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BIOSYNTHESIS OF AMINO ACIDS ACCORDING TO SOIL FERTILITY

IV. TIMOTHY HAY GROWN WITH TRACE ELEMENTS

by WILLIAM A. ALBRECHT and JOHN DE JONGE

Department of Soils, University of Missouri, Columbia, Mo.

INTRODUCTION

For the successfull management of the production of feed and food, the fuller comprehension of the functions of the soil's inorganic elements catalyzing the synthesis, by plants, of the amino acids required by various forms of life is becoming more essential. The quality of food commodities relative to the proteins and protein-like substances has become a much more serious matter than their quantity.

The production of carbohydrates (or fats) as bulk by plants results readily from the growth of most any kinds of them. Such occurs on soils with less specific or complete arrays of the inorganic fertility supplies offered. But the synthesis by plants of the necessary amino acids, while guaranteeing both the quality and quantity of those in the daily protein required by warm-blooded animals and man, looms up as a most significant matter in the (a) choice of the crops with high potentialities for protein, and (b) management of the soil fertility (organic as well as inorganic) more completely supplied and more specifically balanced than we yet surmise.

Crude protein may be all too "crude"

Research along the above line of thinking is already supporting the validity of it [8 9 7 2 5]. The list of essential inorganic elements is extending itself. It now includes many which were formerly neglected but unwittingly applied, such as sulfur and chlorine, and those needed only in trace amounts. Among the latter, more are becoming essentials according as refinements in chemical methods and bioassays classify them in that category. Boron, for example, suggests itself as an essential element for warm-blooded animals including man [3]. Selenium and chromium have now been given functional essentiality, while cadmium has been found in conjunction with zinc in a protein fraction of horse kidney, given the name of metallothionein [12]. Some experiments prompted the theory that a more fitting balance of the major nutrient elements in the soil for the plant's higher production of its required proteins may improve the root's activities in taking up the trace elements from the soil [1 2 5]. It may also modify the catalytic services by the trace elements in the plant's synthesis of compounds essential in its own growth, protection and reproduction.

With nitrogen in commercial fertilizers going into such extensive use on non-legumes in the hope of higher concentrations of protein, resulting from it in the forages, there is the prevailing belief that the increase in crude protein (nitrogen multiplied by 6.25) must represent higher quality as feed. More of protein so obtained and classified may be making it all the more crude and even dangerously so [4].

Some observations

That such may be the fact was demonstrated by the death of cattle consuming the fodder from corn (maize) in 1954 when the drought and the extreme temperatures (113°F) destroyed the enzymes in the bleached leaves (duplicating the pattern of nitrogen deficiency in the leaf) in Missouri (U.S.A.) in early July, which was the beginning of an extended, hot, dry season lasting well into September. In early August there was a rainfall of near two inches. Maize fodder cut and fed in July was not lethal to livestock. But that which was cut and fed in September was fatal with symptoms diagnosed as nitrite—nitrate poisoning. The filling of a silo at that season was fatal, because of the inhalation of nitrous fumes, to a workman entering after a night following the previous day's filling operation [4].

Chemical tests of the lower sections of the stalks of maize, injured by the drought, revealed as much as 0.65 per cent of nitrogen in the nitrite—nitrate forms in the dry matter when the combined total nitrogen in all forms was only 1.00 per cent. Were this amount of total nitrogen considered as 6.25 per cent crude protein of feed value, this would be far from the truth when by that mathematical evaluation the fodder contained 4.06 per cent of poisons (0.65 times 6.25 = 4.06).

Timothy (*Phleum pratense*) was once a prominent hay crop for horses in eastern United States. But it has been reported that proprietors of livery stables in earlier days learned that their horses fed on timothy hay fertilized with Chile saltpeters could not hold up under a long day's travel. Timothy was feed also for other farm animals, especiallly when grown with red clover, which crop combination was once an extensive one in farm practice.

With the dwindling acreage given to red clover because of costly seed and numerous failures of seedlings without careful soil treatments ahead of them, the question whether fertilization of timothy (and other non-legume forages) with nitrogen and other essentials including trace elements would improve its feeding value, was considered for test.

That timothy hay is not of the rabbits' choice equal to that of red clover hay was indicated in some bioassays of these two hays. It was observed that if one handled the red clover hay in the large room at some distance from the rabbit hutches, those animals responded immediately by moving to the empty hay racks on one side of them. But if one handled the timothy hay similarly, they remained indifferently quiet, regardless of the time period since the previous feeding. Since timothy is not of marked choice by this test animal, the question of whether the soil treatments might shift its rank upward in that category seemed a big one.

In applying commercial nitrogen in fertilizers more widely for non-legumes on our humid soils, we dare not assume the crop's synthesis of this element into crude protein, much less into an array of amino acids making complete proteins on the more highly developed soils. As agronomists fertilizing such soils for a grass agriculture, we need to appreciate the responsibility of providing a fertility balanced in all other respects before nitrogen is applied for the production of more protein in the crop to make more and better feed thereby.

Chemical composition of timothy hays used in bioassay

In some experiments studying the value of nitrogen as a soil treatment for possible improved feeding values in terms of the amino acids in a non-legume, some timothy was grown on Clarksvill gravelly loam * with a basic treat-

TABLE 1

Inorganic elements in timothy hay

Treatments (pounds per acre)	P %	Ca %	K %	Mg %	Na %	S %	Mn *ppm*	Fe *ppm*	B** *ppm*	Zn** *ppm*	Co** *ppm*
No Nitrogen	.175	.280	1.43	.210	.070	.146	147	355			
40 Nitrogen	.123	.217	1.18	.210	.045	.125	147	364			
40 Nitrogen + 30N* . .	.129	.204	1.24	.268	.055	.127	134	295			
40 Nitrogen + 60N*** .	.129	.187	1.16	.222	.045	.123	119	419	3.7	10.4	.06
40 Nitrogen + B	'128	.235	1.11	.275	.055	.128	134	345	5.5	4.8	.08
40 Nitrogen + Z	.120	.192	0.97	.262	.060	.150	60	295	5.2	20.8	.02
40 Nitrogen + Mn . . .	.136	.193	1.08	.157	.050	.127	105	537	5.9	14.4	.08
40 Nitrogen + Co . . .	.130	.221	1.03	.275	.050	.141	105	352	6.2	12.4	.03
40 Nitrogen + Cu . . .	.130	.238	1.08	.281	.050	.142	105	337	6.0	22.4	.03
40 Nitrogen + the 5 tracee elements	.152	.240	1.07	.238	.045	.130	60	375	5.9	32.8	.03
Mean Values	.133	.221	.113	.240	.052	.134	112	366	5.5	16.9	.047

* Extra nitrogen fertilizer was applied in the form of solution.
** Determined by spectrographic methods.
*** This 60N applied also with each trace element. See Table 3.

* This is the upland soil of the Ozark area. It is a residual limestone soil with considerable cherty fragments scattered throughout. It is of low productivity, hence not cultivated but kept in grass.

TABLE 2

Nitrogen (%) and amino acid contents (mg/g dry matter) of timothy

Treatments (pounds per acre)	Ni-trogen	Methio-nine	Trypto-phane	Lysine	Threo-nine	Valine	Leucine	Isoleu-cine	Histi-dine	Argi-nine	Phenyl-alanine	Total amino acids
No Nitrogen	.915	.50	2.48	.715	2.60	2.86	12.1	6.35	.81	2.86	2.48	33.76
40 Nitrogen	.884	.39	2.16	.894	2.00	2.64	9.0	5.11	.65	2.68	2.02	27.55
40 Nitrogen + 30N *	1.02	.35	2.24	1.20	2.40	2.76	11.5	5.58	.59	2.88	2.09	31.60
40 Nitrogen + 60N **	1.34	.52	2.90	1.65	2.80	3.69	14.6	7.12	.91	3.75	2.95	40.90
40 Nitrogen + B***	1.47	.70	2.90	1.82	3.26	3.74	14.6	7.05	.80	4.00	2.80	41.67
40 Nitrogen + Zn	1.30	.56	2.86	1.58	3.04	3.23	13.2	7.13	.82	3.45	2.66	38.53
40 Nitrogen + Mn	1.15	.57	3.39	1.40	2.80	3.48	13.5	7.20	.67	3.88	2.81	39.70
40 Nitrogen + Co	1.34	.76	3.39	1.66	3.04	3.56	14.6	7.42	.89	3.88	2.86	42.06
40 Nitrogen + Cu	1.21	.45	2.73	1.68	2.78	3.12	14.2	6.62	.67	2.55	2.53	37.33
40 Nitrogen + the 5 trace elements	1.35	.72	3.61	1.84	3.06	3.59	13.6	7.31	.89	4.00	2.94	41.56

* Extra nitrogen fertilizer was applied in the form of solution.

** This 60 N was applied also with each trace element. See Table 3.

*** Also 60 N with trace elements.

ment of limestone, phosphorus, and potassium over an area extensive enough to permit seven additional treatments of higher applications of nitrogen (40 pounds nitrogen per acre as initial fertilizer plus 60 pounds of it later as solutions) coupled separately with one of the five trace elements, namely boron, zinc, manganese, cobalt, and copper, and then also with all five of these trace elements in a mixture. Many of these salts were sulfates.

The timothy hays were harvested by treated plots and put under chemical analyses of their ash for the elements, phosphorus, potassium, calcium, sulfur, magnesium, sodium, and also for the trace elements, manganese, iron, boron, zinc, and cobalt. The data are given in Table 1. These more complete inorganic analyses were made of the hays from the seven plots given the above heavier application of nitrogen and of those in combinations of the lesser amounts of nitrogen. It was the hays grown under these treatments which were put under careful bioassay later by feeding them to weanling rabbits. These timothy hays were analyzed also for their amino acid contents by microbiological procedures, according to modifications of the methods of Stokes *et al.*[10]. The results, tabulated in their relation to the total nitrogen are given in Table 2, for their respective contents of the ten essential amino acids.

RESULTS

According to the data in the Table 2, the separate trace elements as soil treatments, save boron and cobalt, gave a lowered percentage of total nitrogen * in the dry matter, or thereby, a lower total crude protein. This might suggest a more efficient metabolic service for plant growth (as mass) by nitrogen in those three cases of the combination with manganese, zinc, and copper as soil treatments, that is, giving more plant growth per unit of nitrogen and of these fertility trace elements. Cobalt was without disturbing effect on the concentration of the total nitrogen in the timothy hay. Boron increased it by .13 per cent in the absolute, raising it from 1.34 to 1.47, or nearly ten per cent, relatively. The mixture of all five trace elements cannot be considered as a significant modifier of the concentration of the total nitrogen when this was increased only from 1.34 to 1.35 per cent. It is significant to note that the extra nitrogen applied to the soil pushed up the concentration of nitrogen in the dry matter of the timothy giving the percentage series of 0.91, 0.88, 1.02, and 1.34 for the series of applications of 0.0, 40., 70., and 100 pounds of this element per acre.

* Measured by modified Kjeldahl method in order to include nitrates.

TABLE 3

Timothy hays arranged in order of decreasing nitrogen (crude protein) to exhibit their differing orders (a) according to their totals of the ten essential amino acids and (b) according to arithmetical factors by which nitrogen must be multiplied to represent those amino acids

Soil treatments	Nitrogen*	Crude protein*	Acids*	Decreasing order	Factor**	Decreasing order
40 lbs N + 60 lbs N + Boron . .	1.47	9.187	4.167	2	2.83	7
40 lbs N + 60 lbs N + all 5 traces	1.35	8.437	4.156	3	3.07	4
40 lbs N + 60 lbs N	1.34	8.375	4.090	4	3.05	5
40 lbs N + 60 lbs N + Cobalt . .	1.34	8.375	4.206	1	3.13	2
40 lbs N + 60 lbs N + Zinc . . .	1.30	8.125	3.853	6	2.96	6
40 lbs N + 60 lbs N + Copper . .	1.21	7.562	3.737	7	3.08	3
40 lbs N + 60 lbs N + Manganese	1.15	7.187	3.970	5	3.45	1

* As per cent of dry matter.

** Factor by which the nitrogen must be multiplied to give the total amounts of the amino acids listed.

According to the influences of the trace elements supplementing the nitrogen as soil treatments, if one were to use these nitrogen values, the treatment with boron gave a crude protein content 24 per cent higher than that by the soil treatment of manganese comparatively, as 9.18 per cent and 7.18 per cent respectively. If the timothy hays are arranged in order of decreasing concentrations of crude protein for the respective soil treatments, this order is quite different from that according to concentrations of the ten essential amino acids, set up for comparisons in Table 3. Also, if one compares the percentages of the dry matter made up of the ten essential amino acids – measured separately and in total – with the total nitrogen, it is interesting to note the variations in the mathematical factors by which the nitrogen would need to be multiplied to obtain the values of the ten essential amino acids combined. These values, or factors, varied from 3.45 for the soil treated with manganese, the trace element connected with the lowest crude protein, to 2.83 for boron connected with the highest crude protein in this series. This would suggest the lowest crude protein concentration as the most efficient in converting its nitrogen or itself into the ten essential amino acids.

Deaths from heat showed proteins of timothy not equal to other proteins

That even such measures of the totals of the essential amino acids alone are not a true index of the nutritional value of the hay was shown in the feeding trials of these hays using weanling rabbits as the bioassay agents during the summer of 1954 which gave disastrous results in the deaths of them in connection with the heat wave of that unusual summer when the tests were made.

That timothy hay, even when fertilized with increasing amounts of nitrogen, is not taken so readily by rabbits was demonstrated in some preliminary trials. Given three hay samples representing fertilization with mounting increments of nitrogen, the rabbits chose hays with more nitrogen only at the beginning of the increased fertilization scale. They reversed this behavior, choosing hay with lesser amounts of applied nitrogen, at the higher increments of the fertilization scale. They exhibited reluctance to eat any grown with nitrogen fertilization at the largest increments applied.

In some additional trials with rabbits offered a constant amount of grain (maize) per day along with four separate lots of hay (fescue, *Festuca elatior* L var. *arundinacea*) given increasing nitrogen fertilization, the consumptions of the hays by these bioassayers of nutritional quality according to soil treatments put the amounts as ratios to grain in the following order as decreases for more nitrogen applied, namely, 1.93, 1.43, 1.16, and 1.00 by using the lowest ratio as 1.00. The amount of fescue hay per 20 rabbits per day as means of choices of 8 soil treatments was 168 g when for red clover for 4 treatments it was 840 g showing the former plant species one of poor choice. It was more so when fertilized with increasing amounts of nitrogen. By using the test rabbits with the same maize and each of the hays separately, the first increment of nitrogen fertilization raised the amount taken but even that was less than of hay given less nitrogen coupled with phosphate fertilizers. Higher applications of nitrogen put the hay in choices below that of no nitrogen treatments [11].

When the first set of four separate soil treatments of increasing nitrogen fertilization under the fescue hay were submitted to the rabbits' choice, the daily consumptions per 20 rabbits varied from a low of 22.7 to a high of 44.5 g per soil treatment with a total of the four at 126.4. For the second set of four soil treatments of increasing nitrogen with some phosphate the daily consumptions varied from a low of 24.0 to a high of 85.4 g with a total of the four at 211 or a mean of the eight of 168.5 grams.

In corresponding trials with alfalfa hay, a legume, grown with increments of nitrogen fertilizers coupled with given amounts of phosphate and also limestone, the amounts consumed for the four soil treatments as ratios of hay to grain were 6.11, 5.42, 2.25, and 1.25 with the first choice of alfalfa being that grown without nitrogen fertilization. The amount of alfalfa hay consumed per 20 rabbits per day as the mean of choices of 8 soil treatments was 651.0 g.

For the first set of four choices the daily consumptions per treatment varied from a low of 51.6 to a high of 256.0 g per day per 20 rabbits with a total of the four at 622.9. For the second set the low was 68.5 and the high 297.2 while the total was 679.4 g.

For the choices of the red clover with but one set of four treatments other than mainly nitrogen, the low was 76.2 and the high 374.1 while the total was 840 g.

While the choice by the rabbits of the fescue hay was improved by the first increment of nitrogen to the soil growing it but was lowered by additional increments, any increment of nitrogen fertilizer for the alfalfa hay, a legume, lowered it in the choice by the rabbits. It is significant to note the differences in plant species and in the total amounts of hays taken, and also the changes in choice by the treatments of the soil growing either species [11].

Timothy hays grown on soils with different treatments fed to lambs over a winter period in some additional studies also showed the low feed values of the hays in the irregularities of the health of these animals. The lowest gains were recorded for the hay grown with only nitrogen as soil treatment. They exhibited increasing lameness first in one hind leg, then in both, then failing locomotion and later death. Irregular growths of the hoofs were commonly visible symptoms [6].

While timothy hay in combination with grain is not the animal's choice, yet if soil treatments using nitrogen in addition to basic other treatments including the trace elements, will improve the plant's production of proteins, it was deemed worthy of test of the protein values of timothy hay under such generous soil improvements.

When the tests using rabbits were set op for the summer of 1954, no record heat wave in Missouri, U.S.A., was anticipated. Nevertheless, while that disaster as deaths of the test animals upset the records of gains or losses in weights by them, it served in an usunual way to demonstrate the low value of the protein in the timothy species grown even under such supposedly complete soil treatments.

The mounting temperatures of the summer heat wave correlated themselves with the increasing number of deaths of the experimental rabbits fed on the lots of timothy hay combined in all cases with wheat of a single lot. There was no apparent correlation between the schedule of deaths and the soil treatments of the timothy hays. At the fortnightly weighing periods, the lots of rabbits were each restored to its initial numbers by adding individuals from the remaining original lot which suffered no heat fatalities. Those in the original lot, in the same location as the test rabbits, were fed on the same wheat grain, but this was combined with green grass growing on the nearby soil, fertilized and watered with the rabbit manure and hutch washings.

Deaths from the heat began on June 11 and amounted to a total of 57 (nearly 75%) for the seven lots by July 17, or the termination date set for the six-week trial. During the heat wave the maxima of temperatures ranged from 88° to 113°F with a mean maximum of 99.4°F during the fortnight closing with July 17.

On that day and forward (with one death the preceding night) the fatal ration of wheat and timothy hay was supplemented with 10 g per rabbit per day of commercial, fat-free, dried skim-milk powder. No more deaths occurred during the extension of the experiment for nine days, when the maximum daily temperatures ranged from 89° to 111°F with a mean maximum of 98.2°F.

As a sequel to the improved animal reactions to the high temperatures because of the extra protein supplied in the skim-milk powder, a repeat of the former test was started promptly on July 26 using maize, oats and the wheat in equal parts by weight, as the grain of the rations along with the roughages of the same timothy hays previously used. This trial, duplicating the preceding one and under the continuing high temperatures exhibited fatalities of the rabbits again until August 23 (during four weeks), when the feeding of the timothy hay was discontinued and red clover hay was fed instead.

No deaths of the experimental rabbits occurred during the extension of the test with the red clover hay for the fortnight from August 23 to September 6, during which the maxima of the continued high temperatures ranged from 79° to 102° with a mean maximum of 97.6°F for those 14 days. For the fortnight preceding the data of change to the red clover hay, the maxima ranged from 70° to 98°F, with a mean maximum of 82.5°F. At the close of this test with too many early disasters to give any reliable data as weights, there were still eight rabbits left of the original lot fed during the entire summer on the wheat-grass ration among which no fatalities had occurred during the extensive record-breaking heat wave.

DISCUSSION

While the low values of crude protein in forages or hays can be improved by fertilizing the soil with nitrogen in conjunction with other elements, there is possible error in believing that such higher concentrations of total nitrogen in crops mean more protein either in quantity or quality.

In terms of the animal's choice, the non-legumes fertilized naturally by nitrogen in urinary and fecal droppings are refused as shown by the tall, green spots in pastures on humid soils. Self-restrictions of consumptions by the animals, ranging from 220 to 10 grams per day per rabbit, as the cited differences between fescue and alfalfa, militate against the animal's getting enough of the amino acids to provide the protein requirements when other factors than these protein parts seem to be responsible. Only the animal can be the final test of what the soil treatments do to make feeds of high quality as nutrition.

That feed values, according to soil treatments, need to be put under bioassay with more than animal survival and gain in weight as the criteria, was demonstrated by the accidental deaths of the rabbits at higher temperatures, but their survival on the same feed at lower ones. The former was a readily evident, clinical situation; the latter an undetected, subclinical one. What the level of health was of the latter remained unmeasured, though very probably a low one.

The chemical compositions as ash and as amino acids added themselves to the many tabulations of modified values of crops because of soil treatments with fertilizers. But they indicated no correlations of suggestive values, of possible help in the interpretation of the animals reactions to the feed, as growth, self-protection and reproduction. Perhaps with more biochemical values resulting from our relating the animal nutrition to the plant nutrition, and the latter in turn, to the soil fertility through bioassays, we may interpret the biosynthesis of amino acids by plants for wiser guidance of soil treatments accordingly.

SUMMARY

Timothy hays grown with intensive soil treatments, including trace elements, (without significant differences in yields of hay per acre), were put under bioassay by weanling rabbits, only to have the high temperatures of a summer heat wave demonstrate by deaths of these test animals, regardless of soil treatments, that the protein intake (and what might accompany it) was not equal to the animal's needs. No deaths resulted in the stock rabbits in the same environment, fed on the same grain but with green grass in addition. Also, ten grams of skim-milk powder per rabbit per day prevented the death of the test rabbits as the extended heat wave permitted

the demonstration. Similarly red clover hay, a replacement of the timothy, demonstrated that it also provided the protein needs to prevent deaths of the rabbits by the heat. According to the bioassays, the timothy suggests its own classification as of low potential as animal nutrition, even under extensive soil treatments growing it.

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