EFFECT OF PENICILLIN AND AUREOMYCIN ON CHICK GROWTH AND THE MICROFLORA OF THE CECA

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INTRODUCTION

Many unidentified factors are needed for maximum growth, hatchability and reproduction in chickens in addition to those nutrients, vitamins and minerals, which are now known to be essential. Since there is an interrelationship among many nutrients and vitamins, and since unknown factors may be involved with growth and reproduction in farm animals as well as in chickens, these problems have been attacked from several different angles. In dealing with an unidentified factor there is no known chemical assay. For this reason, bio-assay using rats, chickens and certain bacteria has been used most widely to determine potency and to test possible sources of the unidentified factor. Growth is used most generally as a measure of the potency of the known source of the unidentified factor.

The amount of an unidentified factor required for maximum growth can not be established until the factor has been identified and standardized. To make a study of an unidentified factor, the ration must first be adequate for all known essential nutrients. The nutritive value of feed ingredients may vary as to type, maturity and methods of processing. To eliminate this variation sufficient amounts of each feed ingredient to be used in each ration must be obtained at one time for the entire feeding trial. With feed ingredient variations reduced to a minimum in the experimental basal ration, the amount of an unidentified growth factor in any supplement can be measured by the increased growth and

efficiency of feed utilization obtained when the supplement is added to the basal control ration.

Objectives of Study

The main objectives of this study were (1) to determine the effect of crystalline penicillin G and aureomycin hydrochloride upon the bacterial flora of the ceca in the chick when a semi-purified ration was fed, (2) to measure the growth response obtained by adding crystalline penicillin G to a practical ration, (3) to measure the growth response obtained by feeding a practical ration supplemented with crystalline penicillin G to chicks with ligated ceca, and (4) to measure the growth response obtained by injecting procaine penicillin G in sesame oil intramuscularly.

REVIEW OF LITERATURE

In recent years many reports have been made which show that certain antibiotics will accelerate the growth rate of chicks. Moore and coworkers (1946) reported that the addition of streptomycin and sulfasuxidine to a semi-purified ration brought about a growth response in chicks. The antibiotics did not cause a sterilization of the intestinal tract of the chicks, but did produce a reduction in the coliform bacteria of the cecal contents. Newell, Peterson and Elvehjem (1947) reported increased growth in chicks fed a practical ration supplemented with a combination of dried penicillin mycelium and fish press water. Later research by McGinnis and coworkers (1951) and Cunha and coworkers (1949) showed that an aureomycin mash (A.P.F. concentrate) produced greater growth in turkey poults and swine than did vitamin B_{12}. Stokstad and Jukes (1950a,b) and Whitehill, Oleson and Hutchings (1950) reported that antibiotics increased the growth rate of chicks fed all vegetable protein rations.

Thayer, Leong and McGinnis (1950) reported data which indicated that an A.P.F. concentrate has a sparing effect on the protein needs of young chicks. Cunha and coworkers (1950) reported similar data with swine. Groschke (1950) reported that aureomycin or streptomycin produced greater growth in chicks than did vitamin B_{12} and that the antibiotics lowered the vitamin B_{12} requirement of the chick. He offered the explanation that the antibiotics stimulated growth indirectly by changing

the intestinal microflora from "undesirable" to "desirable" types of organisms and that the "desirable" types were responsible for the growth effect through intestinal synthesis. McGinnis (1950) presented evidence of synthesis by favorable bacteria by showing that terramycin and penicillin inhibited the growth of clostridia in the intestinal tract of chicks and poults. Cook and coworkers (1952) reported that penicillin caused a reduction in fecal lactobacilli counts and an increase in coliform counts in the poult.

Elam, Gee and Couch (1951a) reported that penicillin caused a significant increase in the total numbers of intestinal microorganisms, including enterococci and penicillin-resistant organisms. Elam, Gee and Couch (1951b) reported that the injection of penicillin stimulated growth in the chick and that the injection of aureomycin failed to have such an effect. The microbial counts, which were made from the feces, showed that the oral or injected penicillin increased the number of yeasts and the number of penicillin and aureomycin-resistant bacteria. Aureomycin increased the number of aureomycin-resistant bacteria and yeast, but did not affect the penicillin-resistant bacteria. Elam, Gee and Couch (1951c) reported that penicillin did not increase the total number of bacteria or the number of enterococci and that there was no change in lactic acid or coliform groups. The numbers of penicillin-resistant organisms, aureomycin-resistant organisms, as well as yeasts, were increased.

On the other hand Wolterink, Ogle and Groschke (1951) reported data which indicated that the action of the antibiotics may be involved in an increased rate of absorption from the intestine. Sunde and coworkers (1951) reported data which showed that one function of antibiotics is to spare vitamin B_{12} and thus indirectly to spare the needs of methionine

and choline in the chick.

Anderson, Cunningham and Slinger (1952) found that the pH of the cecal contents of chickens was reduced when penicillin was added to rations containing different levels of protein. The reduction in pH was associated with an increase in the numbers of lactobacilli, anaerobic and microaerophilic, aerobic, and coliform types of organisms and with a reduction in the number of enterococci.

The intestinal flora of the chick has been reported to vary with the type of carbohydrate in the ration. Johansson, Sarles and Shapiro (1948) studied the effects of various carbohydrates on the microflora of the gastro-intestinal tract. They reported that dextrin-containing rations produced the greatest numbers of microorganisms in all segments of the intestinal tract, as well as the least "spread" between the different bacterial counts. The ceca were found to be the site of the greatest concentration of microorganisms. Shapiro and Sarles (1949) made microbial counts of the intestinal flora of normal chicks from hatching to maturity. They found that newly hatched chicks were relatively free of microorganisms in their intestinal tract and that there was a rapid increase in the microflora 25 hours after the intake of feed. Here again the ceca were the site of the greatest numbers of microorganisms.

Mannering, Orsini and Elvehjem (1944) found riboflavin-deficient rations to be entirely adequate for rats, if the carbohydrate components were either dextrin or starch. Their results led them to conclude that incomplete digestion of dextrin or starch allows some of the carbohydrate to reach the ceca where it can be utilized by vitamin-synthesizing bacteria. Couch and coworkers (1948) used the laying hen as an experimental animal for studying the intestinal synthesis of biotin as reflected by

the biotin content of the egg and by hatchability. They reported that dextrin favored the intestinal synthesis of biotin but that sucrose, lactose and dried whey did not favor such synthesis. Couch and coworkers (1950) concluded that the ceca were not required for the synthesis of biotin when mature hens were fed dextrins or starch as a source of carbohydrate in a biotin-low ration. They found, also, that the removal of the ceca tended to enhance the intestinal synthesis of biotin in the intestinal tract of the laying hen.

The importance of the function of the ceca in the fowl has been reviewed by Olson and Mann (1935). They concluded that the ceca may be removed or occluded with no apparent detriment to the well being of the chicken. The pH of the ceca was lower than the pH of either the ileum or the large intestine. Olson and Mann (1935) also reported that there was a selectivity of the materials which gained entrance into or were emptied from the ceca.

In view of the contradictory results cited above it seemed desirable to study the effect of penicillin and aureomycin on the microflora in the ceca of the chick. If the assumption is made that the microflora of the ceca are responsible for growth stimulation in the chick, the growth stimulation produced by the cecal microflora would be eliminated if the ceca were rendered non-functional. With this in mind, the ceca of chicks were ligated, penicillin was administered, and a study was made to determine the effects of the penicillin on growth.

EXPERIMENTAL PROCEDURE AND RESULTS

General

This experiment was conducted in the battery room on the poultry farm at the Oklahoma Agricultural Experiment Station. Day-old chicks were used in all feeding trials. The chicks were wing banded and weighed at the start of each feeding trial and at weekly intervals throughout the feeding period. The feed was removed from the feeders approximately 18 hours prior to the time the chicks were weighed. A record was kept of the quantity of feed consumed by each lot of chicks between weighings. The chicks were brooded in multi-sectioned electrically-heated battery-type brooders. Each section was equipped with raised wire floors, waterers at each end and feeders on each side.

The basal rations were formulated using the most up-to-date nutritional tables. The rations were adequate in all nutrients known to be required by the chick. The feed for each treatment was mixed one week prior to the beginning of the feeding period. The antibiotic used was added at that time. The antibiotic was first mixed with 100 cc of tap water and then mixed with a small amount of the feed. This quantity of feed was sifted to remove any lumps which may have formed and was then added to the rest of the feed in the mixer. A sample of each ration was taken and a chemical analysis was made for protein, nitrogen-free extract, water, fat, fiber, ash, calcium and phosphorus.

The weight gain data were analyzed according to the analysis of

variance of Snedecor (1946).

Experiment 1 - Changes in the Microflora of the Ceca of the Chick as Related to the Growth Stimulating Effect of Antibiotics

Methods and Materials

This feeding trial was designed to study the effects of penicillin and aureomycin, when added to a semi-purified cerelose ration, on the microflora of the ceca and on the growth of the chick.

A semi-purified basal cerelose ration rich in dextrose was selected since dextrin favors bacterial synthesis as reported by Couch and co-workers (1948). The composition of the semi-purified cerelose basal ration used is shown in Table I.

The basal ration was supplemented with crystalline penicillin G at the rate of 20 mg. (27,600 units) per pound of feed for those chicks receiving penicillin. Aureomycin was added to the basal ration at the rate of 20 mg. per pound of feed for those chicks receiving aureomycin. The chicks treated intramuscularly with penicillin received 12,500 units of aqueous procaine penicillin G intramuscularly at 48 hour intervals, beginning at one-day of age. Injections were made into the leg of the chick using a tuberculin syringe and a 24 gauge needle.

Cross-bred day-old chicks from a New Hampshire X Barred-Plymouth Rock mating were used in this feeding trial. The chicks were distributed at random among eight lots with 33 chicks in each lot. These lots were

TABLE I

SEMI-PURIFIED CERELOSE BASAL RATION*

Ingredient	Percent
Cerelose	45.4
Hi-Pro-Con[1]	41.0
T-Cake (Wheat gluten hydrolysate)	1.0
Vitamin Mixture[2]	5.0
Mineral Mixture[3]	5.0
Soybean Oil	2.5
DL-Methionine	0.1

*Analysis of Semi-Purified Ration: Protein 21.09%, N.F.E. 54.80%, Fat 5.63%, Fiber 1.09%, Moisture 11.08%, Ash 6.73%, Calcium 1.14% and Phosphorus .80%.

[1]Hi-Pro-Con A 50% protein soybean oil meal.

[2]Vitamin Mixture: (5 Pounds): Vitamin A Feeding Oil (6000 IU/gm.) 0.2 lb., Vitamin D_3 (2,000 AOAC/gm.) 0.1 lb., Niacin 1.362 gm., Menadione 0.018 gm., Riboflavin 0.454 gm., Thiamin Hydrochloride 0.227 gm., Pyridoxine 0.227 gm., Folic Acid 0.0227 gm., Biotin 0.0045 gm., Merck A.P.F. No. 3 51.0 gm., Choline Chloride 90.69 gm., Tocopherol Concentrate 0.002 gm., and Cerelose 4.39 pounds.

[3]Mineral Mixture: Calcium Carbonate 29.4%, Dicalcium Phosphate 32.6%, Potassium Phosphate 15.7%, Sodium Chloride 11.34%, Magnesium Sulfate 9.46%, Ferrous Sulfate 1.03%, Manganese Sulfate 0.54%, Potassium Iodide 0.062%, Copper Sulfate 0.028%, Zinc Chloride 0.018% and Cobalt Chloride 0.0037%.

then assigned experimental treatments as follows:

Lot No.	Treatments	Males	Females
1, 6	Basal (semi-purified) Control	17	16
2, 7	Basal - Penicillin 20 mg/lb.	17	16
3, 8	Basal - Aureomycin 20 mg/lb.	17	16
4, 5	Basal - Penicillin (intramuscularly)	19	18

Couch and coworkers (1948) had reported that the ceca were the sites of the greatest bacterial synthesis. Johansson, Sarles and Shapiro (1948) had reported that the ceca contained the greatest concentration of bacteria in the intestinal tract. They also reported that chicks fed a ration rich in dextrose had less spread between different bacterial counts. With these reports in mind the ceca were selected as the sections of the intestinal tract in which to study the microflora. Bacterial counts were made at one, two and four week intervals to see if there was a gross change in the bacterial groups in the ceca.

A composite sample was taken from the ceca of three chicks from those lots which had received penicillin and aureomycin in the ration when the chicks were one week, two weeks and four weeks of age. The chicks treated intramuscularly had samples taken only at four weeks of age.

Three chicks from each lot were sacrificed for sampling. The following technique was employed in removing the ceca. The chick was placed on its right side and an incision was made in the left side with a pair of scissors. The ceca were then grasped with a pair of thumb forceps near the junction of the intestine. They were severed with a pair of scissors and lifted out by tearing the mesentery. The distal end of the ceca was removed with a pair of scissors and the contents expelled into a sterile petri dish. This was accomplished by winding the ceca around the thumb

forceps which were held against the lid of the petri dish. The cecal contents of the three chicks were pooled and the sample was thoroughly mixed with a spatula, which had been cleaned and flamed between samples. A one-half gram sample was taken for dilution and culture.

The media and culture procedures were essentially the same as those outlined by Shapiro and Sarles (1949). The one-half gram sample was placed in 49.5 ml. of sterile tap water in a six ounce dilution bottle which contained a layer of glass beads. These initial 1:100 dilutions were shaken vigorously until thoroughly mixed. Then serial decimal dilutions were made in sterile tap water up to $1:10^{-10}$ dilution and inoculations were made into the following media: (1) Tryptone agar (Johansson, Sarles and Shapiro-1948) for aerobic plate count, (2) "Thioglycolate medium" (Baltimore Biological Laboratories) with agar and glucose added, in shake-tubes for an indication of numbers of anaerobic bacteria, (3) Carrot-Liver Extract (CL) Agar (Garey, Foster and Frazier - 1941) in plates for an indication of numbers of lactic acid bacteria, (4) "S-F" broth of Hajna and Perry (1943) for dilution counts of enterococci, and (5) Difco Desoxycholate Agar plates for the enumeration of coliform bacteria.

Cultures prepared with Desoxycholate, Tryptone Agar, C-L Agar and "Thioglycolate medium" were incubated at 37°C for 24 hours before counts were made. Those prepared with "S-F" broth were incubated at 45°C for 72 hours after which enumerations were made. Five tubes per dilution were used for dilution counts, and the most probable numbers of organisms were determined from the (MPN) tables in Standard Methods for the Examination of Water and Sewage (1946).

Results

The pertinent data on growth stimulation and cumulative efficiency of feed utilization made by the chicks fed a semi-purified cerelose ration supplemented with penicillin or aureomycin are presented in Tables II and III.

TABLE II

AVERAGE GRAMS OF GAIN BY MALES AND FEMALES FED THE SEMI-PURIFIED CERELOSE BASAL RATION AND THE BASAL RATION SUPPLEMENTED WITH PENICILLIN OR AUREOMYCIN IN EXPERIMENT I

TREATMENT	BASAL		PEN.20mg/lb.		AUREO.20mg/lb.		PEN.(IM)	
Lot No.	1	6	2	7	3	8	4	5
TWO WEEKS								
Av. Male Gain	122.5	119.9	120.1	115.0	122.7	110.8	115.5	124.7
Av. Female Gain	101.8	88.4	108.7	108.2	121.3	113.8	113.1	118.0
Lot Mean	111.9	103.9	114.1	111.5	122.0	112.3	114.5	121.3
Treatment Mean	107.7		113.0		117.2		116.5	
FOUR WEEKS								
Av. Male Gain	316.2	338.4	338.4	389.4	347.9	326.0	378.7	406.0
Av. Female Gain	291.3	275.0	306.0	335.4	319.1	312.8	343.7	350.9
Lot Mean	303.9	306.6	325.1	361.2	334.0	319.4	362.4	375.0
Treatment Mean	305.3		342.1		326.9		368.9	

A comparative examination of the data indicates that both penicillin and aureomycin stimulated chick growth and that penicillin greatly improved feed efficiency. Examination of Table IV, analysis of variance, indicates that no significant difference existed between the antibiotic treated chicks and the control chicks at two weeks of age. However, at four weeks of age the chicks fed penicillin or aureomycin made gains that were significantly greater than the controls.

Chicks receiving procaine penicillin G injected intramuscularly made

TABLE III

AVERAGE CUMULATIVE FEED EFFICIENCIES AND ANTIBIOTIC CONSUMPTION

OF NORMAL CHICKS IN EXPERIMENT I

Lot No.	Treatment	Av. Per Chick	One Week	Two Weeks	Three Weeks	Four Weeks	Av. Chick Wt. (Lbs.) (4 wks.)	Lbs. Feed Per Lb. Gain (4 wks.)
1, 6	Basal - Control	Feed[1]	0.136	0.403	0.850	1.371	0.678	2.012
2, 7	Basal - Pen.20mg/lb.	Feed	0.139	0.405	0.862	1.425		
		Antibiotic[2]	3,836	11,178	23,791	39,330	0.753	1.892
3, 8	Basal - Aureo.20mg/lb.	Feed	0.143	0.421	0.872	1.491		
		Antibiotic[3]	2.86	8.42	17.44	29.82	0.718	2.049
4, 5	Basal - Pen. IM.	Feed	0.168	0.465	0.971	1.524		
		Antibiotic[4]	50,000	87,500	137,500	175,000	0.808	1.881

[1]Feed weights in pounds.
[2]Average units of crystalline penicillin G consumed per chick.
[3]Average milligrams of aureomycin consumed per chick.
[4]Units of procaine penicillin G administered intramuscularly per chick.

the greatest gains and made the most efficient use of the feed consumed. The chicks which received the basal ration supplemented with penicillin made greater gains and had better feed utilization than did the control chicks and the chicks fed aureomycin in the ration. The chicks which received the basal ration supplemented with aureomycin made greater gains than did the controls, but they required 0.037 pounds more feed to produce a pound of gain than did the control chicks. They also required 0.157 pounds more feed to produce a pound of gain than the chicks receiving the penicillin supplemented rations.

TABLE IV

ANALYSIS OF VARIANCE - EXPERIMENT I

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F
TWO WEEK GAINS of 206 Chicks:				
Total	205	135,946	--	--
Treatments	3	3,456	1,152	1.86
Basal vs Antibiotics	1	2,737	2,737	--
Aureomycin vs Penicillin	1	203	203	--
Penicillin Oral vs Penicillin IM	1	516	516	--
Lots in Treatments	4	2,859	715	1.15
Sex in Lots in Treatment	8	11,852	1,482	2.39*
Error	190	117,779	620	--
FOUR WEEK GAINS of 184 Chicks:				
Total	183	489,278	--	--
Treatments	3	85,584	28,528	15.80**
Basal vs Antibiotics	1	--	51,286	28.40**
Aureomycin vs Penicillin	1	--	20,618	11.42**
Penicillin Oral vs Penicillin IM	1	--	13,680	7.57**
Lots in Treatments	4	20,180	5,045	2.79*
Sex in Lots in Treatment	8	80,152	10,019	5.55**
Error	168	303,362	1,806	--

*Significant difference at the 5% level.
**Significant difference at the 1% level.

The enumerative data on the bacteriological analysis of the cecal feces of the chicks are presented in Table V. The microbial counts are

TABLE V

SUMMARY OF MICROBIAL COUNTS PER GRAM OF CECAL FECES IN EXPERIMENT I

Lot No.	Treatment	Bacterial Groups		
		Coliform (10^8)	Lactic (10^{10})	Enterococci (10^4)
	AGE 1 WEEK			
1, 6	Basal - Control	14,200	188	500
2, 7	Basal - Penicillin 20 mg/lb.	14,000	200	410
3, 8	Basal - Aureomycin 20 mg/lb.	8,000	392	127,000
	AGE 2 WEEKS			
1, 6	Basal - Control	204	156	240
2, 7	Basal - Penicillin 20 mg/lb.	236	132	102
3, 8	Basal - Aureomycin 20 mg/lb.	220	290	63,000
	AGE 4 WEEKS			
1, 6	Basal - Control	144	126	94
2, 7	Basal - Penicillin 20 mg/lb.	156	152	92
4, 5	Basal - Penicillin (IM)	144	158	154
3, 8	Basal - Aureomycin 20 mg/lb.	216	344	63,000

expressed as the average number of bacteria per gram of cecal feces on a wet weight basis. Note the similarity in the bacterial counts of chicks supplemented with penicillin and the control chicks fed the basal ration. Differences in the microbial counts in cecal feces between penicillin and aureomycin appeared most prominent at one week of age. The aureomycin appeared to depress the coliform group and favored the lactic acid and enterococci groups. Since this study on the microflora of the ceca was designed only for gross changes in the microflora, no further study was made.

Experiment 2 - Growth Stimulating Effect of Penicillin Fed to Chicks with Ligated Ceca

Methods and Materials

This feeding trial was designed to study (1) the growth stimulating effect of penicillin when added to a practical corn-soybean oil meal broiler ration and (2) the growth effect of penicillin injected intramuscularly into chicks with normal and ligated ceca.

Cross-bred day-old chicks from a Silver Oklabar X New Hampshire mating were used in this feeding trial. The chicks were distributed at random into seven lots as follows:

Lot No.	Treatment	
15	Basal (corn-soybean) Control	normal ceca
16	Basal - Penicillin 20 mg/lb.	normal ceca
17	Basal - Penicillin I.M.	normal ceca
18	Basal - Penicillin I.M.	normal ceca
19	Basal	ligated ceca
20	Basal - Penicillin 20 mg/lb.	ligated ceca
21	Basal - Penicillin I.M.	ligated ceca

The chicks which received penicillin in the feed were fed the basal corn-soybean oil meal ration supplemented with 20 mg. (27,600 units) of crystalline penicillin G per pound of feed. The chicks which received penicillin intramuscularly received 12,500 units of procaine penicillin G in sesame oil at 48 hour intervals beginning at one day of age. Injections were made into the leg of the chick using a tuberculin syringe and a 24 gauge needle. Chicks in Lot 18 received a single injection at three days of age to test the residual effect of a single injection of

procaine penicillin G. The formula of the corn-soybean oil meal basal ration is presented in Table VI.

TABLE VI

PRACTICAL CORN-SOYBEAN OIL MEAL BROILER RATION*

Ground yellow corn	55.0 lb.
Pulverized oats	5.0 lb.
Corn gluten meal	5.0 lb.
Fish meal (60% protein)	5.0 lb.
Soybean oil meal (44% protein)	25.0 lb.
Calcium carbonate	1.0 lb.
Steamed bonemeal	2.0 lb.
Merck A P F No. 3	0.2 lb.
Viadex[1]	0.2 lb.
Riboflavin mix[2]	4 gm.
Choline chloride	32 gm.
Calcium pantothenate	0.3 gm.
Manganese sulfate	6.5 gm.

*Analysis of the Practical Corn-Soybean Oil Meal Ration: Protein 23.16%, N.F.E. 52.02%, Fat 2.65%, Fiber 3.11%, Moisture 10.89%, Ash 8.34%, Calcium 1.43% and Phosphorus 0.82%.

[1]Viadex 400(AOAC) D and 2000(IU) A per gram.

[2]Riboflavin 1 gm./ounce.

The surgical technique for cecal ligation has been described for fowls by Durant (1926) and for turkeys, with special reference to the control of blackhead, by Durant (1930). The feed was removed from the feeders 36 hours prior to the ligation of the ceca. The chicks were five days old when this was done. Cecal ligation was performed on the chicks in lots 19, 20 and 21. The down was plucked from the left side in the flank region. The chick was then placed on its right side and was restrained by an assistant. A vertical incision approximately one-fourth inch in length was made, posterior to the last rib and ventral to the border of the ilium. This incision was made through the skin, muscle and

peritoneum. A small wound spreader was inserted through the incision to retract the edges of the incision. With a pair of thumb forceps the ceca were grasped near the junction of the small intestine and the ceca were lifted through the incision. A ligature was placed at the junction with the small intestine using a taper point needle and cotton suture (#40 cotton thread). The wound spreader was then removed and the edges of the incision were united with a single cotton suture. Occasionally a wind-puff formed. To relieve this condition a small incision was made in the skin and the air allowed to escape. This procedure was repeated if the wind-puff reappeared.

At the end of the five week feeding period the chicks were slaughtered, examined for functioning ceca and sex was determined. The data of those chicks which had one or both ceca functioning were not included in the calculations.

Results

The pertinent data on growth stimulation and cumulative efficiency of feed utilization made by chicks, with normal and ligated ceca, fed a practical corn-soybean oil meal ration supplemented with penicillin are presented in Tables VII and VIII.

A comparative examination of the data shown in Table VII indicates that when penicillin was added to the ration, or penicillin was injected intramuscularly, the growth rate was stimulated and feed efficiency was improved in chicks with normal and ligated ceca. Those chicks with normal and ligated ceca that were fed penicillin in the basal ration made the greatest gain in body weight and the most efficient use of the feed consumed. The surgery necessary for cecal ligation suppressed the growth

rate slightly. With penicillin in the ration, chicks with ligated ceca made greater gains and utilized feed more efficiently than did chicks with normal ceca fed the basal ration. Examination of Table IX, analysis of variance, indicates that a significant growth stimulation effected by penicillin occurred as early as two weeks of age in chicks with normal and ligated ceca. A single injection of 12,500 units of penicillin at three days of age had no residual effect on the growth rate or feed efficiency in chicks. The results reported here indicate that the ceca are not essential for the growth promoting action of penicillin.

TABLE VII

AVERAGE CUMULATIVE FEED EFFICIENCIES AND ANTIBIOTIC CONSUMPTION OF CHICKS WITH NORMAL AND LIGATED CECA IN EXPERIMENT 2

Lot No.	Treatment	Number of Chicks	Av. Per Chick	One Week	Two Weeks	Three Weeks	Four Weeks	Five Weeks	Av. Chick Wt. (Lbs.) (5 wks.)	Lbs. Feed Per Lb. Gain (5 wks.)
15	Basal - Control (normal ceca)	23	Feed[1]	0.141	0.380	0.836	1.314	2.075	0.929	2.233
16	Basal - Pen.20mg/lb. (normal ceca)	29	Feed	0.142	0.418	0.905	1.398	2.195		
			Units	3,919	11,538	24,978	38,585	60,582	1.025	2.122
17	Basal - Pen. IM* (normal ceca)	29	Feed	0.139	0.381	0.840	1.361	2.202		
			Units	50,000	87,500	137,500	175,000	225,000	1.009	2.182
18	Basal - Pen. IM** (normal ceca)	13	Feed	0.142	0.403	0.918	1.379	2.194		
			Units	12,500	—	—	—	—	0.982	2.234
19	Basal (ligated ceca)	16	Feed	0.119	0.328	0.739	1.192	2.168	0.844	2.568
20	Basal - Pen.20mg/lb. (ligated ceca)	20	Feed	0.131	0.391	0.880	1.383	2.204		
			Units	3,616	10,792	24,288	38,171	60,830	1.024	2.152
21	Basal - Pen. IM* (ligated ceca)	20	Feed	0.132	0.371	0.852	1.329	2.079		
			Units	50,000	87,500	137,500	175,000	225,000	0.934	2.225

*Received 12,500 units of procaine penicillin G in oil every 48 hours during the feeding period.
**Received only one injection of 12,500 units of procaine penicillin G in oil.

[1]Feed weights in pounds.

TABLE VIII

AVERAGE GRAMS OF GAIN BY MALES AND FEMALES WITH NORMAL AND LIGATED CECA

WHEN FED A PRACTICAL CORN-SOYBEAN OIL MEAL RATION IN EXPERIMENT 2

TREATMENT	Basal	Pen.20mg/lb.	Pen.IM*	Pen.IM**	Basal	Pen.20mg/lb.	Pen.IM*
Lot No.	15	16	17	18	19	20	21
CECA	Normal	Normal	Normal	Normal	Ligated	Ligated	Ligated
No. Chicks	23	29	29	13	16	20	21
TWO WEEKS							
Av. Male	94.6	105.0	103.3	95.9	81.3	101.6	98.1
Av. Female	85.5	94.1	98.9	101.6	86.7	102.9	89.5
Lot Mean	90.7	100.5	101.2	98.1	83.7	101.9	94.5
FIVE WEEKS							
Av. Male	450.5	490.1	482.1	450.4	391.1	472.5	460.7
Av. Female	384.5	420.8	429.6	437.8	352.3	452.6	391.9
Lot Mean	421.6	465.5	456.7	445.5	374.1	464.9	429.8

*Received 12,500 units of procaine penicillin G in oil every 48 hours during the feeding period.
**Received only one injection of 12,500 units of procaine penicillin G in oil.

TABLE IX

ANALYSIS OF VARIANCE - EXPERIMENT 2

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F
TWO-WEEK GAINS OF 150 Chicks:				
Total	149	30,271	---	---
Treatments	6	5,477	913	5.37**
(18) vs (15,16,17,19,20,21)	1	---	25	---
(15,16,17) vs (19,20,21)	1	---	569	3.35
(15) vs (16,17)	1	---	1,882	11.07**
(16) vs (17)	1	---	2	---
(19) vs (20,21)	1	---	2,456	14.45**
(20) vs (21)	1	---	544	3.20
Sex within Treatments	7	1,670	239	1.41
Error	136	23,124	170	---
FIVE-WEEK GAINS OF 150 Chicks:				
Total	149	527,215	---	---
Treatments	6	119,410	19,902	9.39**
(18) vs (15,16,17,19,20,21)	1	---	299	---
(15,16,17) vs (19,20,21)	1	---	17,507	8.26**
(15) vs (16,17)	1	---	25,428	11.99**
(16) vs (17)	1	---	1,121	---
(19) vs (20,21)	1	---	12,660	5.97*
(20) vs (21)	1	---	62,395	29.43**
Sex within Treatments	7	117,303	16,758	7.90**
Error	136	290,502	2,120	---

*Significant difference at 5% level.
**Significant difference at the 1% level.

DISCUSSION OF RESULTS

The growth stimulating effect of antibiotics has been generally accepted. Presumably the effect is due to the action of antibiotics on the intestinal microflora. In turn these changes are responsible for the increased growth rate and nutritional efficiency in chicks. This theory of the mode of action of antibiotics was based upon the findings of Moore and coworkers (1946) who first observed that streptomycin, sulfasuxidine or a combination of streptomycin and sulfasuxidine stimulated growth, but failed to sterilize the intestinal tract of chicks. However, the treatments did produce a marked reduction in the coliform bacteria of the cecal feces. Elam, Gee and Couch (1951b) found that the oral or parenteral administration of penicillin effected growth response, but that aureomycin administered parenterally did not stimulate growth in the chick. These workers also reported changes in the intestinal microflora as a result of the injection of penicillin or aureomycin. In a later report Elam, Gee and Couch (1951c) found that the injection of penicillin or bacitracin had no effect on the intestinal microflora, although growth was stimulated. Data presented herein have shown that penicillin, given orally or injected intramuscularly, stimulated growth and had no effect on the microflora of the ceca. Aureomycin added to the ration stimulated chick growth and depressed the growth of coliforms, but favored the growth of lactic acid and enterococci types of bacteria.

It is apparent that growth stimulation can be obtained by adding antibiotics to the ration, at least under most conditions, with or without

changes in the intestinal microflora. If the assumption is made that the growth effect from some or all antibiotics is due to changes brought about in the microflora of the ceca, it could be argued that such response would not occur if the ceca were rendered non-functional. Data reported in Table III and VII have shown that penicillin administered orally and parenterally to chicks with non-functional ceca produced a significant growth response.

Elam, Gee and Couch (1951b) reported an increased growth rate from the parenteral administration of penicillin, bacitracin or inactivated penicillin without change in the fecal microflora. From their data they proposed that the antibiotic might act as a metabolite within the body of the chick.

Anderson, Slinger and Pepper (1952) reported that the feeding of certain microorganisms, particularly E coli isolated from the ceca of chicks, caused an increased growth. Romoser, Shorb and Combs (1952) also reported that penicillin caused a change in the cecal microflora from a predominate lactobacilli type to a predominate coliform type. Viable coliform organisms, E coli and Aerobacter aerogenes, when isolated from the ceca and fed to chicks, stimulated growth both with and without the addition of an antibiotic to the ration.

Slinger and coworkers (1951) reported that aureomycin and penicillin enhanced the utilization of protein but did not lower the protein requirement. Norris (1952) indicated that antibiotics might influence certain biochemical systems so that an increased synthesis of essential nutrients occurred in the gastrointestinal tract.

The possibility exists that the injected antibiotics may be excreted into the intestinal tract either through the bile or urine. It is also

possible for very minute amounts to be found in the secretion of the glands of the intestinal mucosa. However, it is unlikely that these very minute amounts could change the microflora of the intestinal tract.

It is apparent that under certain conditions growth responses can be obtained from antibiotics without change in the microflora of the ceca. The stability of penicillin is a very important factor, overlooked by many workers who have concluded that the growth response effected by penicillin was due to a change in the intestinal microflora. Berk, Shepard and Glasser (1947) reported that pure crystalline penicillin when kept dry retained its antibiotic potency for limited periods, but was rapidly inactivated by small amounts of water. McDermott and coworkers (1946) reported that about one-third of orally administered penicillin given to man was absorbed. The absorption occurred chiefly from the duodenum. The remaining two-thirds was not absorbed, presumably because it had passed beyond the absorbing area. The unabsorbed penicillin was either destroyed by the intestinal bacteria or small amounts escaped in the feces. A small amount was inactivated by the gastric acidity.

The crystalline penicillin used in this experiment was first mixed in water and then mixed into the ration, which was prepared one week prior to each feeding trial. During this period some of the penicillin may have lost its antibiotic activity. Another possibility that has not been considered is that fact the *E coli* produces an enzyme "penicillinase", which destroys the antibiotic activity of penicillin in the gastrointestinal tract as reported by Abraham and Chain (1940). Together with the knowledge of the small amount of penicillin consumed per chick, as shown in Tables III and VII, growth effected by penicillin appears to result from causes other than changes in the microflora of the ceca.

SUMMARY AND CONCLUSIONS

A significant growth response was produced by chicks when small quantities of certain antibiotics were added to the ration. Equal growth response was obtained when penicillin was injected intramuscularly.

The character of the microflora of the ceca of chicks was not altered from that of the control chicks when penicillin was fed or injected intramuscularly. When aureomycin was added to the ration the coliform group of bacteria decreased and the lactic acid and enterococci groups of bacteria increased during the first week.

Functional ceca are not essential for antibiotics to produce their effects on the nutrition and the growth in the chick.

The mechanism by which the antibiotics produce their effects on nutrition and growth appears to be as a metabolite rather than intestinal bacterial synthesis of nutrients.

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