A MEASURE OF THE VITAMIN A CONTENT OF ARIZONA SORGHUM GRAINS AND YELLOW CORN

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A MEASURE OF THE VITAMIN A CONTENT OF ARIZONA SORGHUM GRAINS AND YELLOW CORN, AND OF THE VALUE OF ALFALFA AS A VITAMIN A SUPPLEMENT.

by

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A MEASURE OF THE VITAMIN A CONTENT OF ARIZONA SORGHUM GRAINS AND YELLOW CORN, AND OF THE VALUE OF ALFALFA AS A VITAMIN A SUPPLEMENT.

- Introduction -

The sorghum plant is one of the oldest to be cultivated by man. (1) Many wild forms are found abundantly in tropical and subtropical parts of the Old World, and it is believed that the cultivated forms were derived from the wild species independently in Africa and India long before the Christian Era. The story of the domestication of the sorghum plant is lost in the obscurity of the past. Long ago when agriculture was a matter of religion, there arose religious ceremonies in connection with the sowing and reaping of the sorghums. These ceremonies have come down through the ages and are observed in some parts of India at the present time.

The grain sorghums are extensively cultivated in Africa and India where they form the staple food of a large part of the population. From these two centers have come the innumerable varieties of sorghums which are now grown in many parts of the world.

The most valuable varieties grown in the United States were imported from the Orange River district of South Africa about 50 years ago. Because similar climatic conditions prevail in South Africa and the Great Plains of America, the imported plants are well adapted to their changed enviornment. However, varieties obtained from Tropical Africa mature too late to be of wide use in the United States, and more recent importations from Egypt of the dura type seem better suited to conditions in this country.

With the settlement of the semi-arid sections of Central and Western United States there arose a great demand for draught resistant crops, and the value of the sorghum grains was recognized. Their cultivation met with success in Kansas, Oklahoma and Texas, and at the present time they are important grain and forage crops in many other states.

Forms of sorghum grown in the United States may be classified into three groups (2): broom corns, saccharine sorghums, and non saccharine sorghums. This last class commonly known as grain sorghums is grown for both forage and grain, and is divided into the kafir corn and dura groups. Varieties of the former are stocky, leafy, and have erect heads, the stalks and leaves remaining green and succulent until after the grain is ripened, a quality which adds to their forage value. Hegari falls into this class of grain sorghums. (3) Yellow milo commonly known as milo maize or red milo belongs to the dura group, the members of which are taller and less leafy. The seed heads are often goose necked, but recent strains have been developed in which nearly all the seed heads remain erect. This eliminates difficulty in harvesting the grain due to the drooping heads. Since the leaf and stalk are dry when the grain is mature, members of this group do not make a good forage as those of the kafir corn group.

The non saccharine sorghums were introduced into Arizona about forty years ago and have become important agricultural crops. Hegari and yellow dwarf milo have to a large extent replaced other varieties. In the five year period from 1924 to 1928 inclusive, annual sorghum grain production increased from 600,000 bushels to 1,500,000 bushels. (4)

The non saccharine sorghums, hegari and dwarf yellow milo are used in poultry and live stock feeding. Expansion of production of these grains in the state lies in their greater use in the feeding of farm animals and poultry. At the present time there is a wide spread preference for yellow corn among poultry growers and stockmen of the state, rather than for the home grown sorghums. Much of the stock is shipped out of the state for finishing and every year Arizona imports thousands of bushels of yellow corn, which cannot successfully be grown here. The sorghum grains must meet the direct competition of imported yellow corn.

In 1921 at the Agricultural Experiment Station of the University of Arizona, an experiment was undertaken to study the effect of a ration of ground hegari and tankage on the breeding record of swine (5). Two gilts when placed on this ration weighed 71 and 46 lbs. Both grew normally over a period of several months and reached a large size, but both failed to conceive. One of the animals became unthrifty, weakened and died. The other at the end of 16 months had become blind and showed symptoms of nutritional deficiency. She was then turned out on alfalfa pasture, where her condition became very much improved and a few months later she conceived.

Another feeding experiment of interest was carried on in 1923 at the experiment station farm, for the purpose of comparing hegari with yellow corn in rations for feeding young pigs (5). Fourteen young pigs averaging 36 pounds each in weight were divided into two lots, one given a ration of ground hegari and tankage, the other corn and tankage. The corn group thrived very well and finished for the market in 17 weeks, while on the other hand the lot on ground hegari showed some nutritive deficiency, manifested by impaired eyesight, deafness, subnormal growth, and nervous spasms.

There have also been verbal reports of "nutritional roup" in chickens when fed on the sorghum grains.

The general feeling against the use of these grains is based on experiences of this kind in which nutritive failure has resulted when they are substituted for yellow corn in the ration.

A chemical analysis which was made showed little difference in the composition of yellow corn and the sorghum grains.

Grain	Water	Ash	Crude Protein	Carbohydrate N-free extract	Carbohydrate Fiber	Fat
Hegari*	6.88	1.76	13.66	74.55		3.15
Dwarf Milo*	6.64	1.78	13.87	74.34		3.39
Dwarf Milo**	9.38	1.63	12.16	72.09	1.47	3.27
Dwarf Milo***	10.70	2.80	10.70	70.50	2.40	2.90
Average of ** Sorghum Grains	9.52	1.70	13.01	70.95	1.53	3.29
Dent Corn*	7.00	1.58	10.98	74.94		5.50
Dent Corn***	12.00	1.50	9.90	69.70	2.00	4.90

* Analyses made at University of Arizona. Water content low due to dry atmospheric condition.
** Analyses by the Plant Chemical Laboratory of the Bureau of Chemistry, U. S. Department of Agriculture.
*** Analyses from Henry and Morrison.

From such analyses one might even consider the sorghum grains slightly superior to yellow corn due to their higher protein content. It is generally agreed, however, that because of their hard outer coating the starch of the sorghums is less completely digested than that of yellow corn. The difference is less than 10%. Cracking and grinding increases the digestibility and palatability of the grain sorghums (6,7,8).

Chemical analysis alone does not serve as an adequate test of the nutritive value of a foodstuff. Animal feeding experimentation gives more complete information of food values. McCollum and his associates (9) have shown by experimental feeding of animals that grains as a class, including yellow corn, are not adequate as the sole source of protein. "The proteins of cereal grains have approximately 1/3 to 1/2 the value of milk proteins for the support of growth and maintenance in the rat." In more recent work Heller and Green (10) state that "the protein of the grain sorghums is very good, but like that of yellow corn is deficient in certain amino acids which may be supplemented by the addition of small amounts of certain commercial food stuffs, rich in these constituents." In 1919 McCollum also showed that failure of swine to grow at a normal rate on a corn ration was due not entirely to the incomplete nature of the protein but to lack of essential ash constituents as well (11). Therefore, yellow corn as well as the sorghum grains should be supplemented with a good source of protein and materials rich in minerals when used in animal feeding. It is the custom in Arizona to supplement hegari and milo rations with protein feeds such as tankage and cottonseed meal.

The superiority of yellow corn obviously does not lie in the quality of its protein, or in its ash constituents.

However, there is another dietary requirement which must be taken into account when considering the nutritive value of any food. The necessity for a number of chemically unknown dietary essentials called vitamins is of more recent recognition. Need of vitamins in live stock feeding has been demonstrated very strikingly in recent years. All animals need the vitamins though the amount required may vary widely with different species. With the possible exception of vitamin D, none of the six vitamins recognized at the present time can be synthesized by the animal body, so that animals are dependent upon their ration for an adequate vitamin supply.

McCollum (12) has pointed out that vitamin A occurs in greatest concentration in the actively functioning part of the plant rather than in the storage organs. He found grains as a class to be poor sources of vitamin A. On the other hand, Steenbock (13) has called attention to the association of vitamin A with yellow plant pigment, and has shown that yellow corn is a very good source of this vitamin, while the white varieties contain very small amounts or are practically devoid of it. In 1923 Emmett and Peacock (14) showed that nutritional "roup", which is so prevalent in Arizona in poultry fed on hegari and milo, is caused by a lack of vitamin A in the diet.

These observations indicate that the sorghum grains may be deficient in vitamin A and therein may lie their inferiority to yellow corn in the feeding of farm animals and poultry. Up to the present time very little study has been made of the vitamin content of the sorghum grains. Heller and Green (10) have concluded from their work with the sorghum grains that "the vitamin content of most of the members is sufficient for all practical purposes, vitamin A being present in amount sufficient for growth and reproduction, but in all species insufficient for continued rearing of the young."

The purpose of this work was to measure quantitatively the vitamin A content of the sorghum grains, hegari and milo, to compare them with yellow corn, and if found deficient, to study the value of alfalfa as a vitamin A supplement.

EXPERIMENTAL PART I.

Quantitative Measurement of the Vitamin A Content of Hegari, Milo, and Yellow Corn.

Samples of select hegari and dwarf, yellow milo were obtained from the Agronomy Department of the University of Arizona. The yellow corn was a select variety secured from Kansas.

GENERAL METHOD. The method used for quantitative determination of the vitamin A content of hegari, milo and yellow corn, was developed by Sherman and Munsell (15) from that of Drummond and Coward. (21)

Albino rats weighing 40 to 65 grams at 28 to 29 days, were taken from mothers which had been raised on the standard stock ration of 1/3 whole milk and 2/3 whole wheat and sodium chloride equal to 2% of the whole wheat. They were given a diet free from vitamin A but which was adequate in every other respect. The vitamin A free diet used had the following composition:

Cornstarch	67%
Casein	18%
Dried Yeast	10%
Salt Mixture	4%
Sodium Chloride	1%

The cornstarch which serves as the main energy producing food in this ration contains no detectable amounts of vitamin A. Casein, the source of protein was rendered free from this vitamin by extraction with 95% ethyl alcohol as described by Sherman and Munsell (14). Two hundred grams of casein was placed in a round bottom flask and extracted at boiling point for one hour with 500 c.c. of 95% alcohol, over a steam bath under a reflux condenser. It was then filtered hot in a Buchner funnel by means of suction. The casein was extracted three times in this manner, then dried at room temperature. Casein so treated contains no traces of vitamin A when tested by the animal feeding method. The necessary mineral elements are supplied by the Osborne and

Mendel (16) salt mixture and sodium chloride which also adds to the palatability of the ration.

The vitamin B requirement of the animal was met by including 10% of dried yeast in the diet. That yeast contains no detectable amount of vitamin A was shown by Osborne and Mendel (17), who found that dried yeast when fed as the sole source of vitamin A at a level as high as 47.5%, induced no response in growth in animals suffering from vitamin A deficiency.

In this experiment vitamin D, the antirachitic vitamin, was supplied by the store in the body of the rat from the stock ration, and by irradiation of the cornstarch of the basal diet under a mercury vapor quartz lamp for one half hour at a distance of eighteen inches.

The animals on this diet continued to grow for a period of four to five weeks due to the storage of vitamin A in their bodies from the stock ration. This period during which the body store is being exhausted is often called the "depletion period" or "fore period." When the reserves of vitamin A were exhausted the animals became stationary in weight averaging 130 grams, and began to show other signs of lack of the vitamin, such as light sensitive eyes.

At this time the rats were put into individual round metal cages with false screen bottoms to prevent access to excreta. In addition to the basal ration, animals from the same litter were given daily for an experimental period of eight weeks, graded amounts of one of the grains, the vitamin A content of which was to be measured. The yellow corn was eaten very readily, but considerable difficulty was encountered in inducing the animals to eat the full amounts of hegari and milo. It was found that these grains were much more palatable to the rat when cooked for one minute or less in boiling water. The effect of cooking for so short a time was considered negligible as vitamin A from plant sources has been found to be fairly stable to heat even in the presence of air (18,19).

At least one animal from each litter was used as a negative control and continued on the unsupplemented basal ration until death. Weekly records were kept of the food intake and weight of each animal. The general condition of each rat was observed daily, special attention being given to the onset of ophthalmia and any other symptom suggestive of lack of vitamin A. Animals dying during the experimental period were autopsied to determine whether the animal died of vitamin A deficiency or other abnormal condition. At the end of the eight week experimental period all the remaining animals were chloroformed and autopsied to ascertain whether or not any symptoms of vitamin A deficiency were present. Characteristic signs most frequently observed in these animals were: ophthalmia, infections of the glands situated near the base of the tongue, of the sinuses, middle ear, and bladder. The degree of each infection was graded by use of the positive sign (+) from one to four in number.

Any advantage the other members of the litter showed over the negative control was attributed to vitamin A in the test grain. The results of such an experiment may be expressed in terms of Sherman's (20) unit which is defined as "that amount of vitamin A which when fed daily just suffices to support a limited rate of gain of three grams per week or 25 grams in eight weeks in a standard test animal" prepared as above described.

RESULTS. Weight records of the negative control animals appear in table I. The average length of survival of these animals during the experimental period was 37 days.

TABLE I.

Preliminary Period			Experimental Period										Observations and Autopsy Record					
Rat No.	Initial Weight	Final Weight	Weekly weight in grams 1	2	3	4	5	6	7	8	Total Gain	Survival	Eyes	Glands near Tongue	Ears	Sinus	Bladder	Miscellaneous
	grams	grams									grams							
118♂	55	140	139	135	131	126	116	98	*		-42	41	+++	++++	++	++	++	cough
123♀	53	136	135	130	125	120	111	107	106	87*	-49	56	++++	++++	+++			
243♂	65	178	167	155	141	126	110	93	*		-85	43	++		++		+++	cough
248♀	41	135	132	129	126	121	116	*			-19	42	blind	++++		++		
266♂	48	132	132	129	121	109	89	*			-43	37	+++	+		++	++	
275♂	48	124	114	103	92	81	*				-44	24	+++					
280♀	44	120	112	102	90	77	*				-43	27	++				+++	diarrhea
284♂	47	112	106	98	91	84	78	69	*		-43	38	blind	+++	++		++	
293♀	39	99	101	97	90	72	*				-27	28	blind	++			++	hemorrhages
321♂	65	138	138	135	130	125	*				-13	24	blind	+++				diarrhea
328♀	52	120	118	115	110	105	100	81	*		-39	38	blind	+++		+	++	
Avg	51	130	126	120	112	102	100	85			-41	37						

The results of feeding 1, 2, 3 and 4 grams of hegari appear in table II. Chart I shows the tabulated data as graphed growth curves.

TABLE II.

Preliminary Period				Experimental Period											Observation and Autopsy Record					
Rat No.	Initial Weight	Final Weight	Length	Amt. Hegari Daily	Weekly weight record in grams 1	2	3	4	5	6	7	8	Gain or Loss in 8 wks.	Survival days	Eyes	Glands near Tongue	Ears	Sinus	Bladder	Miscellaneous
	grams	grams	days																	
121♂	55	136	38	1	140	145	147	148	148	143	137	120	-16	Whole Pd.	+++	+++		++	++++	
122♂	53	127	38	1	125	119	115	115	113	113	106	91	-36	" "	++++	++++		+	++++	
Avg	54	131	38	1	133	132	131	131	129	128	121	105	-26							
120♂	55	132	38	2	138	143	148	152	158	155	149	140	- 8	" "	+++	++++		++		
124♀	53	122	38	2	120	115	106	*						20 days	+					
265♂	48	138	33	2	140	142	142	145	145	135	122	102	-36	Whole Pd.	++	++++			+++	Urinary Calculi
272♀	46	120	33	2	122	127	133	137	133	131	129	111	- 9	" "	+++	+++	+	++		
Avg	50	128	35	2	133	137	141	145	145	140	133	116	-12							
267♂	48	135	33	3	137	139	141	141	141	139	124	124	-11	" "	+	+			++	
271♀	48	130	33	3	137	146	152	157	153	151	151	151	21	" "	+	++	+	++		
273♀	44	122	33	3	128	135	135	135	136	135	136	136	14	" "	++				+++	
288♂	41	127	34	3	128	128	136	144	145	144	136	128*		51 days	++	+++	+		+	
292♀	41	105	34	3	107	109	111	118	121	124	119	*		51 days		++		+		
Avg	44	124	33	3	127	131	135	139	139	139	133	135	8							
264♂	49	129	33	4	134	136	138	140	142	143	143	143	14	Whole Pd.	+	++				Cough
270♀	48	128	33	4	138	148	153	154	159	163	147	132	4	" "	++	+++		+	++++	
287♂	43	115	34	4	118	127	133	140	133	108*				40 days		+			++	
289♀	44	110	34	4	112	114	115	117	121	126	130	132	22	Whole Pd.		++++				
290♀	43	115	34	4	118	121	124	125	130	133	133	136	21	" "					++	
291♀	42	105	34	4	112	120	120	125	125	133	134	136	21	" "		++	++	++	+++	
322♂	63	160	35	4	164	167	167	171	175	178	181	181	21	" "	++				++	
323♂	60	173	35	4	178	182	186	190	190	198	196	*		54 days	+	+				
326♀	57	132	35	4	136	140	139	141	145	150	153	153	21	Whole Pd.	+				++	
327♀	56	130	35	4	134	137	140	142	144	146	151	153	23	" "					++	
Avg	50	130	34	4	134	139	142	145	146	147	152	146	19							

* Died

The animals receiving one and two grams of hegari showed only slight improvement over the negative controls. All except one lived throughout the experimental period but showed very severe symptoms of vitamin A deficiency. Even the higher levels of hegari failed to protect the animals against infection. All the animals except one on the three gram level, and half of those on the four gram level showed marked ophthalmia, and on autopsy decided symptoms of lack of vitamin A were observed in all. Four grams of hegari daily is not sufficient to provide one unit of vitamin A. It is slightly below the unit level.

The results with yellow milo were very much better than with hegari, as can readily be seen from table III and chart II.

TABLE III.

Preliminary Period				Experimental Period											Observations and Autopsy Records					
Rat No.	Initial Weight	Final Weight	Length	Amt. Milo Daily:	Weekly weight record in grams 1	2	3	4	5	6	7	8	Total Gain	Survival Days	Eyes	Glands near Tongue	Ears	Sinus	Bladder	Miscellaneous
	grams	grams	days	gms									grams							
249♂	48	151	33	1	143	143	147	153	159	163	155	150	-1	Total Pd.	++	++++				
246♀	55	144	35	2	150	157	165	169	172	176	179	179	35	" "	++	+				cutaneous malnutrition
250♂	48	149	*33	2	152	155	159	161	162	164	166	167	18	" "	+			++		
252♂	40	149	33	2	153	161	167	170	170	152	*			38 days	+++	++				
258♂	52	129	33	2	132	136	139	142	144	146	150	150	21	Total Pd.					+	cough
262♀	48	110	33	2	113	115	115	116	117	117	120	120	10	" "		+ +		+		
Avg	49	136	33	2	140	145	149	152	153	151	154	154	21	" "						
245♂	40	130	35	3	136	141	146	153	160	163	163	163	33	" "						cough
255♀	38	123	33	3	129	134	140	144	149	154	157	157	34	" "	+					
259♂	50	124	33	3	127	130	132	134	134	135	136	137	13	" "						
261♀	48	120	33	3	125	131	135	138	140	143	145	145	25	" "						
263♀	48	119	33	3	124	130	136	140	145	149	153	154	35	" "						
Avg	45	123	33	3	128	133	138	142	146	149	151	151	28							
244♂	52	160	35	4	168	175	184	194	204	208	212	212	52	" "					++	
247♀	45	130	35	4	136	143	150	156	161	165	167	165	35	" "						
254♀	38	130	33	4	138	146	152	160	166	169	175	175	45	" "	+			+		
256♂	55	159	33	4	167	175	183	188	192	192	190	189	30	" "						
260♀	50	115	33	4	123	131	139	141	144	147	152	154	39	" "						
320♂	65	160	35	4	168	176	186	197	205	208	213	214	54	" "						
324♀	60	115	35	4	123	131	136	138	146	154	160	160	45	" "						
Avg	52	142	35	4	146	153	161	168	174	178	181	181	43							

* Died

Though two grams of yellow milo did not provide enough of the vitamin to protect the animals against infection, the average total gain for the experimental period was only slightly below the unit level. Three grams of milo contains enough vitamin A to support an average total gain of 28 grams in eight weeks, slightly more than the standard gain. All remained in good condition and were almost completely protected against infection. Four grams of milo promoted gains much beyond the unit level. Yellow milo is approximately twice as rich in vitamin A as hegari.

In the case of yellow corn, results shown in table IV, chart III, .25 grams per day provided enough vitamin A to produce slightly more than 25 grams gain in the eight weeks period. However, there were only two animals on this level and there is need for more evidence on this point.

TABLE IV.

Preliminary Period				Experimental Period											Observations and Autopsy Record					
Rat No.	Initial Weight	Final Weight	Length	Amt. corn daily	Weekly Weight Record in Grams 1	2	3	4	5	6	7	8	Total gain 8 wks	Survival days	Eyes	Glands near Tongue	Ears	Sinus	Bladder	Miscellaneous
	grams	grams	days	grams									grams							
295♂	52	141	34	.05	120	104	87	83	*					29 da	+++	++			+++	
296♂	48	132	34	.05	130	130	128	126	125	124	123	114	-18	Total Pd.	++++					
297♂	48	111	34	.05	114	117	120	122	124	122	101	87	-21	" "	Blind	+++			++++	
300♂	44	128	34	.05	131	133	135	137	125	108	*			47 da.	+++	++++			+	
301♂	43	116	34	.05	118	120	121	121	113	111	94	*		45 "	+++	++++		++		
303♀	45	106	34	.05	108	112	118	118	124	126	123	91*		45 "	++++	+++	+	+	++	
319♂	69	133	35	.05	137	140	140	137	132	110	*			40 "	Blind	++++	++	++	++++	
325♀	57	121	35	.05	127	134	141	145	148	116	*			40 "	+++	+++				
Avg	51	124	34	.05	123	124	124	124	127	117	110	97	-20		+	++++	++	++	+	
276♀	50	121	37	.1	121	123	127	131	138	136	121	104*			Blind	++++		++		
294♂	53	135	34	.1	137	143	148	153	160	163	152	134	- 1	Total Pd.	+++	++		++	+++	
299♂	47	139	34	.1	141	144	148	150	152	156	154	154	16	" "	+++	++		+		
302♀	45	130	34	.1	132	130	135	140	145	149	144	144	14	" "	++++	+++				
Avg	49	131	35	.1	133	135	139	144	149	151	143	134	10							
277♀	47	130	36	.25	133	141	145	150	157	165	168	168	38	" "					++	
279♀	45	130	36	.25	134	134	139	147	152	156	164	164	34							
Avg	46	130	36	.25	133	138	141	148	154	160	166	166	36							
278♀	45	125	36	.50	129	138	147	156	161	164	166	169	45	" "						
281♀	39	120	36	.50	121	129	134	140	150	157	165	173	53	" "						
Avg	42	123	36	.50	125	134	140	148	155	160	165	171	49							
119♂	55	132	38	1	148	158	169	180	192	202	214	224	92	" "						
117♂	55	128	38	1	138	148	158	166	176	186	195	200	72	" "						
Avg	55	130	38	1	143	153	163	173	184	194	204	212	82							

* Died

Animals receiving 0.05 grams of yellow corn as the only source of vitamin A showed very slight improvement over the negative controls, and likewise 0.1 grams gave results far below the unit gain. Growth considerably above the unit level resulted in feeding 0.5 grams of yellow corn daily; and 1 gram contained enough vitamin A for nearly normal growth throughout the experimental period.

Table V gives a summary of the results obtained by feeding the three grains in graded amounts as the sole source of vitamin A in a diet which was otherwise adequate.

TABLE V.

Summary of Results of Feeding Graded Amounts of Hegari, Milo and Yellow Corn.

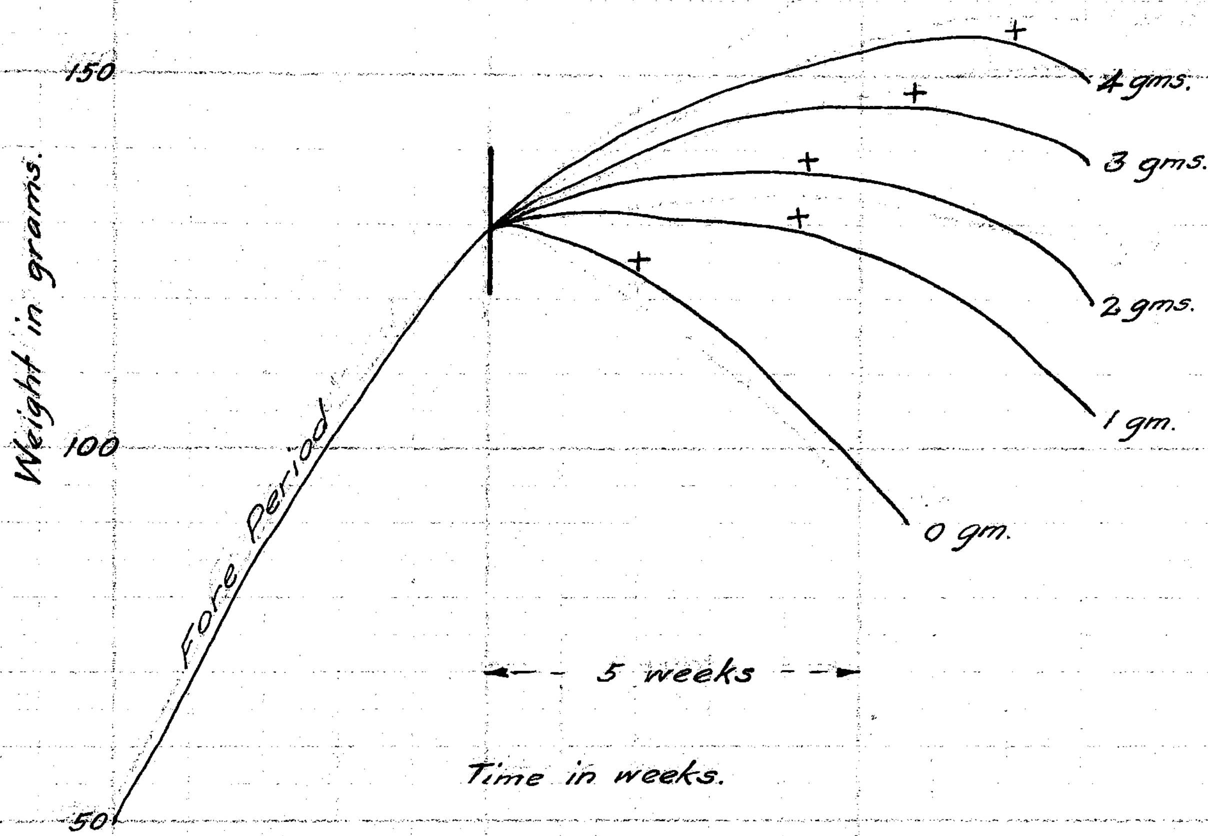
Grain	Amount Fed Daily	No. of Rats	Initial Weight	Weight end of Fore Pd	Final Weight	Total Gain
	Grams		grams	grams	grams	grams
Hegari	0	11	51	130	85	37 da -41
	1	2	54	131	105	-26
	2	4	50	128	116	-12
	3	5	44	124	132	8
	4	8	50	130	149	19
Milo	0	11	51	130	85	37 da. -41
	1	1	48	151	150	- 1
	2	6	49	133	154	21
	3	5	45	123	151	28
	4	7	52	137	181	43
Yellow Corn	0	11	51	130	85	37 da. -41
	0.05	8	51	124	97	-20*
	0.1	4	49	131	134	10
	0.25	2	46	130	166	36
	0.50	2	42	122	171	49
	1.00	2	55	130	212	82

* Weight of only those surviving.

A striking difference in the vitamin A content of yellow corn, milo and hegari is evident from the growth curves of rats receiving 1 gram of one of these grains daily as the sole source of this vitamin. These curves appear in Chart IV. Animals receiving 1 gram of hegari daily showed little improvement over the negative controls; those receiving 1 gram of milo barely maintained their weight; while 1 gram of yellow corn supported nearly normal growth throughout the experimental period.

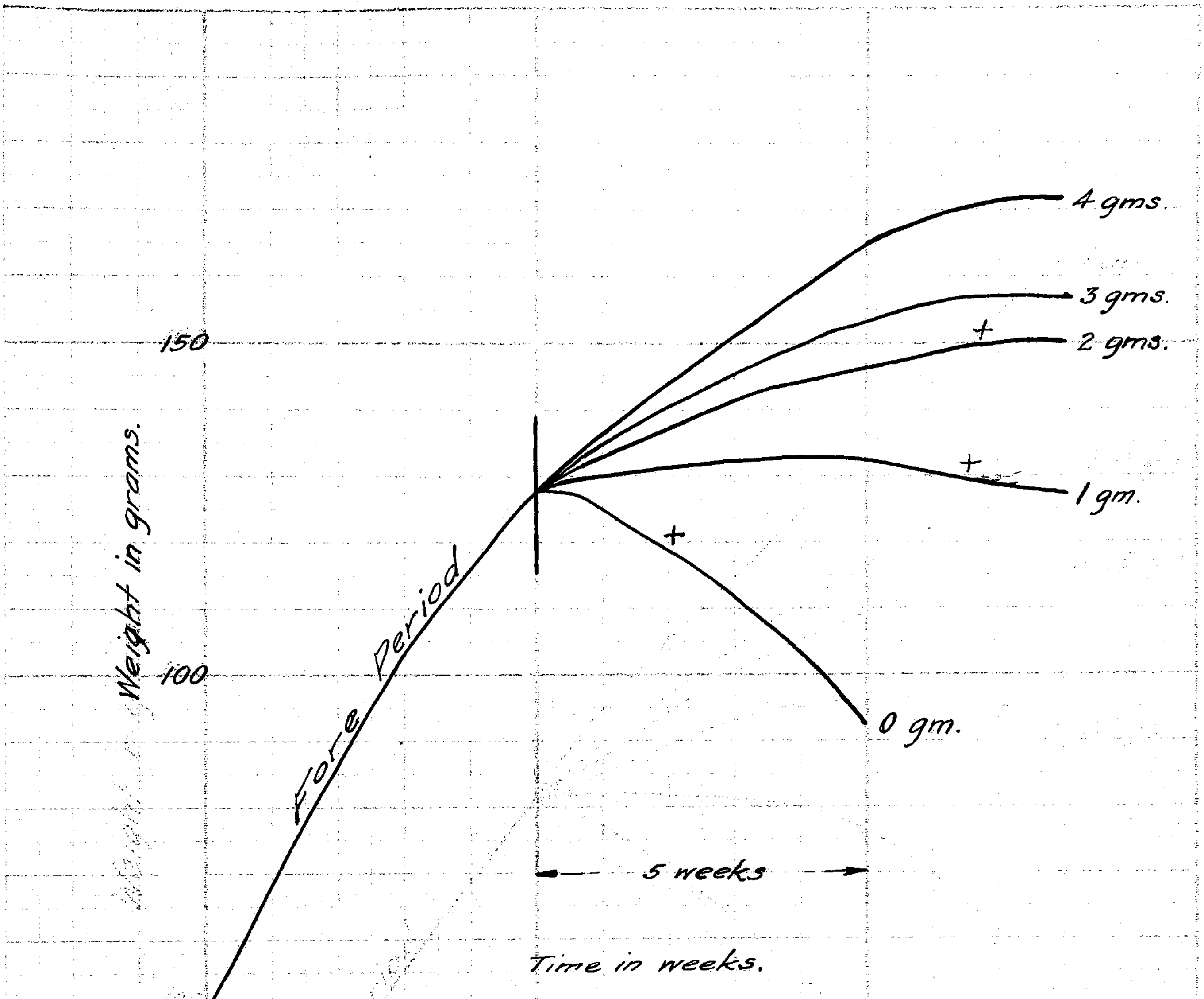
Though more animals should have been used to measure the vitamin A content in a strictly quantitative manner, nevertheless, the results obtained do serve as a basis for comparison of the vitamin A contained in hegari, milo and yellow corn.

CONCLUSIONS: It is evident from the results obtained that yellow corn is at least 15 times as rich in vitamin A as hegari. Dwarf yellow milo contains about twice as much vitamin A as hegari.



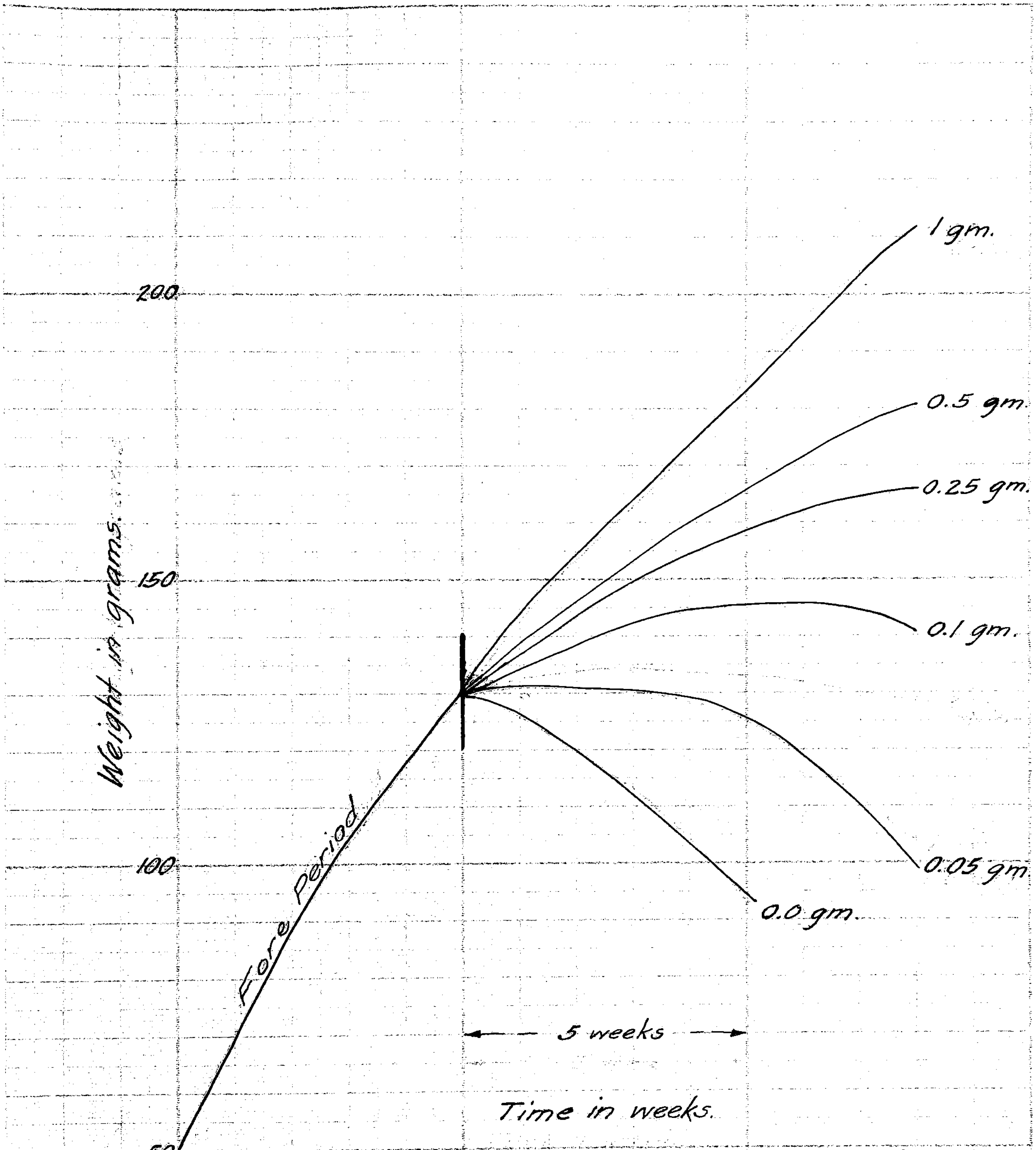
Curves showing average gains in weight of rats receiving 1, 2, 3, and 4 grams of hegari daily.

CHART NO. I



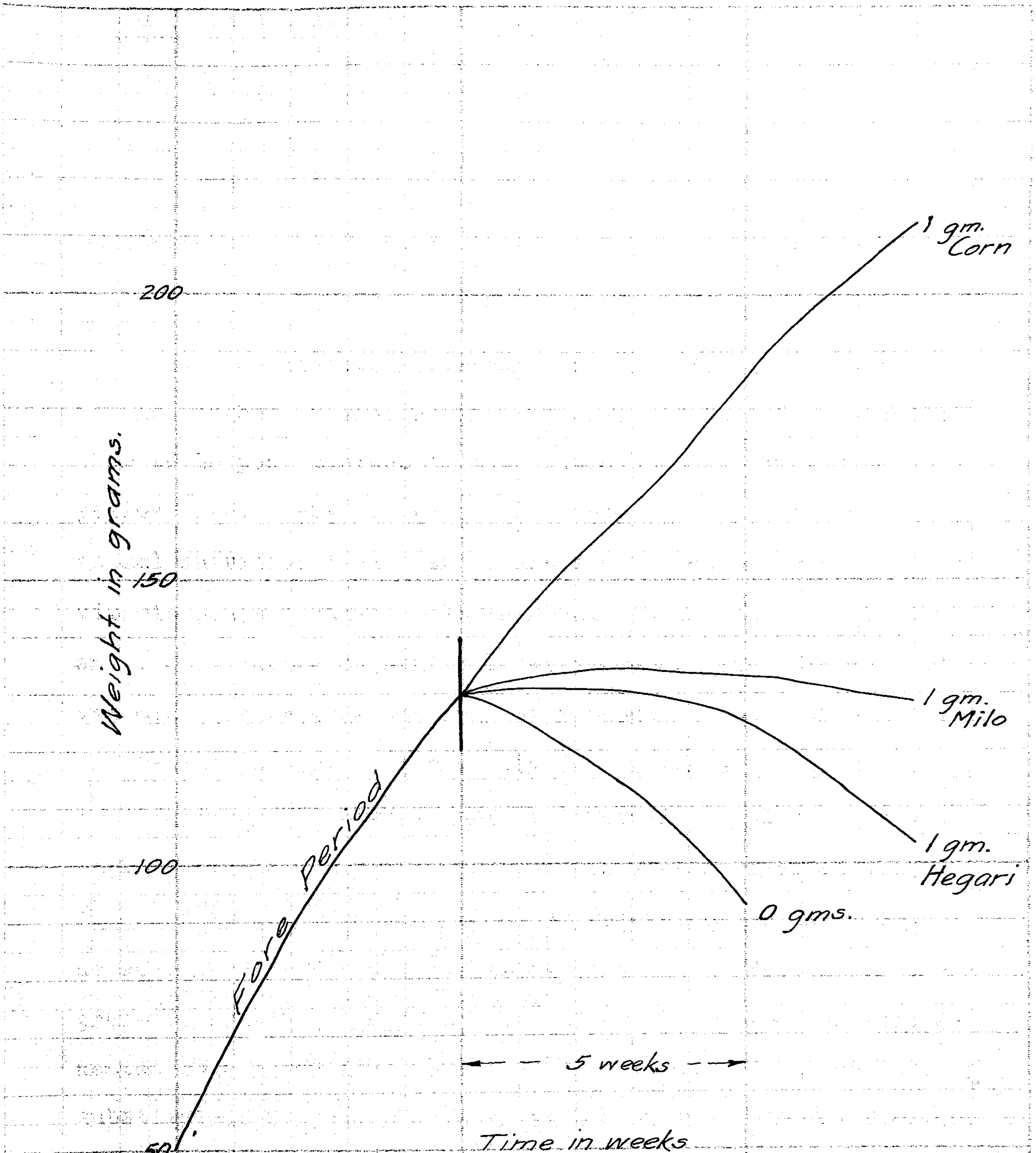
Curve showing average gains in weight of rats receiving 1, 2, 3, and 4 grams of milo daily.

CHART NO. II



Curves showing average gains in weight of rats receiving graded amounts of yellow corn daily.

CHART NO. III



Curves showing average gains in weight of rats receiving 1 gram of hegari, milo, or yellow corn as the only source of vitamin A.

CHART NO. IV

EXPERIMENTAL PART II.

The Value of Alfalfa as a Vitamin A Supplement for Sorghum Grain Rations.

The quantitative measurement of the vitamin A content of the sorghum grains, hegari and milo, in Part I, has shown them to be poor sources of this vitamin as compared with yellow corn. Many phases of the importance of vitamin A in animal nutrition have been emphasized in recent years as this vitamin plays a varied role in normal physiological activity and its influence on health is widespread. Insufficiency of vitamin A interferes not only with normal growth but results in general weakening of the body and increased susceptibility to infection. Vitamin A must be liberally supplied in the diet to maintain a high degree of health and vigor at all ages, to provide for successful reproduction and lactation, as well as satisfactory length of life (20). Because of the great importance of vitamin A, sorghum grains must be supplemented with a foodstuff rich in this vitamin, when they are substituted for yellow corn in rations used in animal feeding.

Alfalfa, which can be successfully grown in Arizona, was selected as a supplement because it is a very good source of vitamin A*, and is widely used in animal feeding. In order to determine how much alfalfa must be included in sorghum grain

*Smith, M.C., Unpublished data from this laboratory.

rations, rats were fed experimental diets which contained 1%, 3%, 5% and 10% of alfalfa leaf meal. These diets were made adequate in protein and mineral elements by including 10% of unextracted casein and 2% of the Osborne and Mendel salt mixture. Vitamin D was supplied by 0.05% of irradiated cholesterol. The following table gives the composition of the various diets containing different percentages of alfalfa leaf meal.

TABLE VI.

Composition of Experimental Diets

Diet No.	Alfalfa Meal %	Grain %		Casein %	Salt Mixture %	Yeast %	Cod Liver Oil %
4	0	Hegari	84	10	2	2	2
19	0	"	86	10	2	2	0
29	1	"	85	10	2	2	0
25	3	"	83	10	2	2	0
21	5	"	81	10	2	2	0
27	10	"	76	10	2	2	0
26	3	Milo	83	10	2	2	0
28	2	Corn	84	10	2	2	0

* 0.05% irradiated cholesterol included in each diet.

Rats at 28 days of age were taken from the stock colony which is reared on Sherman's Diet B, kept in square metal cages in lots consisting of three pairs matched as well as possible as to size and litter. Each of the seven lots was given one of the experimental diets and distilled water ad libitum for the duration of the experimental period, 7 to 9 months. The rations were eaten readily by the animals. A weekly record kept of the amount of food consumed showed no difference in palatability of the rations. All of the animals were weighed weekly.

Females which showed unusual gain and other evidence of pregnancy were separated from the lot and weighed every other day until the young arrived. When the young were born, the weight of the mother was again recorded and a bedding of finely cut pure crepe paper supplied. Each litter was weighed as a lot weekly until four weeks of age, at which time the young were weaned and individual record made of weight, sex, and general condition. The mother was then returned immediately to the lot from which she came.

This experiment was conducted over a period of 7 to 9 months as the results of a short time feeding test may not be dependable when there is only a partial lack of vitamin A. The growth curves of animals receiving different percentages of alfalfa were compared with those obtained on diet 4, in which codliver oil as a source of vitamin A served as the supplement to hegari, which has given optimal results in this laboratory. The average gains made by the males of each lot at various ages were compared.

The influence on reproduction of different amounts of alfalfa in the diet was considered significant, as Evans and Bishop (22) have shown that a diet which contains enough vitamin A for growth may still fail to provide an enough of the vitamin sufficient to meet the added demands of reproduction and lactation. This was very strikingly brought out by Sherman and McLeod (23) who by feeding two series of rats on diets of the same composition, except that one contained skimmed milk while the other contained whole milk, which increased the vitamin A content of the diet, found that the lower intake of vitamin A was sufficient for nearly normal growth but insufficient for successful reproduction. Whereas on the whole milk diet not only did normal growth result but successive generations were very successfully produced and reared. Likewise, Sherman and Muhlfield (24) have found that an increased proportion of milk in the diet resulted in larger number of young born, increase in the percentage of young reared, better maintenance of body weight of mothers during the lactation period, and a higher average weight of the young at weaning.

The criteria for judging the reproduction success of animals on the different experimental diets included: number and size of litters and young born, percentage of young reared, ability of female to maintain her own well being (body weight, etc.), general physical condition and weight of young at weaning, and the number of generations during the experimental period.

In order to observe the influence of each diet on successive generations, one pair from the first litter raised by each female was continued on the diet of the lot from which it had come.

A premortem and postmortem examination of the general health of each animal was made. Premortem evidences of lack of vitamin A consisted of ophthalmia, respiratory infection manifested by coughing and sneezing, and nasal hemorrhages, cutaneous malnutrition including sore tails, thin fur and hairless spots on body, and premature signs of old age. According to Sherman and McLeod (23) animals receiving the more liberal amounts of vitamin A did not show signs of senility as early, and lived more than twice as long as those on a diet containing less of the vitamin.

On postmortem examination careful observation was made of the lungs, bladder, and glands near base of tongue. Steenbock, Sell and Buell (25) were the first to observe that animals kept for several months on a diet poor in vitamin A showed increased susceptibility to lung infection. Sherman and McLeod (23) found that though animals may grow normally on a restricted intake of vitamin A, they exhibit tendency to break down with lung infection in early adult life. Such animals have actually less of the vitamin in their lung tissues than animals on a diet containing generous amounts of the vitamin, according to Sherman and Boynton (26). The presence of vitamin A in lung tissue makes it more resistant to infection.

To further check the adequacy of each diet the survival periods of 28 day old rats upon a vitamin A free diet were used to measure the storage of vitamin A in the body from the various diets. It has been shown that there is an actual storage of vitamin A in tissues of the body, and that a moderate difference in the vitamin A content of diet causes a decided difference in the amount of the vitamin stored (26-27). Large reserves of vitamin A can be built up in the body if the animal is given the opportunity by feeding a diet rich in this vitamin. "Surplus of vitamin A in the body is not simply a reserve asset to be used at some future time, but also actively increases the vigor and ability of the animal to resist disease" (20). Therefore a diet to be optimal must provide enough vitamin A to enable the animal to build up a liberal reserve of the vitamin in its body.

RESULTS: Influence of Diet on Growth. The average gains in the weight of males of each lot at various ages served as a means of comparing the growth induced on diets containing different percentages of alfalfa. These data are expressed in table VII.

TABLE VII.

Average Gains in Weight at Various Ages of Males on Diets Containing Different Percentages of Alfalfa

Diet:	First Generation				Second Generation			Third Generation		
No.	8 wks	12 wks	16 wks	28 wks	8 wks	12 wks	16 wks	8 wks	12 wks	16 wks
19	106	156	203	283	63	143	168			
29	109	163	226	297	101	181	225			
25	108	178	234	319	99	179	229	86	137	
21	119	178	274	307	115	189	226	87		
27	111	177	232	301	115	201	235	92	174	202
26	107	180	230	307	109	181	222	80	170	
28	113	161	226	299	98	184	242	100	155	192

Weights expressed in grams.

All the experimental animals were taken from the stock colony at 28 days of age, and having had equal opportunities for storage of vitamin A from the stock diet, the influence of this reserve was considered the same in each case.

Although the average gains in weight of males on diet containing alfalfa meal in percentages varying from 0 to 10 showed no striking differences, animals on diet 19, containing no alfalfa, consistently made the smallest gains throughout the experimental period. At 16 weeks the average gain in weight of males on diet 19 was 203 grams as compared with 226 grams on diet 29 which contained only 1% alfalfa. At the end of 28 weeks the average gain on diet 19 was 283 grams while animals on all the other diets weighed about 300 grams.

Chart V shows the growth curves of animals reared on diet 4 which has proved to be optimum in this laboratory. These growth curves are slightly superior to any obtained with the alfalfa and grain diets as can readily be seen by comparison with curves on charts VI to XII, inclusive.

Growth of the second generation on diet 19 was markedly inferior to that of the first. The average gain at the age of 8 weeks was 63 grams as compared with 106 grams gain in the first generation; and again at 16 weeks of age the first generation had gained 283 grams, the second generation only 168 grams. On all the other experimental diets animals of the second generation grew at a rate equal to that of the first. Second generation animals on diet 27 made larger gains in weight than those on any other diet except 28, which contained yellow corn.

Animals of the third generation regardless of diet showed retarded growth. On diet 27, which contains 10% alfalfa, the average gain of the third generation at 8 weeks of age was 92 grams, as compared with 115 grams in the second generation;

and again, at the end of 16 weeks, the average gain was 202 grams as compared with 235 grams in the second generations.

With the exception of diet 19, which showed a slightly inferior rate of growth in the first generation, and a markedly inferior rate in the second, the influence on the growth rate of insufficient amounts of vitamin A in the experimental diets was not evident until the third generation.

Influence of Diet on Success of Reproduction.* Data showing the degree of success of reproduction and lactation appear in table VIII.

TABLE VIII.

Average Reproduction Records of Females on Diets Containing Different Percentages of Alfalfa.

Diet No.	Avg. Record per Female			Avg. No. Reared per Female	Per-cent Reared	Avg. Wt. at Weaning grams	No. of Generations
	No. Litters	Size of Litters	No. Born				
19	2.6	7.5	20	3.3	16	30.5	3
29	3.3	6.9	23	9.0	30.9	41.1	3
25	6.0	8.3	50	14.0	28.0	48.3	3
21	5.0	6.8	35	20.5	58.5	50.8	3
27	5.0	8.3	41	27.7	68.0	47.3	4
26	5.0	7.4	37	15.0	40.5	46.7	3
28	5.5	8.1	45	22.0	48.8	46.0	3
4	5.0	9.7	44	40.0	91.9	58.0	4

*In all charts the number of young born is indicated on the curve of females by the figure at the left of the letter Y; the number of young reared by the figure at the right.

Of the three females on diet 19 containing no alfalfa, one failed to rear even her first litter, one produced four litters but reared only a part of the first, and the third gave birth to only two litters and successfully reared but one. The breeding record of the females on diet 19 appears in chart VI. These animals produced the smallest number of litters, the percent of young reared was lowest, and the average weight of the young at weaning was less than that on any diet containing alfalfa. The average number of litters born on diet 19 was 2.6; the average number of young per female, 20; whereas, on diet 25, which contains 3% alfalfa, four litters were born per female in the same length of time, each female producing an average of 32 young. Only 16% of the young on diet 19 were reared, while females on diet 29 succeeded in rearing 30%; on diet 21, 58%; and on diet 27, 68%. Offspring of diet 19 weighed 30.5 grams at weaning as compared with 41 grams on 1% alfalfa; 48.3 grams on 3% alfalfa, and 50.8 grams on 5% alfalfa.

One percent of alfalfa in the diet had a noticeably favorable influence on reproduction, evident from larger number of litters born, greater percent of offspring reared, and larger average size of young at weaning. Two females of this lot failed to raise their later litters. Chart VII gives a graphic representation of the reproductive history of females on diet 29.

An increased percentage of alfalfa in the diet resulted in the birth of a larger number of young per female, a greater number and percent of young reared, and a favorable influence on the number of generations produced. Females receiving 3% alfalfa reared 28% of their young, while 10% of alfalfa enabled the mothers to rear 68% of their offspring. Although the females on diet 27 were more successful in reproduction and lactation than those receiving more limited amounts of alfalfa, their record is markedly inferior to that obtained on diet 4. For example; females on diet 27 reared only 68% of their young, as compared with 91.9% reared on diet 4. Then, too, the average size of the litter and number of young born was smaller on 10% alfalfa than on the optimal diet.

Females on all the diets sustained loss of their body weight while suckling young. This was very noticeable the third week of lactation when there frequently occured a decided loss in the weight of the female. Comparing the general shape of the curve of females on diet 4 during the lactation period with that of females from the alfalfa grain diets, the superiority of the former is evident. Even 10% of alfalfa in the hegari diet was not sufficient to protect the female against loss of weight during the lactation period.

The second generation was less successful in reproduction and lactation than the first, as evidenced by lower percent of young reared, smaller weight of young at weaning, and poor physical state of the young. Though the record of the third generation on these diets is limited, there are indications that reproduction and lactation in the third generation is inferior to that of the second. The third generation females on diet 27 produced several litters but failed to raise any of their young. Each generation was less successful than the preceding one in reproduction and lactation.

Influence of Diet on General Health. Infection of the eyes occurred in the males of the first generation of every lot except that receiving 10% alfalfa. Ophthalmia appeared earliest in animals on the hegari diet containing no alfalfa. One percent of alfalfa in the diet delayed the onset of ophthalmia 9 weeks; 5%, 15 weeks. The time of appearance of ophthalmia is marked in all the charts with a positive (+) sign.

The females showed eye infection more frequently during pregnancy and the lactation period. Ophthalmia was observed in the females on diets 19 and 29 after having their second litter; on diet 25, after the third litter, and on diets 21 and 27, after the fifth litter. The higher percentages of alfalfa protected the females for a longer time against infection, but the added demands for vitamin A during pregnancy and lactation brought about a gradual depletion of the store in the body, which together with insufficient amount of vitamin A in the diet, lowered the animals resistance to infection.

On all of the experimental diets ophthalmia appeared very frequently in the late litters during the third week of life, and disappeared during the fourth week when the young ate more freely of the mother's diet. Late litters were markedly inferior to earlier litters from the same female. Moreover, ophthalmia appeared earlier in the second generation than in the first, and still earlier in the third generation, showing increased susceptibility to infection with each succeeding generation.

The males and females on diet 28 containing yellow corn, showed unmistakable signs of ophthalmia toward the end of the experimental period. Late offspring all had ophthalmia at time of weaning. Such data indicate that yellow corn does not contain enough vitamin A for maintenance in health of the rat.

Evidences of cutaneous malnutrition including sore tails, thin fur and entire lack of hair on parts of the body, were observed in many of the young, even those receiving as much as 10% of alfalfa in the diet. This is added evidence that 10% of alfalfa in the sorghum grain ration fails to provide enough vitamin A for maintenance of a high degree of health and vigor.

It was also observed that animals on diets containing 3% and 5% alfalfa showed distinct signs of old age at a time when they should have been in the prime of life. Their fur was coarse and yellow, lusterless and very thin. This was in marked contrast with animals receiving 10% alfalfa which appear to be in much better physical condition.

Postmortem examination revealed infection in all of the animals on diets 19 and 29. Though these animals had been on the deficient diets for only seven months, the degree of infection was very marked. At least 50% of the animals from all the experimental diets showed lung infection. However, animals which had received 5% of alfalfa in their diet showed very advanced cases of lung disease, while those on 10% alfalfa showed only a slight degree of infection. Nevertheless, 10% of alfalfa in the diet does not provide a sufficient amount of vitamin A to maintain a high degree of resistance to infection in the rat.

While older rats on a vitamin A deficient diet are more susceptible to lung infection, young animals under the same conditions more frequently show ophthalmia (20). This infection of the eyes was prevalent among the second and third generations, but only the older animals of the second generation showed lung infection.

Influence of Diet on Storage of Vitamin A. Table IX shows the influence of different percentages of alfalfa in the diet on the storage of vitamin A in the body of rats at time of weaning, as evidenced by survival on a vitamin A free ration.

TABLE IX.

Average Records of Storage of Vitamin A on Diets Containing Different Percentages of Alfalfa.

Diet No.	No. of Rats	Initial Weight	Maximum Weight	Total Gain	Onset of Ophthalmia	Survival
		grams	grams	grams	day	days
19	8	34	51.0	2.2	4th	23.3
29	8	48.8	79.3	10.1	15th	39.6
25	8	48.8	87.1	21.6	18th	43.0
21	4	61.0	112.2	25.2	19th	47.0
27	8	51.3	105.6	23.0	31st	53.8
26	4	54	101.5	19.0	18th	46.5
28	2	56	139.5	37.5	35th	59.0
B*	11	51	130.0	38.0	40th	72.0

*Stock Diet

Young rats reared upon diets containing different percentages of alfalfa, when placed on a vitamin A-free diet at weaning showed remarkable differences in body reserves of vitamin A as indicated by maximum weight attained, time of appearance of ophthalmia and length of survival period.

The animals from the different diets continued to grow and increase in weight for varying lengths of time and reached different maximum weights. The inferiority of diet 19 was again apparent from the fact that 28 day old rats from this diet when deprived of vitamin A, showed ophthalmia in less than a week, made very little gain in weight, and survived on an average of only 23 days. One percent of alfalfa in the previous diet delayed the appearance of eye disease about two weeks, and increased the time of survival 16 days.

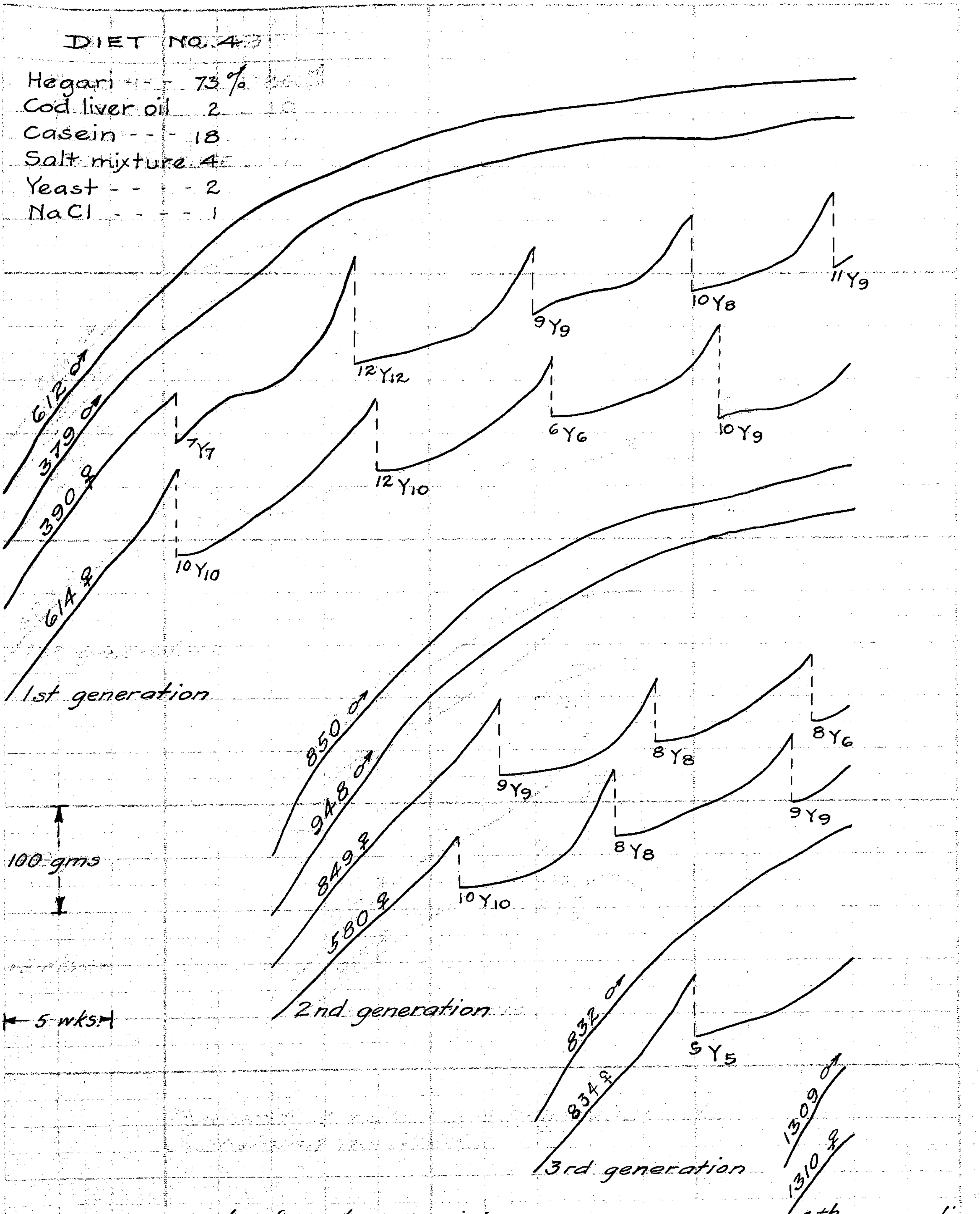
With the higher percentages of alfalfa in the previous diet, animals attained a greater maximum weight before the decline came, and the onset of ophthalmia was delayed. An increase from 1% to 10% of alfalfa in the previous diet delayed the appearance of eye disease 16 days and increased the life of the animal two weeks.

Comparing the survival records of animals from the alfalfa grain diets with those of animals from diet B, which has proved adequate in all respects for growth reproduction, and lactation, it is evident that none of the experimental diets in this work permitted as much storage of vitamin A as diet B. Again, it is evident that even the highest level of alfalfa feeding (10%) used in this study, proved inadequate to compensate for the low vitamin A content of hegari.

CONCLUSIONS: The sorghum grains, hegari and milo, are inadequate as the only source of vitamin A in animal feeding rations. This inadequacy is manifest by an inferior rate of growth, interference with normal reproduction and rearing of the young, and increased susceptibility to infection, with general decline in health and early appearance of old age. With each succeeding generation there is a gradual decrease in the body reserves of vitamin A, resulting in a general weakening of the strain.

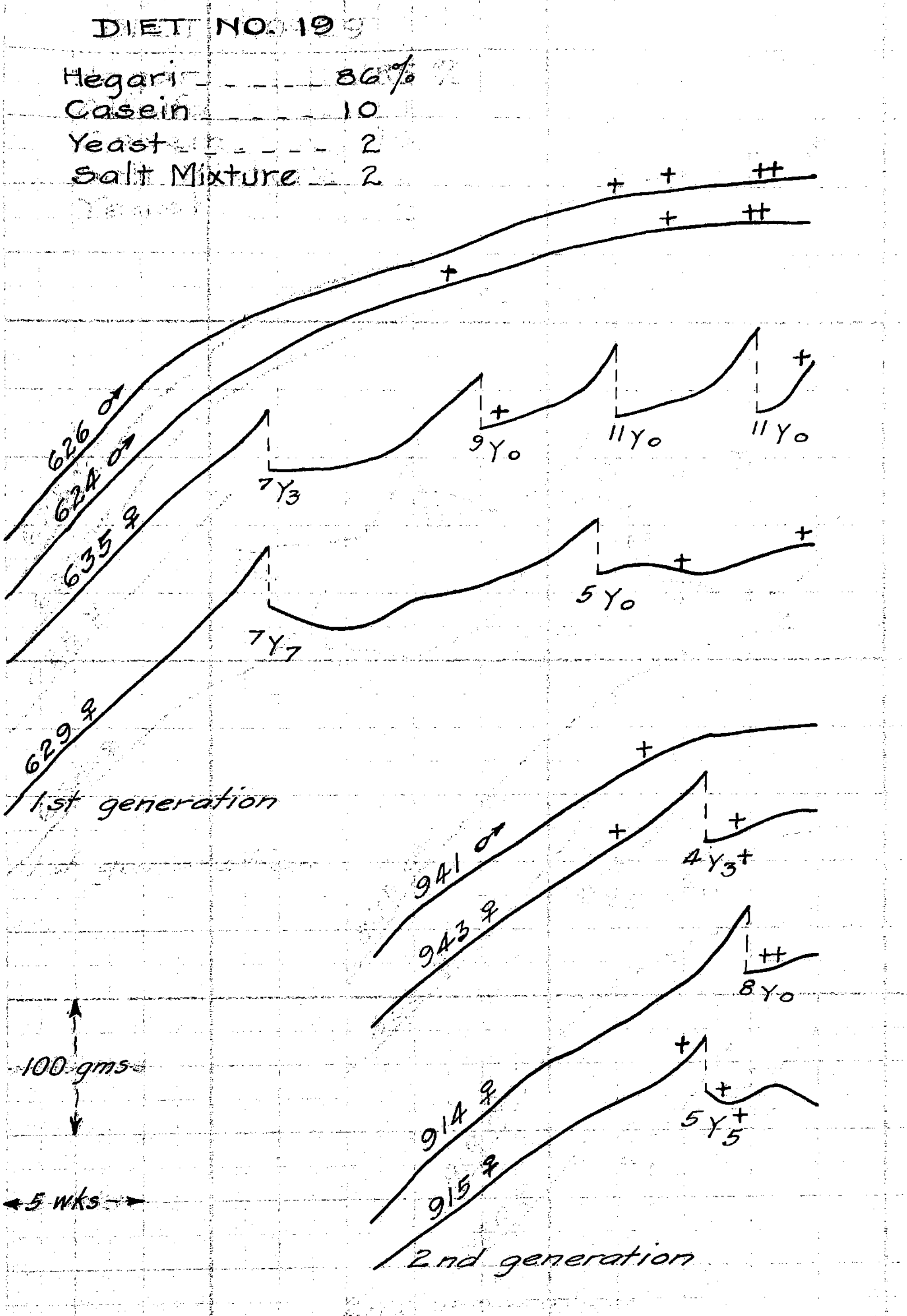
When judged by the same criteria, yellow corn is a much better source of vitamin A than the sorghum grains, hegari and milo, although it does not contain enough of this vitamin to promote normal nutrition in the rat.

Alfalfa leaf meal constitutes a good supplement for vitamin A in the sorghum grain ration, as evidenced by a favorable influence on growth, reproduction and lactation, and resistance to infection, when it comprises 1, 3, 5, and 10 percent of the ration. However, the highest percent fed (10%) of alfalfa leaf meal does not provide enough vitamin A for optimal growth and reproduction,and for promotion of health and vigor throughout successive generations.



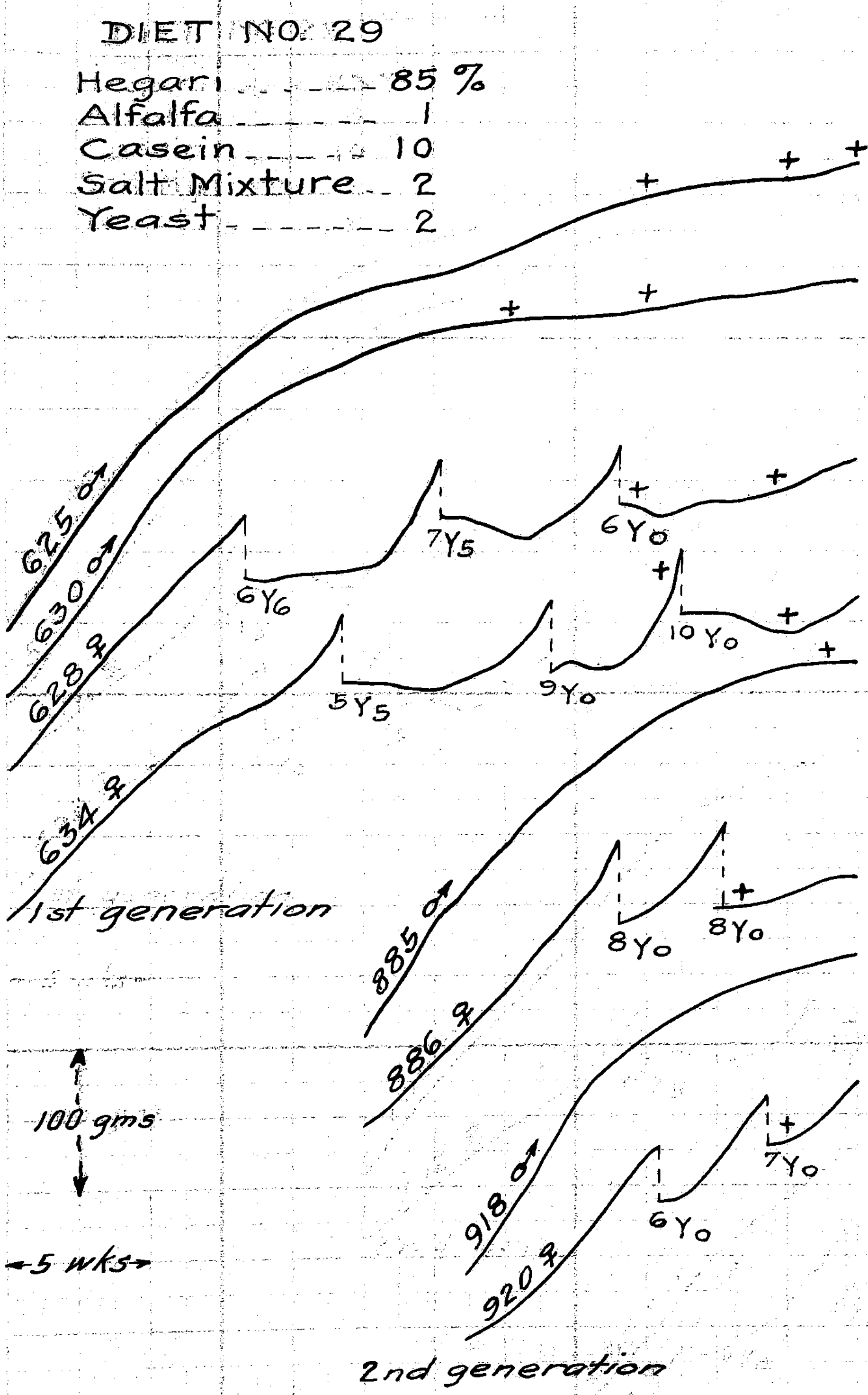
Record of rats receiving an adequate diet.

CHART NO. V



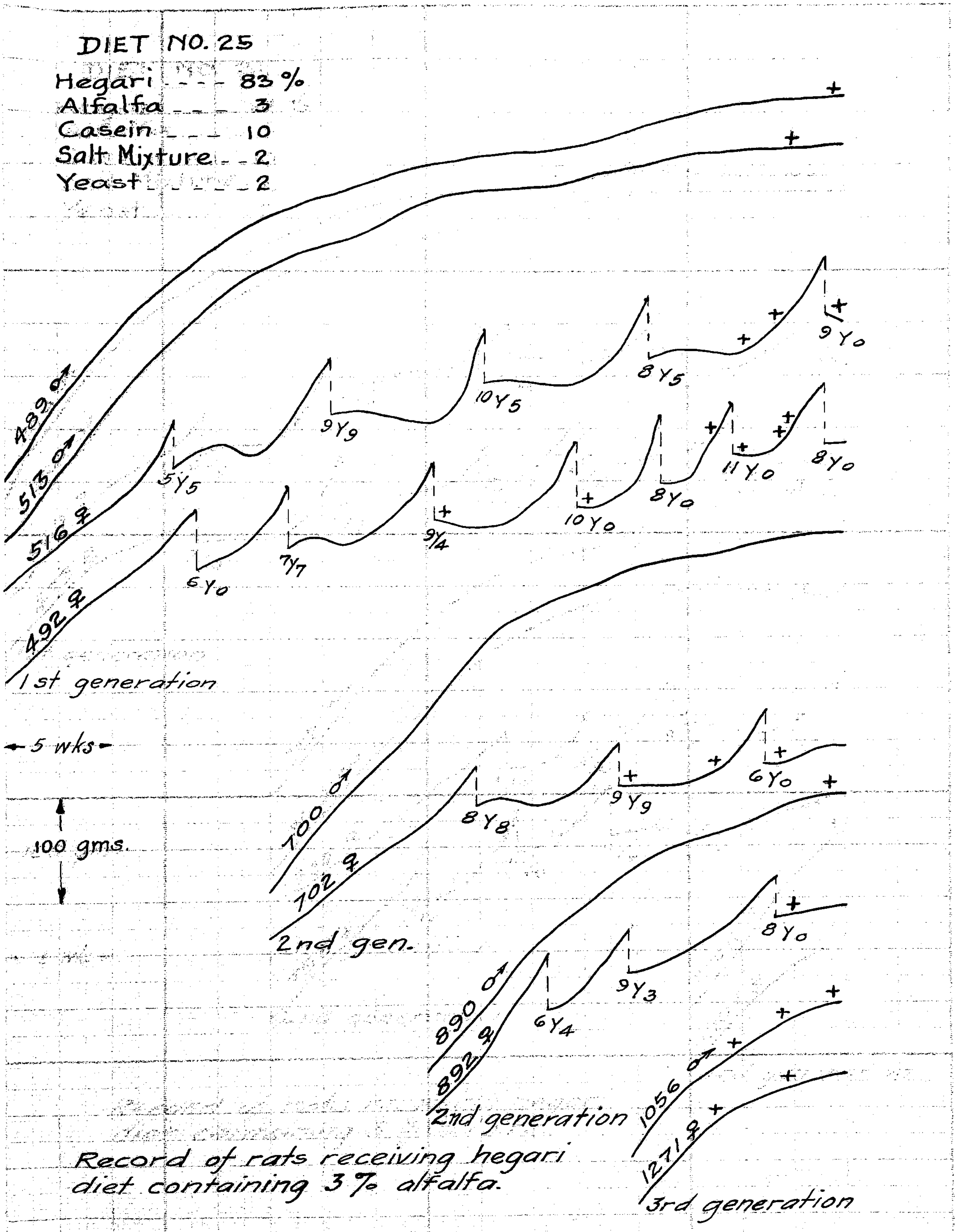
Record of rats receiving hegari diet containing no alfalfa.

CHART NO. VI



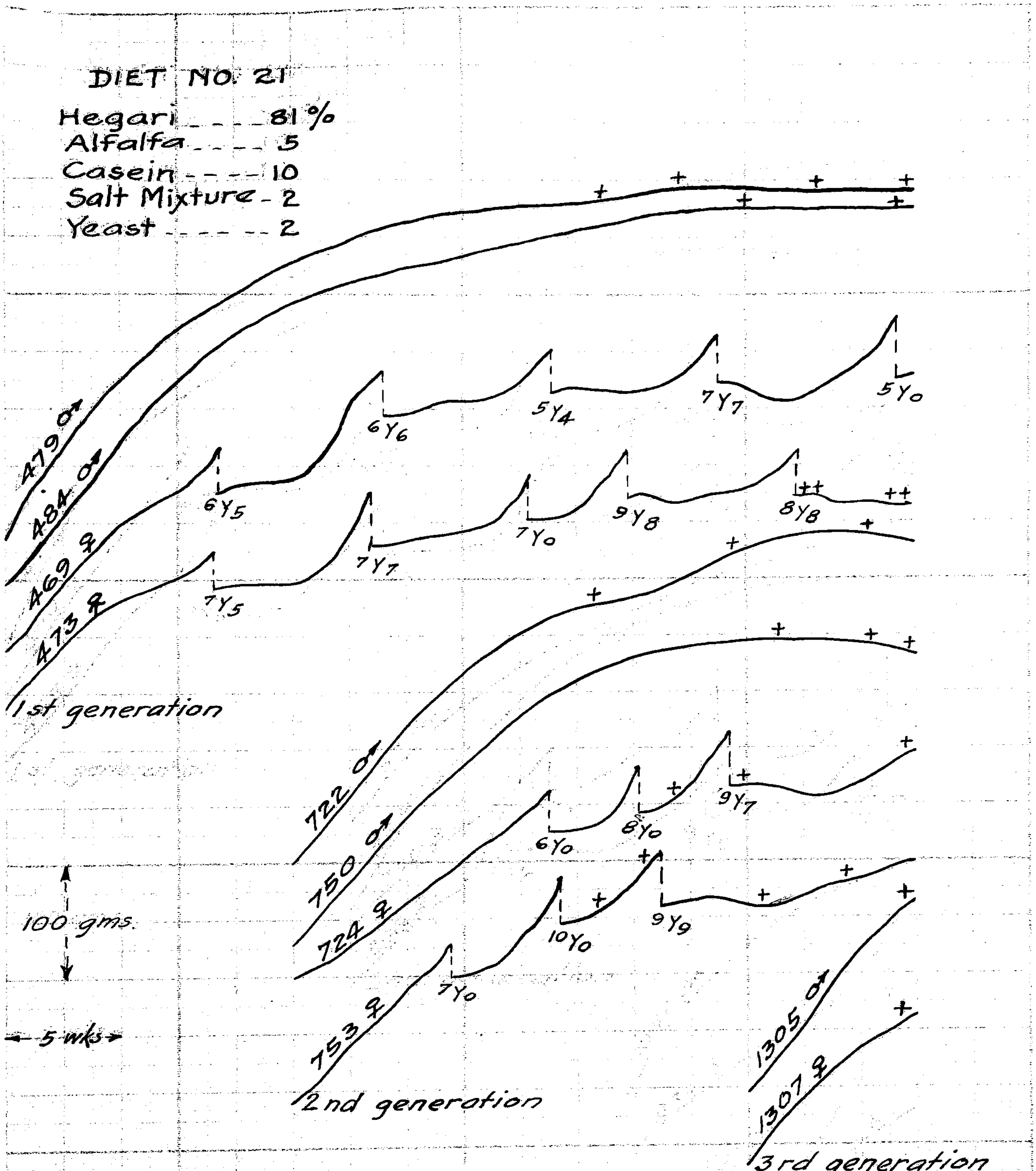
Record of rats receiving hegari diet containing 1% alfalfa.

CHART NO. VII



Record of rats receiving hegari diet containing 3% alfalfa.

CHART NO. VIII



Record of rats receiving hegari diet containing 5 % alfalfa.

CHART NO. IX

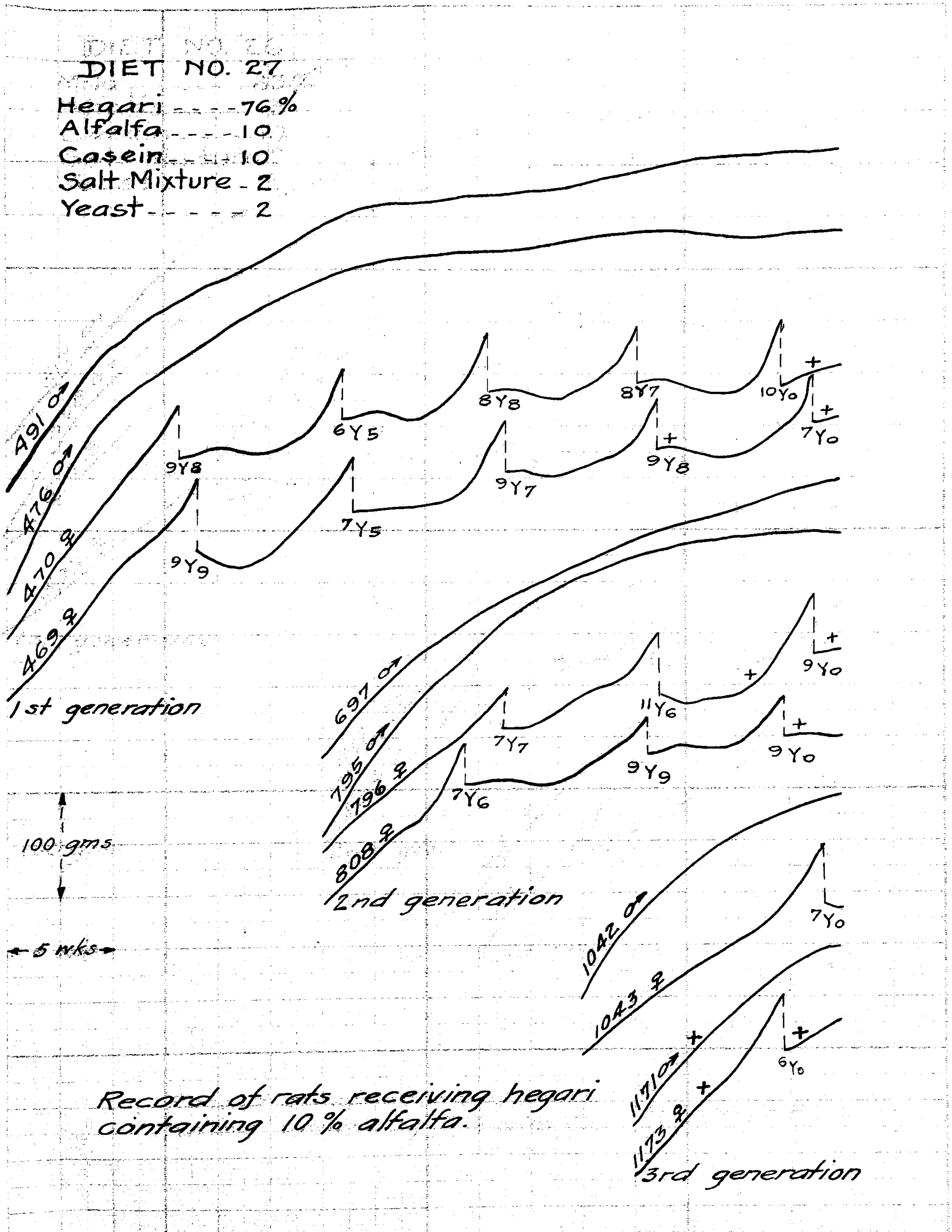

DIET NO. 27
Hegari - - - - 76%
Alfalfa - - - - 10
Casein - - - - 10
Salt Mixture - 2
Yeast - - - - 2
491 ♂
476 ♂
470 ♀
469 ♀
9Y8
6Y5
8Y8
8Y7
10Y0
7Y0
9Y9
7Y5
9Y7
9Y8
1st generation
697 ♂
795 ♂
796 ♀
808 ♀
7Y6
7Y7
11Y6
9Y9
9Y0
9Y0
2nd generation
100 gms
5 wks
1042 ♂
1043 ♀
1171 ♂
1173 ♀
7Y0
6Y0
3rd generation
Record of rats receiving hegari containing 10% alfalfa.

CHART NO. X

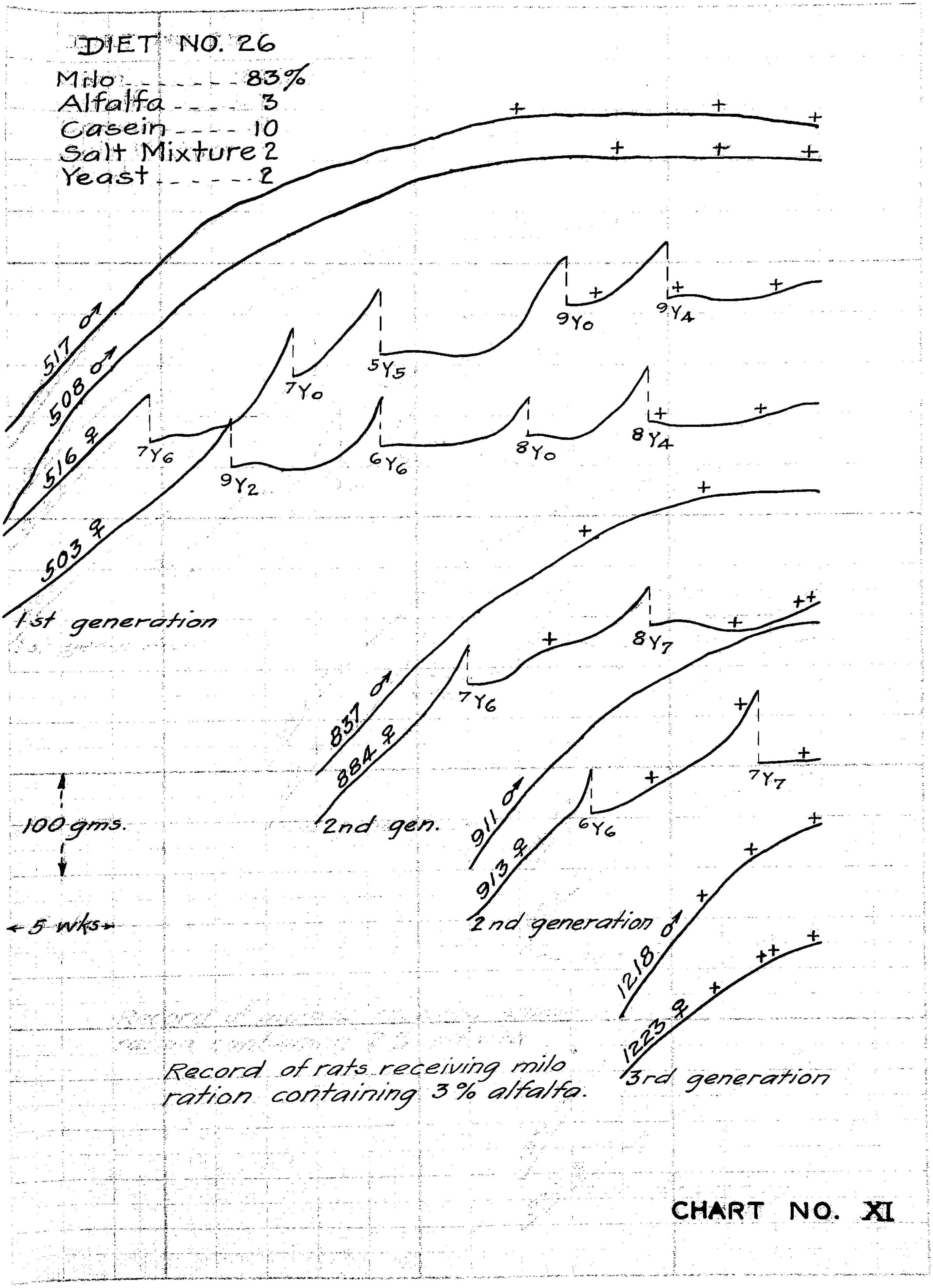

DIET NO. 26
Milo 83%
Alfalfa 3
Casein 10
Salt Mixture 2
Yeast 2
517 ♂
508 ♂
516 ♀
503 ♀
7Y6
7Y0
9Y2
5Y5
6Y6
9Y0
8Y0
9Y4
8Y4
1st generation
837 ♂
884 ♀
7Y6
8Y7
2nd gen.
911 ♂
913 ♀
6Y6
7Y7
2nd generation
1218 ♂
1223 ♀
3rd generation
100 gms.
5 wks
Record of rats receiving milo ration containing 3% alfalfa.

CHART NO. XI

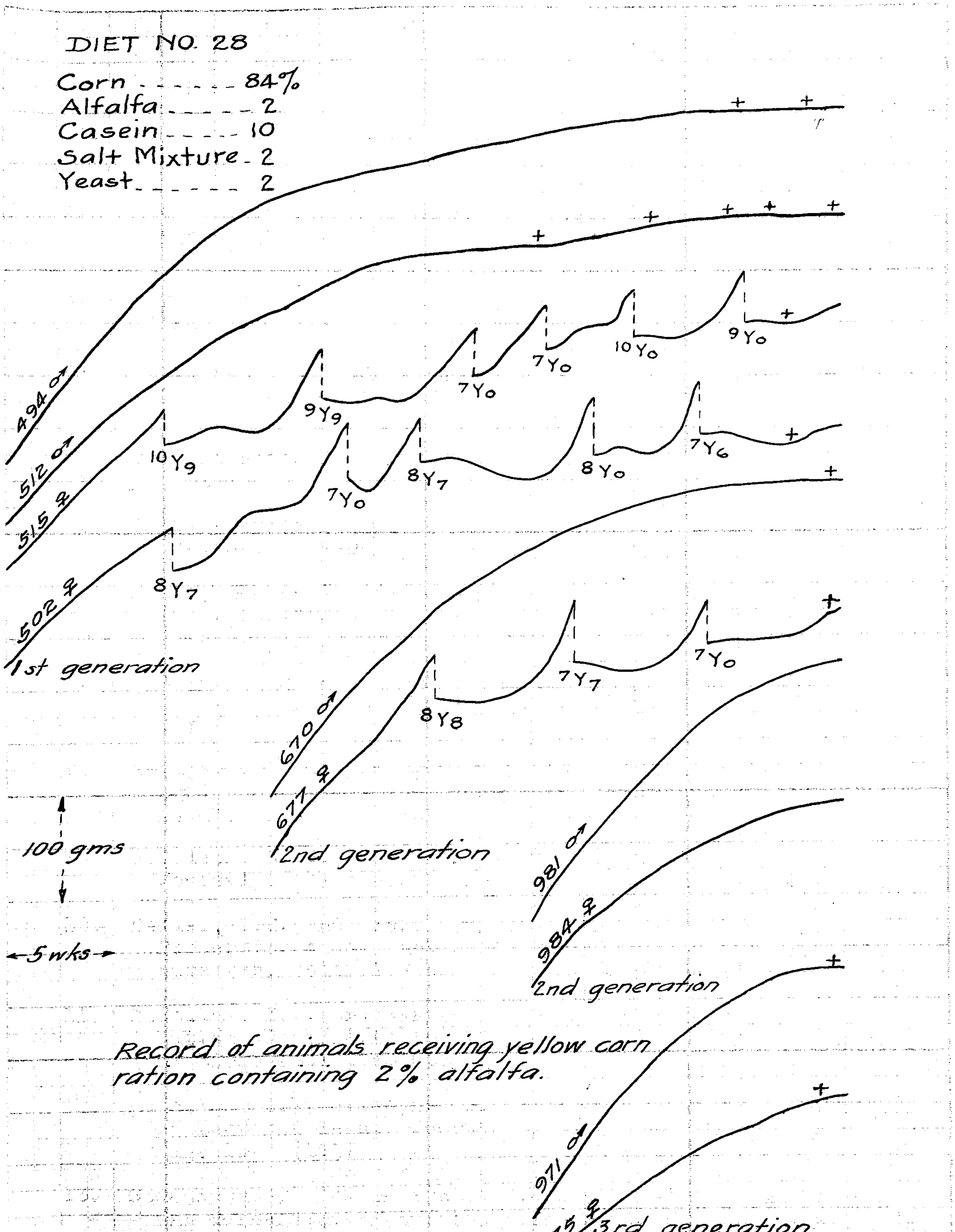

DIET NO. 28
Corn 84%
Alfalfa 2
Casein 10
Salt Mixture . 2
Yeast 2
494 ♂
512 ♂
515 ♀
502 ♀
1st generation
10 Y9
9 Y9
7 Y0
7 Y0
10 Y0
9 Y0
7 Y0
8 Y7
8 Y0
7 Y6
8 Y7
670 ♂
677 ♀
2nd generation
8 Y8
7 Y7
7 Y0
981 ♂
984 ♀
2nd generation
971 ♂
975 ♀
3rd generation
100 gms
5 wks
Record of animals receiving yellow corn ration containing 2% alfalfa.

CHART NO. XII

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