The effects of proteins from skim milk and other sources on tomatoes
by Frederick C Dawson

A THESIS Submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of Master of Science in Chemistry
Montana State University

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It is pointed out that phosphorus is more greatly concentrated in the actively growing top of the plant. Also pointed out is the accumulation of nitrate in greater quantities in the older portion of the plant, Nitrogen detected by the Kjeldahl determination is shown to be more highly concentrated in the growing tops.

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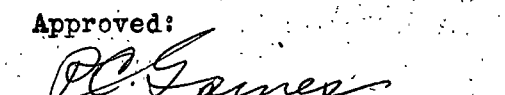

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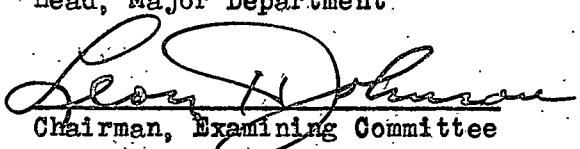

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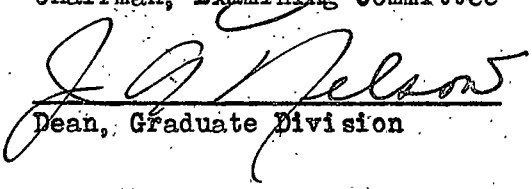

Dean, Graduate Division

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I. ABSTRACT

The response of Bonny Best tomatoes to soil application of skim milk and selected proteins has been studied. The studies were directed at obtaining information on plant growth and on the distribution in the plant of phosphorus, nitrate-nitrogen, and Kjeldahl nitrogen. The actively growing top of each plant was pinched off and subjected to chemical analysis separately from the remainder of the plant. Roots were not analyzed.

It is pointed out that phosphorus is more greatly concentrated in the actively growing top of the plant. Also pointed out is the accumulation of nitrate in greater quantities in the older portion of the plant. Nitrogen detected by the Kjeldahl determination is shown to be more highly concentrated in the growing tops.

II. INTRODUCTION

From a consideration of the nitrogen and phosphorus content of skim milk, it was decided to use skim milk as a fertilizing material for plants. It was also thought that small quantities of trace elements found in milk might be of some value. Thus, in 1946, the Department of Horticulture of the Montana Agricultural Experiment Station conducted exploratory experiments to determine the effect of soil application of skim milk upon the growth of Bonny Best tomato plants.

The preliminary experiments and subsequent experiments conducted by Dr. V.E. Iverson of the Agricultural Experiment Station, (4) (5) showed that milk treated tomato plants possessed larger and more fibrous root systems, larger and taller stems, and greater leaf areas. Studies on the fruit indicated increased early yields, and a more fleshy fruit with less seeds.

III. EXPERIMENTAL PROCEDURE

As a continuation of the previous work, the present work was set up to accomplish the following objectives:

1. To ascertain the component or components of milk which contributed to the accelerated growth.
2. To determine changes in chemical constitution of the treated plants
3. To investigate other protein materials which may give the above mentioned results.

To achieve these objectives, experiments were conducted as follows:

1. An exploratory experiment to determine proteins which give best

growth results as well as to establish technique.

2. A second experiment using milk and selected proteins from the first experiment.

The first experiment used a stock soil consisting of 25% sand, 25% well rotted manure, and 50% loam. The tomatoes used were the Bonny Best variety. Transplants were made on the twenty-ninth day of November, ten days after planting of the seeds. Transplants were made into four-inch pots, one plant per pot. Applications of selected foods were started the following day. Ten plants constituted the number of plants given a particular treatment. Treatments were the following: control, skim milk, nitrogen plus phosphorus, gelatin, casein, lactalbumin, egg albumin, powdered whole egg, wheat gluten, wheat starch, and lactose.

Treatments were administered to the soil at one week intervals over a period of five weeks. A total of 150 ml. of solution per plant was administered in increments of 10 ml. the first week, 20 ml. the second week, 30 ml. the third week, 40 ml. the fourth week, and 50 ml. the fifth week.

Analysis was performed on all materials used as treatments, to determine the nitrogen and phosphorus content. All solutions were then made up so that the nitrogen and phosphorus content of milk was equaled. Enough protein material was taken to equal either the nitrogen or phosphorus content of milk whichever concentration was attained first by the least amount of protein material with addition of either potassium phosphate (K_3PO_4) or ammonium nitrate to make all concentrations of nitrogen and phosphorus equal to that found in milk. In the case of both lactose

and wheat starch, since the extremely low content of nitrogen and phosphorus would necessitate such great quantities of solid material, 100 grams of the solid were used and both K_3PO_4 and NH_4NO_3 were added plus water to make 1500 ml. solution. The solution containing only nitrogen and phosphorus compounds were made up using $(NH_4)_2HPO_4$ and NH_4NO_3.

Table I, page 7, shows the weight of material taken for each treatment. Also shown on Table I is the amount of nitrogen and phosphorus containing compounds required to bring all concentrations of nitrogen and phosphorus up to that of milk.

Also included in the first experiment were thirty plants in a mixture of 50% peat and 50% vermiculite along with thirty plants in vermiculite alone. In each of these variations ten plants were used as controls, ten plants were given treatment of nitrogen plus phosphorus, and ten plants were given treatments of skim milk.

Plants growing in vermiculite and vermiculite-peat received, in addition to the above applications, a solution containing complete nutrients. (7) Applications of 50 ml. per plant were made every other day for the duration of growth of the plants.

Plants were harvested on January 15th, 1951. Thus they were allowed to grow a total of 48 days from date of first treatment. It was felt this was necessary to allow a greater differentiation in the growth between the various treatments.

Plants were removed from the pots, the soil washed from the root systems. The roots and tops were separately wrapped, and dried at 105 degrees centigrade to constant weight.

TABLE I

TABLE SHOWING WEIGHT OF MEDIA USED, THEIR NITROGEN AND PHOSPHORUS CONTENT, AND NITROGEN AND PHOSPHORUS COMPOUNDS ADDED TO MAKE CONCENTRATIONS OF NITROGEN AND PHOSPHORUS EQUAL TO THAT OF MILK

Media	% Nitrogen	% Phosphorus	Wt. of Media	K_3PO_4	NH_4NO_3	$(NH_4)_2HPO_4$
Skim Milk	0.64	0.10		0.00	0.00	0.00
Casein	14.33	0.74	69.033	7.199	0.00	0.00
Egg Albumin	11.92	0.09	82.991	10.206	0.00	0.00
Lactalbumin	14.92	1.60	64.343	8.225	0.00	0.00
Dried Egg	7.43	0.76	133.143	3.765	0.00	0.00
Gelatin	15.59	0.02	63.454	10.635	0.00	0.00
Gluten	13.10	0.16	75.511	9.852	0.00	0.00
Wheat Starch	1.40	0.05	100.000	0.00	19.671	6.183
Lactose	0.00	0.00	100.000	0.00	23.551	6.400
Nitrogen plus Phosphorus			0.00	0.00	23.551	6.400

*Nitrogen and Phosphorus content stated in percent. All other values are stated in grams and represent amounts of materials in 1500 ml. of solution. Specific Gravity=1.019

As a result of observations on plant size and dry weights, the following materials were selected to be used in the second experiment: skim milk, nitrogen plus phosphorus solution, gelatin, egg albumin, lactalbumin, casein, gluten, and wheat starch.

Plants were again transplanted ten days after planting of the seed. Transplants were made individually into four-inch pots. Tomatoes used were the Bonny Best variety. Treatment commenced the day following transplanting on the 25th of January, 1951. Treatment consisted of 10, 20, 30, 40, and 50 ml. portions administered at weekly intervals exactly as in the first experiment. Soil was of the same composition as was used previously with one exception. This exception being a 10% addition per pot of soil in which had previously been grown a tomato plant and which had been subjected to treatment with milk. In the remainder of this paper this soil will be referred to as inoculated soil.

Also included in this experiment were a series of control plants and milk treated plants grown in soil without this inoculation. Nine plants constituted a series which received a particular treatment.

On March 8, 1951, the plants were removed from the soil. In each series three plants were cut off even with the ground and subjected to pressure to extract the plant sap. The press used was a laboratory model Carver press. The remaining six plants of each series were handled in the manner here described. The roots were severed from the rest of the plant, wrapped in cheese cloth, dried, and weighed. The top of the plant was pinched off just below the top two branches. These two parts were wrapped separately, dried, and weighed together. Drying was carried

out at 105 degrees centigrade to constant weight.

In the remainder of this paper "tops" shall be construed to mean that portion of the plant which was pinched from the very uppermost part. "Bottoms" shall mean the remainder of the plant exclusive of the roots.

After complete drying and weighing, the plants were ground in a small laboratory Wiley mill to pass through a forty mesh screen.

Analyses were carried out on the plant sap, tops, and bottoms to determine the amount of phosphorus, nitrate-nitrogen, and Kjeldahl nitrogen.

The sap pressed from the tomato plants was prepared for analysis according to the following procedure. Twenty-five ml. of the sap were accurately pipetted into a 50 ml. centrifuge tube. To this was added 10 ml. of 3N trichloroacetic acid and the protein which precipitated was centrifuged out. The supernatent liquid was used for analysis. Aliquot size for each analysis was 0.1 ml.

The Kjeldahl determination is an adaptation of the Official Method of the A. O. A.C.(1) used by the Chemistry Experiment Station at Montana State College.

The alteration consisted of the use of approximately 12.5 grams of a catalytic mixture of K_2SO_4 and HgO in each digestion flask. This mixture contains 1000 grams K_2SO_4 and 50 grams HgO. Intimate mixing is obtained in a ball mill. A mixed indicator (6) is used in the titration. For this work 0.5 grams of bottoms and 0.1 grams tops were used as sample.

For the nitrate-nitrogen a colorimetric method was used. (3) The

procedure employed follows.

In the case of the bottoms, a 0.1 gram sample was placed in a 125 ml. erlenmyer flask. Twenty-five ml. of distilled water were accurately pipetted into the flask and approximately 0.5 grams of magnesium oxide added. The flask was securely stoppered and thoroughly shaken for a period of ten minutes. The contents were then filtered through number 2 Whatman filter paper. A 1.0 ml. aliquot was then taken for the analysis.

In the case of the tops, a 20 mg. sample was placed in a 50 ml. flask, 5 ml. water pipetted into the flask, approximately 0.1 grams MgO added and the flask stoppered and shaken as before. For the tops a 2 ml. aliquot was taken.

Analysis of the sap did not require the above procedure. Samples of 0.1 ml. of the sap were pipetted accurately and carried through the analysis as described below.

The aliquot was transferred to a 250 ml. flask with a 24/40 ground glass connection. Three or four glass beads, 15 ml. of 80% by weight sulphuric acid, and 1 ml. of a 2% in acetone solution of 3,4 Xylenol were added. These materials were agitated ten minutes, allowed to set twenty minutes, and then diluted with 115 ml. distilled water. The solution was then subjected to distillation until about 85 ml. of distillate was collected. The receiving flask was a 200 ml. volumetric flask which contained 10 ml. of a 2% NaOH solution. The flask was filled to volume with distilled water, mixed, and filtered through a number 2 Whatman paper into which had been placed a piece of cotton. The filtrate was collected in colorimeter tubes being careful to discard the first tubeful.

The color was read in a Coleman Spectrophotometer Model 11 at a wave length of 432 millimicrons. Distilled water was used as the blank. A standard curve, Figure 1, page 12, plotted on log paper was used to interpret the readings.

For the phosphorus analysis a colorimetric method (2) was again employed. A modification, adapted for use in the Montana State College Chemistry Experiment Station served for this work.

Samples which amounted to 0.1 grams of bottoms and 20 mg. of tops were placed in long necked 250 ml. florence flasks. Approximately 25 ml. of an ashing solution were added along with a few glass beads. The ashing solution was a mixture in the proportions of 1000 ml. distilled water, 1000 ml. concentrated nitric acid, 100 ml. sulphuric acid of specific gravity 1.84, and 150 ml. of 70-72% perchloric acid. The flask with contents was placed on a hot plate and boiled until the heavy fumes of perchloric acid had ceased to be evolved and fumes of SO_3 appeared. About 1 ml. of liquid remained in the flask. Contents of the flask were washed into a 100 ml. volumetric flask. The flask was then filled to the mark.

Two analyses were run on the plant sap. The first analysis was carried out using all steps described above. This is referred to as "Ashed" on Table III. The second analysis omitted the preceding steps and is referred to on Table III as "Non-ashed". Both analyses were subjected to the operations described below.

Aliquots which equaled 5 ml. for the bottoms, 20 ml. for tops, and 0.1 ml. of sap were introduced into a 25 ml. graduated test tube. To this sample was added a 2 ml. of 10N sulphuric acid, 2 ml. of amidol solution

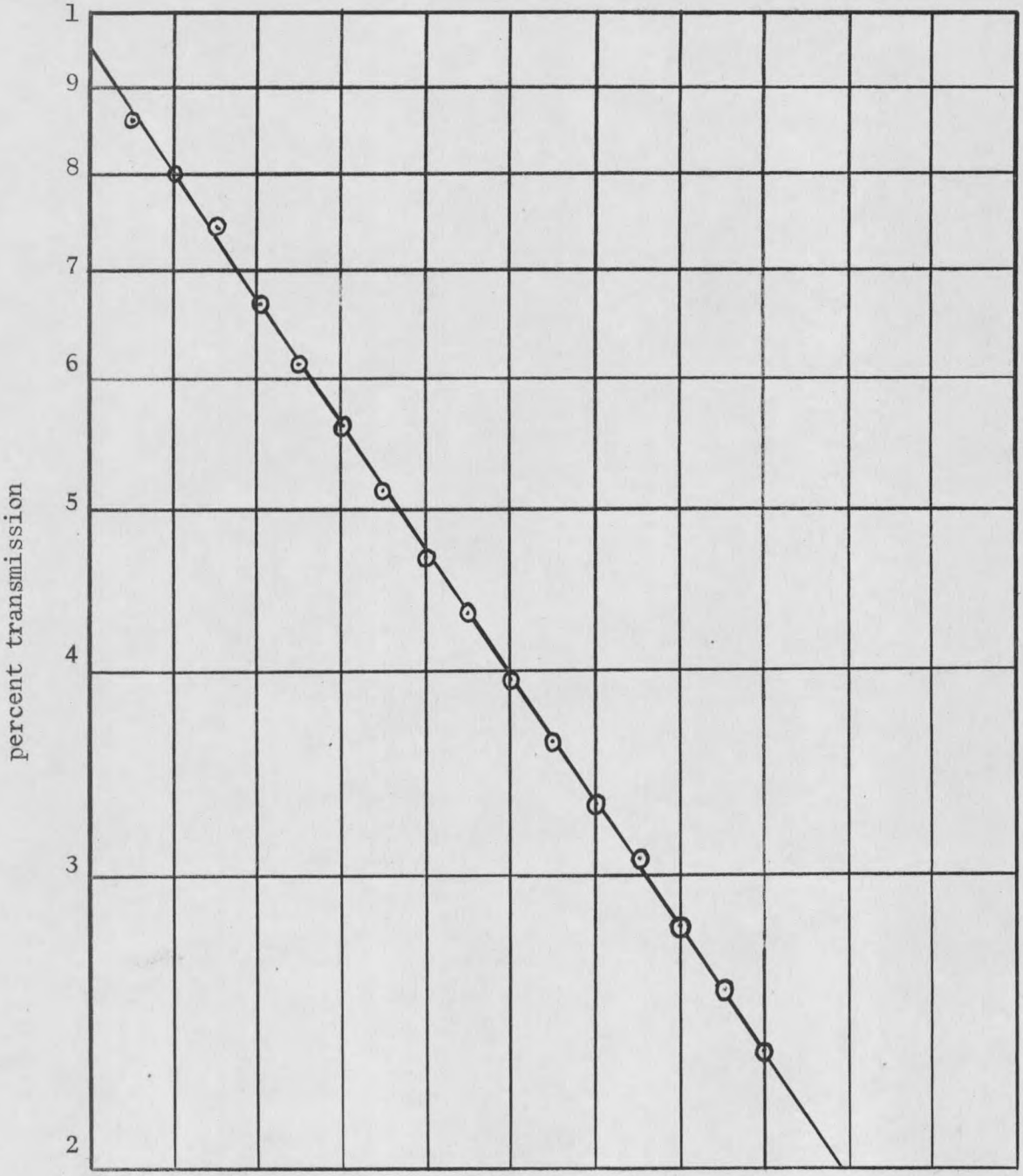

Figure 1

STANDARD CURVE FOR NITRATE-NITROGEN
concentration NO_3-N/200 ml.
in micrograms

and 1 ml. of 8.3% ammonium molybdate in that order. Distilled water was then added to the 25 ml. mark and the solution transferred to colorimeter tubes. Mixing of the solution was accomplished by pouring back and forth several times. A period of one-half hour was allowed to elapse between addition of the molybdate solution and reading in the spectrophotometer. Again the Coleman Model 11 was used with readings taken at wave length of 650 millimicrons. A standard curve, Figure 2, page 14, plotted on log paper was used to interpret the readings.

The amidol solution consisted of 117 grams of potassium meta bisulphite and 5 grams of 2,4 diamino phenol dihydrochloride diluted to 500 ml. It is necessary to filter this solution before use. If kept under refrigeration it is of value for a period of two weeks.

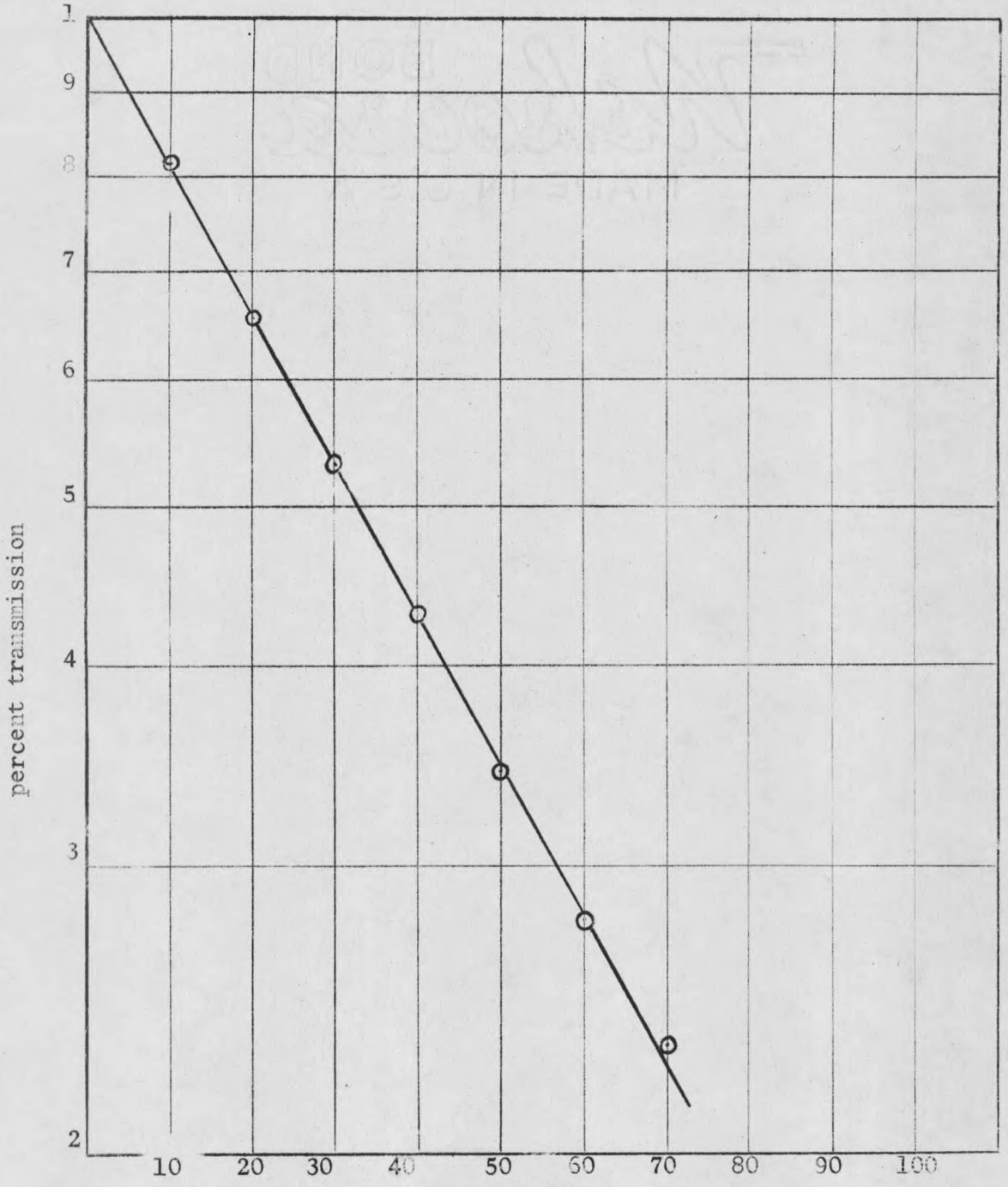

Figure 2

STANDARD CURVE FOR PHOSPHORUS

concentration per aliquot
in micrograms

IV. EXPERIMENTAL RESULTS

All results set forth in the tables and graphs which follow were obtained from the second planting.

The results obtained from weighing the roots and upper part of the individual tomato plants are set forth in Table II, page 16. Average weights of each treatment are illustrated by Figure 3, page 21 .

The results of analysis of the plant sap for nitrate-nitrogen and phosphorus are shown on Tables III and IV, page 17. Figures 4 and 5, pages 22 and 23 , are graphs of the average values for each treatment. Since the ashed and non-ashed phosphorus values show no significant difference, no distinction is made in Figure 5 between these two analyses.

The analysis for nitrate nitrogen gives values shown by Table V. The average values for each treatment is represented by Figure 6.

The results obtained in the phosphorus analysis are set forth in Table VI and Figure 7 in the same manner as described above.

Table VII and Figure 8 give results obtained by Kjeldahl analysis.

TABLE II

TABLE OF DRY WEIGHTS OF ROOTS AND TOPS*

ROOTS

Treatment	Plant Number 1	2	3	4	5	6	Average
Non-inoculated Control	0.63	0.70	0.63	0.47	0.47	0.65	0.59
Inoculated Control	0.15**	0.54	0.39	0.59	0.45	0.55	0.50
Non-inoculated Milk	0.82	0.44	1.09	0.73	0.53	0.69	0.72
Inoculated Milk	0.78	0.90	0.83	1.09	0.90	0.84	0.89
Nitrogen plus Phos.	0.60	0.52	0.35	0.39	0.32	0.99	0.53
Gelatin	0.50	0.61	0.41	0.41	0.65	0.39	0.50
Wheat Starch	0.50	0.40	0.66	0.65	0.52	0.28	0.50
Egg Albumin	0.46	0.44	0.71	0.49	0.48	0.48	0.51
Lactalbumin	0.77	0.52	0.42	0.45	0.54	0.47	0.53
Casein	0.73	0.54	0.46	0.47	0.86	0.75	0.64
Gluten	0.67	0.56	0.44	0.42	0.40	0.77	0.54

TOPS

Non-inoculated Control	1.63	2.06	2.10	1.33	1.11	2.51	1.79
Inoculated Control	0.65**	0.92	1.41	2.27	1.95	2.12	1.55
Non-inoculated Milk	3.57	3.86	3.98	2.97	2.79	3.21	3.40
Inoculated Milk	2.77	3.15	2.62	3.94	3.40	3.13	3.17
Nitrogen plus Phos.	4.44	2.38	1.90	1.91	1.84	4.32	2.80
Gelatin	3.01	3.22	2.70	2.78	3.61	2.83	3.03
Wheat Starch	2.52	3.84	3.39	2.97	4.36	1.38	3.08
Egg Albumin	3.00	2.80	3.76	3.01	2.87	3.56	3.17
Lactalbumin	3.60	3.06	2.55	2.43	2.73	3.34	2.95
Casein	3.53	3.29	3.11	2.87	4.00	4.00	3.47
Gluten	3.28	3.05	2.97	2.54	2.55	3.49	2.96

* All values are stated in grams
**omitted from average

TABLE III.

TABLE OF NITRATE NITROGEN ON PLANT JUICE

Treatment	Percent
Non-inoculated Control	0.062
Inoculated Control	0.066
Non-inoculated Milk	0.147
Inoculated Milk	0.148
Nitrogen plus Phosphorus	0.165
Gelatin	0.171
Wheat Starch	0.140
Egg Albumin	0.133
Lactalbumin	0.146
Casein	0.160
Gluten	0.168

TABLE IV.

TABLE OF PHOSPHORUS ON PLANT JUICE*

Treatment	Non-ashed	Ashed
Non-inoculated Control	0.031	0.028
Inoculated Control	0.022	0.019
Non-inoculated Milk	0.027	0.026
Inoculated Milk	0.027	0.025
Nitrogen plus Phosphorus	0.042	0.043
Gelatin	0.035	0.034
Wheat Starch	0.045	0.045
Egg Albumin	0.025	0.022
Lactalbumin	0.023	0.021
Casein	0.031	0.030
Gluten	0.035	0.037

*All values are stated as percent.

TABLE V

TABLE OF NITRATE NITROGEN ON GROWING TOPS AND ON BOTTOMS*

TOPS

Treatment	Plant Number 1	2	3	4	5	6	Average
Non-inoculated Control	0.250	0.750	0.450	0.525	0.120	0.375	0.412
Inoculated Control	1.075	0.425	0.600	0.525	0.300	0.325	0.542
Non-inoculated Milk	0.700	0.500	0.750	0.938	0.600	0.675	0.694
Inoculated Milk	1.063	0.713	0.788	0.475	0.838	0.925	0.800
Nitrogen plus Phos.	0.825	1.025	0.875	0.975	0.475	0.838	0.835
Gelatin	0.413	0.663	0.663	0.813	0.613	0.587	0.625
Wheat Starch	0.625	0.938	0.875	0.838	0.762	0.975	0.835
Egg Albumin	0.900	0.613	0.850	1.088	0.700	0.838	0.831
Lactalbumin	0.438	0.688	0.375	0.525	0.538	0.638	0.533
Casein	1.450	1.000	1.150	1.250	0.700	0.100	1.108
Gluten	1.250	0.400	0.550	0.800	0.900	0.650	0.758

BOTTOMS

Non-inoculated Control	0.338	0.575	0.795	0.425	0.675	0.575	0.564
Inoculated Control	2.325	0.350	0.850	0.775	0.463	0.688	0.908
Non-inoculated Milk	1.850	1.950	1.275	1.850	1.875	1.775	1.763
Inoculated Milk	2.300	2.000	1.700	1.675	1.575	2.050	1.883
Nitrogen plus Phos.	2.175	1.800	2.000	2.575	2.300	1.600	2.075
Gelatin	2.950	2.600	2.525	2.425	1.875	2.075	2.408
Wheat Starch	1.950	1.500	1.875	1.950	1.825	1.700	1.800
Egg Albumin	2.075	1.950	1.725	1.900	2.175	2.375	2.033
Lactalbumin	1.650	2.300	2.100	2.325	2.050	2.000	2.071
Casein	2.050	2.200	2.425	2.300	2.225	2.175	2.229
Gluten	2.500	2.450	2.375	2.800	2.750	1.925	2.467

*All values are stated in percent.

TABLE VI

TABLE OF PHOSPHORUS ON GROWING TOPS AND ON BOTTOMS*

TOPS

Treatment	Plant Number 1	2	3	4	5	6	Average
Non-inoculated Control	0.705	0.700	0.748	0.735	0.778	0.750	0.736
Inoculated Control	0.688	0.850	0.825	0.675	0.800	0.820	0.776
Non-inoculated Milk	0.830	0.848	0.925	0.908	0.885	0.808	0.867
Inoculated Milk	0.853	0.835	0.803	0.855	0.855	0.783	0.830
Nitrogen plus Phos.	1.290	1.225	1.178	1.200	1.175	1.083	1.192
Gelatin	0.855	0.863	0.833	0.775	0.873	0.968	0.861
Wheat Starch	0.865	1.143	1.053	0.925	1.270	1.175	1.072
Egg Albumin	0.855	0.963	0.965	1.003	0.873	0.873	0.922
Lactalbumin	0.798	0.830	0.753	0.773	0.830	0.820	0.800
Casein	1.045	0.993	0.908	1.025	0.968	0.963	0.983
Gluten	0.925	0.943	0.980	1.015	0.955	0.968	0.964

BOTTOMS

Non-inoculated Control	0.685	0.590	0.565	0.603	0.825	0.530	0.633
Inoculated Control	0.640	0.655	0.568	0.403	0.473	0.530	0.545
Non-inoculated Milk	0.622	0.696	0.634	0.670	0.738	0.742	0.684
Inoculated Milk	0.616	0.682	0.688	0.684	0.670	0.692	0.672
Nitrogen plus Phos.	1.060	1.102	1.136	1.114	0.940	1.100	1.090
Gelatin	0.794	0.842	0.768	0.698	0.794	0.778	0.779
Wheat Starch	0.778	0.994	0.944	0.802	1.094	1.018	0.938
Egg Albumin	0.658	0.728	0.686	0.686	0.728	0.658	0.691
Lactalbumin	0.660	0.696	0.676	0.686	0.664	0.640	0.670
Casein	0.774	0.798	0.702	0.794	0.746	0.730	0.757
Gluten	0.768	0.752	0.778	0.790	0.802	0.728	0.770

* All valuesare stated in percent.

TABLE VII

TABLE OF KJELDAHL NITROGEN ON GROWING TOPS AND ON BOTTOMS*

TOPS

Treatment	Plant Number 1	2	3	4	5	6	Average
Non-inoculated Control	4.573	5.254	4.791	3.853	4.245	4.833	4.591
Inoculated Control	4.974	3.993	5.240	5.366	5.128	5.254	4.993
Non-inoculated Milk	5.534	5.814	5.814	5.674	5.744	5.534	5.686
Inoculated Milk	5.632	5.814	5.562	5.968	5.184	5.660	5.637
Nitrogen plus Phos.		6.476		6.660	6.304	6.304	6.436
Gelatin	6.094	5.604	5.940	5.856	5.674	6.234	5.900
Wheat Starch	5.954	5.562	5.534	5.814	5.884	5.783	5.755
Egg Albumin	5.548	5.786	5.508	5.968	5.548	5.730	5.681
Lactalbumin	5.814	5.814	5.688	5.534	5.814	5.814	5.871
Casein	5.800	5.352	5.674	6.066	5.702	5.674	5.711
Gluten	5.982	5.954	5.814	6.080	6.122	5.814	5.961

BOTTOMS

Non-inoculated Control	2.870	3.122	3.178	2.803	2.195	2.232	2.733
Inoculated Control	3.362	2.073	3.080	3.287	3.136	3.315	3.042
Non-inoculated Milk	3.819	3.766	4.046	3.830	3.794	4.032	3.878
Inoculated Milk	3.744	3.816	4.012	3.990	3.556	3.598	3.786
Nitrogen plus Phos.	4.858	4.830	5.001	5.194	4.662	4.852	4.900
Gelatin	4.231	4.217	4.130	4.158	4.155	4.354	4.208
Wheat Starch	4.158	4.715	3.990	4.438	4.441	4.886	4.438
Egg Albumin	3.968	3.879	3.786	4.040	3.990	3.872	3.992
Lactalbumin	4.054	3.962	3.940	3.990	3.996	4.186	4.021
Casein	4.371	4.066	4.382	4.676	4.214	4.292	4.334
Gluten	4.357	4.326	4.298	4.284	4.351	4.466	4.347

*All values are stated in percent.

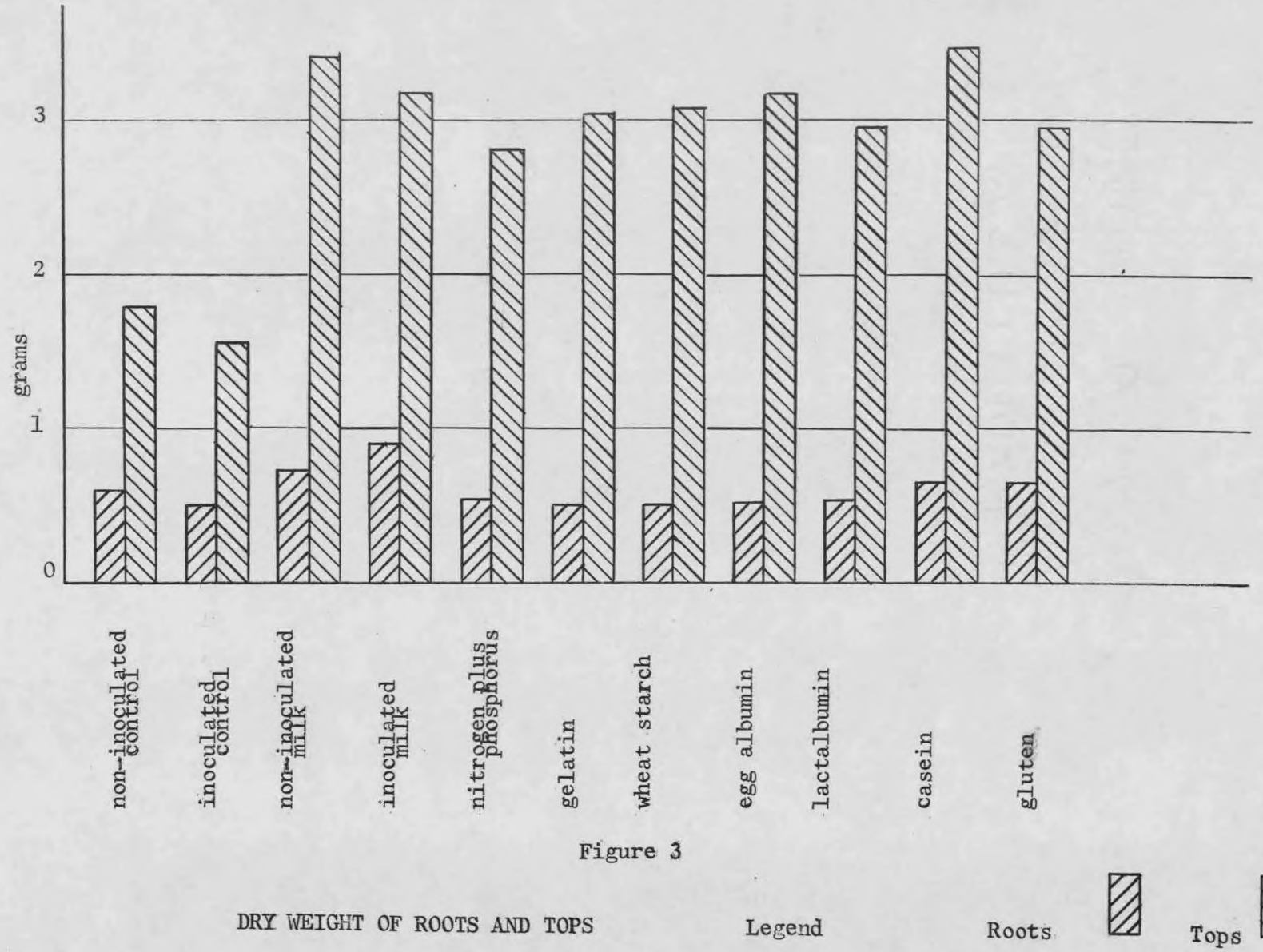

Figure 3

DRY WEIGHT OF ROOTS AND TOPS

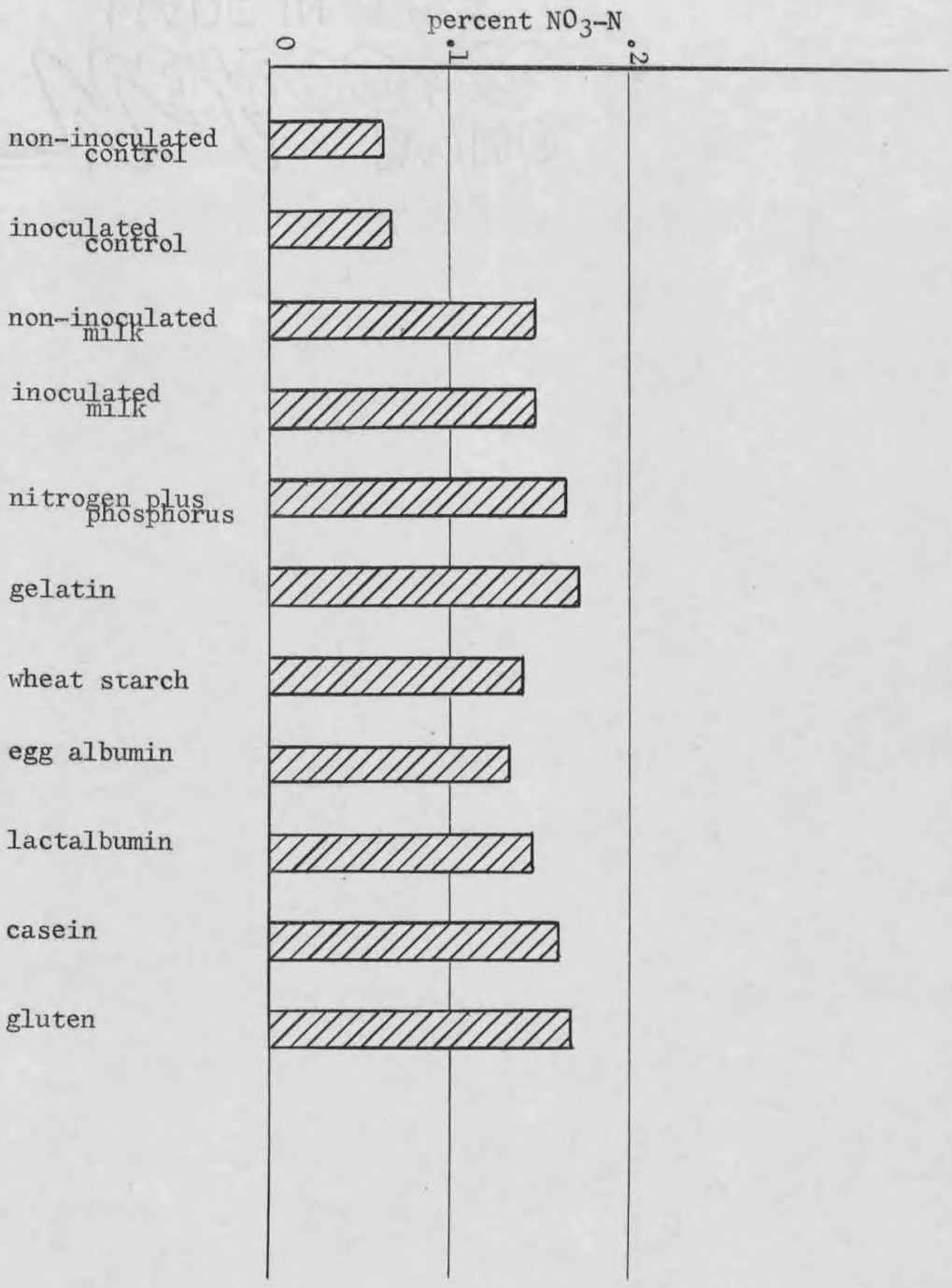

Figure 4

GRAPH SHOWING CONCENTRATION OF NITRATE-NITROGEN IN SAP