THE MODE OF ACTION OF ANTIBIOTICS IN STIMULATING GROWTH OF CHICKS

BY H. EYSSEN, M.D., AND P. DE SOMER, M.D.

(From the Department of Microbiology, Rega Institute for Medical Research, University of Louvain, Louvain, Belgium)

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Despite the widespread use of antibiotics as growth agents in young animals, the mechanisms underlying their mode of action are poorly understood. It is generally accepted, however, that the growth-stimulating effect is mediated by the activity of the antibiotics against harmful intestinal bacteria.

Many antibiotics, such as the tetracyclines, bacitracin, penicillin, oleandomycin, and virginiamycin, which differ widely in their chemical and physical properties, are effective growth agents (1–5). The only known property which these substances have in common is their antibacterial action against Gram-positive microorganisms. Furthermore, bacitracin is a potent growth agent when given by mouth, although it is not absorbed from the intestines (6). The activity of oral bacitracin indicates that the site of action of the antibiotics is primarily restricted to Gram-positive intestinal bacteria.

The mode of action by which inhibition of the Gram-positive intestinal bacteria accelerates animal growth remains largely unknown, although several hypotheses have been proposed. A vitamin-sparing effect has been suggested (7–11). Production of toxic substances by the gut flora is another possible explanation (12, 13). One striking feature, however, is the increased efficiency of feed utilization during antibiotic treatment. It has also been shown that the small intestines of animals given antibiotics are thinner than those of controls and are similar to the intestines of germ-free animals (14). These findings support the hypothesis that one or more intestinal bacteria cause a thickening of the intestinal wall and, perhaps, consequent interference with absorption of nutrients. In this event the vitamin-sparing action of antibiotics would also be explained.

A review of the many experiments carried out to check this hypothesis has been presented by Jukes (15) and by Luckey (16). In different studies, conflicting data on the absorption of amino acids, vitamins, and minerals were reported. These are probably due to the irregular and usually low growth responses, ranging from 0 to 10 per cent, when antibiotics are added to practical type diets. In previous work from this laboratory on the growth-promoting effect of virginiamycin in chicks, a purified casein-sucrose diet according to Stokstad *et al.* (17) was used, and a growth response of 20 to 30 per cent was obtained regularly in short term laboratory experiments over a 2 week period

(5). Therefore this diet was chosen in our study for establishing the extent to which antibiotics promote the absorption of nutrients and the efficiency of feed utilization. In these experiments the influence of virginiamycin on the fecal excretion of fats and carbohydrates was studied in chicks under different environmental and dietary conditions.

TABLE I

Composition of Diet

	Gm.
Casein (crude)	20
Gelatin	8
Sucrose	58.5
Calcium triphosphate	2
Calcium gluconate	5
Salt mixture*	2
Vitamins in glucose‡	1
Corn oil and vitamin A, D, E§	3
DL-methionine	0.15
L-cystine	0.15
Choline chloride	0.2

* 600 mg sodium chloride; 600 mg dipotassium phosphate; 450 mg monopotassium phosphate; 250 mg magnesium sulfate monohydrate; 40 mg manganese sulfate; 50 mg ferric citrate; 2 mg cupric sulfate; 0.6 mg potassium iodide; 0.8 mg potassium bromide; 1.4 mg zinc acetate; 1.6 mg aluminium sulfate; 0.4 mg cobalt acetate tetrahydrate; 0.2 mg nickel sulfate; 0.05 mg sodium molybdate. Water to dissolve trace minerals up to 2 gm.

‡ 1 mg thiamine hydrochloride; 1 mg riboflavin; 5 mg niacinamide; 1 mg pyridoxine; 5 mg calcium pantothenate; 0.5 mg pteroyl-glutamic acid; 0.5 mg vitamin K; 0.02 mg biotin; 5 μg vitamin B_{12}; 100 mg inositol; glucose up to 1 gm.

§ 1,500 u vitamin A acetate; 200 u vitamin D_3; 10 mg vitamin E acetate; corn oil up to 3 gm.

Materials and Methods

All animals were 1-day-old male broiler chicks obtained from a commercial poultry farm. They were divided into lots of 20 to 25 chicks each and the group weights equalized. Experiments were carried out in electrically heated conventional batteries with raised wire screen floors under continuous illumination, feed and water being supplied *ad libitum*.

In the conventional animal rooms, referred to as "old quarters," the batteries were washed with water prior to introduction of the chicks. Dropping trays were removed and scrubbed every day. Water troughs were rinsed and refilled daily throughout the experiment.

As antibiotics do not stimulate growth of chicks in "new quarters," (18–21), some experiments were run in disinfected rooms never previously occupied by chicks. Conventional batteries were scrubbed with a 1 per cent benzethonium chloride solution (hyamin 1622, Rohm & Haas Company, Philadelphia), after which the room and the batteries were sterilized by formaldehyde gas for 3 days. After ventilation for another 3 days the animals were introduced. Feed and water troughs were washed daily and steam-sterilized at 120°C. Floors and other removable parts of the cages were replaced daily and were washed and disinfected by immersion in a 1 per cent benzethonium chloride solution for 24 hours at 60°C before reuse.

The composition of the casein-sucrose diet is given in Table I. This diet largely corresponds to diet 3 described by Stokstad *et al.* (17). In the starch diet sucrose was replaced by cornstarch. The growth-stimulating antibiotic used in these studies was virginiamycin at a concentration of 20 parts per million (ppm). This antibiotic is active only against Gram-positive bacteria. Its growth-promoting effect has been described previously (4, 5).

Feces used for fatty acid determination were collected on glass plates placed underneath the wire floors. The plates were removed every 24 hours and the feces dried under reduced pressure over P_2O_5. Fatty acid determinations were made by the method of Van de Kamer *et al.* (22); the results are expressed in milligrams fatty acids per gram dry feces.

For sugar determination, freshly voided feces were collected in ice-cooled tubes to reduce bacterial fermentation to a minimum. Determinations were made with the anthrone reagent according to Roe (23). Control experiments carried out with Nelson's modification of the Somogyi method yielded values in the same range (24).

An intestinal tracer, chromic oxide (Cr_2O_3), was added to the diet in a concentration of 2 gm per kg feed. Fecal chromic oxide was determined by the method described by Edwards and Gillis (25).

RESULTS

Influence of Virginiamycin on the Daily Body Weight Gain.—In order to ensure that absorption studies were made at the time of maximal antibiotic effect, preliminary experiments were conducted, in which the antibiotic effect was studied from day to day up to the age of 2 weeks.

In each experiment one group of 24 chicks was fed the basal diet to which had been added virginiamycin at the rate of 20 mg/kg, while the control group received the basal diet only. Fig. 1 shows a typical experiment. In the animals fed the sucrose diet, the difference in body weight becomes evident on the 6th day and increases progressively until the 14th day. Virginiamycin is less effective on a starch diet. In Fig. 2 the growth of the same series of chickens is expressed as daily weight gain during the same period. In the birds fed the antibiotic-free sucrose diet, a sudden depression of weight gain is observed after the 5th day. Weight gain remains low for 3 days and thereafter tends to become normal. Little or no growth depression is seen in the antibiotic-treated animals. The period of poor weight increase is also less obvious when a cornstarch diet is used.

The therapeutic effect of 20 ppm virginiamycin indicated that the period of depressed growth in the birds fed the sucrose diet must be ascribed to the development of an infectious condition caused by Gram-positive microorganisms. Further proof of this was obtained from experiments in new quarters, (Fig. 3). The period of poor weight gain was not seen in experiments using the sucrose diet in disinfected rooms, nor was virginiamycin effective in stimulating growth. It was possible, however, to contaminate these birds by feeding fresh feces from control chickens reared in the conventional old quarters. An amount of 50 mg of feces per bird, on 2 consecutive days, resulted in a significant growth depression. The body weight data are summarized in Table II.

It appears, therefore, that the poor growth of chicks fed the basal sucrose diet in old quarters is due not to a direct influence of the carbohydrate itself,

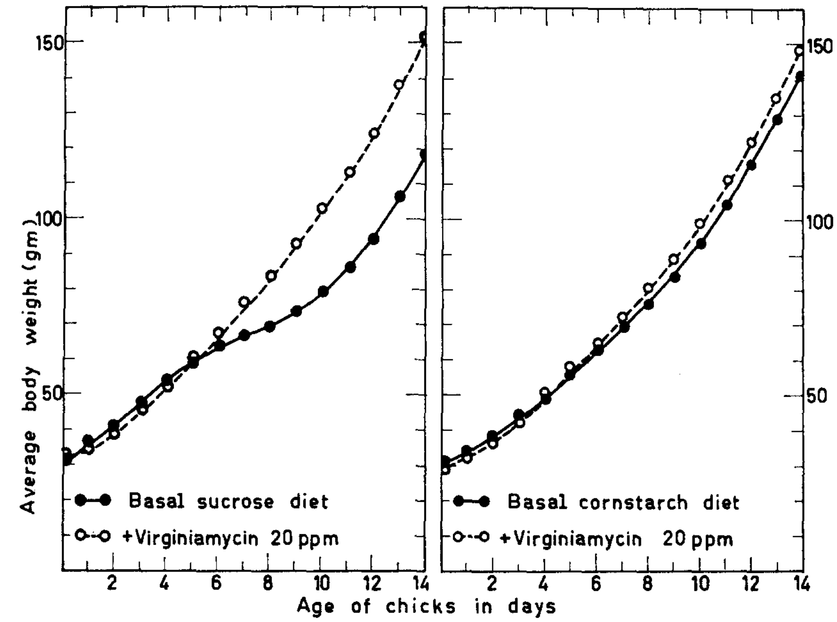

FIG. 1. Effect of virginiamycin on growth of chicks fed either a casein-sucrose or a casein-starch diet.

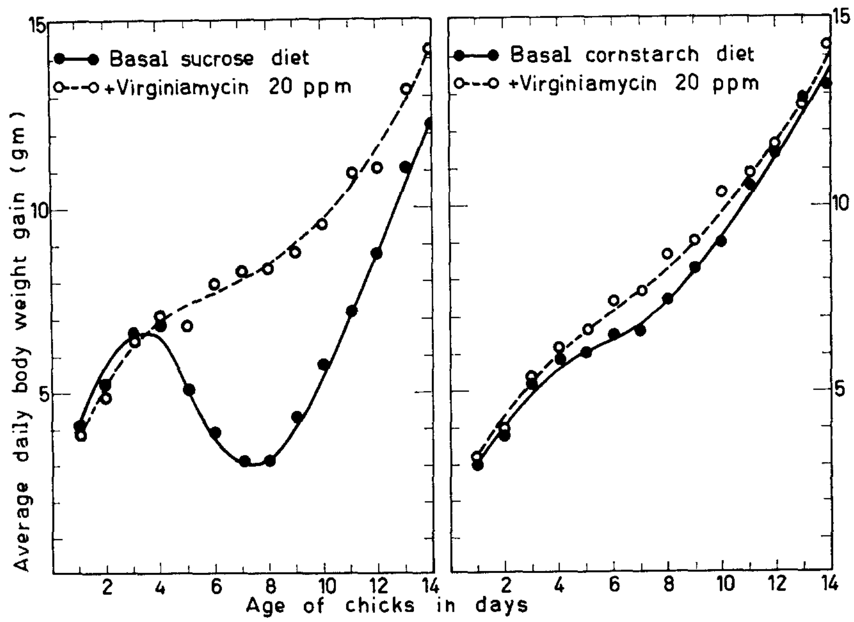

FIG. 2. Effect of virginiamycin on the daily weight gain of chicks reared in "old quarters."

but to an indirect effect depending on the presence of a growth-depressing bacterial population. In absence of this flora, virginiamycin is no longer effective in improving growth.

Influence of Virginiamycin on Efficiency of Feed Utilization and on Intestinal Absorption.—Further experiments were conducted to determine whether the

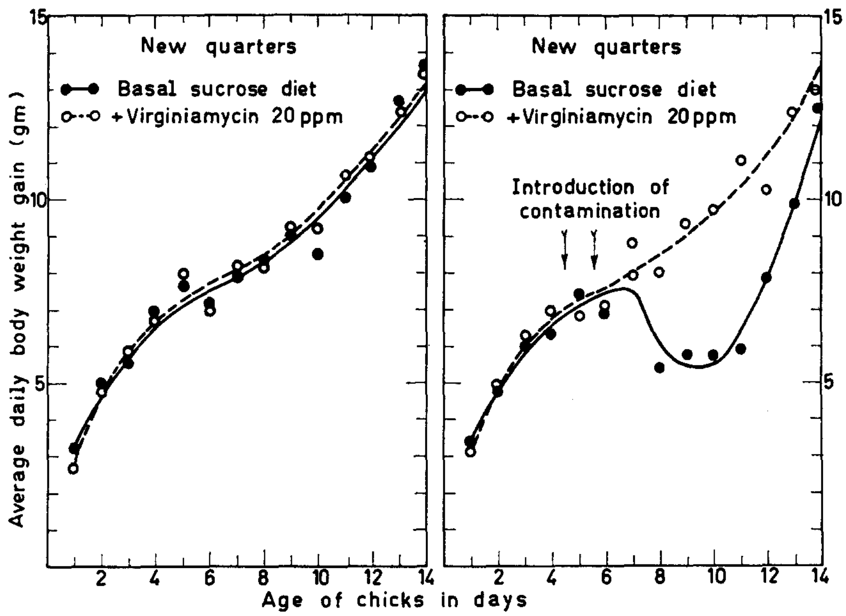

FIG. 3. Effect of virginiamycin on the daily weight gain of chicks fed a casein-sucrose diet in new quarters. Left figure: before introduction of the growth-depressing flora. Right figure: after contamination by feces from birds reared in "old quarters."

period of poor growth was due to a lack of appetite and low feed consumption or to impaired efficiency of feed utilization. In most experiments there was no difference in daily feed consumption between control chickens and those on the virginiamycin-supplemented rations. In other series the difference at the time of minimal weight gain was only 5 to 10 per cent.

An example of evolution of the feed efficiency is presented in Fig. 4. In the antibiotic-fed group and in the control animals, the gram feed intake per gram weight gain ratio is the same during the first 5 days of the experiment with the sucrose diet. From the 6th to the 9th day, however, during the period of poor growth, the feed conversion was profoundly disturbed, but only in the control group. Values as high as 3.0 to 3.5 gm feed per gm weight gain were observed. When a starch diet was fed, only a small, but consistent, difference was found between control and antibiotic fed animals.

TABLE II

Influence of Environment and Type of Dietary Carbohydrate on the Response of Chicks to Virginiamycin

Environment	Diet	No. of animals	Average body weight			Weight increase over basal
			Start	1 wk.	2 wk.	
			gm	*gm*	*gm*	*per cent*
Old quarters	Basal starch	20	31	73	142	—
	+ virginiamycin 20 ppm	20	31	72	149	+6*
Old quarters	Basal sucrose	24	31	67	119	—
	+ virginiamycin 20 ppm	24	31	76	152	+37
New quarters	Basal sucrose	24	31	74	148	—
	+ virginiamycin 20 ppm	24	31	75	150	+2*
New quarters contaminated‡	Basal sucrose	24	31	74	127	—
	+ virginiamycin 20 ppm	24	31	76	148	+22

* not significantly different at 0.05 level of probability ($p > 0.05$).
‡ 50 mg feces from birds in old quarters per chick on the 4th and the 5th day.

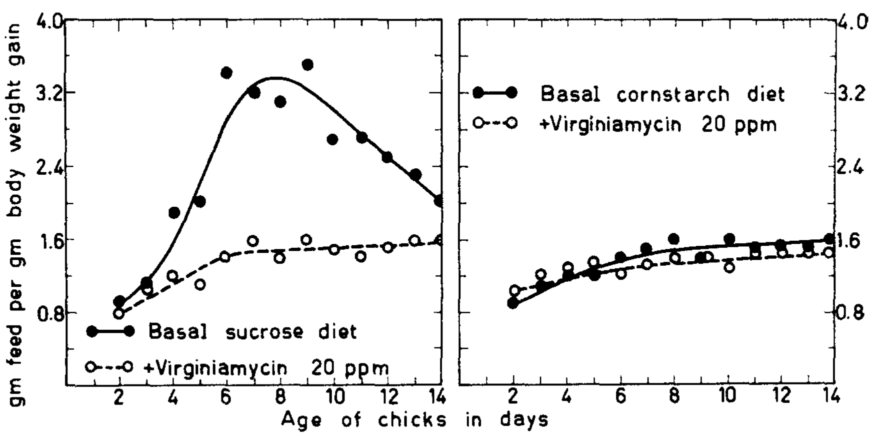

FIG. 4. Effect of virginiamycin and of the type of dietary carbohydrate on the feed conversion of chicks reared in conventional animal rooms.

In parallel experiments with the sucrose diet in clean quarters the feed efficiency values did not deviate from the 1.2 to 1.5 range in the control animals and were not further improved by virginiamycin.

The temporary growth depression associated with disturbance of the feed efficiency might be explained by a malabsorption syndrome from bacterial origin. Therefore, the fecal excretion of lipids and carbohydrates was investigated.

Fig. 5 shows that chicks receiving a sucrose diet without antibiotics lost from 80 to 105 mg of fatty acids per gm dry feces, daily, from the 6th to the 9th day of the experiment. Subsequently the absorption showed progressive improvement. Virginiamycin reduced this steatorrhea to 40 mg/gm but did not completely normalize the absorption. Gas-liquid chromatography of the

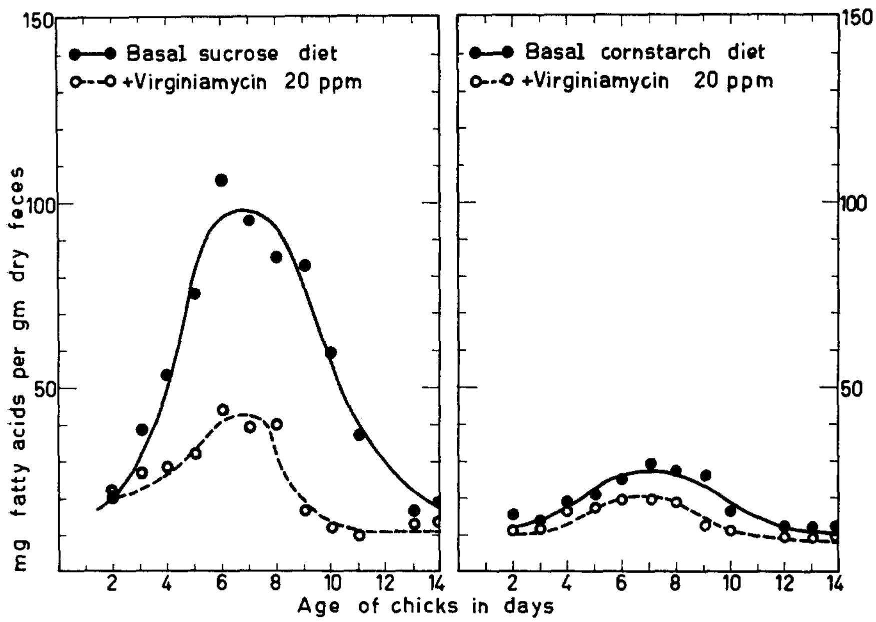

FIG. 5. Effect of virginiamycin on the fecal fat excretion of chicks fed a casein-sucrose or a casein-starch diet.

fecal fatty acids revealed the typical fatty acid spectrum of the dietary corn oil, indicating the fecal fat to be of alimentary origin. On a starch diet, a maximum of fat excretion also occurred on the 8th day, but did not exceed 30 mg/gm feces.

In preliminary investigations on fecal carbohydrate excretion no significant influence of virginiamycin was seen if results were expressed as milligrams of carbohydrate per gram dry feces. Thereafter, chromic oxide, a non-absorbable tracer, was added to the diet at a rate of 2 gm per kg and values were expressed as milligrams of soluble fecal carbohydrate per gram feed consumption. These experiments showed that the control animals during the period of growth depression also lost twice as much carbohydrate as did the virginiamycin-fed birds.

These results seemed to indicate a polymalabsorption syndrome. The addition of chromic oxide to the diet permitted complete balance studies. From these investigations it became evident that the period of poor growth and steatorrhea coincides with a period of increased output of fecal material. Fig. 6 illustrates these data. At the 8th day the control chicks produced 300 mg feces per gm feed consumption, as compared to 175 mg by the virginiamycin-treated birds. As there are virtually no unabsorbable constituents in the diet, the quantity of feces is correlated with the total loss of nutrients either directly

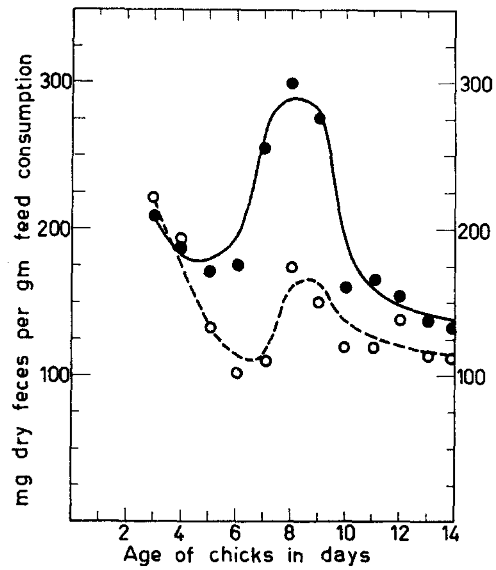

FIG. 6. Effect of virginiamycin on the quantity of feces per gram feed consumption in chicks on a casein-sucrose diet.

from the feed taken in, or indirectly from desquamations and secretions by the gut wall. The bacterial mash does not seem to play an important role, as no significant influence of virginiamycin on total bacterial counts was found.

DISCUSSION

Since the initial discovery in 1946 that antibiotics stimulate chick growth (26), several fundamental observations have been reported.

Antibiotics do not stimulate growth of germ-free chicks (27, 28) or of chick embryos (29), and the activity of oral bacitracin localizes their site of action in the intestinal tract (2). The response to antibiotics is also conditioned by the composition of the diet fed, and vitamin deficiencies sometimes act as stress factors increasing the response (7). Moreover, the nature of the dietary carbohydrate is of fundamental importance. Stokstad *et al.* reported that the response to chlortetracycline was greatest on a diet containing sucrose (17). This antibiotic was virtually inactive on a starch diet. Our experiments with virginiamycin are in agreement with these findings. The most likely explanation for the observed differences between the sucrose and the starch diet seems to be that sucrose provides a favourable intestinal environment for the development of a harmful flora.

Coates *et al.* found that environmental factors also influence the response to antibiotics (18). Chicks kept in a new or clean environment did not benefit from dietary antibiotics. The "subclinical disease," however, could be introduced to clean quarters by feeding feces from birds kept in old quarters (30). Similar results were obtained in the present study.

Our investigations indicate that dietary antibiotics exert their major effect during a limited critical period from the 5th to the 9th day of life. At that time, chicks given no antibiotics develop a syndrome of malabsorption of fats and carbohydrates resulting in a temporary growth depression and poor efficiency of feed utilization. The therapeutic effect of virginiamycin proves that the malabsorption is due to Gram-positive microorganisms and that antibiotics stimulate growth by improving the absorption of nutrients. The malabsorption has been demonstrated only for fats and carbohydrates, but by analogy with human cases of steatorrhea one may suppose that the malabsorption also includes amino acids, vitamins, and minerals. This could explain the vitamin-sparing effect of antibiotics reported by several authors (7–11). Our data are also in agreement with the results of Edwards *et al.*, who found that chickens in disinfected rooms have lower methionine, zinc, and magnesium requirements than those in a contaminated environment (31). Indeed, the malabsorption syndrome was not observed in clean quarters.

The present study has also raised several questions which are as yet unanswerable. The first question is why the malabsorption does not develop until the 5th day of life. It is not impossible that the harmful flora requires several days for full development or that toxic metabolites must reach a given threshold value. A temporary protection afforded by maternal antibodies is another possible explanation. It also remains obscure why spontaneous improvement in growth rate and intestinal absorption occurs after several days. Onset of antibody production is a possible mechanism. By analogy it might be presumed that the detoxicating functions of the liver are not activated until the 10th day of life. Replacement of the primary noxious flora by a secondary harmless population may be another mode of action. It should also be emphasized, however, that our experiments were conducted in batteries with raised wire screen floors. Conceivably in experiments on litter floors, where the animals have access to their feces, the continuous reinfection might give rise to a persistent state of intestinal malabsorption. This requires further study.

The identity of the causative microorganism remains unknown. As previously noted, it must be a Gram-positive species sensitive to virginiamycin and other narrow spectrum antibiotics. In this respect it may be of importance that *Clostridium welchii* has been found to reduce chick growth (27) and that Lev *et al.* reported a close correlation between the number of *Clostridium welchii* in the feces and the response to antibiotics (32). Gnotobiotic studies with mono- and polycontaminated chicks are in progress. In these experiments the influence of mono- and mixed flora's on daily weight gain and fecal fat excretion is under investigation.

SUMMARY

A study was made of the effect of antibiotics on growth of chicks and on intestinal absorption of fats and carbohydrates.

Around the 8th day of life, chicks fed an antibiotic-free casein-sucrose diet developed a transitory syndrome of malabsorption of fats and carbohydrates, associated with disturbance of the efficiency of feed utilization and poor weight increase. Administration of virginiamycin, at a level of 20 ppm, suppressed this period of malabsorption and resulted in improved feed conversion and increased weight gain.

The temporary growth depression and malabsorption were not observed in disinfected rooms in new quarters. Under these conditions virginiamycin did not stimulate growth nor was the efficiency of feed utilization improved by the antibiotic. However, the growth-depressing flora could be introduced to the new quarters by feeding each bird 50 mg of fresh feces collected from chicks in old quarters.

Both the intestinal absorption and the growth-promoting effect of virginiamycin were influenced by the type of carbohydrate in the basal diet, and have been found to be most pronounced when sucrose was fed as the sole source of carbohydrate. The malabsorption was less obvious when cornstarch was substituted for sucrose. In this case virginiamycin had only a limited effect on growth and on feed conversion.

The present investigations suggest that antibiotics stimulate growth of chicks by their antibacterial action against Gram-positive microorganisms which interfere with the absorption of nutrients. Furthermore, the growth-promoting effect seems to be most pronounced during a limited period of a few days around the 8th day of life.

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