats

Rat number		3939♂	3937♂R	3944♂L	3941♀L	3945♂L	3970♀B	3970♀	Consumption of the whole group Total	Average
Weekly consumption	1st wk.	31	21	25	27	27	19	21	171	24.4
	2nd wk.	32	23	20	17	19	13	16	140	20.0
	3rd wk.	28	15	18	12	10 died	8 died	18	91	18.2
	4th wk.	17	2 died	12	5 died			0	29	14.5
Total consumption		108	61	75	61	56	40	55	456	65.1
Av. Weekly consumption		27.0	18.6	18.8	16.5	21.0	14.8	17.5	134.2	19.2