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Quantitative Relation of EDTA to Availability of Zinc for Turkey Poults.* (28385)

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Addition of disodium ethylenediaminetetraacetic acid (EDTA) to a diet containing isolated soybean protein has been shown to increase the availability of zinc for turkey poults(1). Scott and Zeigler(2) showed that EDTA would also improve zinc availability for chickens and that 300 mg of EDTA per kg of diet was more effective than 150 mg/kg. Lease *et al.*(3) found that zinc in sesame seed meal could be made more available to chicks by the addition of EDTA to the diet.

The mechanism by which EDTA improves the availability of zinc is not known. The present experiments were conducted to measure the relative effectiveness of EDTA and zinc as dietary supplements to a zinc-deficient diet for turkey poults.

Experimental. Broad Breasted Bronze turkey poults were fed a practical stock diet for 5 days before being divided into groups of 10 birds each and fed the experimental diets. The basal diet (Table I) contained isolated soybean protein and was supplemented with zinc oxide or EDTA as indicated in Table I. The zinc content of a similar basal ration was 25.5 mg/kg(1). The birds were housed in electrically heated cages which were coated with an epoxy resin to reduce the amount of zinc available in the environment. The experiments were continued for 17 days and the poults were weighed twice weekly and their legs were scored for perosis.

Results and discussion. When poults were fed the basal diet alone growth was very poor and a mild degree of perosis was observed. As zinc was added to the diet, growth was improved in proportion to the amount of zinc added to the level of 30 mg/kg. In Experiment 3, 50 and 200 mg/kg of zinc were also added to observe the effect of higher levels on the severity of perosis. Perosis increased as the level of zinc was increased to about 15 to 30 mg/kg and then decreased as higher levels of zinc were added, probably because at very low zinc levels growth was not sufficient to allow the expression of this deficiency symptom. It was only when reasonably good growth could be obtained that zinc became limiting for bone formation.

EDTA was added in varying levels, both to the basal diet alone and to the basal diet containing 7 and 10 mg/kg of added zinc.

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TABLE I. Experimental Diet Deficient in Zinc.

	g/kg
Cornstarch	425
Isolated soy protein*	330
Cellulose, powdered†	50
Mineral mixture‡	63
Vitamin mixture§	20
Soybean oil, degummed	35
$CaHPO_4$	30
$CaCO_3$	25
DL-Methionine	4.5
Choline chloride‖	10
Erythromycin	1
Inositol	1
BHT¶	1
Vit A (10,000 I.U./g)	0.5
" D (1,500 I.U./g)	3
" E (44 mg/g)	2
Biotin	.4 mg/kg
Vit B_{12}	10.0 μg/kg

* ADM C-1, Assay Protein, Archer Daniels Midland Co., Cincinnati, Ohio.
† Solka Floc, Brown Co., New Hampshire.
‡ Minerals were supplied in g/kg of diet as follows: NaCl (uniodized), 10.0; $MnSO_4 \cdot H_2O$, 0.3; $FeSO_4 \cdot 7H_2O$, 0.65; $CuSO_4 \cdot 5H_2O$, 0.08; $Cu(Ac) \cdot 4H_2O$, 0.02; KI, 0.009; $Al_2(SO_4)_3 \cdot 18H_2O$, 0.25; $MgSO_4 \cdot 7H_2O$, 4.0; KCl, 3.0; K_2HPO_4, 5.0; Na_2MoO_4, 0.009.
§ Vitamins were supplied in mg/kg of diet as follows: riboflavin, 10; thiamine·HCl, 10; pyridoxine·HCl, 10; Ca pantothenate, 30; niacin, 120; folic acid, 5; menadione, 10.
‖ 25% in wheat middling carrier.
¶ Butylated hydroxytoluene.

Growth increased linearily as the level of EDTA was increased. Perosis, also, was more severe than observed in the basal diet alone.

By comparing growth response obtained with the EDTA supplements to that observed by addition of zinc alone, it was possible to calculate the amount of zinc equivalent to 100 mg/kg of EDTA (Table III). The results show that 100 mg/kg of EDTA were approximately equivalent to 8 mg/kg of added zinc in the poult diet and that this equivalent activity was constant over the range of 50 to 300 mg/kg of EDTA. If one were to assume that one atom of zinc would combine with one molecule of EDTA then 100 mg/kg of EDTA would be equivalent to 19 mg/kg of zinc. Thus, even though there appears to be no change in the equivalent values of EDTA and zinc at the various levels of added EDTA, it appears that EDTA is about one-half as effective as the theoretical combining power of EDTA and zinc.

The effectiveness of EDTA in the presence of 7 or 10 mg/kg of zinc was calculated by comparing the growth response of the zinc plus EDTA supplemented group to that fed zinc alone and subtracting the level of the zinc supplement (mg/kg) from the response (equivalent to mg/kg zinc) noted for the combination (Table III). Under these conditions 100 mg/kg EDTA were equivalent to approximately 19 mg/kg zinc. The enhanced effectiveness of EDTA in the presence of a small amount of zinc probably indi-

TABLE II. Effect of Zinc and EDTA in the Diet upon Body Weight Gain and Perosis Score of Turkey Poults.

Dietary supplement		Gain and perosis score of poults—Exp No.			
Zinc (mg/kg)	EDTA (mg/kg)	1 (g)	2 (g)	3 (g)	4 (g)
0	0	56 (.6)*	57 (.6)*	53 (.6)*	46 (.7)*
7	0	109 (1.1)	103 (1.3)		
10	0			114 (1.3)	101 (1.0)
15	0	173 (1.6)	152 (1.7)		
30	0	208 (.9)	198 (1.6)	203 (2.1)	225 (1.1)
50	0			187 (.7)	
200	0			225 (.1)	
0	50	91 (1.0)	80 (1.2)		
0	100	110 (1.2)	118 (1.6)	87 (1.2)	93 (.6)
0	200	126 (.9)	158 (1.1)	147 (1.3)	134 (1.3)
0	300			174 (1.4)	
7	50	179 (1.0)	168 (1.5)		
7	100	189 (1.7)	174 (1.6)		
10	100			197 (1.8)	210 (1.0)
7	200	202 (1.3)	233 (1.2)		
10	200			248 (2.8)	

* Perosis score; 0 = normal, 4 = severely perotic.

TABLE III. Comparison of Zinc and EDTA in Promoting Growth of Zinc Deficient Poults.

Level of EDTA tested (mg/kg)	Zinc (mg/kg) equivalent to 100 mg/kg EDTA in diet—Exp 1	2	3	4
50	9.6	7.0		
100	7.4	9.5	5.5	8.5
200	4.7	8.5	8.8	7.7
300			7.8	
50 (with 7-10 mg/kg Zn)	17.6	27.0		
100 (with 7-10 mg/kg Zn)	16.0	15.0	19	17.5

cates that EDTA not only increases the availability of the zinc already present in the diet, but improves the availability of added zinc as well.

Summary. Various levels of EDTA and zinc were added to the poult diet containing isolated soybean protein. One hundred mg of EDTA/kg were equivalent to approximately 8 mg/kg zinc in supplementing the basal diet. When 7 or 10 mg of zinc/kg were added to the diet, 100 mg of EDTA/kg were equivalent to approximately 19 mg of zinc/kg. The data indicate that EDTA improves the effectiveness of added zinc as well as increasing the utilization of zinc already present in the basal diet. Perosis increased as the zinc level of the diet increased to approximately 30 mg/kg and decreased as zinc was added above this level.

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Lysogeny and Phage Resistance in *Pseudomonas aeruginosa.** (28386)

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Pseudomonas aeruginosa, strain Ps-7, has been shown to produce a proteolytic enzyme which contributes to the virulence of this organism in experimental infections of the cornea(1). Further studies regarding the physiology of this organism have revealed that this strain is lysogenic for at least 3 bacteriophages designated 7v, 7m and 7s. Phage 7v can be isolated from clear plaques which appear spontaneously in areas of confluent growth when Ps-7 is streaked on solid media. Phages 7m and 7s were isolated from plaques which were formed when supernatant fluids from centrifuged broth cultures of Ps-7 were mixed with a series of indicator strains of *Ps. aeruginosa* and plated by the soft agar layer method of Adams(2). Since it is well established that the presence of prophage in a lysogenic bacterium can influence many host characteristics, it was of interest to study the bacteriophages associated with strain Ps-7. Before detailed studies could be undertaken, it was important to search for non-lysogenic strains of *Ps. aeruginosa* which might serve as hosts for these phages and to compare the phages 7v and 7m with the host ranges of other *Ps. aeruginosa* bacteriophages described in the literature(3,4). Data concerning host range patterns of 12 bacteriophages and lysogenicity patterns of 95 strains of *Ps. aeruginosa* are presented here. Some of the properties of phage 7s have been reported elsewhere(5).

Materials and methods. Bacterial strains and bacteriophages. A total of 95 strains of *Ps. aeruginosa* for use in bacteriophage host range determinations and for lysogenicity assays were collected from various New Orleans hospitals. Five bacteriophages designated 12175-B1, -B2, -B3, -B4 and 12055-

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