
THE EFFECT OF CALCIUM ON THE B VITAMIN AND INORGANIC CONTENT OF OATS

DAVID G. BOSTWICK* and THOMAS A. McCOY,
The Samuel Roberts Noble Foundation, Ardmore

There is no question that climatic conditions influence the vitamin content of plants more than any other environmental factor. However, the effect of the inorganic nutrients available to the plant is often appreciable and several recent articles have stimulated interest in relating the health of animal and man to the fertility of soil (1, 2, 4, 11). While these articles present evidence which suggests that such a relation exists, the intimate relations of inorganic nutrition to the nutrient content of plants are not yet fully understood. Watson and Noggle (13) studied the effects of various deficiencies on the riboflavin and ascorbic acid content of oats and Lyon, *et al* (6, 7) conducted some work on the effects of trace element deficiencies and toxicities with reference to some of the B vitamins. In a previous paper (8), the six major elements were studied with respect to niacin, carotene, and chlorophyll content in oats. However, it covered only a survey of general effects on the nutrient

*Deceased, November 19, 1950.

content of the plant. The present paper deals with the effect of increased and reduced amounts of calcium on the B vitamin and inorganic content of oats.

EXPERIMENTAL PROCEDURE

On November 2, 1948, Wintok oats No. CI 3424 were germinated in flats containing pure quartz sands, which had been previously leached with hydrochloric acid and washed with distilled water. When the seedlings were eleven days old, they were transplanted into pure quartz sand in 4-inch glazed soil jars and the treatments were watered with their respective nutrient solutions. The composition of the different nutrient solutions used are listed in Table I.

TABLE I

*Composition of the Nutrient Solutions**

TREATMENTS	0.1 CA	0.2 CA	0.5 CA	1.0 CA	1.5 CA
Ca (NO ₃) ₂	.5	1.0	2.5	5.0	5.0
CaCl ₂	0	0	0	0	2.5
NaCl	10.0	4.5	8.77	7.5	3.75
Na NO ₃	9.0	8.0	5.0	0	0
K NO ₃	5.0	5.0	5.0	5.0	5.0
K H ₂ PO ₄	1.0	1.0	1.0	1.0	1.0
Mg SO ₄	2.0	2.0	2.0	2.0	2.0

*Concentration of salts are reported in millimoles per liter.

The calcium concentration varied in the following manner: 0.1, 0.2, 0.5, and 1.5 times that of the control solution. The control solution was the one described as Solution 1, Method B, of Hoagland and Arnon (5). The methods of raising the plants, harvesting the plants, preparation and methods of analysis for the inorganic ions and niacin are the same as those used in earlier work (8).

During the first three weeks of the growing period, the 0.1 calcium oats showed slight calcium deficiency symptoms. As the growth continued, the typical deficiencies other than the twisting of leaves and the stunted appearance of the plant were not evident. On January 22, 1949, the oats were harvested for both inorganic analysis and vitamin analysis. The plants used for vitamin analysis were prepared in the following manner: The plants were homogenized in a Waring Blendor with sodium acetate-acetic acid buffer (pH 4.5). For the hydrolysis they were transferred to a 250 ml. beaker containing the enzyme suspension and covered with toluene. The enzyme suspension used was the same as the one described for the release of all B vitamins (3) with the addition of hog kidney conjugase. The conjugase was introduced to hydrolyze the pteroylglutamic acid conjugates. After the hydrolysis (37° C. for 24 hours), the samples were steamed for twenty minutes in the autoclave to inactivate the enzymes and remove the toluene. Afterwards, the samples were filtered and washed. The filtrates were made up to a volume of one liter and aliquots were stored in the refrigerator under toluene until used.

Thiamine was assayed by the yeast fermentation method of Schultz, Atkin, and Frey (12). Pteroylglutamic acid and vitamin B₆ analyses were made using the method of Rabinowitz and Snell (10) and riboflavin, biotin, and calcium pantothenate were assayed by McCoy and Snyder's method (9).

RESULTS AND DISCUSSION

The results of the analyses of the oat plants are summarized in Table II. The percentage of calcium in the plant tissues follows the same trend as the nutrient solution but not in the same ratio. It appears from this study that calcium does not significantly affect the absorption of phosphorus, potassium, and magnesium.

TABLE II

TREATMENTS	Composition of Oats					T _{0.5S-x}	N
	0.1 CA	0.2 CA	0.5 CA	1.0 CA	1.5 CA		
	ELEMENTS % (DRY WEIGHT)						
Ca	.87	.87	1.04	1.46	2.46	.37	40
K	5.76	5.54	5.50	5.65	5.63	.22	35
Mg	.48	.50	.42	.52	.49	.05	40
Na	.94	.94	.63	.42	.11	.06	38
P	.40	.40	.33	.41	.35	.04	40
N	4.01	3.88	3.89	3.71	3.82	.11	43
	VITAMINS (γ/GM FRESH WEIGHT)						
Thiamine HCl	1.25	1.26	1.34	1.42	1.63	.07	43
Pteroylglutamic acid	.23	.18	.19	.10	.15	.03	40
Riboflavin	6.59	5.63	4.76	4.70	10.97	.49	40
Ca Pantothenate	3.49	3.55	3.98	3.98	4.00	.27	38
Niacin	4.28	4.58	4.42	4.37	4.56	.21	40
Biotin	.054	.053	.054	.059	.058	.004	44
Vitamin B ₆	2.31	1.75	2.99	2.23	2.46	.13	35

The nitrogen concentration of the plant material decreases with increased calcium content up to the control but the 1.5 calcium treatment contains approximately the same amount of nitrogen as the 0.2 and 0.5 treatments. This fact should be remembered when considering the vitamin content of the plants.

The thiamine concentration of the plant material increases directly with the calcium content of the oats, while the pteroylglutamic acid content follows an opposite tendency. With the exception of the 1.5 calcium treatment, it appears that the riboflavin content is reduced as the calcium content increases. The 1.5 calcium treatment contains a significantly larger amount of riboflavin than any of the other groups. The niacin and biotin content does not appear to be particularly affected by the different treatments. The calcium pantothenate concentration appears to increase with increasing amounts of calcium with one exception that being the 0.2 calcium treatment. The vitamin B₆ content of the tissues is very low in the 0.2 calcium treatment and exceptionally high in the 0.5 calcium treatment. There appears to be little difference between the remaining treatments. Considering the over-all effect of the calcium, it appears that increasing amounts of calcium will increase the amount of calcium pantothenate and thiamine and decrease the riboflavin and pteroylglutamic acid content. The remaining vitamins, niacin, biotin, and vitamin B₆ are apparently not affected.

SUMMARY

Oat plants were grown in sand culture with five different concentrations of calcium in the nutrient solutions. When the plants were twelve weeks old, they were harvested and analyzed for calcium, magnesium, potassium, sodium, phosphorus, nitrogen, and all of the B vitamins except para-amino-benzoic acid. Decreasing concentrations of calcium in the nutrient solutions resulted in corresponding decreases of calcium in the plant tissue. The reduction of calcium in the nutrient solution resulted in an increase in the concentration of nitrogen. It appears that riboflavin and pteroylglutamic acid have an inverse relation to the calcium in the nutrient solution and that thiamine and calcium pantothenate have a direct relation to the calcium concentration. An increase in calcium above the controlled treatment resulted in a significant increase in riboflavin and thiamine.

The authors would like to express their appreciation to Mr. Henry Kathrein, Mr. Joseph Q. Snyder, Mr. Spencer Michael Free, Jr., Mr. Brown Scott, Mr. Bobbie Thompson and Mr. Ruble Langston who assisted in much of the routine work involved in this experiment.

REFERENCES

1. ALBRECHT, W. A. 1943. Soil fertility and the human species, Chem. Eng. News 21: 221-227.
 2. ———. 1948. Climate, soil, and health. I. Oral Surg., Oral Med., and Oral Path. 1: 199-214.
 3. ASSOCIATION OF VITAMIN CHEMISTS, INC. 1947. Methods of vitamin assay. New York: Interscience Publishers, Inc.
 4. GILBERT, F. A. 1948. Mineral nutrition of plants and animals. Norman, Oklahoma: Univ. of Oklahoma Press.
 5. HOAGLAND, D. R. and D. I. ARNON. 1938. The water-culture method of growing plants without soil. Calif. Exp. Sta. Cir. No. 347.
 6. LYON, C. B., K. C. BRISON, and G. H. ELLIS. 1943. Effects of micro-nutrient deficiencies on the growth and vitamins content of the tomato. Botan. Gaz. 104: 495-514.
 7. ——— and ———. 1948. Influence of toxic concentrations of micro-nutrient elements in the nutrient medium on vitamin content of turnips and tomatoes. Botan. Gaz. 109: 506-520.
 8. MCCOY, T. A., S. M. FREE, JR., R. G. LANGSTON, and J. Q. SNYDER. 1949. Effect of major elements on the niacin, carotene, and inorganic content of young oats. Soil Sci. 68: 375-380.
 9. ———, and J. Q. SNYDER. 1950. The use of *Streptococcus faecalis*, A.T.C.C. No. 6057, as a test organism in microbiological assay. Proc. Oklahoma Acad. Sci. 31: 100-104.
 10. RABINOWITZ, J. C. and E. E. SNELL. 1947. The Vitamin B₆ Group XI. An improved method for assay of Vitamin B₆ with *Streptococcus faecalis*. J. Biol. Chem. 169: 631-642.
 11. ROUNTREE, L. G. 1941. The health of registrants and the President's plan of rehabilitation, Science 94: 552-553.
 12. SCHULTZ, A. S., L. ATKIN, and C. N. FREY. 1942. Determination of vitamin B₆ by yeast fermentation method. Ind. Eng. Chem., Anal. Ed. 14: 35-39.
 13. WATSON, S. A. and G. R. NOGGLE. 1947. Effect of mineral deficiencies upon the synthesis of riboflavin and ascorbic acid by the oat plant. Plant Physiol. 22: 228-243.
-