Effects on Growth of Chicks and Poults of Feeding Yeast Isolated from Streptomycin-Fed Poults.* (19861)

F. H. KRATZER, M. P. STARR, D. M. REYNOLDS, C. R. GRAU.

From the Departments of Poultry Husbandry and Bacteriology, University of California, Davis and Berkeley.

It has been shown repeatedly that the addition of antibiotics to rations which are believed to contain adequate amounts of all the known nutrients, results in a distinct growth response by chicks or turkey poults,(1-4). Concomitant with this growth increase, when streptomycin was fed to poults, a 5- to 10-fold increase in the yeast population of the intestinal contents was noted, as compared with the control birds which did not receive the antibiotic in their diet. Although cultivatable yeasts represent a very small component of the intestinal microflora, a closer examination of their possible role in the antibiotic growth response was prompted because commercial yeast cultures have been reported to produce a small growth response when added to normal diets(5). The following investigations were undertaken to determine whether the feeding of live cultures of yeast, originally isolated from streptomycin-fed poults, could increase the growth rate in a manner similar to that of the antibiotics themselves.

Preparation of the cultures. Yeasts were isolated from the intestinal contents and feces of poults which had been fed a ration containing 50 mg of streptomycin per kilo of diet. In one procedure, samples of intestinal contents were obtained, immediately after sacrificing, from the region just anterior to the ileocecal junction. In another, changes in the fecal flora were being studied by periodic culture from hatching until 5 to 6 weeks. Droppings from these poults were transferred to sterile containers immediately after defecation. Both types of samples were cultured in the same manner. Quantitative platings were made using 2 specialized media: wort agar; and acidified potato-dextrose agar (pH 3.5). Microscopic examination of preparations from individual colonies showed that many yeasts were present. A representative collection of these yeasts was assembled from the quantitative plates, and the cultures were purified by restreaking several times from aqueous suspensions. Most of these cultures were, superficially at least, alike in microscopic morphology and colony appearance and, for economy in futher work, a single representative culture was studied most intensively. This organism designated as E-16, was grown in mass culture for poultry feeding trials, and subjected to the usual microbiological determinative tests. Occasional use was made of an unidentified yeast-like fungus, designated G-1-B, which was isolated from a commercial yeast supplement. Unless otherwise qualified, however, the results detailed here are based on studies with yeast strain E-16. *Mass cultivation.* For the poultry feeding trials, the yeasts were grown in a yeast-peptone broth† distributed in 1 liter volumes in Fernbach flasks. They were incubated at about 30°C on a rotary shaking machine for several days. When the population had attained a maximum cell volume, the yeast was harvested by means of a Sharples supercentrifuge. The moist cake was stored at 4°C or if dried material was desired, lyophilized in a freeze-dry apparatus (8). Contrary to expectations, the viability of the yeast was not reduced appreciably by lyophilization. Approximately 20 g (wet weight) of cells per liter of medium were produced in this way. *Determinative Tests.* The typical culture (E-16), was subjected to the usual taxonomic examination. with results as follows: Moist creamy growth on potato-

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† Formula for yeast-peptone broth:

Yeast extr.	5 g	K_2HPO_4	2
Trypticase	5	$CaCl_2$	.2
$(NH_4)_2HPO_4$	1	Glucose (cerelose)	20
$MgSO_4 \cdot 7H_2O$	.5	Water *q.s.*	1 liter

dextrose agar; colonies entire and convex. Dull, pasty growth on yeast-dextrose-$CaCO_3$ agar; somewhat spreading colony. Mycelium present in subsurface growth after several days at room temperature. Many budding cells along the hyphae; some pseudomycelium and true mycelium. Surface growth on corn meal agar consists of small, pasty colonies with a subsurface mycelium, no chlamydospores observed after five days at 25°C. Surface growth on blood agar (37°C) shows small, entire colonies. A mycelial fringe is noted after 6 days with budding cells on the mycelium. Acid and gas are produced from glucose; no acid or gas from lactose or raffinose. No ascospores observed on carrot or potato slices, on plaster of paris, or in tomato juice with a paper wick. Subcutaneous injection of a dense suspension of yeast cells into guinea pigs produced no lesions. On the basis of these tests and observations, yeast strain E-16 is tentatively identified‡ as *Candida tropicalis*.

Procedure for growth tests. Samples of yeasts were tested for growth-promoting activity in 4 different series of experiments, using both chicks and poults. The chicks used were S.C. White Leghorn cockerels unless noted otherwise; the poults were of the Bronze variety not segregated by sexes. The birds were housed in electrically heated batteries with raised wire floors. Some of the experiments were started when the birds were one day of age, while in others the control ration was first fed for a week or 10 days. In Series 1, the yeast preparations were mixed in the feed at the start of the experiment with no attempt to prolong the life of the microbes. In Series 2, the yeast was mixed daily in the feed with the aid of a small amount of water. Lyophilized yeast was used in Series 3. Daily additions of yeast were made in the experiments with poults (Series 4). The basal ration used in Series 1 and 2 was a practical type of chick ration (Table I). The ration in Series 3 was of a purified type which contained methanol-extracted soybean oil meal as the protein source(6). A practical poult-starting ration was used for the fourth series of experiments (Table I). The APF supplement which was used in some trials contained 7 mg of aureomycin per g.

TABLE I. Basal Rations Used in Experiments with Chicks and Poults.

Ingredient	Series 1 & 2 (chicks), %	Series 4 (poults), %
Corn, yellow, ground	30	20
Wheat, ground	13.5	10
Barley "	10	13.8
Alfalfa meal, dehydrated	4	5
Wheat mill run	15	10
Soybean oil meal	10	25
Fish meal	7.5	7.5
Meat scrap		2.5
Liver meal	2	
Dried whey	2.5	2
Dried skim milk	2.5	
Ground limestone	1.5	2
Bone meal, special steamed	1.2	1.5
Salt	.5	.5
Mn sulfate	.025	.025
Feeding oil (2250 A, 300 D)	.25	
Dry "A" (10000 units/g)		.075
" "D" (2000 ")		.01
Riboflavin	1 mg/lb	
Butyl fermentation product		.25

Results. In the first series of chick tests (Table II) there was no response to yeast G-1-B in either the presence or absence of streptomycin. Growth was slightly reduced by yeast E-16 alone, but its addition to the ration containing streptomycin produced a slight gain. In these trials the feed was mixed only at the start of the experiment.

In an attempt to keep the yeast viable for a longer period, daily mixing was used in the second series (Table III). In Exp. 6, there was a slight growth increase with all of the yeasts tested. Since some of this may have been due to stimulation in feed consumption by water added to the feed, in the remaining trials control groups were included with water added in amounts equivalent to that used in mixing the yeast in the feed. Yeast E-16 gave a very slight increase in growth above the water-mixed control in 2 experiments. Pasteurizing and lyophilizing had little effect upon its growth-promoting activity.

In Series 3 (Table 4), yeast E-16 was lyophilized and mixed in the feed at the start of the experiment at a level equivalent to 2% of the moist yeast. The results show a slight increase in percentage gains for 2 of 3 trials.

‡ We are indebted to Mr. Sidney Saperstein and Professor Herbert G. Johnstone for aid in this matter.

TABLE II (Series 1). Effect of Antibiotics (50 mg/kg), APF Supplement (.25%) and Yeast (1%) upon Growth of Chicks.
(Feed mixed at start of experiment only.)

Supplement	Avg gain (g) Exp. 1	Exp. 2	Exp. 3*	Exp. 4*	Exp. 5*
0	205	237	130	253	195
Streptomycin	220	223	124		
APF supplement			136	259	214
Yeast E-16		204	121†	244	
Yeast E-16 (lyophilized)			129		
Yeast G-1-B	203			231	198
Brewer's yeast			129		
Streptomycin + yeast E-16		249			
Streptomycin + yeast G-1-B	222				
APF supplement + yeast G-1-B					227
No. of chicks/group	15	20	20	20	20
Duration, days	22	20	21	28	28

* Avg of male and female gains.
† Chicks inoculated with yeast E-16 orally at start of exp., none in feed.

TABLE III (Series 2). Effect of Antibiotic (50 mg/kg), APF Supplement (.25%) and Yeast (1%) upon Growth of Chicks.
(Feed mixed daily.)

Supplement	Avg gain (g) Exp.6*	Exp.7*	Exp.8	Exp.9*
0	202	263		130
				124
Water		275	218	133
Aureomycin	200		267	
APF				136
Streptomycin	218	279	239	
Yeast E-1	211			
Yeast E-16	217	283	229	129
Yeast E-16 L†				127
Yeast E-16 P				137
Yeast E-16 L & P				122
Yeast E-34	213			
" G-1-B	220			
Streptomycin+E-1	236			
" +E-16	224			
APF + yeast E-16				144
No. of chicks/group	20	20	28	20
Duration, days	20	22	28	28

* Avg of male and female gains.
† L = lyophilized; P = pasteurized.

The difference, however, was not great enough to be statistically significant even at the 5% level. Liver concentrate increased growth consistently, and yeast added to it caused no further increase in growth. The response due to aureomycin was slightly less than was required for significance at the 5% level. Yeast and liver concentrate gave increases which were not statistically significant when added to the aureomycin-fed groups.

In the 4th series, yeast E-16 was tested with poults (Table V). In 2 trials it failed to increase growth above the control ration which was mixed with water daily.

Discussion. The mechanism through which antibiotics accelerate the early growth of chicks and poults is obscure. Although there are many changes in the microflora of the intestinal tract when antibiotics are fed, the observation that the yeast population increased was of particular interest. This information, coupled with the fact that under certain conditions a commercial live yeast culture had been noted to increase growth in chicks pointed to the possibility that antibiotics might owe their activity to the stimulation of intestinal yeast growth. It might be postulated that the yeast supplied a nutrient essential for chicks and poults. Although the birds fed yeast showed a slight increase in some trials, antibiotics had an even greater and more consistent effect. Testing of the hypothesis regarding a possible role of intestinal yeast in the antibiotic growth effect is far from completed; however, the present results suggest that the postulate is incorrect.

These experiments were started before the relative value of the different antibiotics in promoting growth had been established. In previous work(4), streptomycin was shown to give a definite growth response, although it was less than that obtained with penicillin. McGinnis(7) has shown that streptomycin was relatively ineffective when fed at 5 mg per kilo of feed but at 25 mg per kilo it pro-

TABLE IV (Series 3).
Effect of Lyophilized Yeast and Liver Concentrate upon Growth of Chicks.

Supplement level	%	Avg daily gain (%) — Without aureomycin: Exp. 10	Exp. 11	Exp. 12	Avg	Plus aureomycin: Exp. 10	Exp. 11	Exp. 12	Avg
0		6.28	6.12	6.03	6.14	6.45	6.34	6.17	6.32
Lyophilized yeast	2	6.32	6.40	5.94	6.22	6.44	6.40	6.39	6.41
Liver concentrated	.5	6.48	6.37	6.22	6.36	6.59	6.49	6.35	6.48
Lyophilized yeast + liver concentrate	2 .5	6.24	6.50	6.28	6.34	6.36	6.35	6.21	6.31

Least difference for significance at 5% level, .21 and at 1% level, .30.

TABLE V (Series 4). Effect of Antibiotic and Yeast Supplements upon Growth of Poults.

Supplement	Level	Avg of ♂ and ♀ gains (g) Exp. 13	Exp. 14
0		434	659
Streptomycin	50 mg/kg	464	
Procaine penicillin	50 "	538	669
Yeast E-16	1%	433	640
Streptomycin + yeast E-16	50 mg/kg 1%	502	
No./group		17	13
Duration, days		28	31

duced gains equal to aureomycin and terramycin. The level of streptomycin used here should have been adequate to give its maximum growth response.

The few strains of yeast isolated for these trials represent only a minute segment of the total microbial population; hence the present study does not provide conclusive evidence that alterations in the yeast or other microbial flora are not responsible for the antibiotic growth increase.

Summary. A 5- to 10-fold increase was noted in the yeast population of the intestinal contents of turkey poults when they were fed a ration containing streptomycin. When the yeasts were isolated and fed to other poults and chicks there was no consistent increase in growth, although very slight increases were noted in some trials. The growth increase observed with the feeding of antibiotics apparently cannot be simulated by feeding the particular strains of yeast isolated.

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Induction of Precocious Opening of Immature Rat Vagina with Neostigmine.* (19862)

C. FREDERIC FLUHMANN.

From the Department of Obstetrics and Gynecology, Stanford University School of Medicine, San Francisco.

It has been possible to affect the genital cycle of adult polyestrous animals and to re-establish function in senile females by various drugs which act on the autonomic nervous system, but it has been generally held that the ovaries of immature rats or mice cannot

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