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BIOSYNTHESIS OF AMINO ACIDS ACCORDING TO SOIL FERTILITY

III. BIOASSAYS OF FORAGE AND GRAIN FERTILIZED WITH "TRACE" ELEMENTS *)

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That fertilization of the soil brings about improvement in the nutritional values of feed as measured by animal growths, was reported by Albrecht and Smith [1] [8]; McLean [3]; and Kendall, Nevens and Overman [2]. When the problem of nutrition points to the proteins, not only in totals measured by nitrogen but also in the proper balance of their constitutent essential amino acids, then, the synthesis of these nitrogenous compounds by plants (and microbes) for higher nutritional values in forages and grains for animals may well be considered as a responsibility of proper soil management. That the concentrations of various amino acids in plants are influenced by the different inorganic nutrient elements and the ratios of their concentrations in the medium in which the plants are grown was shown by Sheldon, Blue, and Albrecht [5] [6] [7]. The work reported here was another attempt to study this relation of the amino acid array in a forage (alfalfa, or lucerne) and in a grain (corn or maize) to the soil treated with "trace" as well as major elements of fertility values. It was an attempt, also to make bioassays of the forage and the grain by means of rabbits with even amino acid- and other nutritional-supplements in the rations for these test animals.

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EXPERIMENTAL METHODS

The forage crop of alfalfa (lucerne) was grown on a residual limestone soil (Newtonia silt loam) in Southwest Missouri, (U.S.A.), given four soil treatments. These were: (1) magnesium sulfate (100 pounds/acre) plus a trace element mixture at the same rate; (2) magnesium sulfate only; (3) no treatment; and (4) trace elements (100 pounds/acre). The mixture of the trace elements contained manganese sulfate, 55 pts; copper sulfate, 20 pts; borax (sodium tetraborate), 10 pts; zinc sulfate, 10 pts; and cobalt sulfate, 5 pts.

Chemical analyses of the samples of four alfalfa hays grown on the soils given these different treatments were made for the elements nitrogen, calcium, magnesium, phosphorus, potassium, sodium, and copper. The hays were subjected to microbiological analyses for nine of the ten amino acids essential for the white rat (phenyl alanine was not determined). They were then subjected to a bio-assay of their nutritional values by feeding them to four groups of young, white, New Zealand rabbits. Some of the rabbits were fed *ad libitum* and some were *pair fed*. All of them were given a supplement of ten grams of oats per head per day.

The grain crop of corn (maize) was grown on one of the planosols (Cherokee silt loam) also in Southwest Missouri (U.S.A.) in a test using each of seven different combinations of the five major elements (nitrogen, phosphorus, potassium, calcium, and magnesium) as fertilizers in conjunction with each of three rates, viz. 0, 100, and 300 pounds per acre of the trace element mixture. Tests were made of (a) the hardness of the grains on grinding, (b) the rabbits' preference for them in lots of seven, (c) the amounts of each of the ten essential amino acids they contained according to microbiological assays, and (d) nutritional values by feeding some of them from different soil treatments to rabbits, with and without supplements of the commonly deficient three amino acids, viz. lysine, tryptophane, and methionine, and of vitamins and casein.

RESULTS FROM TRIALS OF THE FORAGE

In the chemical studies of the alfalfa (lucerne) hays, the major differences in their relation to the fertility treatments of the soil growing them were found for the concentrations of phosphorus and for some of the amino acids. Although the concentrations of total nitrogen in the alfalfa varied but little with the soil treatments, the concentrations of the tryptophane and the methionine in the hay were increased by the addition of magnesium and trace elements in combination (Table 1). The fact that these two amino acids are most commonly deficient in many forages gives particular significance to this discovery. When fed the hays grown on the soil treated with magnesium plus the trace elements, the rabbits made better gains by 15 per cent than those by the rabbits fed the hay grown with no soil treatments (Table 2). Twenty-two per cent less hay was required per unit of gain in the former case than in the latter (Table 2).

TABLE I

Concentrations of nitrogen, phosphorus, and two amino acids in alfalfa hay according to treatments of the soil growing it

Treatments of the Soil	Concentrations			
	Nitrogen (%)	Phosphorus (%)	Tryptophane (mg/g)	Methionine (mg/g)
Magnesium and trace elements	3.05	0.247	2.52	5.44
Magnesium	3.20	0.224	—	—
No treatment	3.12	0.218	1.86	4.57
Trace elements	3.19	0.211	—	4.20

TABLE II

Gains in weights and alfalfa hays consumed per gram of gain by rabbits according as hays fed them were grown on soils given different treatments

	Gains (G/Animal)			Hay		
	ad lib. Feed	Paired Feed	Av.	Offered G/Animal	Wasted G/An.	Consumed G/G Gain
Magnesium and trace elements	879	809	849	4536	1105	4.04
Magnesium	742	748	744	5050	1561	4.69
No treatment	751	711	740	5084	1491	4.86
Trace elements	684	723	699	4840	1420	4.89

It is significant that the forage grown on the soil given the treatments consisting of magnesium alone, or of only the mixture of the five trace elements (manganese, copper, boron, zinc, and cobalt), gave (a) no significant increase in the concentrations of the commonly deficient amino acids in the forage; (b) no greater gains in rabbit weights; and (c) no significantly greater gains per unit of feed consumed than those from the forage grown on the soil given no treatment. However, when two of these treatments of the soil were combined, i.e. applying the magnesium along with the five trace elements, then the forage was improved decidedly (a) in its concentrations of tryptophane and methionine, (b) in the gains in the weights of rabbits in a given time, (c) and in the gains per unit of feed consumed.

What this all would mean in terms of the fecundity of the rabbits was not studied. It suggests, however, that research in plant nutrition would be more remunerative if, instead of using each element singly as a soil treatment and then these in various combinations of a few of the essential elements, all the known elements were applied in combination and then in successive trials an individual one, or combinations of them, were witheld in the manner similar to that used in studies in human nutrition testing the amino acids (4). It suggests still further, that the soil treatments can improve the nutritional values of the forages for animals, and that these improvements probably come via the better balance of the amino acids and other complex compounds of synthetic origin within the plants.

RESULTS FROM TRIALS WITH THE GRAIN

While, naturally, one cannot know how corn tastes to a rabbit and cannot design methods of measuring the differences in tastes, it seemed that possibly the hardness of the corn grain might be a factor in the rabbits' preference for that from one soil treatment over another when their selection from a lot was permitted. Accordingly, the respective hardnesses of the samples of corn from the twenty-one different soil treatments were determined by running each through a Wiley laboratory mill and sieving it with a nest of 10-, 40-, and 80-mesh sieves to find the percentages of the samples passing through each.

There was the least variation in hardness of the grain for the seven combinations of the major nutrient elements without additions of the trace elements as soil treatments. For this lot from 14.7 to 21.3 per cent passed through the 10-mesh sieve. But when 100 and 300 pounds, or increasing amounts, of trace elements supplemented the major nutrient elements applied to the soil, then there was greater variation in the hardness of the grains, namely, from 14.3 to 30.7 and from 8.1 to 33.7 per cent, respectively, passing the 10-mesh sieve.

But when these samples of grain were submitted to the rabbits in lots according to the seven major nutrient elements as the only variable while the soil treatment with trace elements was a constant, the variations in the amounts consumed showed the maximum taken over the minimum amounting to 1.25 times for zero trace elements, 2.3 times for 100 pounds of these applied to the soil, and 2.05 times for their application at 300 pounds per acre in combination with the major nutrients. That there was no correlation between the hardness as measured by grinding and the selection by the rabbits as shown by amounts consumed in these lots, was clearly demonstrated by the fact that these animals consumed exactly the same amounts of the hardest and of the softest of the samples. Surely, then, the differentiation by the rabbits between these grains was not according to their hardness as measured by the extra labor of grinding of mastication, but was according to the other differences invoked by differences in the soil treatments.

It was significant that as an average, in this preference testing of the lots of grain when the major nutrients as soil treatments varied with a single trace element treatment in combination, the amounts of grain consumed as a lot were highest for the soil treatments using no trace elements, the next highest were for the 300 pounds of this treatment, and lowest for the soil treatment with 100 pounds of trace elements as supplementary fertilizer. When the heaviest applications of the trace elements to the soil (300 lbs/A) were considered, it was significant that the rabbit preference, in terms of maximum grain consumption, singled out this soil treatment of the trace elements, in some cases of it in combination with or without the major elements accompanying it. This latter choice was confirmed by field observations when the grain on the plot with only 300 pounds of trace elements was taken by rodents much more than that on any other plot, and by the fact that among the twenty-one bags in storage, the mice cut into only the bag representing this particular soil treatment of trace elements alone and in their heavy applications.

In a second trial, in which the major nutrient elements were nearly a constant soil treatment while the trace elements were the variable and the consumption of grain represented the rabbits' choice between 0, 100, and 300 pounds of the trace elements as soil treatments, the preference by them was (a) for the corn fertilized with 100 pounds of trace elements when these three rates each were combined with only dolomite or with dolomite and starter fertilizers; (b) for no trace elements when in conjunction with most complete major nutrient fertilization; (c) for 100 pounds of these when the complete fertilization used superphosphate in place of rock phosphate; and (d) for 100 pounds of trace elements when in combination with more complete major treatments omitting potassium; but for 300 pounds of trace elements when these were used alone. As an average, the preference pointed to the 100 pounds of trace elements in these three sets of combinations according to 0, 100, and 300 pounds of trace elements.

While the selections of the grains by the rabbits, or their preferences for consumption, presented marked discriminations according to the treatments of the soil growing the grains, such preferences pointed to no particular physiological factor as possibly responsible. Consequently, the corn grains were fed to lots of rabbits (4–7 per lot) for six weeks with the corn supplemented by amounts of (a) each of the pure amino acids separately; namely, tryptophane, .11–.12 per cent by weight; methionine, .23–.30 per cent; and lysine .20 per cent, (b) vitamins *) and (c) casein 5 per cent. The gains in weight by the rabbits, and the corn required per gram of gain, as given in Table 3, show clearly that the soil treatments growing the corn exerted a decided effect on the nutritional efficiency of the grain as measured by the gains in weight of the rabbits.

Of the treatments using only the major elements on this soil, the dolomitic, or magnesium, limestone was most singular in giving corn of high nutritional efficiency when it required but 5.48 grams to make a gram of gain in weight of rabbit. The treatment next

*) With each 400 g corn there were added

5000 units	—	Vitamin A
1000 units	—	Vitamin D
2.5 mg	—	Vitamin B_1 (Thiamine hydrochloride)
2.5 mg	—	Vitamin B_2 (Riboflavin)
50.0 mg	—	Vitamin C (Ascorbic acid)
20.0 mg	—	Nicotinamide (Niacinamide)
5.0 mg	—	Pantothenic Acid (Calcium salt)
0.5 mg	—	Vitamin B_6 (Pyridoxine hydrochloride)
1.0 mcg	—	Vitamin B_{12} (Cyanocobalamin)

TABLE III

Concentrations of total nitrogen, lysine, tryptophane and methionine in corn (maize) according to soil treatments growing it, and gains in weights by rabbits consuming it with and without supplements

No. Soil treatment	Treatment	Nitrogen %	Lysine mg/g	Tryptophane mg/g	Methionine mg/g	Supplement Fed	Gain/Rab. G/6wks	G Corn/ G Gain
1	Magnesium limestone	1.61	10.0	0.60	5.7	None	291	5.48
2	Ditto 1 plus starter fert. (a)	1.58	8.8	0.74	5.0	—	—	
3	Ditto 2 plus nitrogen, pot. rock phos. and trace ele. (b)	1.81	7.0	0.67	5.9	Tryptophane Methionine (c) Lysine	281	5.59
4	Ditto 3 plus extra trace ele. (d) and superphos.	1.79	10.0	0.69	4.9	None	129	13.67
5	Ditto 4	1.79	10.0	0.69	4.9	Methionine	204	6.53
6	Ditto 3 minus potassium	1.72	7.0	0.56	5.7	None	175	8.28
7	Ditto 6	1.72	7.0	0.56	5.7	Tryptophane	260	5.31
8	None	1.78	7.9	0.62	5.7	None	202	7.97
9	Ditto 8	1.78	7.9	0.62	5.7	Vitamins (e)	233	6.79
10	Ditto 8 plus trace elements	1.62	7.9	0.59	5.4	None	212	5.72
11	Ditto 10	1.62	7.9	0.59	5.4	Casein (f)	344	4.30

(a) The starter fertilizer used as the soil treatment consisted of 250 lbs per acre of a 4–12–4.
(b) The complete soil treatment consisted of the starter fertilizer, after plowing under per acre 400 lbs ammonium nitrate, 200 lbs potassium chloride, 1500 lbs rock phosphate and trace elements, 100 lbs.
(c) These three amino acids were added in pure form since in respect to the commonly considered needs for rats the corn was seriously low in these amino acids.
(d) The trace elements in total amounted to 300 lbs per acre, while other treatments were the duplicate of treatment No. 3.
(e) This was a composite of many vitamins. (See footnote page 341).
(f) Purified casein was a supplement to the extent of five per cent of the ration.

in the decreasing order of efficiency was the trace elements only, when 5.72 grams of corn were required per gram of gain. Corn grown without any treatment required almost eight grams, 7.97, to make one gram of rabbit gain. When heavy soil treatments of major elements were used omitting potassium, it required 8.28 grams of corn for one of gain. But when the major five elements, including superphosphate in place of rock phosphate, and the extra trace elements were the soil treatments growing the corn grain, it required 13.67 grams of corn per gram of gain in body weight.

That these efficiencies could be improved by the addition of (a) some of the more commonly deficient amino acids, (b) the more complete protein (casein), and (c) the vitamins, is clearly indicated by the greater nutritional efficiency of the corn when these supplements were fed along with it. That the trace elements alone and the dolomitic limestone, all not commonly considered effective for increasing the yields as bushels per acre, served to improve the nutritional efficiency is not only startling, but also significant. It suggests that we have been led astray from nutritional services as the objective in growing this grain. That the fertilizer treatments with major elements, commonly considered most beneficial for increased yields as bushels of grain per acre, should have been so inefficient in giving the grain nutritional values is even more startling. That grain so grown should compel an animal to eat 2.5 times the amount to make the gain possible when eating corn grown on the same soil treated with only dolomitic limestone, points clearly to our past management of the soil for the delivery of vegetative bulk but not necessarily for nutritional efficiency by that mass produced.

These results show that by means of bioassays of our forages and grains, we shall be able to manage our soils more judiciously to produce these feeds for efficient nutritional services, and to recognize the benefits by magnesium, by the trace elements and, by other aspects of soil fertility not yet, or only recently, given attention as beneficial additions to the soil.

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