EFFECTS OF SUBTHERAPEUTIC DOSES OF ANTIBIOTICS ON POULTRY INTESTINAL BACTERIA

by

Roger W. Kelley

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APPROVED:

W. E. C. Moore, Chairman

L. V. Holdeman

L. M. Potter

N. R. Krieg

J. G. Ferry

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Blacksburg, Virginia

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ABSTRACT

SECTION I. INTRODUCTION - PURPOSE OF DISSERTATION

Subtherapeutic doses of antibiotics stimulate the growth and feed efficiency of many animals. This growth stimulation effect is mediated by the antibiotic's effect on the intestinal flora. The presence of an intestinal flora results in a growth depression (compared to germ-free animals) that is partially alleviated by low concentrations of antibiotics.

Despite many years of work by numerous research groups, the site of the growth stimulation effect in the intestinal tract is unknown and very little is known about the actual species of the intestinal flora that are affected by antibiotic treatment. Most bacteriological studies have been restricted to facultative or aero-tolerant bacteria. Inadequate methods for culture of anaerobic bacteria have resulted in the isolation of only a small percentage of the total bacteria in the intestinal tract. Also, no attempts have been made to compare the numerically predominant bacterial species present in untreated vs. antibiotic-fed animals.

The purpose of my dissertation research was to determine if growth stimulation of poultry by antibiotics was correlated to changes in the numbers or occurrence of the predominant species of the intestinal flora. The dissertation is divided into two main sections. The first is an extensive review of the effects of antibiotics on poultry and the second section contains the experimental results.

SECTION II. LITERATURE REVIEW

INTRODUCTION

Subtherapeutic doses of antibiotics stimulate the growth of many animals including poultry, swine, calves, and humans. Yet despite over 35 years of research, the mechanism of growth stimulation is unknown. There have been many reviews on the effects of antibiotics on the nutrition and growth of animals (Jukes and Williams, 1953; Braude et al., 1953; Stokstad, 1954; Jukes, 1955; Taylor, 1957; Luckey, 1959; Francois, 1962; Visek, 1978); however, most are out of date or concentrate on only a few of the possible mechanisms. This review will be limited almost exclusively to research with poultry. The differences in physiology and diet between animals such as poultry and ruminants is extreme, and this is reflected in their vastly different intestinal bacterial populations. Therefore, it frequently is not valid to compare effects of antibiotics between different species of animals, and previous attempts to find a unifying mechanism of growth stimulation have foundered in the inconsistencies and conflicting results. The research efforts were well summarized by Luckey (1959): "It is apparent that conflicting data are absent only when there is no more than one report on the topic."

An early observation in poultry nutrition was that animal protein supplements increased the growth of chicks on all-vegetable protein diets (e.g., Nestler et al., 1936; Christiansen et al., 1939; Hammond and Titus, 1944; Heuser et al., 1946; Rubin and Bird, 1946). It was later found that Streptomyces aureofaciens fermentation broths also stimulated growth. The growth stimulation was initially believed to

be due to the vitamin B_{12} in the mixtures. However, the growth response of poultry fed a diet supplemented with B_{12} was not sufficient to account for the stimulation by fermentation broths (Carlson et al., 1949; Stokstad et al., 1949). Reports that the growth response was due principally to chlortetracycline (produced by S. aureofaciens) and that streptomycin also was effective, resulted in an explosion of research (Stokstad and Jukes, 1950a; Groschke and Evans, 1950; Whitehill et al., 1950). It was soon shown that antibiotics stimulated growth of turkeys (Stokstad and Jukes, 1950b), pigs (Carpenter, 1950; Jukes et al., 1950; Luecke et al., 1950), and other animals. It also was discovered that almost all of the common antibiotics such as penicillin, chlortetracycline (CTC; trade name aureomycin), oxytetracycline (OTC; trade name terramycin), and streptomycin were effective in stimulating growth. Almost immediately, antibiotics began to be used routinely as feed supplements and assumed an important economic role in the meat and pharmaceutical industries.

Scattered reports of growth stimulation by antimicrobials had been made prior to 1950. Growth stimulation in chickens and turkeys was reported with para-aminobenzoic acid, sulfasuxidine, streptomycin, and arsonic acid derivatives (Briggs et al., 1944; Moore et al., 1946; Morehouse and Mayfield, 1946; Bird et al., 1949), and Harned et al. (1948) found that CTC stimulated the growth of chicks. However, the significance of these findings was not realized by other researchers and sometimes not even by the authors themselves.

One possible mode of action of antibiotics was a direct effect upon the host. However, studies with germfree animals soon confirmed

that the primary effect of the antibiotics was on the intestinal microflora. Growth increases did not occur when antibiotics were fed to germfree chicks and poults (Luckey, 1952; Forbes et al., 1958, 1959; Forbes and Park, 1959; Gordon et al., 1958; Coates et al., 1963). In fact, the growth of germfree animals usually was equal or superior to that of conventional animals. Thus, antibiotic treatment can be regarded as not stimulating growth rates, but restoring them to "normal" levels.

The basic mechanisms suggested for growth stimulation are: i) antibiotics cause an increase in the number of organisms that produce various vitamins or growth factors that are stimulatory for the host; ii) antibiotics suppress bacteria that compete with the host for nutrients; or iii) antibiotics directly or indirectly inhibit organisms that produce subclinical infections.

This review will discuss: i) some of the variables affecting the growth response to antibiotics; ii) briefly summarize poultry intestinal physiology; iii) review evidence for the effects of antibiotics on the utilization of various nutrients; iv) discuss specific effects of antibiotics on the intestinal flora; and v) present some of the possible mechanisms by which inhibition of the intestinal bacteria might benefit the host.

PARAMETERS OF GROWTH RESPONSE TO ANTIBIOTICS

Age Specificity and Duration of Response

The maximum growth response to antibiotics occurs in the first four weeks of life, and usually the response peaks within the first

two weeks. By the third to fourth week the weight gain due to antibiotic treatment stabilizes and then begins decreasing (e.g., Scott and Glista, 1950; Sieburth et al., 1951; Branion and Hill, 1952; Heuser and Norris, 1952; Heywang, 1952; Johnson, 1952; MacGregor et al., 1952; Slinger and Pepper, 1954a; Wisman et al., 1954; Griffin, 1979). In other words, after two to four weeks of age, antibiotic-treated birds are unable to maintain their initial growth response and their weight begins to approach that of untreated birds.

The first four weeks of life are the most critical for antibiotic treatment. If birds are started on antibiotics after this period, there is usually no growth response. Griffin (1979) found a maximum growth response to zinc bacitracin in the first week of life. No significant growth response occurred if birds were started on zinc bacitracin when 7-days-old or older. If bacitracin was withdrawn at 21 days of age or before, there was no detectable growth advantage by eight weeks of age. Birds fed CTC, penicillin, or oleandomycin beginning at five or eight weeks of age showed no increase in growth (MacGregor et al., 1952; Scott and Jensen, 1952; Lillie et al., 1953; Sherman et al., 1959).

Although there is a consensus that the maximum effect of antibiotics occurs in the first four weeks, there are conflicting data concerning growth responses after this period. Several investigators found that when antibiotics were discontinued at four or five weeks of age, the chicks' weight at eight weeks was equal to that of chicks fed antibiotics for the full eight week period (Heuser and Norris, 1952; Lillie et al., 1953; Griffin, 1979). On a larger time scale, MacGregor et al. (1952) found that discontinuation of penicillin at

eight weeks did not affect the final weight at 20 weeks compared to poults continuously fed penicillin. In contrast, other researchers have found that chicks or poults fed antibiotics for only four to eight weeks had lower adult body weights than birds kept on antibiotics (Berg et al., 1950; Scott and Jensen, 1952; Atkinson et al., 1954; Sherman et al., 1959). Some of the conflicting results are probably due to the different diets employed. Matterson and Singsen (1951) found that antibiotic treatment of birds on a plant protein diet resulted in an increasing rate of weight gain through seven weeks of age (the end of the experiment), whereas the rate of gain of birds on a diet supplemented with fish meal peaked at three weeks. Wisman et al. (1954) and Atkinson et al. (1954) also reported a delay in maximum growth response with poorer quality diets. Regardless of the duration of antibiotic treatment, as long as birds are kept on antibiotics for at least four weeks they usually still weigh more at market age (Johnson, 1952; MacGregor et al., 1952; Scott and Jensen, 1952; Wisman et al., 1954; Sherman et al., 1959).

Antibiotics and other Antimicrobial Agents

Almost all antibiotics stimulate growth, although some are more effective than others. In general, for an antibiotic to be a successful growth promotant in poultry it should be active primarily against gram-positive bacteria, presumably because the predominant intestinal bacteria are lactobacilli. Antimicrobial agents such as arsanilic acid and arsonic acid derivatives also stimulate growth in poultry, although the response is usually less than with CTC or penicillin (Morehouse and

Mayfield, 1946; Bird et al., 1949; Morehouse, 1949; Stokstad and Jukes, 1950a; McGinnis et al., 1951; Anderson et al., 1952; Elam et al., 1953; Combs et al., 1954; Pepper et al., 1954; Pepper and Slinger, 1955). Copper supplements are effective for growth stimulation of swine (Barber et al., 1960) but do not produce consistent responses in poultry (Coates and Harrison, 1959; Slinger et al., 1962; Alvares et al., 1964b).

One of the more interesting antibacterial agents is acrylic acid. During a study of the intestinal flora of Antarctic birds, Sieburth (1959a) found that the gastrointestinal contents had antibacterial activity. This was suggested to be due to the presence of marine algae in the diet of the prey of the birds (Sieburth 1959ab). The active factor in the antibacterial effect of the algae was later identified as acrylic acid, which was shown to have antibacterial properties both *in vitro* and *in vivo* (Sieburth, 1960). Feed trials with chicks resulted in suppression of *E. coli* and an increase in "*Aerobacter aerogenes*". [*Aerobacter aerogenes* is in quotes because it is no longer a valid species. Strains formerly identified as *A. aerogenes* are either *Enterobacter aerogenes* or *Klebsiella aerogenes*.] Acrylic acid also appeared to cause an increase in the growth rate of the chicks (Sieburth, 1961). These feed trials were extended by White-Stevens et al. (1962). Although sodium acrylate alone did not increase the growth of chicks significantly, it had an additive effect on growth when it was fed with CTC. There was no additive effect with OTC or penicillin. The *in vivo* suppression of *E. coli* by acrylate also was noted in pigs, but it was not associated with a significant growth increase (Kershaw et al., 1966; Cole et al., 1968).

Despite an antimicrobial spectrum as broad as any of the more efficacious antibiotics, chloramphenicol (trade name chloromycetin) usually does not stimulate, and may slightly depress, the growth of chicks and poults (Branion and Hill, 1951; Heuser and Norris, 1952; Lillie and Bird, 1953). Bunyan et al. (1977) found no effect of 5 or 25 ppm chloramphenicol on growth of chickens, but it significantly depressed growth at 100 ppm. Reid et al. (1954) and Whitehill et al. (1950) were among the few to report a stimulatory effect. Hauser et al. (1956) reported that 25 ppm stimulated growth, but less than other antibiotics at the same concentration.

Proposed explanations for the lack of effect of chloramphenicol include: i) it does not reach the target site because over 90% of an oral dose is absorbed by the gastric mucosa; ii) its antimicrobial spectrum is too wide, resulting in the inhibition of both beneficial and harmful bacteria; or iii) it doesn't affect the relevant portion of the flora. The antimicrobial spectrum of chloramphenicol is similar to that of the tetracyclines which are very effective in eliciting a growth response. Thus, the first possibility seems most likely. Smith and Crabb (1960a) found that chloramphenicol treatment of calves caused an increase in resistant coliforms but, unlike tetracycline, it did not decrease the bacterial counts. Nor was any chloramphenicol detected in the feces, whereas CTC was easily demonstrated. Yacowitz and Bird (1953) fed chicks a single 50 mg dose of chloramphenicol. Most of the drug was absorbed or destroyed (89%). Of the proportion that was detected, 63% was in the urine and feces while 28% was found in the gizzard and duodenum. Very little antibiotic activity was

detected in the ileum or ceca. Thus, as in mammals, chloramphenicol appears to be absorbed in the anterior portion of the poultry gastrointestinal tract.

Another possible explanation for chloramphenicol's failure to stimulate growth is that it might have a direct inhibitory effect on the host. This possibility was given strong support recently by the work of Al-Hussainy et al. (1979). They found that chloramphenicol at doses of 10-200 mg/kg body wt in rats or rabbits caused damage to the intestinal mucosa. The number of villi decreased and some became thinner and elongated. Goblet cell activity (mucin secretion) increased and glucose absorption was reduced by 50%. These changes could explain why chloramphenicol usually fails to stimulate growth in low doses and depresses growth in higher ones.

It is not possible to rank antibiotics such as penicillin, CTC, or OTC according to their efficacy. Although apparent differences in growth stimulating ability may be reproducible at one brooding facility, these differences frequently do not occur at other locations. Some generalizations can be made, however. The arsenicals and the aminoglycosides (e.g., streptomycin, gentamycin, or kanamycin) are usually less stimulatory than penicillin or tetracyline. One reason for poor growth stimulation by aminoglycosides may be that they are inactive under anaerobic conditions, the normal state for the intestinal tract (Bondi et al., 1946; Williamson and White, 1956). They also are sensitive to even mildly acidic conditions, and it is difficult to separate the effects of the anaerobic environment from the reduction in pH due to atmospheric CO_2 (Geiger et al., 1946;

Verklin and Mandell, 1977). Also, the aminoglycosides do not inhibit gram-positive bacteria as effectively as penicillin or bacitracin.

Dose-response Relationship

Maximal growth response occurs at concentrations of only 10 to 50 ppm of most antibiotics. At or below the level of 25 ppm, there usually is an increased growth response with increased antibiotic concentration (Davis and Briggs, 1951; McGinnis et al., 1951; Saxena et al., 1952; Slinger et al., 1954b; Waibel et al., 1960). Higher concentrations do not further increase growth (Stokstad and Jukes, 1950ab; Whitehill et al., 1950; Davis and Briggs, 1951; Heywang, 1952; Slinger et al., 1954c; Begin, 1971). Nor is there any evidence for an additive effect of antibiotics (Davis and Briggs, 1951; McGinnis et al., 1951; Sieburth et al., 1951; Johnson, 1952; Williams and Hill, 1952; Saxena et al., 1952; Elam et al., 1953; Sherman et al., 1959; Bare et al., 1964a; Marusich et al., 1974; Griffin, 1979). A lack of correlation between growth response and antibiotic concentration has also been observed (Reynolds et al., 1951a; Sherman et al., 1959; Foster, 1978; Yates and Schaible, 1962). Heuser and Norris (1952) reported no consistent effect of concentration of CTC, OTC, bacitracin, or penicillin on growth response at levels ranging from 1.25 to 50 ppm.

Some of the discrepancies regarding optimal concentrations may be accounted for by variation in the basal diets. Reynolds et al. (1951b) reported that on a diet without added vitamin B_{12} CTC gave a maximum response at 25 ppm, but, when extra B_{12} was added, the maximum response occurred at 5 ppm.

Species Specificity

Antibiotics are effective in stimulating the growth of many animals including poultry, swine, cattle, rats, and mice and men. Not all animals, however, are stimulated by antibiotics and responses may range from a marginal effect to an overtly deleterious one.

Branion and Hill (1952) tested the effects of 50 ppm of penicillin, CTC, OTC, and streptomycin on the growth of goslings. By two weeks of age, the antibiotics stimulated growth by 7.4 to 16.9%. By four weeks of age, the antibiotic-fed birds still weighed more than the untreated birds, but the percent difference was less. After four weeks, the birds were transferred from batteries to a grass range. When weighed at six weeks, the OTC-fed birds weighed less than the control birds and, for the period from four to eight weeks, gained 29% less weight than the controls. The data show that most of the growth increase due to the antibiotics occurred in the first two weeks of life, after which the untreated birds had a faster growth rate, although at eight weeks the antibiotic-fed birds still weighed more (except with OTC). Slinger et al. (1953b) also studied the effect of penicillin on goslings during the first two months of life. Penicillin increased growth through eight weeks of age, but the increase was statistically significant only with male birds at two weeks. Penicillin had no stimulatory effect if grass was available. Lindblad et al. (1955) conducted two experiments with antibiotics and goslings. In the first, neither 25 ppm penicillin nor 50 ppm arsonic acid stimulated growth at weeks one through four and penicillin decreased weight non-significantly. In a second experiment, neither arsonic acid (50 ppm)

nor CTC (25 ppm) had any effect. At 100 ppm, CTC significantly increased growth for weeks one and two only. Penicillin (25 or 100 ppm) also stimulated growth significantly at weeks one and two. By week five, however, the controls were heavier than any of the antibiotic-fed groups. These studies suggest that antibiotics can stimulate growth of geese during the first 7 to 14 days of life but that continued treatment may be deleterious to the bird. Antibiotics did not affect the growth of ducks up to six weeks of age either (Branion et al., 1953).

The guinea pig is the most striking exception to the stimulatory effect of antibiotics on growth. Penicillin, streptomycin, OTC, and CTC are all highly toxic at low concentrations. Roine et al. (1955) reported that as little as 0.1 mg of CTC caused signs of severe illness and one to three mg caused death. Early studies attributed death to an increase in Listeria (Roine et al., 1955) or an overgrowth of coliform bacteria (DeSomer et al., 1953; Eyssen et al., 1957). More recently it has been shown that antibiotics induce a lethal enterocolitis in guinea pigs and hamsters which is caused by Clostridium difficile and its toxins (Bartlett et al., 1977 and 1978; Rifkin et al., 1978; Rehg, 1980).

Oral vs. Parenteral Antibiotics

There have been conflicting reports concerning the efficacy of injected antibiotics. The first published reports found no growth response in chicks when CTC, penicillin, or streptomycin were injected (Groschke and Evans, 1950; McGinnis et al., 1950; Whitehill et al., 1950). However, other research groups reported that penicillin, CTC, and bacitracin stimulated growth of chicks and poults when injected,

and the growth response, in some instances, equalled that with oral antibiotics (Dixon and Thayer, 1951; Elam et al., 1951ab; Fell and Stephenson, 1953; Reid et al., 1954). Differences in the injection schedule probably are not the reason for the conflicting results because antibiotics injected at intervals from once per day to once per week were capable of eliciting a growth response. Elam et al. (1951b) reported that injected bacitracin was as effective as an oral dose. Injected penicillin also increased growth of chicks, but oral penicillin was superior. Oral penicillin increased the numbers of penicillin-resistant bacteria significantly. Parenteral penicillin caused only a nonsignificant increase in the numbers of resistant bacteria, but when the drug was mixed with aluminum monostearate in sesame oil to slow the release of the drug in the body there was a significant increase. Similar results were found by Reid et al. (1954) with turkey poults, except that CTC caused a larger growth response when injected. They also found an increase in antibiotic resistance from injected CTC and penicillin, but not from bacitracin. The results of studies by Elam et al. (1951b) and Reid et al. (1954) suggest that at least some of an injected dose is excreted into the intestines where it causes a growth response and an increase in resistant bacteria. In mammals, the majority of injected penicillin is excreted in the urine but a variable percentage is excreted in bile into the intestinal tract (Rake and Richardson, 1946). Injected CTC also is excreted in bile (Kelly and Buyske, 1960; Eisner and Wulf, 1963).

GASTROINTESTINAL ANATOMY AND PHYSIOLOGY

The intestinal tract of chickens and turkeys is divided anatomically into the crop, proventriculus, gizzard, small intestine, ceca, and large intestine. The primary site of the growth stimulating effect of antibiotics is not known; however, most evidence suggests that antibiotics strongly affect the nutrition of the host. Before discussing interactions between antibiotics and specific nutrients, it is necessary to briefly review the anatomy of the poultry intestinal tract and the role each section plays in digestion and absorption.

Crop

The occurrence of a crop varies with the species of bird. Ducks and geese have only a slight enlargement of the esophagus compared to the large sac of chickens and turkeys. The crop acts primarily as a food storage area and the only major digestive process that occurs is starch digestion. Salivary and crop bacterial amylases are probably more important for total starch degradation than pancreatic enzymes because pancreatectomy reduces digestion by only 26% (Ariyoshi et al., 1964). On a commercial-type diet, removal of the crop from chicks does not affect weight gain (Fisher and Weiss, 1956).

Values for the pH at different sites in the poultry intestinal tract are listed in Table 1. The largest variation occurs in the crop where the observed pH is dependent upon the length of time since the last meal. Following a meal the pH drops from 5.5 - 6.0 to around 4.9 within four to five hours. The decrease is due to production of lactic acid by the crop lactobacilli because no equivalent decrease

TABLE 1. The pH of the poultry intestinal tract.

Bird (no. tested)	Site								Reference[a]
	Crop	Proventriculus	Gizzard	Duodenum	Jejunum	Ileum	Ceca	Colon	
Chicken (20)	4.5[b]	4.4	2.6	5.8-6.0	5.8-5.9	6.3-6.4	5.7	6.3	1
Turkey (4)	6.0	4.7	2.2	5.8-6.5	6.7-7.0	6.8	5.9	6.5	1
Chicken (12)	4.8 (2.6-5.4)[c]	4.6 (3.6-5.0)	3.9 (2.9-4.4)	6.2 (6.0-6.4)	6.0 (5.7-6.4)	7.4 (6.4-7.8)	6.7 (6.4-7.0)	- (6.6-7.6)	2
Chicken (20)	4.2 (3.7-4.6)	-	2.7 (2.1-3.1)	5.7 (5.3-6.0)	5.4 (4.9-5.8)	6.2 (6.0-6.4)	5.7 (4.7-6.1)	-	3
Chicken (80-118)	4.1-7.8	0.3-3.0	0.4-3.8	5.6-7.7	6.0-7.9	5.7-8.5	5.6-8.1	5.3-8.4	4
Chicken (6)	4.9	-	4.2	5.8	6.2-7.0	7.8	7.0	-	5
Chicken (11)	3.2	-	3.2	5.8	5.7	7.3	5.3	7.6	6
Chicken (500)	6.5 (4.2-7.8)	1.5 (0.2-3.1)	2.9 (0.4-5.4)	6.4 (5.2-7.6)	6.6 (5.5-7.7)	7.3 (5.7-8.2)	6.9 (5.7-8.1)	7.0 (5.2-8.4)	7
Chicken (25)	4.5	4.4	3.2	6.3	-	-	5.5	6.4	8
Chicken (42)	5.8 (4.0-6.4)	4.7 (3.1-6.1)	4.1 (3.0-5.0)	6.3 (5.6-7.1)	7.0 (6.1-8.0)	7.6 (6.9-8.4)	7.1 (5.8-8.2)	7.4 (6.3-8.2)	9
Chicken (30)	4.7 (4.4-4.8)	4.5 (4.3-4.6)	2.9 (2.8-3.0)	6.1 (6.0-6.4)	6.3 (6.0-6.6)	6.6 (6.4-6.8)	6.1 (5.8-6.5)	6.8 (6.6-7.2)	10

[a]References: 1 = Farner, 1942; 2 = Wiseman et al., 1956b; 3 = Lev and Briggs, 1956b; 4 = Herpol and van Grembergen, 1961; 5 = Smith, 1965; 6 = Bergaoui and Vervaeke, 1980; 7 = Herpol, 1966; 8 = Buckner et al., 1944; 9 = Olson and Mann, 1935; 10 = Hewitt and Schelkopf, 1955.

[b]Mean value; - = not tested.

[c]Range of pH values is given in parentheses.

occurs in germfree birds (Smith, 1965; Ivorec-Szylit and Szylit, 1965 and 1973; Szylit et al., 1980).

There is no obvious effect of age upon intestinal pH except possibly in the ileum. The lowest mean values in Table 1 are those reported by Lev and Briggs (1956b) in studies of birds less than one month old. Timms (1968) measured the pH in the ileum of six birds. At 18 days the average pH was 7.1 (range 6.3 - 7.8), at 7 weeks it was 7.3 (6.8 - 7.6), and by five months it was 7.6 (7.4 - 8.0). Herpol (1966), however, sampled a much larger number of chickens and found no effect of age on the pH at any site in the digestive tract. In the ceca, antibiotic treatment is associated with a significant decrease in the pH of the contents (Anderson et al., 1952, 1953ab; Branion et al., 1953; Hill et al., 1953b). The reason for the decrease is not known.

Proventriculus, Gizzard, and Small Intestine

Food passes from the crop to the proventriculus where hydrochloric acid and pepsinogen are secreted, but the residence time is very short (< 1 minute). From the proventriculus the food proceeds to the gizzard, a muscular stomach whose primary function is to grind food. Some peptic digestion also occurs in the gizzard. Duodenal contents periodically reflux into the gizzard, thus re-exposing partially digested food to more pepsin degradation (Dziuk and Duke, 1972; Oguro and Ikeda, 1974ab; Sklan et al., 1978). As long as the food is quite moist and soft, the removal of the gizzard has no deleterious effect on digestion (Fritz et al., 1936). The small intestine can be subdivided into the

duodenum, upper ileum, and lower ileum. The duodenum is clearly demarcated by a duodenal loop. The ileum, however, usually is arbitrarily divided into two approximately equal length segments separated by the yolk stalk (Meckel's diverticulum; some authors refer to the upper ileum as the jejunum). As with other monogastric animals, most digestion and absorption occurs in the small intestine (Hudson et al., 1971; Boorman and Freeman, 1976).

Ceca and Large Intestine

Most birds possess two bilateral ceca which are filled by retroperistalsis of contents from the colon. The cecal contents are semiliquid, with a pH usually ranging from 5.5 to 7.0, and contain large numbers of bacteria (10^{10} to 10^{11} per gram wet weight of contents). In addition to the dietary colonic contents, urine also is transported into the ceca. This is discussed in more detail in the section on uric acid.

The highest concentrations of volatile fatty acids in the intestinal tract are found in the ceca. Annison et al. (1968) reported that total volatile fatty acids in adult chickens ranged between 101 and 242 μmoles/g of cecal contents. The average proportions of volatile fatty acids were acetic, 56%; propionic, 29%; butyric, 10%; isobutyric, 1%; isovaleric and 2-methylbutyric, 2%; and valeric, 2%. Similar proportions were reported by Shrimpton (1963). In a study of twelve two-week-old turkeys, volatile fatty acids averaged 534 μmoles/g of cecal contents (see Table 2).

TABLE 2. Short chain fatty acids (SCFA) in the gastrointestinal tract of two-week-old poults (μmoles/g wet weight)[a]

SFCA	Crop[b] (7)	Gizzard (4)	Duodenum (14)	Lower Ileum (18)	Ceca (12)
Acetic	139 (7)[c] (30-476)	21 (3) (0-51)	44 (6)[d] (0-265)	82 (18) (18-265)	378 (12) (3-1709)
Formic	5 (1) (0-33)	-	31 (4) (0-194)	47 (9) (0-208)	9 (3) (0-54)
Propionic	-	-	5 (2) (0-44)	5 (4) (0-41)	52 (9) (0-172)
Isobutyric	-	-	-	0.7 (1) (0-13)	-
Butyric	-	-	-	5 (6) (0-33)	91 (10) (0-464)
Isovaleric	-	-	-	-	1 (2) (0-8)
Valeric	-	-	-	-	3 (3) (0-19)
Total VFA[e]	144 (30-476)	21 (0-51)	80 (0-503)	137 (18-372)	534 (6-2279)
Lactic	256 (6) (0-505)	2 (1) (0-7)	20 (2) (0-139)	220 (17) (0-494)	56 (3) (0-363)
Succinic	24 (6) (0-68)	0.5 (1) (0-2)	- -	12 (11) (0-62)	12 (4) (0-121)
Fumaric	-	-	-	-	5 (1) (0-66)
Total SCFA	424 (132-813)	23 (0-51)	115 (0-602)	370 (53-838)	608 (104-2399)

[a] Kelley (unpublished data).

[b] Number of birds sampled.

[c] The first number is the mean value for the number of birds sampled followed in parentheses by the number of birds that had detectable levels of the short chain fatty acid. The range of values is listed below the means. Detection limit ranged from 0.1 to 1.0 μmole/g wet weight of contents.

[d] Seven of fourteen birds had no detectable products.

[e] VFA = volatile fatty acids.

The function and contribution of the ceca to poultry nutrition are not well understood. They have been suggested to be important in cellulose, protein, or carbohydrate digestion, vitamin synthesis, absorption of nitrogen, or water re-absorption. A major problem in studying the role of the ceca in digestion is that cecectomized birds usually grow as well as normal birds (Mayhew, 1934; Beattie and Shrimpton, 1958; Nesheim and Carpenter, 1967; Thompson and Boag, 1975). Several early nutritional studies reported that cecectomy was associated with a decrease in digestibility of crude fiber, but growth was not depressed [see Halnan (1949) and Thornburn and Willcox (1965) for reviews of earlier work]. Thornburn and Willcox (1965) found no effect of cecectomy on starch or pentosan digestion. Cellulose digestion was decreased in one bird but not another. Generally though, there was a decrease in crude fiber digestibility. Much of the earlier work should probably be repeated because there have been significant methodological improvements in assays of fiber digestibility. The fiber digestion that does occur is probably mostly of hemicelluloses. Barnes et al. (1972) did not detect cellulolytic bacteria in chicks up to six weeks old. Of 48 strains of cecal anaerobes tested in pure culture, none was able to hydrolyze xylan or cellulose and only one hydrolyzed dextran. Several strains were able to hydrolyze pectin (Barnes and Impey, 1972). Other researchers also have failed to isolate cellulolytic bacteria from the cecal contents of birds, including pheasants (Barnes et al., 1973) and geese (Mattocks, 1971).

The cecal contents contain approximately 10 to 15 times more biotin, 6 to 8 times more folic acid, and twice as much riboflavin and niacin compared to the contents of the small intestine (Couch et al., 1949 and 1950; Sunde et al., 1950b). The host, however, does not seem to benefit from these vitamins. On a vitamin B_{12} deficient diet, cecectomy of laying hens did not affect the B_{12} concentrations in their eggs for up to 36 weeks. Jackson et al. (1955) injected radiolabelled B_{12} into the ceca of chicks and detected the absorption of only 8% of the dose, although a large percentage of injected methionine and sulfate was absorbed. Sunde et al. (1950b) found that cecectomized birds were less affected than normal birds by a biotin-deficient diet. Results from germfree and conventional chicks on vitamin deficient diets support the conclusion that cecal vitamins are of minimal benefit to the host (Coates et al., 1968).

The importance of the ceca to the bird seems to depend upon the type of diet. On a commercial-type ration, cecectomy does not inhibit or stimulate growth, thus suggesting that the ceca play a minor role in poultry nutrition. However, on diets of poor nutritional quality, the ceca may have a net positive effect. Ordinarily, protein and carbohydrates are almost completely digested and absorbed in the small intestine. For example, on a diet containing cereal starches little or no starch is detected in the ceca, but when raw potato starch is used, a substantial portion of the ingested starch passes through the small intestine and into the ceca (Masson, 1954). When birds are fed raw soybeans (which are poorly digested compared to heated preparations), there is an increase in proteolytic activity in the cecal

contents, indicating that at least some of the protein passes into the ceca. On a raw soybean diet, cecectomized birds gain less weight and have a lower feed efficiency than normal birds, suggesting a net positive effect of the cecal flora (Nitsan and Alumot, 1963). Similarly, the digestibility of heat-damaged protein is significantly lower in cecectomized birds (Nesheim and Carpenter, 1967).

The final segment of the poultry intestinal tract is divided into the large intestine and cloaca. The ureters empty into the cloaca which acts as a reservoir for wastes. Some water and electrolyte absorption occur in the cloaca and large intestine but the amount is limited. The large intestine in poultry is quite short and probably is not important in the overall nutrition of the host.

Transit Time

Most researchers have reported the transit time of contents through the intestinal tract to be two to four hours (Hillerman et al., 1953; Henry et al., 1933; Stokstad et al., 1953; Jukes et al., 1956a; Tuckey et al., 1958; Aylott et al., 1968; Dal Borgo and McGinnis, 1968; Duke et al., 1969). The transit times vary depending on the diet fed, the marker used, and the age of the birds. The values represent the time required for the first appearance of the marker and are misleading because they do not indicate the average time that the majority of the feed particles are exposed to host and bacterial metabolism. Sklan et al. (1975) reported a residence time in the crop ranging from 3 to 4½ hours, but food can remain in the crop for up to 24 hours (see Heuser,

1945, for a review of earlier work). The time for total clearance of a marker ranges from one to three days (Duke et al., 1969; Harwood, 1937).

The effects of antibiotics on transit time vary with the conditions of the test and the diet. CTC or penicillin have been reported to decrease (Jukes et al., 1956a; Dal Borgo and McGinnis, 1968), increase (Hillerman et al., 1953; Stokstad et al., 1953) or have no effect (Tuckey et al., 1958) on the transit time. The type of carbohydrate also can influence the transit time (Monson et al., 1950; Stokstad et al., 1953; Tuckey et al., 1958; Dal Borgo and McGinnis, 1968).

INTERACTION OF ANTIBIOTICS AND POULTRY NUTRITION

Vitamins

Water soluble vitamins. Many researchers have noted that antibiotics stimulate the growth of animals fed vitamin deficient diets, and may lower the dietary requirement for some vitamins. These effects could occur by: i) improved absorption of vitamins; ii) increased production of vitamins by the intestinal flora; or iii) decreased utilization or destruction of vitamins by the intestinal bacteria. There is no question that the intestinal bacteria of animals produce vitamins *in vivo*. However, the importance of these vitamins to hosts that do not practice coprophagy is debatable. Most researchers have tested the effects of antibiotics on vitamins indirectly. Varying concentrations of vitamins were fed with or without antibiotics and the growth responses of the birds were recorded. Of greater value are studies such as those of Waibel et al. (1952b) and Teeri et al. (1959). These authors determined the concentrations of vitamins in eggs as

affected by antibiotics, thus providing a more direct indication of an increase in absorption. Some of the reported effects of antibiotics on vitamin utilization are summarized in Table 3.

The vitamins most consistently affected by antibiotics are biotin and folic acid (Table 3). Although a few researchers have reported that antibiotics improve utilization of niacin (Pepper et al., 1953; Slinger et al., 1953a), most have found no effect of antibiotics on niacin deficiency in poultry (Nelson and Scott, 1953; Wharton et al., 1958; Table 3). Many contradictory reports also exist regarding the effects of antibiotics on vitamin B_{12} (Oleson et al., 1950; Davis and Briggs, 1951; Reynolds et al., 1951b; Stokstad and Jukes, 1951; Elam et al., 1951a; Hsu et al., 1952; Peterson et al., 1952).

Slinger and Pepper (1954b) reported that maximum weight gains of turkey poults occurred at lower concentrations of biotin when penicillin was added to the diet. Similarly, growth of chicks on a folic acid-deficient diet was stimulated by both CTC and a bacitracin-penicillin mixture (Monson et al., 1954b). Antibiotic treatment also was associated with an increase in the amount of folic acid produced by intestinal coliforms. In the control birds, cecal isolates produced more extracellular folic acid than did ileal strains. However, antibiotic treatment caused a two- to three-fold increase in average folic acid production by isolates from the ileum. Production of folic acid by cecal isolates increased, but not as much. Increased folic acid production was detected in the ileum and ceca by the second day of antibiotic treatment and in the duodenum by the third day. Other researchers have also noted an increase in folic acid-synthesizing coliforms following

TABLE 3. Effects of antibiotics on water soluble vitamin utilization by poultry

Bird	Antibiotic	Increased absorption/utilization of or decreased requirement for:	No effect on:	Reference[a]
Chicken	CTC	folic acid, nicotinic acid, riboflavin		1
Chicken	CTC		ascorbic acid, riboflavin	2
Chicken	CTC	pyridoxine	niacin, pantothenic acid, riboflavin	3
Chicken	CTC or penicillin	thiamine	pyridoxine (effect of penicillin not tested)	4,5
Chicken	CTC or bacitracin-penicillin mixture	folic acid		6
Chicken[b]	OTC	biotin, folic acid, pantothenic acid, vitamin B_{12}	niacin, riboflavin	7
Turkey	OTC or penicillin	biotin, pantothenic acid		8
Chicken	penicillin	biotin, folic acid, pyridoxin	pantothenic acid, riboflavin, thiamine	9
Chicken[b]	penicillin	biotin, folic acid		10
Chicken	penicillin, bacitracin, streptomycin or sulfasuxidine	folic acid, B_{12}	niacin, pantothenic acid, pyridoxine	11
Chicken[b]	bacitracin		biotin, folic acid, niacin, pantothenic acid, riboflavin, vitamin B_{12}	7

[a] 1 = Biely and March (1951); 2 = Squibb et al. (1952); 3 = Jukes and Williams (1953); 4 = Waibel et al. (1952a); 5 = Waibel et al. (1953); 6 = Monson et al. (1954b); 7 = Teeri et al. (1959); 8 = Slinger and Pepper (1954b); 9 = Coates et al., (1951a); 10 = Waibel et al. (1952b); 11 = Monson et al. (1952).

[b] Based on analysis of vitamin content of eggs.

antibiotic treatment (Rhodes et al., 1954; Wiseman et al., 1956a). Monson et al. (1954b) fed chicks a semi-synthetic diet with folic acid as the major limiting nutrient. Supplementation of the diet with a large concentration of folic acid (5 mg/kg feed) caused a growth increase that was equal or greater than that caused by antibiotics alone. Therefore, it was concluded that the increased growth resulting from antibiotic supplementation of folic acid-deficient diets was due to an increase in production of folic acid by intestinal coliforms. An interesting result regarding folic acid production by coliforms was that a much higher percentage (60 to 100 vs. 21 to 30) of isolates from antibiotic-treated birds were able to grow in a defined medium; i.e., the coliforms from the control birds were nutritionally more fastidious.

Eyssen et al. (1962) studied the response of chicks to virginiamycin and suggested that inhibition of the gram-positive flora in the crop and duodenum was responsible for growth stimulation. The hypothesis was based on the observation that the highest concentrations of antibiotic were found in these two sites. The flora in the crop and duodenum was composed primarily of lactobacilli which required multiple B vitamins (Eyssen, 1962). The vitamin requirements of the four predominant species of lactobacilli in the poultry intestinal tract are shown in Table 4. Eyssen et al. (1962) suggested that inhibition of lactobacilli by antibiotics resulted in increased availability of dietary vitamins which in turn stimulated growth. To test this hypothesis, a mixture of B vitamins was administered to chicks. No growth response was noted when up to five times the normal vitamin concentration was fed or injected. Also, treatment with the vitamin supplement

TABLE 4. Vitamin requirements of poultry intestinal lactobacilli.[a]

Vitamin	L. acidophilus	L. fermentum	L. plantarum	L. salivarius
Folic acid	+	-	-	+
Niacin	+	+	+	+
Pantothenic acid	+	+	+	+
Pyridoxin	-	-	-	-
Riboflavin	+	-	-	+
Thiamine	-	+	-	-
B_{12}	-	NR[b]	-	-

[a]Data from Rogosa (1974).

[b]NR = not reported.

plus virginiamycin resulted in no greater growth response than occurred with the antibiotic alone. Therefore, it was concluded that a sparing effect of B vitamins (i.e., enhanced absorption, decreased utilization by bacteria, or increased bacterial production) was not the mechanism of growth stimulation by virginiamycin (Eyssen, 1962).

Fat soluble vitamins. Burgess et al. (1951) and Coates et al. (1952b) found that penicillin increased the liver concentration of vitamin A, while Squibb et al. (1952) reported that CTC had no effect on vitamin A concentrations. Germfree and conventional chicks had equally low survival times on diets with no vitamin A, suggesting a lack of microbial synthesis or cecal absorption of vitamin A (Rogers et al., 1971; Coates, 1973). In fact, the intestinal bacteria may have a net deleterious effect on vitamin A nutrition because liver concentrations of vitamin A are higher in germfree than conventional chicks (Coates, 1968). There is very little synthesis or absorption of vitamin K in the ceca of poultry. Some vitamin K is formed in the excreta and may be available to the bird by coprophagy (Griminger, 1957; Nelson and Norris, 1961).

Minerals

As with vitamins, antibiotics seem to enhance the effects of many minerals. Growth responses are usually larger with mineral-deficient diets. Penicillin and CTC increase absorption and/or utilization of calcium (Migicovsky et al., 1951; Gabuten and Shaffner, 1954; Lindblad et al., 1954; Brown, 1957), manganese (Slinger et al., 1951b; Pepper et al., 1952 and 1953), phosphorous (Lindblad et al., 1954), and magnesium

(Sullivan, 1964) in chicks and poults. Supplee and Combs (1960) reported that maximum growth of chicks occurred at lower potassium concentrations with antibiotics, and feed efficiency improved; i.e., the antibiotic lowered the host requirement.

Very little work has been done on the effects of intestinal bacteria on mineral requirements. Calcium absorption is greater in germfree than in conventional chicks (Edwards and Boyd, 1963a) and Reddy et al. (1969) reported that calcium and magnesium, but not phosphorus, are absorbed better by germfree than by conventional rats. This might be due to the thinning of the intestinal wall associated with germfree life. It also could be due to effects of bacterial metabolism. For example, conjugated bile acids stimulate absorption of calcium and bacterial deconjugation of the bile might lower the net calcium absorption. In contrast, iron absorption is usually less efficient in germfree animals. Reddy et al. (1965) suggested that this might be due to the higher oxidation-reduction potential in the intestines of germfree animals because iron is most readily absorbed in the reduced ferrous form.

Protein

Many authors have suggested that the growth response with antibiotics is due to increased efficiency of protein utilization or a decrease in protein requirements (e.g., Machlin et al., 1952; West and Hill, 1955; Schumaier and McGinnis, 1969). Slinger et al. (1952a) reported that the percent growth stimulation of chicks by CTC and penicillin decreased with increasing protein concentration. CTC and

20% protein gave the same response as 23% protein without CTC. Similarly, 23% protein plus CTC gave results equal to that of 26% protein alone. Penicillin was not capable of compensating for a 3% difference in protein levels. McGinnis (1951) reported that penicillin enhanced protein utilization by turkeys, but there was no decrease in the concentration of protein required for maximal growth. At four weeks of age, poults fed penicillin and 24% protein grew as well as those fed 28% protein without antibiotics. Biely et al. (1952) also found that penicillin or CTC did not affect the protein requirement of chicks. These data suggest that antibiotics do not lower protein requirements per se because weights continued to increase with increasing protein. It appears, however, that antibiotics may enhance utilization because the effect of antibiotics was equivalent to an additional three to four percent dietary protein.

Protein composition also may be a factor in growth stimulation. Slinger et al. (1951a) found that penicillin and CTC increased the incidence of white feathers in poults, an indication of lysine deficiency. These authors suggested that the deficiency was due to increased amino acid requirements as a result of an increased growth rate. When the diet was increased from 20 to 28% protein, no lysine deficiency occurred. The authors later reported that the lysine deficiency symptoms were not always repeatable (Slinger et al., 1954c). Draper (1958) found that penicillin stimulated absorption of radiolabelled lysine by chicks, but addition of supplemental lysine to poult diets did not affect growth (Potter and Shelton, 1976). Biely et al. (1952) found no decrease in lysine or tryptophan requirements

in chicks fed CTC or penicillin. On low tryptophan diets, the antibiotics had no effect on weight gain, but did decrease mortality from 75 to 25%. Nicotinic acid supplements also decreased mortality [tryptophan is a precursor of nicotinic acid]. Thus, the antibiotics might have increased absorption of tryptophan, or nicotinic acid, or stimulated bacterial synthesis of the vitamin. Jones and Combs (1951) reported that CTC spared tryptophan but not lysine. Lotenkov and Podluzhnaya (cited in Hudson et al., 1971) also reported that CTC increased the absorption of amino acids.

Carbohydrates

Early studies with rats showed that dextrin, and to a lesser extent starch, alleviates the effects of vitamin B-complex deficient diets much better than diets containing mono- or disaccharides such as glucose, lactose, or sucrose (see Johansson and Sarles [1949] for a review). The addition of dextrin appears to result in increased vitamin synthesis by rat intestinal bacteria. The general conclusion has been that "the incomplete digestion of dextrin or starch allows some of the carbohydrate to reach the cecum where it can be utilized by vitamin-synthesizing bacteria." (Johansson and Sarles, 1949). In contrast, almost all of the dietary carbohydrates such as glucose and sucrose are absorbed in the small intestine.

Similar results have been found with poultry. Dextrin increased the weight of chicks more than cerelose (glucose monohydrate), starch, sucrose, or lactose (Monson et al., 1950 and 1952). Other researchers confirmed that starch gave a much better growth response than sucrose

or a glucose-fructose mixture. Antibiotic treatment (CTC or virginiamycin) did not affect the growth of birds on a starch diet but increased the weight of sucrose-fed birds to a level equivalent to that obtained with starch (Stokstad et al., 1953; Eyssen and DeSomer, 1963a). Monson et al. (1952) reported that streptomycin, penicillin, or bacitracin increased the growth of chicks fed dextrin. Penicillin and bacitracin also increased growth of birds fed sucrose, but growth was still less than on a dextrin basal diet. As with rats, the use of dextrin as carbohydrate source reduced the growth depression effect of a folic acid-deficient diet in chickens (Luckey et al., 1946; Monson et al., 1950). It was also superior to sucrose and/or lactose with biotin-deficient diets and resulted in more biotin in the eggs and a higher percent hatchability (Couch et al., 1948; Sunde et al., 1950b). Monson et al. (1952) studied the effects of dietary carbohydrates on liver concentrations of B-vitamins. Dextrin, cerelose, starch, sucrose, or lactose had no effect on liver concentrations of B_6, B_{12}, niacin, or pantothenic acid. There was a nonsignificant increase in hepatic biotin in animals fed dextrin, cerelose, or starch vs. those fed sucrose or lactose. The folic acid concentration was highest in birds fed lactose, but growth was poorest. Cecal concentrations of vitamins did not correlate with liver concentrations.

Monson et al. (1954b) noted that a dextrin diet was associated with increased folic acid production by chicken coliforms. But when antibiotics were added to the dextrin diet, there was no correlation between liver folic acid concentration and the growth response of the chicks. Nor did the doubling of the dietary level of the water soluble

vitamins increase growth (Monson et al., 1950). It was concluded that the superior growth effect of dextrin was not due to production of known vitamins. Monson et al. (1954a) later reported that with high concentrations of protein, growth on sucrose was similar to that with dextrin. They concluded that the improved growth with dextrin at lower protein concentrations was due to more efficient protein utilization. One possible explanation is that the transit time through the intestinal tract is longer with dextrin than sucrose or lactose, thus affording the host more time to digest and absorb the proteins and vitamins in the diet (Monson et al., 1950; Stokstad et al., 1953).

Uric Acid and Urea

Uric acid usually comprises 75-80% of the nitrogen excreted in urine; ammonia accounts for 10-15%; urea 2-6%; and the remainder is in creatine and miscellaneous amino acids, although the exact proportions may vary with diet (Nesheim and Carpenter, 1967; O'Dell et al., 1960). Despite its metabolic importance, very little is known about the interactions between uric acid and the intestinal flora. Akester et al. (1967) and Skadhauge (1968) have shown that urine flows back into the ceca but not into the small intestines of chickens so that uric acid is available as a nitrogen source for cecal bacteria. Uric acid-utilizing bacteria occur in high numbers in the ceca of poultry. Barnes et al. (1972) reported that counts of uric acid-degrading bacteria ranged from $10^{7.9}$ to $10^{10.6}$ per gram in chickens and turkeys. This comprised 30 to 50% of the cultivable flora (but recovery of the microscopic counts was only ca. 20%), and represented all major groups of anaerobes.

None of the strains required uric acid for growth and all were capable of using ammonia as a nitrogen source (Barnes and Impey, 1974). Uric acid is degraded, by anaerobic bacteria, to ammonia, CO_2, and acetic acid (Mead, 1974; Vogels and van der Drift, 1976; Potrikus and Breznak, 1980). Aerobic bacteria usually degrade uric acid to urea and CO_2 (Schefferle, 1965; Vogels and van der Drift, 1976).

Bare et al. (1964ab) reported that addition of uric acid to the diet (1 to 2%, w/w) caused a significant growth depression at one to four weeks of age, but 0.5% uric acid had no effect. Antibiotic treatment completely offset the growth depression of uric acid. Analysis of gut contents showed a decreased concentration of uric acid when antibiotics were administered. There was no increase of uric acid in the serum, indicating that the intestinal bacteria were responsible for the decrease in concentration.

Compared to conventional birds, germfree chicks excrete more nitrogen in the form of urea, uric acid, amino acids, and peptides (Miller, 1967; Salter and Coates, 1971; Salter, 1973; Salter et al., 1974). This suggests that bacteria might be involved in the recycling of nitrogen. Urease activity in the gut occurs primarily in the ceca and is of bacterial origin. Little or no urease activity is detectable in the proventriculus, gizzard, or small intestine (Delluva etal., 1968; Lee, 1977; Bergaoui and Vervaeke, 1980). Some urease activity may occur in the crop, but it is of dietary origin, e.g., from soybean meal (Lee, 1977).

Lee and Blair (1972) used a synthetic diet containing only the essential amino acids and showed that conventional chicks were capable

of using dietary urea to synthesize amino acids. Using the same diet, Okumura et al. (1976) found that 2% added urea significantly stimulated the growth of conventional but not germfree chicks. The benefit of urea is dependent on the type of diet. With a defined diet containing only essential amino acids, urea can improve growth (Featherston et al., 1962; Farlin et al., 1968; Lee and Blair, 1972; Allen and Baker, 1974; Baker and Molitoris, 1974; Okumura et al., 1976). No increase in growth occurs on complete diets with natural protein sources even though the urea is usually utilized (Moran et al., 1967; Blair and Lee, 1973; Davis and Martindale, 1973; Kagan and Balloun, 1976). Although the amount of urea and uric acid degradation increases on low protein diets or following starvation, it yields only a negligible portion of the total nitrogen requirement (Emmanuel and Howard, 1978). Addition of urease to a diet containing urea, and low in nonessential nitrogen, increased the concentrations of amino acids in the liver and serum of chicks, but it had no significant effect on growth (Lee, 1977). Lee (1977) suggested that the beneficial effects of released ammonia on amino acid synthesis might be offset by the toxic effects of ammonia itself. The role of ammonia in growth stimulation by antibiotics will be discussed further in the mechanisms of action section.

Non-Antibiotic Feed Supplements

A common observation in poultry nutrition has been that animal protein supplements such as fish products, liver products, skim milk, or dried whey increase the growth of birds fed an all plant protein diet. Because most diets in early poultry nutrition work were

deficient in vitamin B_{12}, it was at first thought that this was the major nutrient present in additives such as fish meal. However, even with supplementary B_{12}, animal protein additives gave a growth response (e.g., Carlson et al., 1949; Combs and Shaffner, 1950; Sunde et al., 1950a; Singsen and Matterson, 1952). Other nutrient supplements such as dried brewer's yeast, dried distiller's solubles, corn fermentation solubles and grass juice also have been shown to stimulate growth.

The improved growth of birds given animal protein supplements might be due to suboptimal concentrations of one or more amino acids in the plant protein, a decreased digestibility of plant protein, or the presence of growth inhibitory substances in the plants (discussed in the next section). The growth response is probably a combination of the above factors as well as the presence of as yet unidentified growth factors. Antibiotics produce a larger percent growth response in birds on plant protein diets, but the birds are heavier when animal protein is also present (Matterson and Singsen, 1951; Branion and Hill, 1951; Heuser and Norris, 1952; Branion and Hill, 1953; Elam et al., 1953; Stokstad et al., 1953; Wisman et al., 1954; Menge and Lillie, 1960). In other words, there is an additive effect of antibiotics and other supplements such as fish meal. Antibiotics either spare some of the unknown growth factors or increase their intestinal synthesis. However, antibiotics alone do not elicit a maximal growth response.

Potter et al. (1977) found that 4% fish meal gave a growth increase equal to that obtained with 55 ppm of zinc bacitracin, but the effect was additive. Supplemental methionine (0.4%) could account for most of the growth stimulation by fish meal, but there was still a

significant additive effect of fish meal and zinc bacitracin. Thus, fish meal contained unidentified growth factor(s) not accounted for by methionine or zinc bacitracin. Monson (1969) suggested that taurine (derived from cysteine) was one of the growth factors in fish meal, and Chang and Waibel (1970) obtained equal responses to fish meal or taurine alone. However, Potter (1972) observed no growth stimulation with up to 250 ppm added taurine.

Some of the reported inconsistencies in response to growth factors may result from carryover of nutrients from the dam to her progeny. Increased response to growth factors and vitamins has been noted in birds hatched late in the hatching season, from older dams, or from nutritionally deficient dams (Kratzer, 1952; Menge et al., 1952; Scott and Jensen, 1952; Atkinson et al., 1955; Waibel, 1958). Kohler and Graham (1952) reported that better growth of control birds and poorer response to growth factors occurred during the periods in the hatching season when the dams had access to "good range". Penicillin treatment of the dams may give a slight advantage to their offspring (Slinger et al., 1952b, 1953a, 1954b; Jennings, 1957), but the effect is not cumulative over several generations (Coates and Davies, 1959).

Growth Inhibitors in Feed

Even diets that are nutritionally well balanced may adversely affect the growth of poultry because toxic compounds occur in many plants that are used for feed. Studies with germfree animals have demonstrated that the intestinal flora can have a major impact on the degree of growth inhibition by some of the compounds. Most research

has concentrated on the inhibition of growth by raw soy, navy or kidney bean meals (navy and kidney beans belong to the same species of bean, Phaseolus vulgaris).

Raw bean meals significantly depress the growth of poultry and the two toxic factors that account for most of the growth depression are protease inhibitors and phytohemagglutinins (lectins). Soybeans contain multiple protease inhibitors that bind to trypsin and chymotrypsin and cause an inhibition of proteolysis (Ham et al., 1945; Alumot and Nitsan, 1961; Nitsan and Alumot, 1965; Gertler et al., 1967). In addition to the amino acid deficiencies resulting from the poorly digestible protein in raw bean meals, undigested protein in the small intestine may interfere with the absorption of other nutrients (Sklan et al., 1979). Sulfur-containing amino acids are the limiting amino acids in most beans and growth depression has been attributed to a methionine deficiency due to the poor digestibility of raw bean meals. Supplementary amino acids, especially methionine or cystine, partially alleviate the growth depression (Hayward and Hafner, 1941; Almquist et al., 1942; Almquist and Merritt, 1953; Fisher and Johnson, 1958; Linerode et al., 1961). However, even with supplemental amino acids, growth of animals fed raw beans is still less than when heated beans plus amino acids are fed (Hayward and Hafner, 1941; Liener et al., 1949; Saxena et al., 1962; Gertler et al., 1967). Nor does did the addition of proteinases and peptidases to the diet affect the weight of chicks (Brambila et al., 1961; Linerode et al., 1961; Kakade and Evans, 1965b). Besides decreasing protein digestion, raw soybeans and purified trypsin inhibitor cause pancreatic hypertrophy in the chick (and rat) (Chernick et al., 1948;

Nesheim et al., 1962; Nitsan and Alumot, 1964 and 1965; Lepkovsky et al., 1965; Rackis, 1965; Garlich and Nesheim, 1966; Gertler et al., 1967). The mechanism of pancreatic hypertrophy has been reviewed by Liener and Kakade (1980). Lyman and Lepkovsky (1957) proposed that growth depression by raw beans was due to the loss of amino acids secreted by the hyperactive pancreas, especially because pancreatic secretions have a high concentration of sulfur-containing amino acids. However, pancreatic hypertrophy is probably not a major cause of growth depression because hypertrophy occurs even when there is little or no growth depression (Booth et al., 1960; Saxena et al., 1963a; Khayambashi and Lyman, 1966; Gertler et al., 1967; Sambeth et al., 1967).

In poultry, growth depression by raw soybeans is age dependent. After six weeks of age no growth inhibition by raw bean meals occurs, even though there is pancreatic hypertrophy (Alumot and Nitsan, 1961; Nesheim et al., 1962; Bornstein and Lipstein, 1963; Saxena et al., 1963a; Nitsan and Alumot, 1964 and 1965). In older birds, increased trypsin production by the hypertrophied pancreas may be capable of overcoming the effects of the trypsin inhibitors (Nitsan and Alumot, 1965). When older birds are placed on a raw bean diet, their growth returns to normal faster than younger chicks (Bornstein and Lipstein, 1963).

The anti-trypsin activity of soybean fractions does not always correlate with growth depression (Borchers et al., 1948; Rackis et al., 1963; Saxena et al., 1963ab; Garlich and Nesheim, 1966; Gertler et al., 1967) and it is evident that the poor digestibility of raw bean meals, combined with the effects of the trypsin inhibitors, accounts for only

a portion of the growth depression due to raw soybeans (Liener, 1953; Birk and Gertler, 1961; Rackis, 1965; Garlich and Nesheim, 1966; Gertler et al., 1967; Kakade et al., 1973). The other major toxic factor in raw beans is lectins (phytohemagglutinins). Lectins are common components of plants and have many biological activities including agglutination of blood cells, induction of mitosis, and depression of phagocytic activity. Many of the biological, biochemical and immunological properties of lectins have been reviewed elsewhere (Lis and Sharon, 1973; Liener, 1974; Jaffé, 1977 and 1980). Feed trials have shown that purified lectins inhibit the growth of rats (Liener, 1953; Honavar et al., 1962) and chickens (Wagh et al., 1963; Hewitt et al., 1973). Some of the confusion in the literature regarding the relative importance of lectins in growth inhibition by raw beans (Liener and Pallansch, 1952; Birk and Gertler, 1961; Jaffé and Gaede, 1959; Kakade and Evans, 1965a; Stead et al., 1966; Turner and Liener, 1975) probably is due to the occurrence of multiple types of lectins in beans. Both toxicity of the lectins and their hemagglutinating abilities against blood cells from different species may vary considerably (Jaffé, 1980). Also, purified lectin preparations may be composed of subunits that vary in their biological activity (Allen et al., 1969; Yachnin and Svenson, 1972).

Jayne-Williams and Burgess (1974) tested different fractions of raw navy bean meals on the growth of Japanese quail. The fraction with the highest phytohemagglutinin (PHA) activity resulted in a mortality rate equal to that of raw beans. PHA content might explain the differences in toxicity of soy and navy beans to quail. Navy

beans caused 100% mortality within two weeks while soybeans only depressed growth about 6%. Raw navy beans were found to have 1000-fold greater PHA activity (and a lower concentration of trypsin inhibitors) than soy beans (Jayne-Williams and Burgess, 1974). Other researchers also have reported soybeans to be less toxic than P. vulgaris beans (Jaffé and Vega Lette, 1968). The differences in oral toxicity also might be due to the greater susceptibility of soybean lectin to pepsin digestion compared to kidney bean lectin (Liener, 1958; Jaffé, 1980).

Both lectins and protease inhibitors are heat labile and the growth depression by raw bean meals can be eliminated by heat treatment. Growth depression also can be alleviated by antibiotic treatment, thus suggesting an important role for the intestinal flora (Braham et al., 1959; Linerode et al., 1961; Dal Borgo and McGinnis, 1968; Nitsan and Bondi, 1965; Kakade et al., 1970). Proof of the importance of the intestinal flora was provided by studies with germfree animals. Raw beans depressed growth of both germfree and conventional chicks, but germfree chicks were affected much less (Coates et al., 1970; Hewitt et al., 1973). Similar results were found with Japanese quail, although the quail seem to be more sensitive to growth inhibition than chickens (Jayne-Williams and Hewitt, 1972). A comparison of raw and autoclaved navy bean diets showed minimal differences in their effects on intestinal bacteria in the quail. Counts of total anaerobes and streptococci were very similar at all levels of the intestinal tract, although the ratio of Streptococcus faecalis to S. faecium shifted slightly with diet. Counts of lactobacilli were 10 to 100-fold lower in the crop and small intestine in birds fed raw

beans, but cecal counts were not affected by diet. Coliform counts were similar in the crop and small intestine, but about 300 times higher in the ceca of birds fed raw beans (Jayne-Williams and Burgess, 1974). Jayne-Williams and Hewitt (1972) isolated 149 strains of intestinal bacteria from the Japanese quail. When these strains were associated with germfree birds there was 100% mortality on raw bean diets, the same as with conventional birds. Testing different combinations of the 149 strains, it was found that the coliforms were primarily responsible for the deaths. When birds were associated with the 149 strains minus the coliforms, no deaths occurred although growth was depressed. In contrast, monoassociation with a single E. coli strain resulted in 50 to 75% mortality.

Several possible hypotheses were suggested by Coates et al. (1970) to account for the diminished effect of raw beans on germfree chicks: i) the microflora might convert an innocuous dietary substrate into a toxic one; ii) decreased host protein digestion might cause a shift in bacterial populations to more proteolytic species which in turn might inhibit the host; or iii) the bacteria might aggravate a non-microbial effect on the host. Of the hypotheses, the first one seems unlikely because parenterally administered lectins are quite toxic. Also, fermentation of raw bean meal by a strain of E. coli known to result in high mortality did not increase the bean meal's toxicity to germfree birds (Jayne-Williams and Burgess, 1974). Evidently a cooperative effect is occurring between the PHA and the intestinal bacteria, especially coliforms. The effect is not highly specific because coliforms other than E. coli (e.g., Klebsiella aerogenes) also

were effective, and even coliforms from other animals were capable of causing a high mortality rate in gnotobiotic birds. Some strains of streptococci and lactobacilli resulted in the death of gnotobiotic birds, but the mortality was much lower than with coliforms (Jayne-Williams and Burgess, 1974).

The PHA in raw beans does not appear to directly cause the proliferation of coliforms, because the counts of E. coli in different areas of the intestinal tract of monoassociated birds were the same on raw, autoclaved, or no-bean diets. A possibly important difference, however, was a much higher count of bacteria in the livers of birds fed raw beans (Jayne-Williams and Burgess, 1974). The increased rate of translocation could be due to PHA depression of phagocytic activity or direct affects on the intestinal mucosa or both. Lectins have a strong binding affinity for intestinal epithelial cells and may interfere with absorption (Jaffé, 1960; Jaffé and Vega Lette, 1968; Jayne-Williams, 1973; Etzler and Branstrator, 1974; Pope et al., 1975). Lorenzsonn and Olsen (1982) found that lectins bound to rat intestinal epithelia and caused increased shedding, shortening of the villi, acceleration of cell loss, and a reduction of cell area.

The type of cereal grain in the diet also may influence the growth of poultry. Many authors have noted a growth depression of chicks and poults by barley. The inhibitory effect of barley is probably due to the presence of β-glucans which are poorly digested and thus lower the total energy content of the diet (Arscott et al., 1955; Fry et al., 1958a,b; Moran and McGinnis, 1965 and 1966). The β-glucans also produce a stable, highly viscous gel in the intestines

which could interfere with the absorption of various nutrients (Burnett, 1966). Enzyme supplements containing β-glucanases partially eliminate the depression of a barley diet (Rickes et al., 1962; Moran and McGinnis, 1965 and 1967). A similar phenomenon has been reported by Vohra and Kratzer (1964) who found that the addition of polysaccharides to the diet depressed the growth of chicks. In the case of pectin, the growth depression could be alleviated by pectinase supplements. Antibiotic treatment and enzyme supplements have an additive effect on growth inhibition by barley (Moran and McGinnis, 1965). Antibiotics probably offset the physical effects of barley by increasing nutrient absorption that ordinarily would be depressed by the β-glucans without directly affecting the fate of the barley. Many other plant compounds also may affect growth. Besides β-glucans, barley contains tannins and trypsin inhibitors that may contribute to growth depression (Liener, 1980; Liener and Kakade, 1980). Tannins occur in many plants and appear to decrease the digestibility of proteins and amino acids and increase endogenous protein excretion (Vohra et al., 1966; Rostagno et al., 1973). Amylase inhibitors are found in some plants used in feed preparations (Liener, 1980a; Jaffé et al., 1973). When the purified inhibitors are fed, starch digestibility is decreased in rats, dogs, and men (Puls and Keup, 1973).

EFFECTS OF ANTIBIOTICS ON INTESTINAL FLORA

Normal Flora

Knowledge of the normal flora is crucial to understanding the effects of antibiotics on intestinal bacteria. This is especially

important for young birds because the maximum effect of antibiotics occurs during the first four weeks of life.

Chicken. The intestinal flora of newly hatched chicks varies greatly between birds before they are allowed access to feed. Bacterial counts are highest in the ceca and can range from 10^3 to 10^{10} bacteria per gram of contents. The predominant cecal flora also varies and may consist primarily of E. coli, Clostridium paraputrificum, or a mixture of coliform bacteria and streptococci (Shapiro and Sarles, 1949; Lev and Briggs, 1956a; Barnes et al., 1978). Within four hours after the first feeding, bacterial counts in the crop and ceca rise sharply, but the numbers in the small intestine are about equal to those found in the feed. By 16 to 24 hours post-feeding the total counts stabilize in the small intestine and ceca (Shapiro and Sarles, 1949; Lev and Briggs, 1956a).

Several researchers have studied the development of the intestinal flora as the chick ages. No effect of age upon the concentrations of coliform bacteria or lactobacilli was observed, although a 1000-fold variation in counts between birds was common (Shapiro and Sarles, 1949; Slinger et al., 1954a; Lev and Briggs, 1956b; Timms, 1968; Barnes et al., 1972 and 1978). The only consistent observation was a decrease in counts of enterococci as the chicks matured (Shapiro and Sarles, 1949; Slinger et al., 1954a; Timms, 1968; Barnes et al., 1978). Barnes et al. (1972) examined the cecal flora of two to 6½-week-old chicks using anaerobic nonselective media. Neither total microscopic nor cultivable flora counts changed with the age of the chick. Colonies were picked from the isolation medium and identified. Clostridium

clostridiiforme (formerly "Bacteroides clostridiiformis"), and species of Gemmiger and Fusobacterium were found in all age groups. Bifidobacteria did not appear until three weeks of age, and it was not until 6½ weeks that they were present in all of the birds. Anaerobic cocci were 30% of the cultivable flora of two-week-old birds, but the numbers decreased with age until by 6½ weeks they were only 9% of the flora. Bacteroides species were not detectable in the cecal flora until after four weeks of age at which point they accounted for about 25% of the flora.

Shirasaka (1970b) studied the intestinal flora of nine chickens at 11 weeks of age. From the crop to the lower ileum, the proportions of the flora were : lactobacilli > bifidobacteria > streptococci > coliforms > bacteroides. In the ceca, the proportions changed to: lactobacilli = bifidobacteria > bacteroides > coliforms > streptococci. The occurrence of bifidobacteria varied. They were found in 1 of 9 birds in the gizzard, duodenum, and jejunum; 2 of 9 in the ileum; and 6 of 9 in the ceca.

Salanitro et al. (1974 a,b) studied the cecal flora of 12 five-week-old chicks. Unlike previous studies, colonies were randomly picked from a nonselective medium and identified. Of ca. 600 isolates, 34% were anaerobic gram-positive rods, 20% cocci, 16.5% anaerobic gram-negative rods, and 13% clostridia. Barnes and Impey (1970) reported similar values from the ceca of five-week-old chicks: anaerobic gram-positive rods 40%, peptostreptococci 15%, and bacteroides 40%. Most of the isolates of Salanitro et al. belonged to undescribed species, although in one of the studies (1974a), half of the gram-positive rods

were P. acnes. Other identified isolates included Eubacterium rectale, Clostridium ramosum, Gemmiger sp., E. coli, and Bacteroides hypermegas. C. clostridiiforme was the predominant gram-negative rod. This species and B. hypermegas also were the predominant anaerobic gram-negative rods reported by Barnes and colleagues.

The major studies on poultry intestinal flora are summarized in Table 5. Most of the differences between studies are probably due to the high amount of bird-to-bird variation. This occurs especially in the ceca. In one of the studies reported by Salanitro et al. (1974a), anaerobic gram-positive rods averaged 36% of the flora, but the range of values for six birds was 17.8 to 64.6%. For the other groups, the averages were: cocci, 14% (range 0-49.2%); bacteroides, 19% (range 1.5 to 41.8%); and clostridia, 16% (range 0 to 32.7%). Shapiro and Sarles (1949) found up to a 1000-fold difference in bacterial counts between birds sampled at the same site on the same day. The largest study on variation was done by Ochi and Mitsuoka (1958b) who studied 30 adult chickens ($\geq$ 15 months of age). Lactobacilli (facultative) predominated in the ceca of 8 of 30 birds, anaerobic gram-positive rods (which may have included anaerobic lactobacilli) in 14 of 30, and coliforms in 2 of 30. The flora of the other birds usually was a mixture of lactobacilli and anaerobic gram-positive rods. However, lactobacilli comprised less than 2% of the cultivable flora in 9 of 30 birds. Populations in the small intestine were much more consistent. Lactobacilli were 70 to 100% of the cultivable flora in the duodenum of 29 of 30 birds. The other bird had ca. 70% anaerobic gram-positive rods

TABLE 5. Poultry intestinal flora.

Flora	Turkey[a]		Chicken						
	2 weeks (4)[b]	2 weeks (8)	Two weeks (6)[c]				Five weeks (6)		Adult (5)
	Duodenum	Lower ileum	Duodenum	Upper ileum	Lower ileum	Ceca	Ceca[d]	Ceca[e]	Feces[f]
Total cultivable bacteria	6.49×10^9	2.2×10^{10}	1.6×10^5	5.7×10^7	1.6×10^8	1.2×10^{11}	ND[g]	5.6×10^{10}	7.94×10^{10}
% Recovery	60.2	77.1	ND	ND	ND	45	60	60	ND
Total facultatives	ND	ND	1.3×10^5	1.8×10^7	8.7×10^6	7.1×10^8	ND	ND	ND
% of cultivable flora									
Gram positive rods	98.5 (4/4)[h]	80.3 (8/8)	45.7 (6/6)	56.8 (6/6)	66.8 (6/6)	60.6 (6/6)	36.1 (6/6)	32 (6/6)	25.5 (5/5)
Eubacterium	-	8.8 (7.8)	26.4 (4/6)	22.6 (2/6)	7.8 (2/6)	60.6 (6/6)			
Lactobacillus	98.5 (4/4)	72.3 (8/8)	19.0 (4/6)	33.8 (5/6)	59.0 (5/6)	-	-	-	
Gram-positive cocci	-	15.2 (3/8)	41.8 (6/6)	9.8 (2/6)	17.8 (3/6)	14.9 (5/6)	14.1 (3/6)	26	10.0 (5/5)
Gram-negative anaerobic rods	5.9 (1/4)	15.4 (3/8)	3.7 (4/6)	-	0.5 (1/6)	17.0 (5/6)	18.6 (6/6)	14	50.1 (5/5)
E. coli	2.0 (1/4)	14.8 (4/8)	5.4 (1/6)	33.0 (3/6)	14.7 (2/6)	-	4.9 (5/6)	-	0.01 (5/5)
Clostridium sp.	-	2.0 (1/8)	1.8 (2/6)	0.4 (1/6)	-	2.1 (1/6)	15.7 (5/6)	10	-
Gemmiger sp.	-	7.0 (1/8)	1.5 (1/6)	-	-	3.4 (5/6)	5.2 (4/6)	7	ND
Miscellaneous	-	-	3.1 (3/6)	-	0.2 (1/6)	-	5.4 (4/6)	11	-
% Anaerobes[i]	74.3	52.4	38.6 (15.1)	24.3 (49.6)	9.0 (88.7)	99.3 (99.3)	77.	63-83	81.6
% Facultatives	25.7	47.6	61.4 (84.9)	75.7 (50.4)	91.0 (11.3)	0.7 (0.7)	17.5	12-29	4.5

Footnotes for Table 5.

[a]R. W. Kelley, unpublished data. Total counts are bacteria per gram dry wt. of contents.

[b]Age of birds (number of birds sampled).

[c]Salanitro et al. (1978). Total counts are bacteria per gram dry weight of intestines and contents.

[d]Salanitro et al. (1974a). Birds were reared at a commercial facility. Total counts are bacteria per gram dry wt. of contents.

[e]Salanitro et al. (1974b). Birds were reared at the lab. Total counts are bacteria per gram dry wt. of contents.

[f]Mitsuoka and Kaneuchi (1977).

[g]Not done or not reported.

[h]Number of birds/total number sampled that had bacterial group in sample.

[i]% of cultivable flora based on identification of isolates from nonselective media. Numbers in parentheses are % of flora based on total counts.

and 30% lactobacilli. In the middle small intestine, lactobacilli predominated in 27 birds, coliforms in two, and streptococci in one.

The crop and the ceca contain the highest bacterial counts in the avian intestinal tract. The proventriculus and gizzard both have a low pH and most authors consider them to have no indigenous flora. Some researchers have suggested that the duodenum also has no autochthonous flora, but merely consists of washout from the crop. Lev and Briggs (1956b) studied young ($\leq$ 30 days old) chicks and found that total counts and levels of lactobacilli (which were equivalent) decreased going from the crop to the gizzard. Bacterial counts in the crop ranged from 10^7 to 10^9/g, while in the gizzard they were between 10^3 and 10^5 per gram of contents. Once in the duodenum, there was a 1000-fold increase in bacterial numbers. Shirasaka (1970b) studied 11-week-old birds and also found a drop from an average of $10^{8.8}$ in the crop to $10^{7.7}$ in the gizzard. In the duodenum, counts increased to $10^{8.2}$. The data suggest that bacteria are growing and reproducing in the duodenum, although the counts were still lower than in the crop (Lev and Briggs, 1956b). The exception to replication in the duodenum may be the bacteroides which did not increase in numbers from the gizzard to the lower ileum (Shirasaka, 1970b).

Fuller and Turrey (1971) were the first to examine the bacteria adhering to the intestinal mucosa of poultry. Light microscopy revealed the highest numbers of adherent bacteria to be in the crop and ceca. Adherent cells were also seen on tissue sections from the ileum. No cells were seen on tissue from the proventriculus, gizzard, duodenum, jejunum, or cloaca, but cultural studies demonstrated that all of

these sites had an adherent flora (the detection limit for light microscopy was ca. 10^6 bacteria). Salanitro et al. (1978) used scanning electron microscopy and found adherent bacteria in the duodenum, ileum, and ceca. The ability to adhere to the mucosa is dependent on the source of the bacteria. Lactobacilli isolated from mammals were unable to adhere to crop epithelial cells (Fuller, 1973).

Turkey. One of the first and certainly the best study to date of turkey intestinal flora was that of Harrison and Hansen (1950a). They also were one of the few research groups working with poultry flora to incubate their cultures at 41°C, the body temperature of chickens and turkeys. The mean total anaerobe count from the cecal contents of twelve adult turkeys was 1.3×10^{10} bacteria per gram wet weight. E. coli was the only coliform isolated and no salmonella, shigella, or proteus strains were detected. Anaerobic lactobacilli averaged 50% of the flora with the predominant species being "Lactobacillus bifidus" (Bifidobacterium species judging by the photomicrographs). The proportion of fermentative and nonfermentative anaerobic cocci varied considerably among birds. Anaerobic gram-negative rods were less than 1% of the flora. After "L. bifidus", L. acidophilus was the next most common species of lactobacillus. L. plantarum and L. fermentum were isolated in lower numbers.

Other avians. Few studies have been done with birds other than chickens or turkeys, and most have been restricted to the facultative bacterial flora of birds such as geese, swans, and ducks (Faddoul and Fellows, 1966; Geldreich and Kenner, 1969; McBee and West, 1969; Mitchell and Ridgwell, 1971; Hussong et al., 1979; Winsor et al.,

1981). Sieburth (1959a) studied the intestinal microflora of Antarctic birds under very adverse laboratory conditions. None of the birds contained enterococci and the penguins had very low ($< 10^2/g$) concentrations of coliforms. Even when no enterococci or coliforms were detected, anaerobic bacteria were always present (Sieburth, 1959a; McBee, 1960).

One of the more interesting studies was a comparison of duck and chicken intestinal flora by Shirasaka (1970a,b). From the crop to the rectum, the ducks had 100 to 1000-fold higher counts of streptococci and 10 to 100-fold lower counts of lactobacilli than chickens. Lactobacilli were the predominant bacteria in chickens in all areas of the tract, but in ducks the streptococci were at the same concentration as lactobacilli from the crop to the jejunum, and were 10 to 100 times higher in the ileum, ceca, and rectum. This pattern of increased streptococcal counts occurred even when the birds were kept in the same location and fed the same diet. Total bacterial counts were higher in the chicken except in the ceca where the counts were equivalent in the two species. The work of Shirasaka confirmed and extended the data reported by Smith (1965), i.e., streptococci are numerically dominant in ducks whereas lactobacilli are predominant in chickens. The fact that the dominant flora was different even when the birds were on the same diet and under the same environmental conditions suggests a physiological basis for the differences. Ducks do not have a crop, only a slightly enlarged esophageal pouch (Farner, 1942; Smith, 1965). The absence of a crop may be the reason for the lower total counts of bacteria in the small intestine, and the lack of a constant inocula of

large numbers of lactobacilli from the crop may give streptococci a competitive advantage in the duck. The other observed difference between the duck and chicken intestinal tract is that the pH is higher in the small intestine of the duck (Farmer, 1941; Smith, 1965). Thus the intestinal environment of the duck might be more suitable for the growth of streptococci than in the chicken.

Effects of Antibiotics on Intestinal Bacteria

There have been many studies on the effects of antibiotics on poultry intestinal flora, but comparison of results between studies is difficult due to numerous methodological problems. Some of the minor variables that account for the occasionally conflicting or confusing results include the species of the bird, the age of the bird, diet, and the type of antibiotic and dosage used. The major variables are the site sampled, the isolation procedures, and the isolation media.

There is no question that the intestinal flora varies depending upon the site sampled (e.g., Table 5). This variation has been obscured by researchers who pooled gut contents, e.g., the small intestine, duodenum to ileum. An extreme case involved the mixing of the entire gut contents, including ceca (Hauser et al., 1956). Another problem with many bacteriological studies was the analysis of the fecal flora (Elam et al., 1951a; March and Biely, 1952; Rosenberg et al., 1952; Reid et al., 1954; Slinger et al., 1954a; Baldwin et al., 1976). The fecal flora is usually very similar to that found in the lower ileum because the large intestine in poultry is quite small. However, the ceca contract and evacuate an average of twice a day (Johansson et al.,

1948; Clarke, 1979) and it is not clear in most studies whether fecal droppings, cecal droppings, or both, were being analyzed. Another factor is exposure of the feces to air with the resultant increase in counts of facultative and aerobic bacteria. Most specimens were exposed to air for a maximum of one hour, but in one instance they represented an eight hour accumulation of excreta (Baldwin et al., 1976). This greatly increases the chance of cecal and fecal droppings being analyzed together as well as ensuring post-host population changes.

A major problem in any normal flora study is the large amount of variation within and between hosts. Partly in an effort to minimize this variation, and partly to save time, many investigators have pooled the contents from two to five birds (Elam et al., 1951a; Sieburth et al., 1951, 1954; Anderson et al., 1952; March and Biely, 1952; Romoser et al., 1952; Rosenberg et al., 1952; Slinger et al., 1954a). This practice may lead to considerable error if a small number of birds are used. Frequently there is up to a 100-fold difference in counts of an individual bacterial species between birds. Thus, if one of three birds has a 100-fold higher count of coliforms than the other two, the pooled average will be very misleading.

Almost without exception, investigators who studied the effects of antibiotics have relied on selective media and made no attempt to identify the species of predominant bacteria. Researchers who identified each isolate have so far restricted themselves to normal flora studies (Harrison and Hansen, 1950ab; Mitsuoka, 1969; Salanitro et al., 1974ab, and 1978). Also, frequently the isolation media or procedures were not adequate. Many early researchers used thioglycolate

or similar media for the isolation of anaerobes (Elam et al., 1951a; Sieburth et al., 1951 and 1954; Anderson et al., 1952 and 1956; Romoser et al., 1952; Eisenstark and Sanford, 1953; Reid et al., 1954; Slinger et al., 1954a; Baldwin et al., 1976). This usually resulted in isolation of only one to ten percent of the total bacterial flora. Another common practice was the aerobic incubation of lactobacillus selective media. Many intestinal strains of lactobacilli are obligately anaerobic, especially on primary isolation.

The effects of antibiotics on intestinal bacteria, excluding those studies which analyzed feces, are summarized in Table 6. The only consistent results between studies seem to be a decrease in lactobacilli in the crop; a decrease in lactobacilli, total aerobes, and total anaerobes in the small intestine (probably all three are equivalent because lactobacilli are the predominant small intestinal bacteria); and a decrease in enterococci in the ceca.

MECHANISMS OF ACTION

Many mechanisms of growth stimulation have been proposed since the effect of antibiotics was discovered. Chargaff (1945) stated, in reference to blood coaggulation, "It is true, the number of theories of clotting does not greatly exceed that of the workers in the field; but there have been many workers in the field...". Although there is evidence for increased nutrient utilization by antibiotic-fed birds, the effect of the antibiotics on any single nutrient is inadequate to account for the growth response. This has led to the search for bacterial pathogens that may be responsible for growth depression. After

TABLE 6. Effect of antibiotics on poultry intestinal bacteria

Site/Bird[a]	Age	Antibiotic[b]	Lactobacilli	Coliforms	Enterococci	Total anaerobes	Total aerobes	References[c]
Crop								
Chickens	4 wks	Pen 5 ppm	increase	increase	ND[d]	increase	increase	1
	6 wks	CTC 10 ppm	decrease	increase	decrease	ND	decrease	
	average counts wks 1-4	Pen 10 ppm	decrease	decrease	ND	no change	no change	
	"	CTC 10 ppm	decrease	increase	ND	no change	no change	
Chickens (5)	3 wks	CTC 50 ppm	decrease	ND	ND	ND	ND	2
		Virginiamycin 10-100 ppm	decrease	ND	ND	ND	ND	
Small intestine								
Chickens	4 wks	Pen 5 ppm	decrease	decrease	ND	decrease	decrease	1
	6 wks	CTC 10 ppm	decrease	increase	decrease	ND	decrease	
	average of wks 1-4	Pen 10 ppm	decrease	decrease	ND	decrease	decrease	
	"	CTC 10 ppm	decrease	decrease	ND	decrease	decrease	
Turkeys	1-7 wks	Pen 9 ppm	decrease	increase	ND	ND	ND	3
Chickens	4 wks	CTC 44 ppm	decrease	no change	ND	increase	ND	4

[a]Numbers in parentheses are the numbers of individual birds sampled. If there is no number listed, the authors pooled the intestinal contents of two or more birds.

[b]Pen = penicillin; CTC = chlortetracycline; OTC = oxytetracycline.

[c]1 = Sieburth et al., 1954; 2 = Eyssen et al., 1962; 3 = Cook et al., 1954; 4 = Anderson et al., 1956; 5 = Anderson et al., 1952; 6 = Sieburth et al., 1951; 7 = Eisenstark and Sanford, 1953.

[d]Not done.

TABLE 6. Continued

Site/Bird[a]	Age in weeks	Antibiotic[b]	Lactobacilli	Coliforms	Enterococci	Total anaerobes	Total aerobes	References[c]
Ceca								
Chickens	10	Pen 10 ppm	increase	increase	decrease	increase	increase	5
Chickens	4	Pen 5 ppm	increase	decrease	ND	decrease	decrease	1
	6	CTC 10 ppm	decrease	increase	decrease	ND	increase	
Turkeys	2-3	Pen 100 ppm	ND	decrease	decrease	no change	no change	6
	"	OTC 100 ppm	ND	decrease	increase	no change	no change	
Turkeys	1-7	Pen 9 ppm	no change	no change	ND	ND	ND	3
Chickens	4	CTC 44 ppm	decrease	no change	decrease	no change	ND	4
Chickens (10)	8	CTC 2-20 ppm	no change	decrease	no change	no change	no change	7

discussing the probable location of the growth stimulation effects of antibiotics, evidence that the external microbial environment affects the bird's growth response will be reviewed followed by a discussion of individual bacterial groups and their metabolic products.

Site of Action

The site of growth stimulation by antibiotics might occur in one or more of the following areas: the crop, proventriculus, gizzard, small intestine, ceca, or large intestine. The transit time through the proventriculus is too fast for there to be any significant effect of antibiotics at this site. It is also unlikely that the effect occurs in the large intestine or cloaca. The large intestine in poultry is short compared to most monogastric animals and the only known role in the host's nutrition is water and electrolyte absorption. This leaves the crop, gizzard, small intestine, and ceca. Most attention has concentrated on the ceca because they are the site of the highest numbers of intestinal bacteria. As discussed previously in the section on cecal physiology, there is little evidence for vitamin absorption and the presence of the ceca are not essential for growth on a commercial-type diet. Dixon and Thayer (1951) reported that if the ceca were ligated growth stimulation by antibiotics still occurred. To my knowledge no attempt has been made to repeat this initial observation.

Crucial to the discussion of the site of the growth stimulation effect is whether or not the antibiotic survives host and bacterial degradation to reach that site. Except for penicillin, very little is

known about the fate of ingested antibiotics in poultry. Yacowitz and Bird (1953) placed a 40 mg dose of CTC, OTC, or penicillin in the crop of four to five-week-old chicks. Four hours later the chicks were killed and the antibiotic concentrations were measured in various areas of the tract. Of the original dose, only 14% of the penicillin, 16% of the CTC, and 41% of the OTC were recovered in the gastrointestinal tract, urine, or feces. It is difficult to evaluate the significance of the antibiotic concentrations in the tract because the birds were given a single large dose after 24 hours starvation. Sieburth et al. (1954) fed chicks 5 ppm penicillin until one month of age and then sampled different areas of the intestinal tract. The original feed, under the assay conditions used, had 1.8 ppm, the crop 0.75 ppm, the small intestine 0.21 ppm, and the ceca less than 0.018 ppm of penicillin. These findings were similar to those of other researchers who also found little or no penicillin activity in the small intestine or ceca. Katz et al. (1974) could not detect penicillin in the small intestines of six-week-old chicks fed 55 ppm procaine penicillin. When the concentration of penicillin was increased to 110 ppm, small amounts could be detected in the duodenum but none was found in the rest of the small intestine. They concluded that penicillin was destroyed primarily in the upper gastrointestinal tract from the crop to gizzard. Jeffries et al. (1977) fed chicks 25 ppm benzyl penicillin for five weeks. Only 1.6 ppm was detected in the crop and less than 0.01 ppm in the remainder of the intestinal tract, gizzard to ceca. On a diet containing 250 ppm benzyl penicillin, 4.81 ppm was found in the crop, 0.2 to 0.3 ppm in the small intestine, and no penicillin (< 0.01

ppm) in the ceca. The results of Jeffries et al. indicate an almost total inactivation of penicillin. This may have been due to their use of benzyl penicillin which is more acid labile than procaine penicillin, the most commonly used form of penicillin for feed supplements. Jeffries et al. (1977) also administered a single large dose of 500 mg penicillin and found little absorption into the blood, liver, or kidney. Thus, the data suggest a rapid degradation of penicillin in the crop, proventriculus, and gizzard, probably due to the acidic conditions in these organs. The obvious conclusion is that the probable site of the growth-stimulating effect is the crop or gizzard because little or no penicillin is detected in the small intestine. One note of caution concerns the apparent absence of penicillin. Autoclaved penicillin showed no detectable anti-bacterial activity but when fed to chicks it produced a growth increase and an increase in antibiotic-resistant bacteria (Elam et al., 1951b). Another possible problem is the age of the birds. All of the above researchers tested four to six-week-old birds; however, the maximum growth response to antibiotics occurs in the first two to four weeks. Bare et al. (1965) fed chicks a diet containing 44 ppm procaine penicillin and sampled the ileum and ceca at weekly intervals. In the ileum, penicillin was detected at 11.3, 6.2, 0.9, and 0 ppm for the first four weeks respectively. In the ceca, it was present at 5.9, 0.8, 0, and 0 ppm for the same time period. Thus, in one and two-week-old birds, significant quantities of penicillin reach the lower intestinal tract. By the third week the host and/or its flora has changed and penicillin is no longer detectable.

There has been less work with other antibiotics. Jeffries et al. (1977) fed chicks 25 ppm kanamycin or streptomycin. At five weeks of age, very low levels of antibiotic were found from the crop to ileum, but high concentrations (26 to 57 ppm) were present in the ceca. This might be due to absorption in the upper intestinal tract followed by excretion in the urine which in turn is refluxed into the ceca. Bare et al. (1965) fed 11 ppm zinc bacitracin to chicks and reported a slight decrease in intestinal concentrations with age, from 41 ppm in the ileum at one week to 32 ppm at four weeks. In the ceca, the concentration dropped from 54 to 34 ppm. These results are somewhat unusual because they represent concentrations three to five times greater than the dietary intake.

Only a few studies have been done on the effects of antibiotics on bacteria at different sites in the digestive tract. Cook et al. (1954) used 9 ppm penicillin and assayed the gizzard, duodenum, small intestine, ceca, and large intestine of turkeys for coliforms and facultative lactobacilli during the first seven weeks of life. The penicillin had no effect on the counts of lactobacilli in the gizzard or ceca, or on coliform bacteria in the ceca. The lactobacilli decreased in the duodenum, small intestine, and large intestine while the coliform bacteria increased in the small and large intestine (the gizzard and duodenum were not assayed for coliforms). These effects were consistent for four weeks and then began to diminish. Sieburth et al. (1954) reported very similar results with 10 ppm CTC. The coliform bacteria increased in the crop, small intestine, and ceca, while the lactobacilli and enterococci decreased in one-month-old chicks.

The fate of penicillin in the intestinal tract, as well as the limited microbiological data available, suggest that the affected site(s) is the crop, gizzard, or small intestine.

Environment vs. Growth Response

Antibiotic treatment does not always result in growth stimulation, and one major variable is the environment in which the birds are maintained. In general, growth of untreated birds is directly proportional to the cleanliness of their environment. Thus, the cleaner the surroundings are, the smaller the response to antibiotics. Coates et al. (1951b; 1952a) were the first to report the interaction of environment and antibiotic treatment. Three-week-old chicks raised in rooms not previously used for chicken experiments showed no response to penicillin. Their growth was equivalent to that of antibiotic-treated birds in "used" rooms. Similar results were soon reported by other researchers (Lillie et al., 1953; Elam et al., 1954; Morrison et al., 1954). Lillie et al. (1953) found that CTC stimulated growth of chicks by one week of age when the birds were kept in old quarters, but not until three to four weeks of age when they were kept in new ones. When four-week-old antibiotic-fed birds were moved from new to old rooms and taken off antibiotics, there was a "marked but temporary decrease in rate of gain." Hill et al. (1953) reported that penicillin had little effect on three-week-old birds in new rooms, but by four to six weeks of age it caused a slight increase in growth. Similarly, Morrison et al. (1954) found that the maximum response to antibiotics was at four weeks in birds kept in old rooms but not until at least six weeks for those

in disinfected quarters. The poor response of birds in clean quarters to antibiotic treatment may also be affected by the concentration of antibiotic used. With CTC, 200 g/ton gave a growth response in clean quarters but 20 g/ton did not (Hill and Kelly, 1953).

The decreased response in clean environments is due primarily to an increase in the weight of untreated birds. Marusich et al. (1974) reported an average 23.5% growth increase with 50 ppm penicillin in their regularly used rooms. After rigorous cleaning and disinfection procedures, control birds weighed more and the percent response to penicillin decreased. Subsequent trials in the formerly clean room resulted in a progressive decrease in the weight of the controls and an increasing response to penicillin as measured by body weight and feed efficiency. Antibiotic treatment could not compensate completely for the environmental effects because the total weight of treated birds declined with time even though the percent response increased (Coates et al., 1955a).

As with most other aspects of antibiotic treatment, an increased response by birds in old or dirty environments is not always observed. Vogt (1966) found no significant effects of a dirty, normal, or very clean environment on the growth of chicks. In fact, antibiotics did not increase growth except under clean conditions. Potter et al. (1962) found no increase in response to antibiotics by birds kept in old batteries vs. new batteries, although both batteries were kept in a new room. In contrast, Hill et al. (1953) found that the use of old batteries in new rooms decreased growth, although not as much as the use of old batteries in old rooms. The depression of growth in the old

rooms occurred in two of three experiments. Therefore, growth depression is a function of both the room and the type of cage. Chicks and poults raised on elevated wire floors in battery brooders usually show faster weight gains, increased feed efficiency, and reduced response to antibiotics versus those raised in floor pens with litter (Coates et al., 1952a; Saxena et al., 1952; Elam et al., 1953; Morrison et al., 1954; Wisman et al., 1954; Sullivan et al., 1961). The major variable seems to be the degree of exposure of the birds to feces. Direct feeding of fresh feces or intestinal contents significantly depresses the growth of poultry but autoclaved feces have no effect. The growth depression can be eliminated by antibiotics (Elam et al., 1954; Coates and Porter, 1955; Warden and Schaible, 1961 and 1962).

The improved growth of birds raised in a clean environment suggests that specific pathogens may be responsible for growth depression. For example, Elam et al. (1954) reported that the fecal concentration of clostridia was 13-fold higher from chicks raised in regular brooder rooms vs. those raised in clean, disinfected rooms. Similarly, Lev et al. (1957) found that there were fewer C. perfringens in the ceca of one and two-day-old chicks raised in disinfected rooms than in chicks raised in "used" quarters. The interaction between C. perfringens and antibiotic-induced growth stimulation is discussed further in the next section.

Clostridium perfringens

Sieburth et al. (1951) were the first to suggest that the mechanism of growth stimulation by antibiotics was due to the suppression of C.

perfringens. They found that penicillin and OTC reduced the numbers of sulfite-reducers (tentatively identified as C. perfringens) in the ceca of two-week-old turkeys from 2.5×10^4 to less than 100/g. OTC also decreased the numbers of sulfite-reducers in pig feces from 3.4×10^7 per gram to less than 10 per gram. They concluded that antibiotics stimulated growth in turkeys and pigs by preventing an enterotoxemia due to C. perfringens. Several research groups subsequently reported that antibiotics reduced the numbers of C. perfringens, but that growth stimulation was not always correlated with C. perfringens counts (Larson and Carpenter, 1952; Elam et al., 1953). Smyser et al. (1952) found no decrease of C. perfringens counts in chickens by CTC, whereas penicillin produced an increase in numbers beginning at four weeks of age.

Lev and Forbes (1959) and Forbes et al. (1959) reported that implantation of C. perfringens in germfree chicks depressed their growth. The growth decrease was counteracted by penicillin treatment. As was later pointed out (Coates and Jayne-Williams, 1966), this model of infection may be misleading. The depression of growth caused by C. perfringens in germfree chicks was no greater than the normally diminished growth of conventional birds, even though the C. perfringens colonized the gnotobiotic chicks in much higher numbers than in conventional chicks. Also, in conventional chicks C. perfringens is not found in the small intestine, and after one week of age is frequently not detectable ($< 10^2$/g) in the ceca (Lev et al., 1957; Eyssen and DeSomer, 1967; Timms, 1968; Barnes et al., 1972). In contrast, C. perfringens colonized the entire intestinal tract of gnotobiotic

chicks and maintained colonization up to four weeks of age (Lev and Forbes, 1959). Other researchers, however, found no depression of growth of germfree animals by C. perfringens (Eyssen and DeSomer, 1965 and 1967; Coates, 1968; Powell et al., 1974). Penicillin treatment of germfree chicks monoassociated with C. perfringens drastically reduced the numbers of C. perfringens in the lower ileum and ceca, but did not stimulate growth (Wagner and Wostmann, 1959). Eyssen and DeSomer (1967) inoculated germfree chicks with a cecal strain of C. perfringens type A, but there was no inhibition of growth, no increase in small intestinal weight, and no increase in fat excretion. Even when they fed chicks the same strain of C. perfringens used by Lev and Forbes (1959), no growth depression occurred although the strain did colonize the tract. Powell et al. (1974) also failed to find any growth depression of germfree or conventional chicks by C. perfringens, although penicillin treatment did suppress the bacteria in the gnotobiotes. Again, no growth depression occurred when the same strain used by Lev and Forbes (1959) and Forbes et al. (1959) was administered to the chicks. Growth depression did occur using a mixture of human food poisoning strains but it did not respond to penicillin treatment. The authors concluded that the correlation between penicillin treatment and a decrease in C. perfringens was not related to the antibiotic's growth stimulating properties.

Streptococcus faecalis/S. faecium

Antibiotic treatment has been reported to decrease the intestinal counts of enterococci (e.g., Anderson et al., 1952 and 1956; Sieburth

et al., 1951 and 1954; Wagner and Wostman, 1959) and Anderson et al. (1956) reported that feeding S. faecalis to conventional chicks resulted in growth depression. Barnes et al. (1978) also suggested that S. faecalis might be responsible for growth depression because treatment with zinc bacitracin decreased counts of enterococci in the small intestine and ceca of one to three-week-old chicks. S. faecalis was not detected ($< 10^3$) by the second week in the ceca or small intestine of antibiotic-treated birds, but the counts of S. faecium were not affected by antibiotic treatment. The lack of effect on S. faecium was attributed to a greater resistance to bacitracin. Dutta and Devriese (1982) also reported a higher percentage of S. faecium strains to be resistant to bacitracin compared to S. faecalis var. liquefaciens.

S. faecalis var. liquefaciens is a potential pathogen for poultry and is capable of desquamating the intestinal wall. In turkeys, this can result in the penetration of a Eubacterium species with the subsequent development of liver granulomas. Neither organism alone is capable of causing the granulomas (Moore and Gross, 1968). A possible mechanism for growth inhibition by enterococci was suggested by Eyssen and DeSomer (1963ab). A period of fat and carbohydrate malabsorption occurred between five and ten days of age in chicks fed a casein-sucrose diet. It resulted in a decrease in weight gain and a sharp decrease in feed efficiency. Birds fed antibiotics or kept in a clean environment showed no weight loss during this period. The malabsorption syndrome was confirmed by Huhtanen and Pensack (1965a) who found that the predominant bacteria in the duodenum of six-day-old chicks were S. faecalis and a Lactobacillus species. The numbers of enterococci were

drastically reduced by penicillin. When germfree chicks were monoassociated with S. faecalis, growth was depressed although it was still superior to that of conventional chicks plus penicillin (Huhtanen and Pensack, 1965b). The malabsorption syndrome is affected both by the intestinal flora and the hosts' physiology. No malabsorption occurs in germfree chicks. However, the syndrome disappears by the second week of life in conventional and S. faecalis-associated gnotobiotes, even though the counts of S. faecalis remain constant in the gnotobiotic birds (Eyssen and DeSomer, 1967).

Eyssen and DeSomer (1965) monocontaminated germfree chicks with several species of intestinal bacteria, but only S. faecalis produced a consistent growth depression. However, the growth depression was limited and no malabsorption syndrome occurred. When a bacteria-free fecal filtrate and S. faecalis were fed to germfree chicks, malabsorption and significant growth depression occurred. This work was further expanded by Eyssen and DeSomer (1967). The effect of S. faecalis and the fecal filtrate on germfree chicks was additive. S. faecalis depressed growth 6%, the filtrate 10%, and the combination by 16%. The weight of the small intestine and fat excretion also increased in an additive manner (gut weight increased 22% with the filtrate alone and 84% with the filtrate plus S. faecalis). Harrison and Coates (1972) and Harrison and Fuller (1973) also found that a bacteria-free fecal filtrate significantly increased the weight of the small intestine. Unlike the report of Eyssen and DeSomer (1967), Harrison and Coates (1972) found that S. faecalis decreased the intestinal weight. S. faecalis or the filtrate alone depressed growth nonsignificantly or not

at all, but the combination significantly decreased body weight by 8 to 14% (Harrison and Coates, 1972; Fuller et al., 1979). Lev and Forbes (1959) also found no depression of growth of germfree chicks by S. faecalis.

Eyssen et al. (1967) reported that growth rate, intestinal weight, and fat excretion of gnotobiotic birds infected with S. faecalis plus filtrate were similar to those of uninfected conventional birds. The growth depression effect was fairly specific because there was no synergistic effect between the filtrate and lactobacilli, coliforms, or C. perfringens. The active factor in the filtrate was not dialyzable, it was inactivated by heating for 30 minutes at 100°C, and antibiotics had no effect on its inhibitory action. Feces from germfree chicks had no growth-depressing effect. The inhibitory effect produced by the filtrate could be transmitted to birds in adjacent cages, and when the filtrate was fed to a group of birds, their feces were as inhibitory as the original filtrate to other chicks. The growth inhibition factor in the fecal filtrate could be passed through several successive groups of birds without any loss of ability to depress growth. Eyssen and DeSomer (1967) concluded that the active agent in the fecal filtrate was probably a virus, and suggested that the virus infected the intestinal wall.

More information on the probable viral nature of the active agent in fecal filtrates was obtained by Harrison and Fuller (1973). When the filtrate was administered to young chicks (0.2 ml orally), serum 7S immunoglobulin concentrations increased suggesting an antibody response. Also, fecal filtrate from germfree chicks that had received

0.2 ml of filtrate from conventional birds still produced an increase in gut weight in other germfree birds up to two weeks after the original dose. This suggests that the virus is capable of establishing and reproducing itself within the intestinal tract, otherwise it would have been washed out within two weeks. Harrison and Fuller (1973) also noted that as hens aged, their chicks' susceptibility to the growth-depressing effect of the streptococci and fecal filtrate decreased and finally disappeared. Even when growth was not depressed, however, the filtrate consistently increased the weight of the intestines. Harrison and Fuller suggested that increased immune resistance was passed from the dams to the chicks because serum 7S immunoglobulin increases with the age of the hen and antibody levels in the serum are directly proportional to those in the egg.

Some confusion exists concerning the streptococci responsible for growth depression. Huhtanen and Pensack (1965b) and Eyssen and DeSomer (1965, 1967) both reported growth depression to be associated with S. faecalis. But Fuller et al. (1979) stated that, based on the physiological reactions described, the strain used by Huhtanen and Pensack (1965a) could also be identified as S. faecium. Fuller et al. (1979) tested chicken isolates of E. coli, K. aerogenes, L. fermentum, Lactobacillus biovars A and K (described in Fuller, 1973), S. faecalis var. liquefaciens, S. faecium, unidentified species of Streptococcus and Bacteroides, and C. perfringens type A. Even though each of the cultures colonized the intestinal tract of germfree birds from crop to cecum, only a mixture of four S. faecium strains significantly depressed growth without a fecal filtrate. With added filtrate most of the

species significantly depressed the growth of chicks, but the greatest depression occurred with S. faecium (20.6% compared to 13.7% for S. faecalis plus filtrate).

The growth-depressing effect of S. faecium (or S. faecalis) is a synergistic effect between the bacterium and a virus present in the intestinal tract. The virus seems to be primarily responsible for irritation to the intestinal mucosa because it consistently increases the weight of the small intestine. Although S. faecalis var. liquefaciens is capable of desquamation of the intestinal wall, the variability in response of intestinal weight suggests strain-to-strain variation. Damage to the intestinal wall by the virus may result in increased translocation of streptococci to the bird's liver. S. faecalis var. liquefaciens normally is capable of translocation from the intestines to the peritoneum and liver but this ability is restricted to the first four days of life. Translocated bacteria are cleared more efficiently as the bird ages (Fuller and Jayne-Williams, 1968 and 1970). It is not known whether increased numbers of bacteria in the liver are responsible for growth inhibition. Because the growth depression due to S. faecalis and fecal filtrate is reversible by fish solubles (Harrison and Coates, 1972), the mode of action might be by interference with absorption of nutrients. Although the total numbers of streptococci in the tract are not affected by the presence or absence of filtrate, the effect of the filtrate on the numbers of bacteria adherent to the mucosa is unknown. Damage caused by the virus might enhance adherence to the mucosa and thus increase the interference with host absorptive processes. The increase in immunity

with age could mediate adherence or it could reduce the rate of translocation or both.

The connection between S. faecalis/S. faecium and growth stimulation by antibiotics is unclear. The malabsorption syndrome originally described by Eyssen and DeSomer was observed in chicks fed a semi-synthetic casein-sucrose diet. However, if starch was used instead of sucrose little or no malabsorption occurred. It would be interesting to repeat the experiments using a commercial-type diet to determine if the malabsorption syndrome was an artifact of the casein-sucrose diet.

Houghton et al. (1981) studied the effects of penicillin on S. faecalis and S. faecium at different sites of the intestinal tract of three-day-old chicks. Penicillin had no consistent effects on the numbers of S. faecium in the crop. It decreased counts in the duodenum, ileum, and ceca in 15 of 18 trials, but there was a significant growth response in only 3 of the 18 trials. Using the strain of S. faecium (SY1) found by Fuller et al. (1979) to be the most effective in depressing growth of chicks, a significant growth depression of germfree chicks was observed in only 8 of 33 trials. Houghton et al. concluded that penicillin reduced the counts of S. faecium but that neither S. faecium nor S. faecalis was responsible for growth depression because penicillin failed to increase growth in most feed trials.

Enterobacteriaceae

A consistent effect of antibiotic treatment is an increase in the number of intestinal coliforms (Anderson et al., 1952; Romoser et al., 1952; Cook et al., 1954; Rhodes et al., 1954; Sieburth et al., 1954;

Slinger et al., 1954a; Bogdonoff et al., 1959;). Even when the total number of coliforms does not change, antibiotic treatment sometimes results in population shifts from a predominantly E. coli flora to a mixed coliform flora including "Aerobacter" sp. (Wiseman et al., 1956a; Baldwin et al., 1976).

Romoser et al. (1952) noticed an increase in E. coli and "Aerobacter aerogenes" in the ceca of penicillin-fed chicks. When the organisms were fed to other birds "A. aerogenes" did not stimulate growth, but the combination of "A. aerogenes", penicillin, and lactose was more stimulatory than penicillin and lactose alone. Growth stimulation by E. coli was equivalent to that obtained with penicillin and lactose. The combination of all three elements was more effective than any single one; i.e., an additive effect. The authors suggested that penicillin might enhance colonization (Romoser et al., 1953). The ability to stimulate growth was dependent on the diet because on a nutritionally deficient diet, E. coli or "A. aerogenes" stimulated growth, although not consistently. But on a richer diet, neither stimulated growth at all. Penicillin stimulated growth regardless of diet.

Using coliforms isolated from the ceca of chicks fed penicillin, Anderson et al. (1953a) found that one of three E. coli strains stimulated growth significantly. There was an additive effect between the E. coli strains and penicillin and even the strains that did not stimulate growth by themselves, increased growth when combined with penicillin. Another E. coli strain was found that stimulated the growth of poults (Anderson et al., 1953b). The effect of the E. coli

was dependent on viable organisms. Neither the culture filtrate nor killed cells increased growth. There was no consistent effect of the E. coli strains on the cecal flora (Anderson et al., 1953a,b). One confounding variable in the studies of Anderson et al. was that the diet was probably nutritionally deficient. In some of the trials, 10 ppm penicillin produced an average weight increase of 73%.

Warden and Schaible (1960) reported that the administration of E. coli to chicks and poults did not increase weight, with or without zinc bacitracin or OTC. There are several possible reasons why the E. coli might have failed to stimulate growth: i) the source of the strain was not specified, so it might not have been a poultry isolate and thus unable to colonize the intestinal tract; ii) the organisms were inoculated into the crop twice weekly instead of being mixed with the feed as the previous authors had done; and iii) the growth of the E. coli strains may have been inhibited due to the high concentration of antibiotic used (200 g/ton). The same authors (Warden and Schaible, 1962) also added lysed E. coli (a chicken isolate) to the diet at a level of 0.1% on the theory that the bacterial enzymes might be of benefit to the host. This resulted in a significant growth stimulation; however, the basal diet contained 50 ppm arsanilic acid so therefore the growth response might have been an additive effect similar to that observed by Anderson et al. (1953a). Other researchers also have failed to get any growth increase with E. coli strains or with a combination of E. coli and "Aerobacter" strains, with or without antibiotics (Bogdonoff et al., 1959; Edwards and Boyd, 1963b). Although E. coli strains do not stimulate growth, association of E. coli with

germfree chicks does not depress growth either (Forbes and Park, 1959; Forbes et al., 1959; Lev and Forbes, 1959).

Tetracycline treatment in humans frequently results in an increase in Proteus species (e.g., Baker and Pulaski, 1950; Metzger and Shapse, 1950). Sieburth et al. (1951 and 1952a) reported a similar correlation between OTC treatment and Proteus species in turkey poults. No proteus were detected on the basal diet but treatment with 100 ppm OTC increased the counts in turkey feces to 10^4/g. At higher levels of 1000 or 5000 ppm, the counts stabilized at 10^7/g. All of the isolates were identified as P. mirabilis. The increase in Proteus resulted in a displacement of coliform bacteria and at 5000 ppm OTC, no coliforms were detectable. The minimum inhibitory concentration (MIC) of OTC for five E. coli strains was less than 3 µg/ml vs. a MIC of 1000 µg/ml for the Proteus strains (Sieburth et al., 1952a). In contrast, Baldwin et al. (1976) found no Proteus species in turkey feces, even with up to 440 ppm OTC. An oral dose of P. mirabilis had no effect on the growth of chicks (Edwards and Boyd, 1963b).

Lactobacilli

In the decades since Metchnikov first championed the ingestion of lactobacilli to improve human health, there has been extensive research on means of colonizing the intestinal tract. Because lactobacilli are the predominant species in the crop and small intestine of poultry, it was inevitable that the effects of feeding lactobacilli to poultry would be explored. Although Larousse (1970) reported a significant increase in weight due to feeding a Lactobacillus strain, most workers

have found only a nonsignificant weight increase using various poultry intestinal lactobacilli (Tortuero, 1973; Francis et al., 1978; Adler and DaMassa, 1980). The feeding of lactobacilli usually was associated with a decrease in coliform counts. A similar effect has been noted in pigs when lactic acid was fed (Kershaw et al., 1966; Cole et al., 1968).

Ammonia Production

Urea is one source of excreted nitrogen in poultry and an early hypothesis was that antibiotics might increase its utilization. However, added urea does not increase the growth of birds on an adequate diet, nor does urea plus penicillin increase growth beyond that of penicillin alone (Slinger et al., 1952c). The alternative to the previous hypothesis is that bacterial ammonia production might be a cause of growth depression (Francois and Michel, 1955; Michel and Francois, 1955). Francois and Michel (1955) were among the first to report that antibiotic treatment was associated with a decrease in intestinal ammonia concentration (in the pig). Antibiotic treatment also decreased urea hydrolysis in rats (Visek et al., 1959; Holtzman and Visek, 1965). When rats and chicks were immunized against urease, urea hydrolysis in the intestinal tract decreased and growth was stimulated (Dang and Visek, 1960).

Although antibiotic treatment may reduce intestinal ammonia concentrations and urea hydrolysis in rats and chickens, these effects are not always correlated with a growth response (Francois and Michel, 1955; Harbers et al., 1963; Alvares et al., 1964b). It also is important

to note that most of the experiments described have been done with semi-synthetic diets (casein and/or sucrose based) and Alvares et al. (1964a) have shown that the ammonia reduction effect is dependent on the dietary carbohydrate source. It would be worthwhile to repeat some of the experiments with a commercial-type diet to see if there was any detectable effect of antibiotics on ammonia production because addition of urea to poultry diets does not depress growth.

Indole Production

Makino and Umezu (1952) reported that a high level of CTC (500 ppm) rapidly decreased the indole concentration in the intestinal tract of mice (indole is toxic; it is absorbed and then detoxified by the liver). However, Wisman et al. (1957) found that addition of 990 mg indole/kg of feed had no effect on chicks. Nor did CTC treatment (22 ppm) affect indole production or excretion in the intestinal tract.

Nucleic Acids

Using a semi-synthetic diet, Eyssen (1962) found that virginiamycin stimulated growth of chicks by 15 to 21%. Because the diet had no exogenous source of nucleic acids, he suggested that the intestinal lactobacilli might be competing with the host for endogenous nucleic acids (e.g., from desquamated mucosal cells). Addition of 0.05 and 0.2% DNA increased growth 15.4 and 26.9%, respectively. There was also a significant increase when 0.1% of intact or hydrolyzed yeast RNA was added. In a second experiment, 0.2% DNA gave a 13.4% increase and virginiamycin a 14.5% increase. But together, there was a 25.1%

increase. With a commercial diet containing about 0.8% nucleic acids, additional DNA did not stimulate growth but virginiamycin did. In other words, the effects of antibiotic and nucleic acids were additive. Although the chicks' growth might have been limited by nucleic acids, the lactobacilli were not. No change in counts of lactobacilli occurred in the crop, duodenum, or ceca when yeast extract or 0.2% DNA+RNA was added to the diet (Eyssen, 1962).

Naber et al. (1957) found no growth response with nucleic acid supplements unless the birds were on a folic acid deficient diet.

Feed Consumption and Efficiency

The increase in weight gain that occurs with antibiotic treatment is due to an increase in food consumption and an increase in feed efficiency (the ratio of feed consumed vs. weight gained). The increase in feed efficiency is not sufficient to account for the entire weight gain. If the amount of food consumed by antibiotic-treated animals is limited to that consumed by the controls, there is little or no weight increase (Scott and Glista, 1950; Brown et al., 1952; Slinger et al., 1954a; Slinger and Pepper, 1954a and 1955). Sieburth et al. (1952b) reported that restricting feed intake of poults did not eliminate the growth response due to penicillin, however, the birds were on an ad libitum diet with penicillin for one week before feed was restricted.

Slinger et al. (1954a) reported that penicillin increased feed consumption by chicks at four weeks of age. However, when feed consumption was adjusted for body weight, penicillin increased consumption only during the first 2½ weeks. Through seven weeks of age,

feed consumption by antibiotic-treated birds was the same or less than that of control birds (when adjusted to body weight). Similar results were obtained with turkeys. Penicillin treatment increased feed consumption during the first six days of life, had no effect from day 7 to 17, and decreased feed consumption from day 18 to 25. The penicillin-fed birds also had a slower rate of growth compared to untreated birds during days 18 to 25 (Slinger and Pepper, 1954a and 1955). It was concluded that the increased weight of penicillin-fed birds at 24 weeks of age was due to weight increases that occurred in the first three weeks of life. After that time period, treated birds consumed more because they were larger and thus maintained a greater weight than untreated birds.

There have been a large number of papers on the effects of antibiotics on the nutrition and feed efficiency of the host. Although absorption of these nutrients seems to be affected by antibiotic treatment, the amelioration of specific deficiencies is not adequate to account for the observed growth response. Most of the nutritional data could be explained by a common mechanism: antibiotics cause a general increase in the absorptive efficiency of the intestinal tract. There is only one specific mechanism for an increase in feed efficiency that has been well documented and it is discussed below.

Monensin is widely used to increase the feed efficiency of ruminants (Grueter et al., 1976; Perry et al., 1976; Raun et al., 1976; Davis and Erhart, 1976; Potter et al., 1976). A major portion of the ruminant's energy requirement is derived from the absorption and utilization of microbially produced short chain fatty acids (Hungate, 1966). Treatment

of cows with monensin results in an increase in the propionate to acetate ratio as well as a decrease in methanogenesis (Dinius et al., 1976; Richardson et al., 1976; Van Nevel and Demeyer, 1977; Slyter, 1979). The increased propionate concentration is responsible for the observed increase in feed efficiency. Monensin inhibits various acetate-producing rumen bacteria but has little effect on the predominant propionate producers. Although there is some inhibition of methane production, it is inadequate to account for the increased propionate (Chen and Wolin, 1979; Slyter, 1979; Henderson et al., 1981). Monensin is an effective coccidiostat in chickens and turkeys (Shumard and Callender, 1968; Prasad et al., 1971; Reid et al., 1972; McDougald, 1976; Ruff et al., 1976). However, unlike ruminants, the weight and feed efficiency of poultry are not stimulated. In fact, monensin may result in a significant growth depression, especially in older birds (Damron et al., 1977; Damron and Harms, 1981; Stuart, 1978; Marusich and de Young, 1979; Chappel and Babcock, 1979). Other coccidiostats besides monensin also can depress growth of chicks (Keshavarz and McDougald, 1982). Avoparcin increases the feed efficiency of cattle, possibly by increasing propionate concentrations (Johnson et al., 1979). It has a different antimicrobial spectrum than monensin and, unlike monensin, it causes a small increase in growth and feed efficiency of poultry (Roth-Maier and Kirchgessner, 1976; Spoerl and Kirchgessner, 1978; Leeson et al., 1980; Hulan and Proudfoot, 1981).

There is no evidence that antibiotic growth stimulation of poultry is related to shifts in short chain fatty acid ratios. In studies of two-week-old poults, zinc bacitracin produced significant increases in

weight and feed efficiency, but no significant differences in acetate or propionate concentrations occurred at any site in the intestinal tract (Appendix IV).

There is no question that antibiotics increase the feed efficiency of poultry. This could occur through increased absorption of nutrients, a decrease in the loss due to the intestinal flora, or a net increase in microbially-produced metabolites utilized by the host. The last possibility is the most unlikely. Microbially-produced vitamins do not play a significant role in poultry nutrition and antibiotic treatment does not affect the proportions of short chain fatty acids. It is difficult to differentiate experimentally between the other two mechanisms for increasing feed efficiency. One method has been to compare the nutrition of germfree vs. conventional animals.

Effects of Germfree Life on the Intestinal Tract

The small intestine of germfree chickens weighs less than that of conventional birds (Gordon et al., 1958; Coates and Jayne-Williams, 1966; Siddons and Coates, 1972). Because the length is unchanged, it was assumed that the weight decrease was due to a thinning of the wall (Coates and Jayne-Williams, 1966). This was confirmed by histological studies that showed a reduction of the lamina propria, but no change in the muscular outer layers (Gordon and Brückner-Kardoss, 1961).

Similar results have been noted with antibiotic treatment. Gordon et al. (1958) reported that penicillin reduced the weight of the small intestine, but not the ceca, of conventional chicks. Penicillin had no

effect on the weight of the intestines of germfree birds. The weight of the small intestine of penicillin-treated conventional chicks was significantly less than that of untreated chicks, but was still heavier than the intestines of germfree birds. The small intestinal weight was similar in both treated and untreated chicks by eleven weeks of age. Coates et al. (1952a, 1955b) found that penicillin decreased the weight of the small intestine by 17 to 21%. There was no difference in fat or moisture content compared to untreated birds. Chlortetracycline also was found to reduce the weight of the small intestine (17-19%) and ceca (7-13%) (Pepper et al., 1953). [Pepper et al. did not standardize the intestinal weights to the total body weight and therefore erroneously reported that cecal weights increased slightly with antibiotic treatment.] Jukes et al. (1956b) found that both penicillin and OTC reduced the weight of the intestinal tract by 4 to 26%. Tissue sections of the duodenum showed a significant decrease in the size of the lamina propria while the outer muscular layer was not affected. No changes were noted in the length, fat, or moisture content of the intestinal tract. The thinning effect of the antibiotic was minimal during the first week of life, but was significant by the second week.

The absence of the intestinal flora has two main histological effects on the small intestine. The first is a reduction in the weight as discussed above. There is also a reduction in the size of the lymph nodes and reticuloendothelial system, presumably due to decreased antigenic stimulation (Gordon, 1959; Gordon and Brückner-Kardoss, 1961; Sprinz et al., 1961). The other major change is in the villi. They are smaller (and/or the crypts are shallower), more evenly

shaped, and the rate of renewal of epithelial cells is slower in the germfree small intestine (Sprinz et al., 1961; Abrams et al., 1963; Cook and Bird, 1973; Rolls et al., 1978). Because of these differences in the germfree chick, and in other germfree animals, the idea has been proposed that the conventional gut exists in a chronic state of mild inflammation. Thus, the germfree gut might be more efficient at digestion and absorption. For example, in vitro everted sacs of small intestine from germfree chicks absorbed glucose and various B vitamins faster than those from conventional birds (Ford and Coates, 1971). Germfree chicks and rats also absorb fats better than conventional animals (Boyd and Edwards, 1967; Demarne et al., 1973).

As mucosal epithelial cells migrate from the base to the tip of the villi they mature and develop various enzymatic capabilities (Hendrix and Paulk, 1977). Because a slower turnover of mucosal cells presumably would lead to a larger proportion of mature cells, it has been suggested that the germfree intestine would have larger amounts of various digestive enzymes (Reddy and Wostmann, 1966). However, Siddons and Coates (1972) found no difference in the concentrations of maltase, iso-maltase, or sucrase in the small intestinal wall of germfree and conventional chicks. Lepkovsky et al. (1964) were able to detect pancreatic amylases and proteases in the ceca of germfree birds. In conventional birds, the enzymes were degraded by the intestinal bacteria before reaching the ceca. There have been conflicting results with other germfree animals concerning the effect of the intestinal flora on disaccharidase activities (Dahlqvist et al., 1965; Reddy and Wostmann, 1966; Szabo, 1979). The presence of the intestinal

flora does, however, decrease the levels of other digestive enzymes in the intestinal mucosa, including amylases, proteases, phosphatases, and β-glucuronidases (Yolton and Savage, 1976; Kawai et al., 1978; Kawai and Morotomi, 1978; Szabo, 1979; Juhr, 1980; Kawai, 1980).

Effects of Long-term Exposure to Antibiotics

Several researchers have reported that the growth response to a particular antibiotic appears to decrease with time. McGinnis et al. (1958) noted a diminishing response at their brooding facility to penicillin. Introduction of oleandomycin and erythromycin, which had not been used previously, gave much larger responses. Similarly, Nelson et al. (1963) reported a large initial response with tylosin which began to decline after two years of use. Growth response to bacitracin lasted for 1½ years before it began to decrease. Waibel et al. (1954) and Libby and Schaible (1955) suggested that long term use of antibiotics reduced the numbers of growth-depressing bacteria in the environment and that this resulted in the control birds growing better and thus an apparent decrease in the effect of antibiotics. Heth and Bird (1962) compiled the results of eleven years of feed trials with penicillin, tetracycline (CTC or OTC), or zinc bacitracin. The data included the trials reported by Waibel et al. (1954). Over an initial three year period, penicillin showed a gradually decreasing response, as reported by Waibel et al.; however, no significant increase in the weight of the control groups occurred. During a subsequent three year period, penicillin showed no decrease in response, nor did zinc bacitracin. Over a five year period, tetracycline (CTC or OTC) showed

a gradual decrease and during this period basal weights of control birds gradually increased. Coates and Davies (1959) found no change in the response of chicks to penicillin over a five year period.

Antibiotic treatment, whether at therapeutic or subtherapeutic levels, inevitably causes an increase in the number of resistant organisms. It is surprising, therefore, that continued use of antibiotics for many years in the same location still results in a significant growth response. Following antibiotic treatment, a large percentage of the coliforms rapidly become resistant (e.g., Smith and Crabb, 1957; Smith, 1958; Levy et al., 1976); however coliforms comprise a very small percentage of the total intestinal flora. Only a few studies have been done on the development of antibiotic resistance by the anaerobic bacteria in the intestinal tract. Barnes and Goldberg (1962) found a slight increase in the number of resistant gram-negative anaerobes from the ceca of chickens fed CTC. Ahart et al. (1978) found that a higher proportion of coliforms were resistant to antibiotics both before and after antibiotic exposure than were gram-negative anaerobes. Antibiotic treatment (at feed additive levels) of cows and pigs also resulted in a greater increase in the proportion of resistant coliforms. For example, CTC treatment of cows increased the proportion of antibiotic-resistant coliforms from 48 to 87%, but antibiotic-resistant gram-negative anaerobes only increased from 42 to 48%.

Without the selective pressure of antibiotics, resistant strains do not seem to be able to compete as well as sensitive ones and are usually displaced. By five weeks after cessation of antibiotic treatment,

tetracycline or ampicillin resistant strains had been displaced by sensitive strains (Datta et al., 1971). Levy et al. (1977) reported that many chicks still had OTC-resistant E. coli populations up to 2 months after the removal of OTC from the diet. This persistence was evidently due to recurrent exposure and re-infection with resistant bacteria since Levy et al. showed that tetracycline was very stable in the environment. When the chickens were placed in a clean environment, the resistant strains rapidly disappeared. Also, if the birds were placed together with previously untreated birds, the sensitive strains replaced resistant ones within three weeks. This re-infection may explain the persistance of resistant E. coli in pigs for several months after cessation of treatment (Smith and Crabb, 1957). In a herd of CTC-treated calves, 84% had resistant E. coli and 79% had entirely resistant populations. By two months after treatment had ceased, 49% of the calves had resistant strains but these predominated in only 13% of the calves.

One factor in the reduced ability of resistant strains to maintain colonization may be that antibiotic resistance can confer a metabolic penalty. After 24 hours of growth, a CTC-resistant strain of E. coli produced only 64% as many cells as the sensitive parent strain (Peterson and Johansson, 1957). Also, CTC-resistant E. coli were shown to be more susceptible to phagocytosis (in the calf) than sensitive strains (Radisson et al., 1956; MacFadden and Bartley, 1959; MacFadden et al., 1960). MacFadden et al. (1960) proposed that antibiotics stimulate growth by increasing the susceptibility of intestinal bacteria to phagocytosis. Thus, the growth response to antibiotics would be

greatest in young animals because their immune systems are not as efficient as those of older animals. Subminimum inhibitory concentrations (sub-MIC) of antibiotics also can increase phagocytosis and/or intracellular killing (Friedman and Warren, 1974; Alexander and Good, 1968; Nishida et al., 1976; Root et al., 1981). However, the increased susceptibility to phagocytosis, at least of the staphylococci, is very dependent upon the experimental protocol (Lorian and Atkinson, 1980; Atkinson and Amarol, 1982).

The continued occurrence of a growth response, even in the presence of a predominantly resistant flora, may be due to the effects of sub-MIC levels of antibiotics. Antibiotic concentrations ranging from 0.03 to 0.5 times the MIC are capable of decreasing the total numbers and growth rate of various bacteria by 90% (Ezrow et al., 1979; Atkinson and Amarol, 1982). Sub-MIC doses of some antibiotics can inhibit adherence to epithelial cells (Svanborg-Edén et al., 1978; Eisenstein et al., 1979; Ofek et al., 1979; Sandberg et al., 1979; Vosbeck et al., 1979 and 1982). A decrease in the numbers of adherent bacteria could have a major impact on the nutrition of the host and might explain the effects of antibiotics on the ultrastructure of the intestinal mucosa.

ANTIBIOTICS AND HEALTH

There has been much debate in recent years of the possible dangers associated with antibiotics as feed supplements. The primary concern has focused on the increase in antibiotic R-factors and their effect on human health. This and similar anxieties led to the restriction of use of certain antibiotics as feed supplements in

England (Swann, 1969). The Food and Drug Administration of the U.S. also has recommended restrictions on the use of penicillins and tetracyclines (FDA Task Force, 1972).

The general logic sequence of the regulatory commissions has been:

1) antibiotics in feed increase the number of resistant bacteria in animals;

2) the resistant bacteria can either colonize man and cause subsequent disease or transfer their R-factors to human intestinal flora;

3) antibiotic-resistant bacteria are a major human clinical problem.

Concern also has been expressed specifically about the effect of antibiotic supplements on Salmonella species. The conclusions of the Swann Committee report (1969) and the FDA Task Force report (1972) as well as the results of experiments undertaken in response to specific questions raised by the reports have been reviewed and critiqued by several authors (Jukes, 1973; Braude, 1978; Solomons, 1978; van Houweling and Gainer, 1978).

There is no question that use of antibiotics as feed supplements results in a rapid increase in resistant bacteria that can be detected within 24 to 48 hours (Johansson et al., 1953; Reid et al., 1954; Sieburth et al., 1954; Barnes and Goldberg, 1962; Fuller et al., 1960; Ahart et al., 1978; Smith and Crabb, 1957 and 1960a; Levy et al. 1976). There is also little argument that antibiotic resistant human pathogens are a major clinical problem. The controversy arises over the significance and frequency of R-factor transfer between animals

and man as well as direct infection of man by antibiotic-resistant animal bacteria.

Proximity of untreated animals to antibiotic-fed ones can result in the transmission of antibiotic-resistant bacteria; however, the incidence of resistance is much lower than in treated animals (Smith, 1958; Levy et al., 1976). The rise in resistant bacteria following antibiotic treatment is usually due to resistant strains displacing sensitive ones, but in some instances the sensitive strains acquire R-factors and thus maintain their dominance (Smith and Crabb, 1956; Smith, 1958). In the absence of antibiotic treatment of the host, R-factor transfer in vivo is rare even if artificial systems such as gnotobiotic animals are used (Salzman and Klemm, 1968; Jarolmen and Kemp, 1969; Reed et al., 1969; Anderson et al., 1973a,b; Petrocheilou et al., 1976).

In general, intestinal bacteria are host-specific and successful interspecies colonization is infrequent (Smith, 1969; Cooke et al., 1972). Even with germfree animals interspecies colonization is not consistently effective and in gnotobiotes the autochthonous strains may strongly suppress strains from other species (Morishita et al., 1971). There is little evidence for the successful colonization of the human intestinal tract by animal bacteria. Transfer of R-factors to human bacteria occurs, but it does not seem to be an easy process. Smith (1969) was unable to colonize a human or establish R-factor transfer with animal bacteria unless at least 10^9 organisms were administered. The success rate of transfer was increased if the donor strain was given for several days. Even though human strains were better able to

colonize, there was still very little transfer of R-factors and when transfer occurred, the newly resistant strains were rarely detectable for more than four days.

Despite the difficulty in transferring resistance between animals and man described by Smith (1969), it can occur in natural situations. Levy et al. (1976) studied the coliform flora of chickens and farm personnel on a farm where OTC was introduced into the chicken feed (110 ppm). Within 48 hours, over 60% of the bacteria from the treated birds were resistant to OTC and by 2 weeks, 90% of the chickens were excreting OTC-resistant coliforms. Transfer of antibiotic-resistant strains from the treated to untreated birds was slow. It took four months before 30% or more of the untreated chickens in the same barn had a flora with greater than 50% resistant coliforms. It also took three to five months before there was a detectable change in the flora of the farm personnel. However, only four of eleven persons had high numbers of resistant bacteria for two consecutive weeks. These data suggest that transfer of R-factors to humans can occur, but that despite persistent exposure it may take months to occur and the newly resistant bacteria are not stable with time in the intestinal tract. Six months after removal of the OTC feed from the farm there were fewer resistant bacteria in the people than before the experiment started. Other researchers also have reported an increased incidence of antibiotic resistant bacteria among farm personnel with some evidence for R-factor transfer between animal and human enterics (Smith and Crabb, 1960b; Wells and James, 1973; Fein et al., 1974; Dorn et al., 1975; Siegel et al., 1975; Hirsh and Wiger, 1978).

A major concern, which is frequently not discussed by proponents of banning antibiotics, is whether or not the ban would have the desired effects. Although penicillins and tetracyclines have not been available as feed supplements without a prescription in Britain for over 11 years, there has been no decrease in the general level of antibiotic resistance to either antibiotic (Smith, 1975). One possible reason for the lack of decrease is that multiple antibiotic resistance on R-factors is very common. Thus, penicillin or tetracycline resistance could have been retained due to the use of other antibiotics as feed supplements. Another factor is that penicillin and tetracycline are widely used for therapeutic purposes and can result in large increases in the numbers of resistant bacteria. Even in the absence of feed supplements, the intermittent use of therapeutic antibiotics is probably adequate to maintain a high incidence of R-factors. However, the Swann Report (1969) only resulted in a ban on the use of certain antibiotics without a veterinary prescription, and it is evident that penicillins and tetracyclines are still being used as feed supplements under the aegis of "therapeutic treatment" (Braude, 1978).

In summary, it is clear that antibiotic resistance can be transferred between animals and man; however, the transfer does not occur readily and is restricted primarily to persons in frequent contact with the treated animals. Antibiotic resistance has increased in enteric animal pathogens but it is not possible to partition the blame for this increase between therapeutic and nontherapeutic uses of antibiotics. There is no question that there is a potential health

risk from an increase in R-factors due to use of antibiotic feed supplements, but a tighter regulation over the indiscriminate use of antibiotics in human medicine probably would improve human health conditions far more than restrictions on feed antibiotics. It also must seriously be considered whether or not a ban on the use of antibiotics as feed additives would actually reduce the level of antibiotic resistance, as yet an unproven assumption.

SUMMARY AND CONCLUSIONS

Supplementation of the diet with subtherapeutic levels of antibiotics stimulates the growth of many animals, including poultry. Significant growth increases can occur at very low antibiotic concentrations (<10 ppm), but the response does not seem to increase with increasing dosage levels. Nor is there an additive effect with antibiotics.

Growth stimulation is due to the antibiotics' effect on the intestinal bacteria. Growth of germfree animals is not stimulated by antibiotics, and the cleaner the environment the birds are raised in, the better their growth and the less their response to antibiotics. The most effective antibiotics for poultry are those with a broad spectrum of activity against gram-positive bacteria. This is to be expected because lactobacilli are the predominant flora in the crop and small intestines.

The maximum response to antibiotics occurs during the first four weeks of life. If the birds are started on antibiotics after this age, there is little or no growth response. The observed increase in

body weight is caused primarily by an increase in food consumption and feed efficiency. The only antimicrobial agent for which the mechanism of increase of feed efficiency is fairly well understood is monensin. It increases the feed efficiency of ruminants by altering the propionate:acetate ratio. Monensin is not very stimulatory for poultry, and there is no evidence for a shift in the proportions of short chain fatty acids in poultry intestinal contents with other antibiotics.

The magnitude of the growth response is dependent on the nutritional quality of the diet. The better the diet, the lower the response to antibiotic treatment. Yet even with a well-balanced diet plus various growth supplements such as fish meal, antibiotics still elicit a significant growth response.

Although some of the evidence is contradictory and some of the experimental designs have been less than optimal, antibiotic treatment seems to result in increased absorption or availability of vitamins, minerals, and protein. However, improvement in utilization of any single nutrient group cannot account for the entire increase in growth. That antibiotics apparently increase the absorption or availability of so many dietary compounds would seem to argue for a general non-specific improvement in the nutrition of the host. Comparisons of germfree and conventional animals have shown that an intestinal flora decreases the quantity of various digestive enzymes in the small intestine. Another effect of germfree life is a thinner intestinal wall, and the limited evidence available suggests that germfree animals absorb some nutrients more efficiently than conventional ones. Antibiotic

treatment also results in a decrease in the thickness of the intestinal wall.

Microbiological studies of intestinal contents have often yielded conflicting results. The only consistent trends seem to be a decrease in lactobacilli in the crop; a decrease in lactobacilli, total anaerobes, and total aerobes in the small intestine (all of which are probably synonymous because lactobacilli are the predominant small intestinal bacteria); and a decrease of enterococci in the ceca. Even though fairly consistent, most of these studies had serious methodological problems: i) the recovery rate of total anaerobes was usually very low due to the use of inadequate media and anaerobic techniques; ii) the lactobacillus selective media were usually incubated aerobically, thus underestimating the number of lactobacilli up to 100-fold; and iii) bacteriological analysis frequently was done on the pooled intestinal contents of several birds which obscures the effect of bird-to-bird variation. The major problem is that no attempt was made to identify the predominant anaerobes or lactobacilli, so it is not known whether the decrease in lactobacilli in the crop and small intestine is due to a general depression or to the inhibition of specific species.

On the basis of the microbiological studies and the rate of absorption/degradation of antibiotics in the intestinal tract, the site of effect of the antibiotics is probably the crop and small intestine. The actual site of action is probably the small intestine because most of the digestion and absorption occurs there. Only limited amounts of digestion and absorption occur in the crop, but

inhibition of crop bacteria is important because they are a major source of the small intestinal bacteria.

The search for specific bacteria or bacterial metabolites that might be responsible for growth stimulation or depression has been unsuccessful. There is no evidence for antibiotic growth stimulation being due to its effect on Clostridium perfringens, and feeding various strains of coliforms or lactobacilli to birds does not affect growth. The presence of some strains of Streptococcus faecalis (or S. faecium) has been associated with growth depression in chicks on a semi-synthetic (casein-sucrose) diet. Whether this is important on a commercial-type diet is unknown. The only evidence for an effect of a bacterial metabolite is the production of ammonia from urea and uric acid. There is probably a delicate balance between the beneficial effects of host absorption of ammonia for amino acid synthesis and the toxic effects of high ammonia concentrations. Again, on a semi-synthetic diet, ammonia production may be involved in growth depression but experiments need to be done using a commercial diet to determine if it still has a significant effect on growth.

Several researchers have reported that the growth response to antibiotics has decreased after many years of use. Part of this decrease is due to an increase in resistant strains of bacteria and part is due to the improved growth of control groups as poultry diets have been improved nutritionally. Yet despite the antibiotic resistance of a large proportion of the flora, antibiotics still stimulate growth. In general, without the continued selective pressure of antibiotic treatment, resistant bacteria are displaced by sensitive

strains, i.e., they are at a competitive disadvantage. The failure of the antibiotic growth response to disappear is probably due to a balance between the degree of antibiotic resistance and the ability of the strain to successfully maintain colonization in the gut.

The apparent antibiotic resistance of the bacteria can be misleading. Subminimum inhibitory concentrations of antibiotics may increase the susceptibility of bacteria to phagocytosis. They also inhibit the adhesion of bacteria to cell surfaces. A reduction in mucosal populations, both by an inhibition of growth or adhesion, might explain the histological effects of antibiotics on the intestinal mucosa.

The conclusion is that antibiotics stimulate the growth of poultry by a general depression of the bacterial populations in the small intestine. This results in a decrease in competition for nutrients as well as increased absorption by the host.

Section III. EFFECTS OF ANTIBIOTICS ON TURKEY INTESTINAL FLORA.

INTRODUCTION

Despite over 35 years of investigations, there have been no detailed studies on the effects of feed additive levels of antibiotics on the predominant intestinal bacteria. In fact, there have been only a few studies which have attempted to identify the major species in untreated birds (Harrison and Hansen, 1950ab; Mitsuoka, 1969; Salanitro et al., 1974ab, 1978). The purpose of my dissertation was to see if characterization of the predominant intestinal flora would reveal any correlations with antibiotic treatment.

The experiments were performed in three general stages. The first was to select or develop a primary isolation medium that would recover a high percentage of the total intestinal bacteria. The second stage involved the use of various selective media in an attempt to determine the general site of the antibiotic effect. Selective media also were used to detect effects of antibiotics on specific bacterial groups that were not numerically predominant. The last stage was the isolation and extensive characterization of the predominant bacteria in the small intestine of the turkey poult.

COMPARISON OF RECOVERY MEDIA

Introduction

Early studies on the effects of antibiotics on the intestinal bacterial flora suffered from several methodological problems. One major problem was the low recovery of anaerobes due to inadequate techniques for anaerobiosis and the use of nutritionally inadequate isolation media. Use of media such as thioglycolate agar resulted in culturable counts of anaerobes that were 10 to 1000 times lower than microscopic counts (e.g., Anderson et al., 1952, 1956; Eisenstark and Stanford, 1953; Ochi and Mitsuoka, 1958a).

Very few investigators have attempted to develop adequate recovery media for poultry intestinal bacteria. Ochi and Mitsuoka (1958a) compared seven media, including thioglycolate agar, for recovery of chicken intestinal bacteria. The highest counts were on glucose horse blood liver agar plates, however, the count represented only 0.01 to 0.1% of the total bacteria present. Barnes and Impey (1970) compared recovery of chicken cecal bacteria from five-week-old birds using roll tubes and anaerobe jars. They found that M10 (a non-rumen fluid medium developed for rumen bacteria by Caldwell and Bryant, 1966) gave the highest recoveries in roll tubes when supplemented with 5% liver extract and 10% chicken fecal extract. The culture counts were 28.2 and 32.8% of the microscopic count for the two birds tested. Later experiments using the same medium gave similar recoveries (Barnes et al., 1972).

Salanitro et al. (1974b) also compared the recovery of chicken cecal bacteria from five-week-old birds, using media developed for rumen and sludge digestor anaerobes. The highest counts occurred on a

modified rumen fluid medium (M98-5). The average recovery from six birds was 60%, which was higher than that obtained with the original 98-5 medium of Bryant and Robinson (1961), M10, or M10 supplemented with liver and chicken cecal or fecal extracts. The M98-5 medium contained 40% rumen fluid and its omission decreased counts by 50%. The requirement for rumen fluid could not be compensated for by the addition of 5 to 30% chicken cecal extract.

For my purposes it was necessary to perform another media comparison study because: i) there was no way of knowing if results obtained with chicken flora would be valid for turkey flora, the bird to be used in future experiments; ii) most sampling in later experiments would be done on two- to three-week-old birds and their flora is not necessarily the same as a five-week-old bird; and iii) none of the media developed for isolation of human intestinal anaerobes had been compared in the previous studies. Another reason for doing a comparative study was that both Barnes and Salanitro, and in fact almost all researchers studying poultry intestinal flora, incubated their isolation media at 37°C, while the body temperature of poultry is 41 to 42°C (e.g., Whittow, 1976; Harrison and Hewitt, 1978).

Materials and Methods

Animals and diet. One-day-old Large White turkey poults were obtained from a local commercial hatchery. They were not given the usual injection of antibiotics at the hatchery. The birds were raised in Petersime starter batteries with feed and water supplied ad libitum. The pens were under continuous light and the room temperature was maintained at 80°F (ca. 27°C). The diet contained no antibiotics (see Table 7).

Sampling procedures. Three-week-old turkey poults were transported from the brooding facility to the laboratory (10-15 min) and killed by CO_2 asphyxiation. The intestinal tract (duodenum to colon) was removed and placed in a container under a flow of oxygen-free CO_2. The ceca were separated from the intestinal tract and the contents were squeezed into a beaker being flushed with CO_2. A portion of the contents was used for culture dilutions, and the remainder for the dry weight determinations. The contents were mixed, while being flushed with CO_2, before the dilutions were made. Serial ten-fold dilutions were prepared with prereduced diluent (Holdeman et al., 1977) under strict anaerobic conditions according to procedures described by Moore and Holdeman (1974). The ileal contents (from the yolk stalk to the ileo-cecal junction) of three-week-old poults, and the cecal contents of six-week-old birds were sampled similarly in later experiments.

The cultural recovery was determined using six media: M98-5, brain heart infusion agar (BHIA), rumen fluid-glucose-cellobiose agar (RGCA), RGCA + peptone, and the two RGCA media with only 10% rumen fluid. The composition of these media is listed in Table 8. Five

TABLE 7. Composition of diet (% w/w)

Ground yellow corn	58.462
Stabilized fat	1.818
Dehulled soybean meal	35.455
Defluorinated phosphate	3.455
Iodized salt	0.273
Trace vitamin mix[1]	0.091
Vitamin premix[2]	0.264
Methione hydroxy analogue (Monsanto)	0.182

[1]The trace mineral mix contained manganous oxide, zinc oxide, ferrous carbonate, ferrous sulfate, copper oxide, calcium iodate, cobalt carbonate, and calcium carbonate, and supplied in ppm to the diet: manganese, 60; zinc, 60; iron, 20; copper, 2.5; iodine, 1; and cobalt, 0.225.

[2]The vitamin premix supplied the following in grams per 100 lbs of diet: vitamin A (650,000 I.U./g), 0.461; vitamin A and D_3 (65,000 I.U. vitamin A/g and 325,000 I.C.U. vitamin D_3/g), 0.462; vitamin E (227,000 I.U./lb), 1.0; menadione sodium bisulfite, 0.32; thiamine HCl, 0.05; riboflavin, 0.25; calcium pantothenate (D), 0.75; niacin, 3.0; choline chloride (50%), 90.8%; vitamin B_{12} (2000 mg/lb), 0.151; folic acid, 0.05; biotin (1 mg/g), 5.0; pyridoxine HCl, 0.1; ethoxyquin (66.7%), 8.51; sodium selenite premix (0.4 mg selenium/g), 22.7.

TABLE 8. Composition of recovery media (per liter)[ab]

Components	RGCAP-30	RGCAP-10	M98-5	BHIA
Rumen fluid	300 ml	100 ml	400 ml	-
Glucose	0.248 g	0.248 g	0.3 g	-
Cellobiose	0.248 g	0.248 g	0.3 g	-
Maltose	0.248 g	0.248 g	0.3 g	-
Starch	0.5 g	0.5 g	-	-
Yeast extract	0.5 g	0.5 g	-	5.0 g
Glycerol	-	-	0.3 ml	-
Peptone	0.5 g	0.5 g	-	-
Trypticase	-	-	2.0 g	-
$(NH_4)_2SO_4$	1.0 g	1.0 g	(0.14 g)[c]	-
Na_2CO_3	-	-	4.0 g	-
$Na_2S \cdot 9H_2O$	-	-	0.25 g	-
Cysteine-HCl$\cdot H_2O$	0.5 g	0.5 g	0.25 g	0.5 g
Hemin	0.005 g	0.005 g	0.002 g	0.005 g
Vitamin K_1	0.001 ml	0.001 ml	-	-
S2 minerals solution[c]	-	-	50 ml	-
Salts solution[d]	500 ml	500 ml	-	-
Resazurin solution[d]	4.0 ml	4.0 ml	0.4 ml	4.0 ml
Agar	20.0 g	20.0 g	20.0 g	25.0 g
Brain heart infusion broth	-	-	-	37 g
Distilled H_2O	200 ml	400 ml	550 m	1000 ml

[a]Final pH 6.8 to 7.0

[b]RGCA-30 and RGCA-10 had the same composition as RGCAP-30 and RGCAP-10 respectively except that the peptone was not included.

[c]S2 mineral solution (g/1): $KH2PO_4$, 0.82 g; NaCl, 18.12 g; $MgSO_4 \cdot 7H_2O$, 1.82 g; $CaCl_2 \cdot 2H_2O$, 0.59 g; $(NH_4)_2SO_4$, 2.91 g/ $MnCl_2 \cdot 4H_2O$, 0.004 g; $CoCl_2 \cdot 6H_2O$, 0.0004 g.

[d]Formulas for stock solutions are listed in Holdeman et al. (1977).

replicates of each medium were inoculated with 0.1 ml of the 10^{-7} and 10^{-8} dilutions of cecal contents. The media (in roll tubes) were melted and cooled to 50°C before inoculation under oxygen-free CO_2. Duplicate sets, a total of ten roll tubes per medium per dilution per bird, were prepared. One set was incubated anaerobically at 37°C and the other at 41°C.

Microscopic and cultural counts. Direct microscopic clump counts (DMCC) were made from the 10^{-3} or 10^{-4} dilutions of the intestinal contents according to the procedure described by Holdeman et al. (1977). The moisture content was determined by drying a tared sample at 90°C overnight.

Colony counts in roll tubes were made after 2, 5, and 14 days of incubation. Colonies were counted with a dissecting microscope (10-15 X magnification). Microscopic and cultural counts were corrected for the actual sample size (i.e., amount of cecal contents placed in the first dilution tube), and for the moisture content. Thus, all counts were standardized to bacteria per gram dry weight. Counts also were represented as the percent recovery of the DMCC.

Statistical analyses. A three factor analysis of variance with block treatments was used to test the interactions of media and incubation time and temperature. It was followed by Duncan's multiple range test to determine the significance of the various combinations of treatments. Bacterial counts of small intestinal contents were compared by analysis of variance using paired comparisons (Sokal and Rohlf, 1969).

Results and Discussion

The effects of incubation temperatures are shown in Table 9. The mean colony counts and percent recovery were significantly ($p < 0.001$) higher at 41°C than at 37°C. The higher counts at 41°C were to be expected because the mean internal body temperature of domestic poultry is about 41°C and thus the intestinal bacteria would have adapted to 41°C as their optimal temperature.

The media are listed in Table 9 in the order of their cultivable counts and percent recoveries. The highest counts obtained at 41°C were on RGCAP-10, but there were no statistical differences among any of the RGCA-based media. M98-5 and BHIA gave the lowest counts ($p < 0.25$ and $p < 0.05$, respectively).

The effects of length of incubation are shown in Table 10. The counts increased significantly between 2 and 5 days ($p < 0.05$). Depending on the medium they increased from 3.5 to 12.5%. Between 5 and 14 days counts increased from -2 to 6%. The only medium that showed an increase in colony counts at 14 days was M98-5 ($p < 0.1$). Salanitro et al. also reported a nonsignificant increase in colony counts between 6 and 14 days with M98-5.

The data in Table 10 show that RGCAP-10 was the best isolation medium based on mean percent recoveries. However, microscopic counts are prone to a far larger experimental error than cultural counts. Thus, an alternative method for evaluating the media is their rank order of recovery. For each bird, the media were ranked in descending order (one to six) depending on which one gave the highest colony counts, second highest, etc. (Table 11). This procedure has the

TABLE 9. Effects of medium and incubation temperature on recovery of turkey cecal bacteria

Medium	37°C		41°C	
	Count[a]	% Recovery[b]	Count	% Recovery
RGCAP-10	4.33	73.0	5.37	89.1
RGCAP-30	4.69	78.0	4.98	84.1
RGCA-30	4.40	72.5	4.94	81.9
RGCA-10	4.51	75.8	4.79	79.7
M98-5	4.24	70.4	4.57	75.2
BHIA	3.37	55.2	3.39	57.5

[a]Mean colony counts (five replicates) from four three-week-old turkeys times 10^{11} bacteria per gram dry weight. Samples were incubated for five days.

[b]Culture counts/microscopic count X 100.

TABLE 10. Effects of incubation time on recovery of turkey cecal bacteria

Medium	% Recovery on day[a]		
	2	5	14
RGCAP-10	82.4	89.1	90.9
RGCAP-30	68.3[b]	84.1	85.2
RGCA-30	73.8	81.9	79.9
RGCA-10	74.3	79.7	79.8
M98-5	69.7[b]	75.2	79.4
BHIA	44.5[b]	57.5	56.9

[a]Mean percent of DMCC recovered from four birds. Samples were incubated at 41°C.

[b]Mean percent recovery from three birds.

TABLE 11. Rank order of media based on total colony counts. Effects of media, incubation time, and temperature[a]

Medium	Days of incubation					
	2		5		14	
	37°	41°	37°	41°	37°	41°
RGCAP-10	3	1	2	3	4	1
RGCAP-30	1[b]	3[b]	1	2	2	2
RGCA-30	2	5	3	1	3	5
RGCA-10	4	3	4	3	1	3
M98-5	5[b]	2[b]	4	5	5	4
BHIA	6[b]	6[b]	6	6	6	6

[a]The media were ranked from 1 to 6 on the basis of the mean ranking from data on four individual birds.

[b]Data based on three birds.

advantage of reducing the error of bird to bird variation. At 41°C, after five days incubation, RGCA-30 was the best medium based on rank. It was closely followed by RGCAP-30, and then by RGCA-10 and RGCAP-10 which were tied for third place.

Depending on the method used for comparison (average percent recovery vs. average rank value), either RGCA-30 or RGCAP-10 could be considered the best isolation medium. In a later study, the two media were used to isolate bacteria from the ceca of two-week-old turkeys. The results are presented in Table 12. No significant difference was observed between the counts with RGCA-30 or RGCAP-10. Thus, the three best media for recovery of turkey cecal bacteria from three-week-old birds are RGCAP-10, RGCA-30, and RGCAP-30 with little difference among these three. M98-5 and BHIA were the poorest recovery media regardless of the method of evaluation.

As animals mature their intestinal flora also changes. Because media comparison studies by other researchers had been done with samples from older birds, it was important to determine if the optimal recovery medium would change with the age of the bird. The media comparison results with six-week-old turkeys were quite different from those with three-week-old birds (Table 13). M98-5 was the best medium by rank and mean percent recovery, but no significant differences were observed among the top four media. M98-5 was significantly better than RGCA-10 and BHIA ($p < 0.1$ and $p < 0.05$, respectively). These results agreed well with those of Salanitro et al. (1974b) who also found M98-5 to be the optimal recovery medium for chicken cecal bacteria from five-week-old birds. Comparison of incubation temperature was done

TABLE 12. Comparison of RGCA-30 and RGCAP-10 for recovery of turkey cecal bacteria

Medium	Colony counts from bird				Mean
	1	2	3	4	
RGCA-30	8.32[a]	6.55	0.74	8.29	5.97
RGCAP-10	5.64	9.69	0.83	8.37	6.13

[a]Mean colony count (of three replicates from a two-week-old turkey) times 10^{11} bacteria per gram dry wt of cecal contents. Tubes were incubated for five days at 41°C.

TABLE 13. Effects of media on recovery of cecal bacteria from six-week-old turkeys

Medium	Count[a]	% Recovery	Mean rank order
M98-5	4.36	141.1	2.0
RGCAP-30	4.01	126.3	2.8
RGCAP-10	3.91	124.3	3.4
RGCA-30	3.90	123.7	3.6
RGCA-10	3.85	123.6	4.0
BHIA	3.01	97.4	5.0

[a]Mean colony counts (of five replicates) from five turkeys times 10^{11} bacteria per gram dry wt of cecal contents. Roll tubes were incubated at 41°C for five days.

[b]The media were ranked from 1 to 6 on the basis of the total counts for each bird. The data presented are the means of the ranks for the five birds.

with three of the five birds. As with the younger poults, recoveries at 41°C were significantly higher than those at 37°C ($p < 0.002$). The difference in the optimum recovery medium between three- and six-week-old birds is probably due to a shift in bacterial flora with age (Barnes et al., 1972; Salanitro et al., 1974ab, 1978).

Regardless of the age of the bird, the bacterial populations of the ceca are much more complex than those in the small intestine. Lactobacilli are the predominant bacteria in the small intestine of poultry, but are usually not predominant in the ceca. Because lactobacilli have fairly simple nutritional requirements compared to many intestinal anaerobes, it was thought that BHIA might give higher recoveries with small intestinal bacteria than it had with cecal bacteria. The results of three experiments on isolation of small intestinal bacteria are summarized in Table 14. No significant difference was observed between RGCAP-30 and BHIA with duodenal contents and RGCAP-30 was better ($p < 0.25$) in one of two trials with ileal contents. The average recovery using RGCAP-30 was 140.4% from the crop of seven two-week-old poults, 67.6% from the duodena of eight poults, and 78.2% from the lower ileum of 16 birds.

Summary. Incubation of recovery media at 41°C resulted in significantly higher counts than at 37°C ($p < 0.002$). In three-week-old turkey poults, RGCAP-30, RGCAP-10, and RGCA-30 gave the highest recoveries of cecal bacteria. M98-5 was less effective and BHIA was definitely inadequate. However, there was no significant difference between RGCAP-30 and BHIA for recovery of small intestinal bacteria. In older birds (six weeks of age), M98-5 was equal or superior to

TABLE 14. Comparison of RGCAP-30 and BHIA for isolation of turkey intestinal bacteria.

Site	DMCC[a]	RGCAP-30		BHIA	
		Count[b]	% Recovery	Count	% Recovery
Duodenum	2.01×10^{10}	6.49×10^{9}	60.2	6.32×10^{9}	61.9
Lower ileum[c]					
expt. 1	3.64×10^{10}	3.92×10^{10}	98.3	2.88×10^{10}	81.2
2	6.47×10^{9}	4.22×10^{9}	56.0	4.06×10^{9}	55.2

[a]Average of direct microscopic clump counts of contents from four 15-day-old poults. Microscopic and cultural counts are in units of bacteria per gram dry wt of intestinal contents.

[b]Mean colony counts of three replicates. Tubes were incubated for 5 days at 41°C.

[c]The intestinal tract from the yolk stalk to the ileo-cecal junction.

the RGCA-based media. The choice of a primary isolation medium is thus dependent on the site to be sampled and the age of the bird.

USE OF SELECTIVE MEDIA TO MEASURE THE EFFECTS OF ANTIBIOTICS ON INTESTINAL BACTERIA

As discussed in Section I, the actual site of the growth stimulation effect is unknown. Most previous researchers have concentrated on the flora of a single site, usually feces or cecal contents, without any particular justification for choosing that site. Therefore, several selective and nonselective media were used to survey the flora at different sites of the tract in control and antibiotic-treated birds. The other purpose of the selective media was to see if antibiotic treatment had a significant effect on a portion of the flora which was not numerically predominant. Selective media were used to analyze the flora in three separate feed trials.

First Selective Media Experiment

Materials and Methods

Animals and diets. One-day-old Large White Turkey poults were obtained from a local commercial hatchery. They were segregated by sex and birds of each sex were randomized into 24 groups. The birds were wing-banded and assigned to 24 pens with nine birds per pen (a total of 432 birds in the experiment). They were raised to four weeks of age in Petersime starter batteries. Feed and water were supplied ad libitum. The pens were under continuous light and the room temperature was maintained at 80 to 85°F.

The birds were fed a diet containing 26, 28, or 30% protein, with or without zinc bacitracin (see Table 7 for the basal diet of 26% protein). Higher protein levels were attained by adding soybean meal in place of corn. The diets were prepared by first blending identical mixes, dividing them into two equal portions and then adding zinc bacitracin to one of each pair. Zinc bacitracin premix (Baciferm 40, Commercial Solvents Corp., Terre Haute, Indiana) was added in the place of an equal amount of ground yellow corn to a final concentration of 55 mg/kg of feed. The resulting six diets were assigned at random such that each diet was fed to four pens of males and four pens of females.

Feed and mortality records were kept so that average feed consumption for the turkeys in each pen could be determined on a weekly basis. The turkeys were group-weighed by pens at weekly intervals.

Sampling. The birds were weighed at 8 and 15 days of age. The diet and sex showing the greatest growth response were determined and these birds were reweighed individually on days 17 and 18. One control and one antibiotic-fed bird whose weight most closely approached the average weight of the diet group showing the maximum growth increase were chosen for analysis each day for four days beginning on day 17. The weight data from the day 18 weighing were used to select birds on the following two days. The eight birds assayed ranged from 17 to 20 days of age.

The birds were weighed in the morning and transported to the laboratory by 8:30 AM. They were killed by CO_2 asphyxiation and the crop and intestinal tract (gizzard to colon) were removed. Five areas

were sampled: the crop, duodenum, upper and lower ileum (separated at the yolk stalk), and the ceca. The contents were removed and placed in diluent under anaerobic conditions as described in the comparison of media section.

Media. The media used were: RGCAP-30, RGCAP-10, and RGCA-30 for total flora; BHIA for total facultatives; Rogosa SL agar (Difco) for lactobacilli; sulfite-cycloserine (SC; Hauschild and Hilsheimer, 1974) agar for anaerobic sulfite reducers (especially C. perfringens); MacConkey's agar (BBL) for coliforms; M-Enterococcus agar (BBL) for streptococci; Salmonella-Shigella (SS) (Difco) agar; Staphylococci medium no. 110 (Difco); and Littman Oxgall agar (Difco) for yeasts. The compositions of the media are listed in Appendix I. Each dilution was cultured in triplicate. RGCAP-30 was used for counts of non-cecal anaerobes. RGCAP-10 and RGCA-30 were used for recovery of cecal anaerobes. All other media were prepared as plages (20-25 ml/plate). I originally attempted to prepare the Rogosa SL agar in roll tubes. However, even when the agar concentration was increased to 2.5%, the medium would not adhere to the walls of the roll tubes when they were spun. BHIA, MacConkey's agar, Littman Oxgall agar, Staphylococcus medium no. 110, and sulfite-cycloserine agar were prepared one day before the last birds were sampled. The M-Enterococcus agar, SS agar, and Rogosa SL agar were prepared fresh each day. The SC agar plates were placed in an anaerobic glove box overnight before use. The day of use, the SC and Rogosa agar plates were kept in a box which was flushed with CO_2 until they were streaked. After inoculation they were placed in anaerobe holding jars until the jars were sealed.

Each medium was inoculated with 0.1 ml of the appropriate dilution. The agar in the roll tubes was melted and maintained at 50°C before inoculation. The roll tubes were inoculated under a O_2-free 100% CO_2 atmosphere. The inoculum for the plates was spread with a bent glass rod. The SC and Rogosa plates were incubated in anaerobe jars with a 90% CO_2 - 10% H_2 atmosphere; the other plates were incubated aerobically. All media were incubated at 41°C.

Direct microscopic clump counts (DMCC) were made according to the procedure described by Holdeman et al. (1977). The moisture content of the intestinal samples was determined by heating a tared sample at 90°C overnight. Colony counts were performed at three days on the SC and Rogosa plates, at four days for the aerobically incubated plates, and at seven days for the roll tubes. Colonies in roll tubes were counted with a dissecting microscope (10-15 X magnification).

Gas chromatographic analyses were run on the first dilution tube of each site from each bird according to the methods described in the VPI Anaerobe Laboratory Manual (Holdeman et al., 1977).

Statistical analyses. The pen weights and feed efficiency data were tested by three factor analysis of variance (antibiotic X sex X protein level) and by Duncan's multiple range test. Due to the large number of media used and sites sampled, it was possible to sample only two birds per day. Therefore, in order to reduce the possible effect of age on the results, the colony counts, percent dry weights, and the chromatographic products were analyzed by analysis of variance both with and without paired comparisons (Sokal and Rohlf, 1969).

Results and Discussion

Growth response. The effects of diet, sex, and antibiotic treatment at two weeks of age are shown in Table 15. Data on weight gains and feed efficiency (weight gain vs. feed consumed) for weeks one through four are listed in Appendix IIIA. The male poults on a 30% protein diet showed a larger growth response (12.9%, $p < 0.01$) than the other test groups, and consequently were the group sampled. The eight birds sampled were closer to the mean of their groups than any of the other birds.

Colony counts. The mean colony counts from the selective and non-selective media are listed in Table 16. Data on the individual birds are in Appendix IIIB.

SS agar is highly selective for Salmonella and Shigella species, which appear as uncolored colonies that may or may not have black centers. The counts reported in this study are the total counts of colonies on SS agar. No non-lactose fermenting colonies were isolated from the crop, duodenum, upper or lower ileum of any bird. One of eight birds had low numbers (10^2/g) of non-lactose fermenting, H_2S positive bacteria in its ceca. The counts from Littman Oxgall agar have been omitted because it was not sufficiently selective for yeasts.

The only differences that were associated with antibiotic treatment and were statistically significant or approached significance were: i) S. aureus increased ($p < 0.1$) in the crop; ii) total facultatives and total staphyloccoci increased in the duodenum ($p < 0.1$ and $p < 0.05$ respectively); iii) S. aureus counts increased in the upper ileum

TABLE 15. First selective media experiment. Effect of protein and antibiotic treatment on body weights of two-week-old poults[1]

Sex		Protein concentration in diet					
		26%		28%		30%	
		C[2]	Ab[2]	C	Ab	C	Ab
Male		188.4	200.7	197.3	202.4	168.6	239.1
		182.2	221.8	204.1	208.8	212.3	216.4
		196.6	195.5	213.1	195.3	228.7	228.7
		211.5	191.4	207.0	197.8	204.5	234.5
	Mean	194.7[b]	202.3[b]	205.4[b]	201.1[b]	203.5[b]	229.7[a]
Female		197.6	198.0	167.7	202.7	183.5	188.0
		193.5	175.3	178.9	217.8	168.1	201.6
		200.8	161.0	207.6	192.2	198.1	213.6
		192.2	200.7	175.3	189.1	220.8	212.2
	Mean	196.0[b]	183.7[b]	182.4[b]	200.5[b]	192.6[b]	203.8[b]

[1]Average pen weights (in grams) of ten birds. Means not followed by the same superscript are significantly ($p < 0.05$) different.

[2]C = controls and Ab = antibiotic-fed (zinc bacitracin, 55 ppm).

TABLE 16. Effect of zinc bacitracin on recovery of bacteria from the turkey intestinal tract using selective media[a]

Flora	Crop		Duodenum		Upper ileum		Lower ileum		Ceca	
	C[b]	Ab[b]	C	Ab	C	Ab	C	Ab	C	Ab
DMCC[c]	9.61	9.78	8.19	8.83	8.81	8.97	9.56	9.71	11.00	10.80
Total cultivable bacteria	9.63	9.53	8.11	8.42	8.81	8.63	9.51	9.55	10.98	10.83
% Recovery	136.8	63.5	87.2	39.3	83.1	38.8	79.6	47.3	131.7	137.2
Lactobacilli	9.71	9.18	7.92	7.82	7.96	8.35	8.97	8.77	10.14	8.61[d]
Sulfite-reducers	1.82	2.30	3.71	2.03	1.70	1.67	4.45	5.67	6.48	4.69[d]
Total facultatives	8.19	8.30	5.95	7.36[e]	7.50	7.19	8.68	8.90	8.83	8.98
Coliforms	4.87	6.69	4.88	5.19	6.06	6.10	6.60	8.95	7.69	9.10
SS agar	4.54	5.77	2.04	3.56	5.87	5.08	6.06	6.10	6.82	6.48
Total staphylococci	5.74	5.92	2.35	3.58[f]	5.48	3.93	6.10	6.68	5.45	5.13
S. aureus	3.71	4.57[f]	1.96	2.85	1.85	3.53[f]	3.03	2.81	2.84	3.31
Streptococci	7.32	6.93	4.65	4.75	5.97	5.21	7.65	7.56	7.67	6.61

[a] Numbers represent the average values ($\log_{10}$ of bacteria/g wet weight from four 2½ week-old poults.

[b] C = controls; Ab = antibiotic-fed (zinc bacitracin, 55 ppm).

[c] DMCC = direct microscopic clump counts.

[d] $p < 0.25$

[e] $p < 0.05$

[f] $p < 0.1$

0.1); and iv) lactobacilli and sulfite-reducers decreased in the ceca ($p < 0.25$). No significant differences were observed between the other bacterial groups, usually due to a high amount of bird-to-bird variation. Also, no significant differences in total cultivable counts or microscopic counts were detected at any site. The percent recovery decreased in the crop ($p < 0.25$) and in the duodenum and upper ileum ($p < 0.1$).

One of the most interesting differences was the coliform counts on MacConkey's agar. Antibiotic treatment appeared to increase counts in the crop, lower ileum, and ceca. The shifts in counts were not statistically significant but antibiotic treatment did significantly ($p < 0.001$) increase the variation in counts; e.g., the range of counts varied 10,000-fold in the lower ileum with antibiotics but only 66-fold in the controls (Table 17). The high amount of variation in coliform counts may explain many reports in the literature of coliforms increasing with antibiotics (e.g., Anderson et al., 1952; Rosenberg et al., 1952; Sieburth et al., 1954; Cook et al., 1954). All of these authors pooled intestinal contents of several birds, which would make it appear as if antibiotic treatment increased counts (e.g., Table 16). However, when contents from individual birds were cultured it could be seen that this is a very misleading conclusion. One reason coliforms may increase following antibiotic treatment is because they are either naturally resistant to the antibiotics used or rapidly acquire resistance via R-factors. Feed trials using different strains of coliforms have been unable to demonstrate any growth stimulating effect of high

TABLE 17. Effect of zinc bacitracin on coliform counts in the turkey intestinal tract[a]

Bird number	Crop		Lower ileum		Ceca		Lower ileum[b]	
	C[c]	Ab[c]	C	Ab	C	Ab	C	Ab
1	5.05	4.43	6.77	5.94	6.81	6.25	8.49	4.91
2	4.69	5.89	5.15	9.52	7.65	8.90	9.33	8.09
3	4.80	2.48	5.75	5.52	6.47	8.19	8.78	5.13
4	4.86	7.27	6.97	8.33	8.16	9.61	8.20	7.53
Mean	4.87	6.69	6.60	8.95	7.69	9.10	8.91	7.59

[a]The poults were 2½ weeks old when sampled. Counts were made on MacConkey's agar and are represented as bacteria ($\log_{10}$) per gram wet weight of contents.

[b]A second feed trial study.

[c]C = controls; Ab = antibiotic-fed (55 ppm zinc bacitracin).

coliform populations (Bogdonoff et al., 1959; Warden and Schaible, 1960; Edwards and Boyd, 1963b).

Second Selective Media Experiment

The two major purposes for a second selective media experiment were to determine if the differences observed in the first selective media study were repeatable and to find out how well the media were selecting for the organisms desired. In the first selective media study it was not possible to perform further tests on the isolated colonies due to the large number of selective media employed. Per day, 900 plates and 126 roll tubes were inoculated.

An additional goal of this feed trial was to determine the total bacterial counts in different areas of the tract, especially from the crop to the duodenum. Most avian physiology textbooks state that the proventriculus and gizzard do not have an indigenous flora and that bacterial populations are very low due to the low pH. Also it has been suggested that the duodenal flora is due to washout from the crop and that little or no multiplication occurs before the lower ileum. Therefore, in this study, total counts were made from the crop, proventriculus, gizzard, duodenum, and lower ileum contents of each bird.

Methods

The feed trial procedures were similar to those of the first study. Four male poults, two controls and two antibiotic-treated, were sampled on days 15 and 16.

DMCC's and total cultivable counts using RGCAP-30 were made from the crop, proventriculus, gizzard, duodenum, and lower ileum contents of each bird. Crop and duodenum contents were cultured on Staphylococcus medium 110. M-Enterococcus agar was used for the crop, lower ileum, and cecal contents. Total facultatives in the duodenum were measured using BHIA, and MacConkey's agar was used to detect coliforms in the lower ileum. SC agar was used for the lower ileal and cecal contents. Six plates of SC agar were prepared from each dilution and were incubated in anaerobe jars. Three plates were counted after 18-24 hours incubation and the other three after three days. Cecal lactobacilli were isolated using Rogosa SL agar. All media were incubated at 41°C.

The Rogosa agar and SC agar were incubated in anaerobe jars. Colony counts were made at one and three days for SC agar and at two days for the other plated media. Roll tubes were counted at five days.

Results and Discussion

Selective Media. Some of the birds (4 of 8) did not have any food in their crops so it was not possible to obtain counts for comparison with the first study. The data on the remaining sites are summarized in Table 18. Data for individual birds are in Appendix V. Total staphylococci did not increase in the duodenum. The total facultatives decreased significantly with antibiotic treatment in this experiment whereas they increased in the first selective media study. No change in counts on SC agar could be attributed to antibiotic treatment. The only change in bacterial flora that seemed reproducible

TABLE 18. Selective media experiment 2. Effect of zinc bacitracin on turkey intestinal bacteria[a]

Flora	Duodenum		Lower Ileum		Ceca	
	C[b]	Ab[b]	C	Ab	C	Ab
DMCC	8.85	8.49	9.65	9.13	11.06	11.28
Cultivable flora	8.71	8.32	9.55	8.60	ND[c]	ND
% Recovery	75.1	60.9	78.5	45.9	ND	ND
Total staphylococci	<2.60[d]	<4.17	ND	ND	ND	ND
S. aureus	0[e]	0	ND	ND	ND	ND
Total facultatives	7.13	5.66	ND	ND	ND	ND
MacConkey's	ND	ND	8.91	7.59	ND	ND
Sulfite-reducers 1 day	ND	ND	<3.37	<3.20	0	0
Sulfite-reducers 3 day	ND	ND	4.45	<4.82	7.35	7.61
Total streptococci	ND	ND	7.30	6.05	7.45	6.40
"*S. faecalis*"	ND	ND	7.27	5.57	7.38	6.32
Lactobacilli	ND	ND	ND	ND	10.28	9.91

[a]Mean counts bacteria/g wet weight ($\log_{10}$) of four controls and four antibiotic-treated birds at two weeks of age.

[b]C = controls and Ab = antibiotic-fed (zinc bacitracin, 55 ppm).

[c]Not done.

[d]A "<" symbol indicates that at least one of the four birds was below the detection limit.

[e]No colonies were detected in any of the birds (detection limit was < 500/g).

between the two studies was a decrease in cecal lactobacilli upon antibiotic treatment, but the decrease was not statistically significant in this study. Colonies were picked and identified from some of the selective media. They are discussed according to the medium from which they were isolated.

MacConkey's. The high variability of coliform counts noted in the first selective media study (Table 17) was again observed. There was no correlation between counts and response to antibiotics. No non-lactose fermenting colonies occurred on any of the plates. Ten colonies were picked in a randomized manner from the plates of each bird. All of the 80 colonies picked were *E. coli* and comprised three different biovars according to the results of the API-20E strip (Analytab Products, Plainview, N.Y.).

Staphylococcus medium 110. This medium is highly selective for staphylococci, with 7.5% NaCl as the primary selective agent. It stimulates chromogenesis and gives rise to three pigmented colony types: white, pale yellow, and bright yellow-orange. The orange colonies are almost always *S. aureus*, the white ones rarely, and the pale yellow colonies unpredictable (Chapman, 1945, 1946). In this and the previous selective media study, a total count of staphylococci and a count of just the orange colonies, which were designated *S. aureus*, was made.

Of 12 yellow-orange colonies picked from the highest dilutions of crop contents, 11 were identified as coagulase-positive *S. aureus* using the API Staph-Ident strip and Difco serum for the coagulase test. The other isolate was a coagulase-positive *Staphylococcus* sp. whose biochemical pattern did not match that of any known species.

SC agar. Many selective media have been developed to isolate C. perfringens because of its clinical importance. These include sulfite-polymyxin-sulfadiazine (SPS) agar, tryptose-sulfite-neomycin (TSN) agar, and Shahidi-Ferguson-perfringens (SFP) agar with sulfite, polymyxin, kanamycin, and egg yolks. All of the media use sulfite as a differential agent. C. perfringens reduces the sulfite which, in combination with the ferric ammonium citrate, results in a black colony. Harmon et al. (1971) modified SFP by using cycloserine instead of polymyxin and kanamycin. Their medium was designated tryptose-sulfite-cycloserine (TSC) agar. The recovery of C. perfringens was similar on SFP and TSC, but significantly higher than the recovery on SPS or TSN. However, SFP, SPS, and TSN were less selective than TSC (Hauschild et al., 1977 and 1979). They allowed growth of facultative sulfite-reducers and non-reducers (e.g., enterococci). The cycloserine in TSC inhibited these facultatives, especially the sulfite-reducing ones. Hauschild and Hilsheimer (1974) deleted the egg yolks from TSC agar and reported no decrease in recovery of C. perfringens. They designated their modification as SC agar. They compared several media and found, in agreement with Harmon et al., that recoveries on SPS and TSN were low and that SFP, which gave good recoveries, was not inhibitory enough to prevent growth of facultative sulfite-reducers.

SC agar was designed to isolate C. perfringens, but it also supports the growth of other sulfite-reducing clostridia; e.g., C. sporogenes, proteolytic C. botulinum, and C. bifermentans (C. sordellii is inhibited). Therefore it is necessary to run additional tests to confirm the identification of isolated strains. One facet of the

differential process is the rapid growth of C. perfringens. The plates are supposed to be counted within 24 hours because many of the other sulfite-reducing clostridia such as C. sporogenes and C. bifermentans do not produce visible colonies on the medium after incubation for only one day. Because of logistics problems in the first selective media study, it was not possible to count the SC agar until after three days of incubation. Therefore, the counts represented the total number of sulfite-reducers rather than the number of C. perfringens. By three days, plates with large numbers of sulfite reducers produced so much ferrous sulfide that it was sometimes difficult to count colonies due to the diffusion of the black precipitate.

In the second selective media study, counts of black colonies on SC agar were made after one and three days to determine the effects of extended incubation. Black colonies were picked at both time periods. Unlike the results of the first selective media study, there was no change in the numbers of sulfite-reducers in the ileum or ceca that could be attributed to antibiotic treatment.

There were no black colonies from the SC plates streaked with cecal contents by one day (detection limit ca. 500/g) on either diet, but by day three there were large numbers of sulfite-reducers. Cultures from ileal contents from four of eight birds had black colonies after one day of incubation. The black colonies were never predominant, so it was necessary to re-streak them. None of the cecal isolates, which included several Eubacterium and Lactobacillus species, reduced sulfite upon subculture. Many of the ileal isolates from SC plates incubated three days also were species of Eubacterium or

Lactobacillus. The predominant organism isolated was a nonsulfite-reducing Eubacterium species.

Sulfite-reducing isolates from the lower ileum that were picked after 24 hours incubation comprised 3 groups, only one of which was C. perfringens. The other two were previously unnamed species of Clostridium. One of the unidentifiable species produced a stronger and faster sulfite reaction than did the C. perfringens strains. After incubation for three days, the same groups as well as a sulfite-reducing E. coli strain were isolated.

It was apparent that SC agar was not adequate for selection of C. perfringens from poultry intestinal populations. Other species besides C. perfringens reduced sulfite within 18 to 24 hours, and the medium was not inhibitory enough for other species to allow easy isolation and confirmation.

M-Enterococcus agar. This medium was devised by Slanetz and Bartley (1957) and reported to be virtually 100% selective for streptococci. Burkwall and Hartman (1964) modified the medium by adding Tween 80 and sodium carbonate, which resulted in much higher recoveries. Daoust and Litsky (1975) detected no significant difference in recovery of streptococci with M-Enterococcus vs. KF-streptococcus agar or Pfizer selective enterococcus agar. The medium was very selective for streptococci, specifically the fecal streptococci; i.e., S. bovis, S. equinus, S. mitis, and the enterococci S. faecalis and S. faecium. Growth of S. salivarius was slightly inhibited. These results were further substantiated by Pagel and Hardy (1980), who found that >99% of all colonies were streptococci and ca. 93% were "fecal" streptococci. As with many

other streptococcal selective media, tetrazolium chloride is included to allow differentiation between S. faecalis, which reduces the tetrazolium, and other species of streptococci that do not. Differential counts were not made in the first selective media study because there was a continuum of color from white to maroon.

In the second selective media study, a greater effort was made to obtain a differential count. Only those colonies that were entirely dark red were counted as S. faecalis. Ten colonies were picked in a randomized pattern from the M-Enterococcus plates from the ileum and cecal contents (i.e., 20 isolates per bird). The majority (>90%) of the isolates were lactobacilli, most of which resembled L. acidophilus. There was no correlation between species and the color of the colony. Strains of the same species produced colonies ranging from white to dark red (due to the reduction of the tetrazolium). Streptococcus species isolated were S. durans, S. avium, S. faecium, and S. faecalis var liquefaciens. The first two produced white colonies, and the other two produced pink and red ones respectively. S. avium was isolated from the ileum, S. faecium and S. durans from the ceca, and S. faecalis from both sites.

Nonselective media. The counts of the cultivable flora at the different sites of the intestinal tract are listed in Table 19. Bacterial counts between the crop and proventriculus decreased up to 1000-fold, presumably due to the low pH. The effects of the acidity also were apparent in the low numbers of viable bacteria compared to the microscopic count. The recoveries for the four birds that had some feed in the proventriculus were 0.8%, 48.0%, 10.3%, and 2.6%.

TABLE 19. Cultivable flora ($\log_{10}$ bacteria/g wet weight) at different sites in the intestinal tract of two-week-old poults

Site	Control birds				Antibiotic-fed birds[a]			
	1	2	3	4	1	2	3	4
Crop	9.59	9.27	9.86	NC[b]	NC	9.53	NC	NC
Proventriculus	NC	NC	6.64	NC	NC	6.77	7.53	6.13
Gizzard	8.51	8.56	7.85	8.32	8.78	8.23	7.94	8.20
Duodenum	8.78	8.87	7.66	8.83	8.31	8.29	7.48	8.62
Lower ileum[c]	9.61	9.74	9.15	9.52	8.79	8.23	8.39	8.73

[a]Zinc bacitracin, 55 ppm.

[b]NC = no contents

[c]The mean counts of the antibiotic-treated birds are significantly different ($p < 0.01$) from the controls.

The low pH in the gizzard (Table 1) seemed to have little effect on percent of viable bacteria. The average recovery from the gizzard was 66.6% from the controls which was very similar to the recovery rate at other sites in the small intestine.

Viable counts were 2.6 to 117 times higher in the gizzard compared to the proventriculus. Antibiotic treatment had no effect on viable or microscopic counts in or percent recovery from the gizzard. In the control birds, bacterial counts were 1.9 to 3.3 times higher in the duodenum than in the gizzard. It was apparent that although the low pH of the proventriculus drastically reduced the viable flora, the bacteria had multiplied by the time they reach the duodenum. It is not possible to state whether this increase occurs in the gizzard or duodenum because portions of the duodenal contents may periodically reflux into the gizzard (Dziuk and Duke, 1972; Oguro and Ikeda, 1974ab; Sklan et al., 1978).

Antibiotic treatment decreased the viable and microscopic counts in the duodenum ($p < 0.25$; Table 18). It also significantly decreased the viable and microscopic counts in the lower ileum ($p < 0.01$ and $p < 0.025$, respectively). Cecal microscopic counts were not affected by antibiotic treatment.

Third Selective Media Experiment

In the previous two selective media studies the only consistent shift in bacteria associated with antibiotic treatment was a decrease in cecal lactobacilli. Thus, one purpose of this experiment was to repeat this observation and to identify the predominant species

isolated. The second purpose was to determine the effect of zinc bacitracin on the enterococci. Barnes et al. (1978) reported that S. faecalis could not be detected in the ceca or small intestine of chickens fed zinc bacitracin after the first week of treatment. One of the objects of the previous selective media study was to repeat this observation. However, the differential colony counts were not meaningful due to the large numbers of lactobacilli isolated. This was probably because of the presence of Tween 80 in the isolation medium. Therefore, in this trial Tween 80 was omitted in order to increase the selectivity for streptococci.

Methods

The diets, birds, and growing conditions were similar to previous trials. The birds were weighed at two weeks, and the weight data are presented in Appendix VI. At two weeks of age there was a 9.6% increase in weight in birds fed zinc bacitracin. Four poults, two controls and two antibiotic-treated, were sampled on days 15 and 16. Cecal contents were plated on Rogosa agar and M-Enterococcus agar (without Tween 80 and carbonate) and the contents of the lower ileum were plated onto M-Enterococcus agar. The Rogosa agar plates were incubated in anaerobe jars with a hydrogen-carbon dioxide atmosphere. All plates were incubated at 41°C and colony counts were made at two days. Ten colonies were picked in a randomized manner from the Rogosa agar plates from each bird. Differential counts for the M-Enterococcus agar were made (dark red vs. white and pink colonies). Five dark red colonies were picked from plates streaked with ileal contents and five

from the cecal contents of each bird. Ten of the non-red colonies were also picked from the plates from each site (20 per bird).

Results and Discussion

Colony counts of M-Enterococcus agar and Rogosa agar are listed in Table 20. As in the previous studies, there was a decrease in the cecal lactobacilli ($p < 0.25$). Of the colonies picked from the Rogosa agar, 79.7% were lactobacilli and the remainder streptococci. There was no apparent correlation between antibiotic treatment and *Lactobacillus* species.

The results of Barnes et al. (1978) were not confirmed. *S. faecalis* was unquestionably present in both the lower ileum and ceca of two-week-old birds. Antibiotic treatment had no significant effect on *S. faecalis* in the ceca or lower ileum. Of the dark red colonies that were characterized, 76.4% were *S. faecalis*, 7.3% *Streptococcus* species, and 16.4% were *Lactobacillus* species (mostly *L. plantarum*). All of the lactobacilli were isolated from the antibiotic-treated birds. Streptococci comprised 83.6% of the other colonies on the M-Enterococcus agar and included, in order of occurrence: an undescribed *Streptococcus* species, *S. constellatus*, *S. bovis*, and *S. faecalis*. The remaining isolates were *Lactobacillus* species (mostly *L. acidophilus* and *L. plantarum*). There were no effects of antibiotic treatment on the individual species that were isolated from the M-Enterococcus plates.

The results of adjusting the numbers of *S. faecalis* based on biochemical identification of isolates rather than on colonial appearance

TABLE 20. Third selective media study. Effect of zinc bacitracin on turkey intestinal bacteria using selective media[a]

Site	Controls				Mean	Antibiotic-treated				Mean
Ileum										
Total streptococci[b]	6.57	6.59	2.19	6.04	6.34	3.10	5.09	4.69	5.09	4.87
S. faecalis	4.48	5.72	<1.88	3.78	5.14	3.50	2.33	3.49	4.10	3.68
Ceca										
Total streptococci	8.60	7.39	5.96	6.50	8.03	7.61	5.02	5.97	6.48	7.05
S. faecalis	5.76	5.79	4.55	4.61	5.50	7.31	4.51	3.65	2.22	6.71
Lactobacilli	9.78	9.37	7.67	8.34	9.33	8.93	8.54	8.01	7.44	8.52

[a] Colonies per g wet weight of contents ($\log_{10}$).

[b] Total colonies other than *S. faecalis*.

are listed in Table 21. There was still no significant difference in S. faecalis counts due to antibiotic treatment.

Summary of Results with Selective Media

Although there were several differences associated with antibiotic treatment that were observed in the first selective media study, the only change that was reproducible was a decrease in the number of cecal lactobacilli (Table 22). There were no apparent changes in the actual species of cecal lactobacilli, but the number of identified isolates (10/bird) was small. Colonies on Rogosa agar were predominantly lactobacilli with the rest being streptococci.

There were large bird-to-bird variations in coliform counts, especially in antibiotic-treated birds. None of the differences were significant; however, the common practice of pooling the intestinal contents of several birds could easily lead to erroneous conclusions.

No correlation was found between C. perfringens counts and the response to antibiotic treatment. [See Section I, Mechanisms of Action, for further information on the effects of antibiotic treatment on C. perfringens.] SC agar, supposedly one of the best selective media currently available, was completely inadequate for isolation of C. perfringens from poultry intestinal populations.

Staphylococcus medium 110 was very reliable for S. aureus counts and gave no false positive results. The highest counts were in the crop. Other researchers also have isolated S. aureus from the crop of poultry. S. aureus is probably a member of the indigenous crop flora because it occurs in higher numbers than in the feed.

TABLE 21. Third selective media study. Effect of zinc bacitracin on turkey intestinal bacteria using selective media (identification based on biochemical characterization of randomly picked isolates)[a]

Site	Controls				Mean	Antibiotic-treated				Mean
Ileum										
Total streptococci[b]	6.57	6.59	2.19	6.04	6.34	3.10	5.04	4.21	5.09	5.40
S. faecalis	4.36	5.72	<1.88	3.78	5.14	3.50	2.33	3.37	4.10	3.66
Ceca										
Total streptococci	8.60	7.39	<4.96	6.50	8.03	7.61	5.02	5.32	6.48	7.04
S. faecalis	5.76	5.79	4.55	4.61	5.50	6.61	3.80	<0.91	2.22	6.01
Lactobacilli	9.73	8.37	7.67	8.34	9.17	8.76	8.54	8.01	7.24	8.42

[a] Bacteria per g wet weight of contents ($\log_{10}$).

[b] Total streptococci other than S. faecalis.

TABLE 22. Effects of zinc bacitracin (55 ppm) on colony counts from cecal contents of two-week-old poults using Rogosa SL agar

Experiment	Controls	Antibiotic-fed	Significance
1 (3)[a]	1.39×10^{10}	4.07×10^{8}	$p < 0.25$
2 (4)	1.92×10^{10}	8.11×10^{9}	-
3 (4)	2.15×19^{9}	3.31×10^{8}	$p < 0.25$
Mean	1.15×10^{10}	3.18×10^{9}	$p < 0.25$

[a]In parentheses are the number of birds in the control and antibiotic-fed groups in each experiment.

Many investigators have reported that antibiotic treatment is associated with a decrease in enterococci counts (e.g., Anderson et al., 1952 and 1956; Sieburth et al., 1951 and 1954; Wagner and Wostmann, 1959) and the possible role of S. faecalis in growth depression of poultry is discussed in more detail in Section I (Mechanisms of Action). Barnes et al. (1978) reported that S. faecalis could not be detected in the small intestine or ceca of two-week-old chickens fed zinc bacitracin. Huhtanen and Pensack (1965a) also reported a decrease in enterococci in chickens with age, but the decline was more gradual than that reported by Barnes et al. (1978). I was unable to confirm the results of Barnes et al. using turkey poults. S. faecalis was present in both the ceca and lower ileum and the counts were not affected by antibiotic treatment. M-Enterococcus agar was reliable for S. faecalis differential counts, but the identity of the other colonies was unpredictable on the basis of colony color. The majority of non-S. faecalis colonies were streptococci with lactobacilli making up the remainder.

The effect of zinc bacitracin on total counts and percent recovery at different sites in the intestinal tract will be discussed in a subsequent section.

ISOLATION AND CHARACTERIZATION OF THE PREDOMINANT INTESTINAL FLORA

The first selective media experiment was designed to try to pinpoint the area of the intestinal tract that showed the most changes in bacterial populations. Unfortunately, there were no obvious differences between sites. The two areas that showed the greatest changes were the

duodenum and ceca. [Chronologically, the following experiments were done prior to the second selective media study.]

The duodenum was chosen as the site to be extensively characterized for the following reasons: i) there have been many experiments suggesting that antibiotics affect the absorption of various nutrients, and the small intestine is the major site of digestion and absorption; ii) in the first selective media study there was an increase in the total numbers of facultatives with antibiotic treatment, and the percent recovery of total bacteria decreased; and iii) at least with penicillin, the concentration of active antibiotic progressively decreases along the intestinal tract. In a subsequent experiment, the lower ileum was characterized for basically the same reasons.

Materials and Methods

Duodenum

Animals and diets. The feed trial design was identical to the one used in the first selective media study.

Sampling. The birds were weighed by pens at two weeks (15 days old) between 11:30 and 2:30 PM. The group showing the maximal response was determined and each bird in the control and treated groups was then weighed individually. Four control and four treated birds whose weight most closely approached the mean weight of their respective groups were chosen. The birds were transported to the laboratory and killed by CO_2 asphyxiation between 4 and 7:00 PM. The duodenum was removed and its contents placed in diluent under anaerobic conditions. Dilutions were prepared and cultured as previously described (Comparison of

Recovery Media). RGCAP-30 and BHIA were used for nonselective isolation of total anaerobes. Three replicates of each medium were inoculated with 0.1 ml from the dilutions $10^{-3,4,5}$. The roll tubes were incubated at 41°C for five days.

Direct microscopic and colony counts and dry weight determinations were done according to the procedures described in the section on selective media.

Isolation and characterization. Twenty five colonies were picked in a randomized manner from both RGCAP-30 and BHIA according to the methods described by Moore and Holdeman (1974). The colonies from RGCAP-30 and BHIA were picked into Sweet E broth (Holdeman et al., 1977) and BHI broth, respectively. These cultures were re-streaked on BHIA roll tubes and each colony type (if there was more than one) was isolated.

The electrophoretic pattern of soluble proteins from each strain was determined according to the procedures of Moore et al. (1980). One isolate from each unique electrophoretic group was characterized according to the procedures described in the VPI Anaerobe Laboratory Manual (Holdeman et al., 1977).

Lower Ileum

Animals and diets. The feed trial design was the same as the one used in the duodenum study except that the 28% protein diet was omitted and each diet was fed to three pens of males and three of females (216 birds total).

Sampling. The birds were weighed by pens at two weeks (15 days old) between 8:30 and 10:30 AM. The birds were transported to the laboratory and killed by CO_2 asphyxiation by 11:30 AM. The subsequent procedures were the same as those used in the duodenal study except that dilutions $10^{-5,6,7}$ were the ones cultured.

Statistical analyses. The same tests used in the selective media experiment were used for the predominant flora experiments.

API ZYM tests. A 24-hour culture of each strain from the duodenum and lower ileum was inoculated into 5 ml of peptone-yeast extract-glucose broth. The cultures were incubated anaerobically with a 100% CO_2 atmosphere at 41°C for 24 hours. The cultures were centrifuged at 8000 x g for 10 min. The supernatant was decanted and the cells re-suspended in 3-4 ml of sterile 0.85% NaCl to an optical density of 427-479 Klett units of 540 nm, using a Spectronic 20 spectrophotometer. Two drops of the suspension were added with a Pasteur pipette to each cupule of the API ZYM strip. One strip was inoculated with sterile saline as a control. The strips were placed in a moist chamber and incubated aerobically at 37°C for 4 hours. One drop of each of the API detector reagents A and B was added to each cupule on the strip. After 5 minutes at room temperature, the strips were exposed to two 500 watt light bulbs, four inches above the strips, for ten seconds, and then the reactions were recorded by comparison with the color reference chart. Each reaction was recorded on a scale from zero to five, where one is approximately 5 nanomoles of the enzyme and a five is greater than 40 nanomoles.

Antibiotic susceptibility. The minimum inhibitory concentration (MIC) of zinc bacitracin was determined for the strains from the duodenum and the lower ileum.

Zinc bacitracin was diluted according to the procedures described by Ericsson and Sherris (1971). Two and one-half ml from each dilution tube were placed in sterile petri plates. Then, 22.5 ml of sterile Wilkins-Chalgren agar (Wilkins and Chalgren, 1976), which had been autoclaved and cooled to 50°C, was added to each plate. Final concentrations of zinc bacitracin in the plates ranged from 0.125 to 512 µg/ml. Four plates were prepared with 2.5 ml of sterile water instead of antibiotic solution as controls. The plates were dried in a 41°C incubator for one to five hours before use.

Fresh 24 hour cultures of each strain were inoculated into BHI broth and incubated at 41°C for 24 hours. The cultures were then adjusted to one-half the turbidity of the McFarland standard no. 1 with sterile brucella broth. The antibiotic plates were inoculated using a Steers replicator and incubated at 41°C in anaerobe jars for 48 hours. The plates were inoculated beginning with the lowest antibiotic concentration. Two control plates were inoculated before and after inoculating the antibiotic plates. One of each pair was incubated anaerobically as a growth control and the other two were incubated aerobically to detect contamination by facultative bacteria. The three reference strains recommended by Sutter et al. (1979) (Clostridium perfringens ATCC 13124, Bacteroides fragilis ATCC 25285, and B. thetaiotaomicron ATCC 29741) were run with each MIC assay.

The MIC for each strain was defined as the lowest concentration of zinc bacitracin resulting in no growth, a single colony, or a barely visible haze as determined without magnification.

Results and Discussion

Duodenum

Growth response. The effects of diet, sex, and antibiotic treatment at two weeks of age are shown in Table 23. The female poults on a 26% protein diet showed the largest growth response (17.6%, $p < 0.01$). None of the other groups had a significant response to antibiotic treatment. Data on weight gain and feed efficiency for weeks one through four are listed in Appendix VII. The mean weights of the control and treated groups were 131 and 154 g, respectively. The four control birds sampled weighed 128, 129, 130, and 133 g. The antibiotic treated birds weighed 151, 153, 156, and 157 g.

Sampling, isolation, and characterization. As in the selective media studies, antibiotic treatment did not significantly affect the moisture content. The total cultural and microscopic counts are listed in Table 24. The differences in counts and percent recovery were not statistically significant.

The identity of the individual strains are listed in Table 25. The duodenal flora was composed almost entirely of lactobacilli. The only difference that might have been associated with antibiotic treatment was the numbers of Lactobacillus sp. 1, although the difference was not statistically significant. This species, and others that did not vary with antibiotic treatment, are all phenotypically very similar.

TABLE 23. Effects of protein and antibiotic treatment on body weights of two-week-old poults[1]

Sex	Protein concentration in diet					
	26%		28%		30%	
	C[2]	Ab[2]	C	Ab	C	Ab
Male	153.3	161.9	139.2	141.9	137.1	166.1
	145.9	148.0	128.3	153.6	138.2	151.3
	157.5	136.1	137.8	139.4	138.2	155.0
	131.2	165.3	161.2	148.6	160.3	156.5
Mean	147.0^{ab}	152.8^{a}	141.6^{abc}	145.9^{abc}	143.4^{abc}	157.2^{a}
Female	122.0	152.1	131.5	137.5	144.1	132.6
	137.0	145.3	128.9	135.4	123.7	143.9
	133.0	157.6	131.3	116.7	123.0	123.7
	131.9	160.9	129.1	141.5	131.8	140.6
Mean	131.0^{c}	154.0^{a}	130.2^{c}	132.8^{bc}	130.6^{c}	135.2^{bc}

[1] Average pen weight (grams) of ten birds. Means not followed by the same superscripts are significantly ($p < 0.05$) different.

[2] C = controls and Ab = antibiotic-fed (55 ppm zinc bacitracin).

TABLE 24. Recovery of duodenal bacteria from two-week-old turkey poults

Treatment	DMCC[a]	BHIA		RGCAP-30	
		Count[b]	% Recovery	Count	% Recovery
Control	22.5	18.9	83.8	16.1	71.4
	16.0	2.48	15.5	2.94	18.4
	2.07	0.85	40.8	0.82	39.6
	3.70	3.97	107.4	4.12	111.5
Mean	11.1	6.55	61.9	6.00	60.2
Antibiotic[c]	4.52	4.39	97.2	3.47	76.7
	6.37	2.92	45.9	2.56	40.2
	7.87	2.74	34.8	2.03	25.9
	7.32	0.87	11.8	0.94	12.9
Mean	6.52	2.73	47.4	2.25	38.9

[a]Direct microscopic clump count. Microscopic and cultural counts are in units of 10^8 bacteria per gram wet wt of contents.

[b]Mean value of three replicates incubated at 41°C for five days.

[c]Zinc bacitracin, 55 ppm.

TABLE 25. The predominant bacterial species in the duodenum of two-week-old poults

Species	Control birds					Antibiotic-fed birds				
	1	2	3	4	Total	1	2	3	4	Total
L. acidophilus A3	-	4[a]	-[b]	18	22	-	-	8	24	32
L. acidophilus B2	-	-	-	1	1	-	-	1	3	4
Lactobacillus sp. 1	12	-	-	-	12	7	12	-	5	24
Lactobacillus sp. 2	37	30	49	-	116	42	38	40	5	124
Lactobacillus sp. 3	-	-	-	3	3	-	-	-	5	5
L. salivarius subsp. *salivarius*	-	13	-	25	38	-	-	2	6	8
L. fermentum	-	-	-	3	3	-	-	-	1	1
Eubacterium sp.	-	-	-	-	-	-	-	-	1	1
B. fragilis	-	1	-	-	1	-	-	-	-	-
B. uniformis	-	2	-	-	2	-	-	-	-	-
E. coli	-	1	-	-	1	-	-	-	1	1
S. morbillorum	-	-	-	-	-	-	-	-	1	1
Total isolates	49	51	49	50	199	49	50	51	52	202

[a]Number of isolates among 50 colonies picked at random from each of 4 birds on each treatment.

[b]None detected.

At this point it is necessary to digress briefly to discuss the taxonomic status of L. acidophilus. Several recent studies have shown that L. acidophilus strains comprise at least six different DNA homology groups (Gasser and Janvier, 1980; Johnson et al., 1980; Lauer et al., 1980; Sarra et al., 1980). One of these groups was subsequently named L. gasseri (Lauer and Kandler, 1980). Most recently, it has been shown that another of the groups is synonymous with L. crispatus (Cato et al., in press). The tests that vary between groups are listed in Table 26. The greatest difficulty is the distinction between L. crispatus and group A3. These two species also have very similar electrophoretic patterns (Cato et al., in press).

Electrophoretic patterns and biochemical tests (Table 27) were used to identify the duodenal isolates. However, without DNA homology values it is not possible to guarantee that some or all of the A3 strains are not L. crispatus. Many strains were isolated that were most similar to the L. acidophilus groups phenotypically but belonged to undescribed species. Morphologically these strains were small rods to coccobacilli compared to the larger L. acidophilus cells. Most strains liquefied gelatin which is fairly unusual for lactobacilli species. In addition to the biochemical differences, the electrophoretic patterns of these strains were quite distinct from any of the patterns of the L. acidophilus homology groups. The only difference that separated Lactobacillus sp. 1 and 2 was lactose fermentation. Strains of Lactobacillus sp. 2 were consistently lactose-negative. Many of these strains were tested several different times for lactose fermentation. It is quite likely that these two

TABLE 26. Phenotypic characters which vary among L. acidophilus homology groups[a]

Species (homology group)	Fermentation of			Starch Hydrolysis	Milk Curd
	Glycogen	Pyruvate	Trehalose		
L. acidophilus (A1)	+	-	+$^{-}$	-$^{+}$	+
L. crispatus (A2)	v	+	v	+$^{-}$	+
A3	-$^{+}$	-$^{+}$	-	+	+
A4	+$^{-}$	-	-	+	-$^{+}$
L. gasseri (B1)	-	v	+	v	+
B2	-	-$^{+}$	+$^{-}$	-	+

[a]Data are derived from Johnson et al. (1980).

TABLE 27. Differential characters for the predominant duodenal turkey lactobacilli[a]

Species	Fermentation of:				Gelatin liquefaction	Starch hydrolysis	Milk curd	Morphology
	Glycogen	Lactose	Pyruvate	Trehalose				
L. acidophilus A3	+	+	+	-	-	+	+	rods
L. acidophilus B2	-	+	-	-	-	-	+	rods
Lactobacillus sp. 1	-	+	-+	+	+-	v	v	coccobacilli
Lactobacillus sp. 2	-	-	-	+	+	-	-	coccobacilli
Lactobacillus sp. 3	-	-	+	-	-	-	-	rods

[a]Results of other biochemical tests are listed in Table 39.

groups are actually the same species because both Johnson et al. (1980) and Lauer et al. (1980) found several lactose-negative strains of L. acidophilus that were in the same homology group as lactose-positive strains. It is important to note that many of the lactose-negative strains in the above two studies were originally isolated from chickens or turkeys. Lactose fermentation has been shown to be a plasmid-carried character in other lactic acid bacteria, including S. lactis, S. cremoris, and L. casei (McKay et al., 1976; Anderson and McKay, 1977; Hofer, 1977; Klaenhammer et al., 1978; Chassy et al., 1978). Even if Lactobacillus sp. 1 and 2 are the same species, they are probably separate biovars. It may be that for some reason the absence of the lactose plasmid provides a selective advantage for some of the poultry lactobacilli.

Because the duodenal flora was simpler than anticipated, another site in the intestinal tract was sampled. The lower ileum was chosen for most of the same reasons that the duodenum was initially selected.

Lower Ileum

The effects of diet, sex, and antibiotic treatment at two weeks of age are shown in Table 28. The female poults on a 30% protein diet showed the largest average growth response, but the variation in weight of the control birds was fairly large. Therefore, the males on 30% protein were sampled. Their growth response was almost as great as the females and was less variable. Data on weight gain and feed efficiency for weeks one through four are listed in Appendix VIII. The mean weights of the control and antibiotic treated groups were 124.9 and

TABLE 28. Effects of protein and antibiotic concentration on body weights of two-week-old turkey poults[1]

Sex		Protein concentration			
		26%		30%	
		Control	Antibiotic[2]	Control	Antibiotic
Males		105.5	126.0	125.7	130.2
		124.3	122.5	124.7	150.2
		124.5	116.2	124.2	144.1
	Mean	118.1^{bc}	121.6^{b}	124.9^{b}	141.5^{a}
Females		106.0	118.9	113.0	119.5
		111.8	108.0	106.8	122.0
		119.6	122.8	95.2	122.3
	Mean	112.5^{bc}	116.6^{bc}	105.0^{c}	121.3^{b}

[1]Average pen weight (in grams) of nine birds. Means not followed by the same superscript are significantly ($p < 0.05$) different.

[2]Zinc bacitracin, 55 ppm.

141.5 grams, respectively. The four control birds sampled weighed 124, 124, 125, and 127 grams. The treated birds weighed 139, 139, 144, and 146 grams.

Data on the moisture content of the samples are in Appendix VIII. The total cultural and microscopic counts are listed in Table 29. There were no significant differences in moisture contents or bacterial counts. The species that were isolated and characterized are listed in Table 30. There were three species in the lower ileum which appeared to be affected by antibiotic treatment. E. tortuosum and Lactobacillus sp. 2 tended to increase and L. acidophilus A3 decreased in the presence of zinc bacitracin. Once again it was very difficult to separate phenotypically some of the electrophoretic groups.

API ZYM strips were run on all of the strains from the duodenum and lower ileum in an effort to find other characters to separate the groups (Table 31). There was only one consistent difference in the enzyme reactions. All of the strains of Lactobacillus sp. 1 and 2 were positive for acid phosphatase and β-glucuronidase whereas none of the L. acidophilus strains were positive for both characters.

Antibiotic Susceptibility

Bacitracin is a polypeptide antibiotic with a molecular weight of 1411 that is isolated from Bacillus licheniformis. A portion of the molecule is cyclic. It is very soluble in water, methanol, and ethanol, and insoluble in chloroform, ether, and acetone. It is stable between pH 3.0 to 9.0. A pH range of 7.0 to 7.5 results in maximum antibiotic activity.

TABLE 29. Effect of antibiotic treatment and media on recovery of bacteria from the lower ileum of two-week-old poults

Treatment	DMCC[a]	BHIA		RGCAP-30	
		Count[b]	% Recovery	Count	% Recovery
Control	1.84	1.42	77.3	1.42	77.3
	1.80	1.97	109.4	2.08	115.7
	7.54	6.84	90.8	9.87	130.9
	3.15	1.49	47.2	2.18	69.2
Mean	3.58	2.93	81.2	3.89	98.3
Antibiotic[c]	0.50	0.27	52.4	0.45	90.5
	4.15	7.41	178.8	8.94	215.6
	1.14	0.49	42.2	0.53	46.1
	11.0	5.59	50.8	5.45	49.6
Mean	4.20	3.44	81.0	3.84	100.4

[a]Direct microscopic clump count. Microscopic and cultural counts are in units of 10^9 bacteria per gram wet weight of contents.

[b]Mean value of three replicates incubated at 41°C for five days.

[c]Zinc bacitracin, 55 ppm.

TABLE 30. The predominant species from the lower ileum of two-week-old poults. First study.

Species	Control birds				Total	Antibiotic-fed birds				Total
	1	2	3	4		1	2	3	4	
L. acidophilus A3	34[a]	30	9	-	73	-	25	1	-	26[b]
Lactobacillus sp. 1	2	2	-	2	6	-	-	2	3	5
Lactobacillus sp. 2	9	1	-	31	41	2	-	45	45	92
L. fermentum	1	-	-	-	1	-	2	-	-	2
L. plantarum	1	-	3	-	4	-	-	-	-	-
L. salivarius subsp. salivarius	-	15	-	-	15	-	4	-	-	4
Eubacterium sp.	1/1[c]	1/1	3/1	2/2	7/5	10/9	4/1	-	1/1	15/11[b]
E. tortuosum	-	-	-	-	-	3	1	-	1	5
B. hypermegas	-	-	-	1	1	26	5	-	-	31
B. capillosus	-	-	-	-	-	-	1	-	-	1
Fusobacterium sp.	-	-	-	-	-	-	2	-	-	2
E. coli	-	-	17	6	23	3	1	-	-	4
Peptostreptococcus sp.	-	-	3/3	3/3	5/3	5/3	-	-	-	5/3
S. bovis	-	-	15	-	15	-	-	-	-	-
S. intermedius	-	-	-	2	2	-	-	-	-	-
Acidaminococcus sp.	-	-	-	-	-	-	6	-	-	6
C. perfringens	-	-	1	-	1	-	-	-	-	-
Total isolates	48	49	48	47	192	49	51	48	50	198

[a] See legend for Table 26.

[b] $p < 0.25$.

[c] Number of isolates/number of species.

TABLE 31. API ZYM results for turkey intestinal lactobacilli[a,b]

Enzyme	L. acidophilus A3	L. acidophilus B2	Lactobacillus sp. 1	Lactobacillus sp. 2	L. fermentum	L. plantarum	L. salivarius ss. salivarius
Alkaline phosphatase	v	-	-	$-^{+}$	$-^{+}$	$-^{+}$	v
C4 esterase	-	-	$-^{+}$	-	v	-	-
C8 esterase	-	-	$-^{+}$	-	$-^{+}$	-	-
Leucine aminopeptidase	v	-	v	v	-	-	v
Valine aminopeptidase	-	-	v	$-^{+}$	-	-	-
Cystine aminopeptidase	$-^{+}$	-	-	-	$-^{+}$	-	-
Acid phosphatase	v	+	+	+	+	$+^{-}$	+
Phosphoamidase	-	-	v	v	-	$-^{+}$	-
α-galactosidase	v	-	v	v	$+^{-}$	-	v
β-galactosidase	v	-	v	-	+	-	v
β-glucuronidase	$-^{+}$	-	+	+	-	-	-
α-glucosidase	v	-	v	v	+	-	$-^{+}$
β-glucosidase	v	-	v	-	$-^{+}$	-	$-^{+}$

[a] Symbols: +, all strains positive; $+^{-}$, all strains but one positive; -, all strains negative; $-^{+}$, all strains but one negative; v, results varied with strain tested. Only one strain of L. acidophilus B2 was tested.

[b] All strains were negative for C14 lipase, trypsin, chymotrypsin, N-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase.

The primary mode of action results from the binding of bacitracin to polyprenol pyrophosphates, thus eliminating their subsequent dephosphorylation and re-use. This binding is enhanced by divalent cations such as magnesium, zinc, or cadmium and results in inhibition of peptidoglycan and teichoic acid synthesis. Bacitracin also affects bacterial cell membranes, however the details of this activity are controversial (Gale et al., 1972; Storm and Toscano, 1979).

Bacitracin is effective primarily against gram-positive organisms. C. perfringens and other clostridia are usually quite sensitive. Most gram-negatives are resistant, although little work has been done with anaerobes. Finegold and Sutter (1972) reported that B. fragilis and F. nucleatum are very resistant, but that B. melaninogenicus is sensitive to bacitracin.

There is no doubt that antibiotic treatment results in an increase in resistant strains, an increase which is usually quite rapid. Unlike many of the other antibiotics, resistance to bacitracin is not believed to be plasmid-mediated (Jacoby and Low, 1980). Thus, in the presence of low levels of antibiotics, it would seem reasonable that it would take longer for a predominantly resistant population to develop.

The minimum inhibitory concentration of zinc bacitracin was determined for all of the strains from the duodenum and lower ileum. It was anticipated that the antibiotic-treated birds would have a higher proportion of resistant strains. This increased resistance could be manifested by a few highly resistant strains increasing their relative numbers or a general increase in resistance without any significant effect on the actual species and their relative

proportions. The MIC data for the strains from the duodenum and lower ileum are in Tables 32 and 33. It is obvious that there was no correlation between antibiotic treatment with zinc bacitracin and the proportion of the flora that was resistant. It may be that if the birds had been sampled at four weeks instead of two, the anticipated predominance of resistant organisms would have occurred. It is also possible that the growth stimulating ability of bacitracin was due to its effect on a small proportion of the flora that was highly susceptible.

MIC values for some of the species isolated from the turkey intestinal tract are summarized in Table 34. The data confirm the observation of Finegold and Sutter (1972) that B. fragilis and similar Bacteroides species are highly resistant to bacitracin. B. hypermegas was only moderately resistant. Among the gram-positive bacteria, C. perfringens and L. fermentum were fairly sensitive. There was a high degree of variation among strains of the other species.

The bacitracin concentration in the feed used in my experiments was 55 µg/g. There were, however, many strains with an MIC much lower than 55 µg/ml. Thus, a major problem in interpreting the MIC results is the question of how stable bacitracin is in the turkey intestinal tract. Bacitracin is thought to be a nonabsorbable antibiotic. Scudi et al. (1947) administered 1500 units (ca. 30 mg) of zinc bacitracin per kg body weight to two dogs. No bacitracin was found in the urine and less than 0.01 units/ml in the serum within 24 hours; however, less than 5% of the dose was recovered in the stools (collected for 24 hrs).

TABLE 32. Cumulative percent inhibition of duodenal isolates by zinc bacitracin.

Treatment	Minimum inhibitory concentration (µg/ml)										
	0.5	1	2	4	8	16	32	64	128	256	512
Antibiotic-fed bird no.											
1 (46)[a]	0	2.2	100								
2 (49)	0	18.4	100								
3 (51)	0	2.0	9.8	19.6	19.6	19.6	94.1	96.1	96.1	96.1	100
4 (50)	0	2.0	8.0	20.0	20.0	64.0	66.0	76.0	90.0	96.0	98.0
Total (196)	0	6.1	53.1	58.7	58.7	69.9	89.8	92.9	96.4	98.0	99.5
Control bird no.											
5 (49)	0	34.7	89.8	89.8	89.8	91.8	100				
6 (50)	0	0	8.0	8.0	8.0	8.0	68.0	92.0	92.0	96.0	98.0
7 (49)	0	0	40.8	40.8	40.8	40.8	40.8	100			
8 (51)	0	0	5.9	11.8	11.8	33.3	33.3	35.3	80.4	82.4	100
Total (199)	0	8.5	35.7	37.2	37.2	43.2	60.3	81.4	93.0	94.5	99.5

[a]Number of isolates per bird for which data were available.

TABLE 33. Cumulative percent inhibition of lower ileal isolates by zinc bacitracin

Treatment	Minimum inhibitory concentration (μg/ml)												
	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512
Antibiotic-fed													
bird no.													
10 (42)	4.8	4.8	4.8	9.5	9.5	11.9	11.9	11.9	11.9	81.0	92.9	92.9	92.9
12 (39)	0	2.6	2.6	2.6	5.1	5.1	10.3	12.8	33.3	43.6	51.3	97.4	97.4
14 (48)	0	4.2	4.2	4.2	50.0	66.7	89.6	89.6	100				
16 (50)	8.0	8.0	10.0	18.0	90.0	90.0	92.0	92.0	92.0	94.0	96.0	100	
Total (179)	3.4	5.0	5.6	8.9	41.9	46.9	54.7	55.3	62.6	81.6	86.6	97.8	97.8
Control bird no.													
9 (25)	4.0	4.0	4.0	8.0	8.0	14.0	16.0	88.0	92.0	92.0	96.0	96.0	96.0
11 (49)	0	2.0	4.1	8.2	10.2	10.2	10.2	10.2	69.4	73.5	100		
13 (50)	0	0	0	2.0	2.0	2.0	2.0	4.0	16.0	16.0	48.0	56.0	56.0
15 (47)	2.1	2.1	2.1	6.4	23.4	51.1	57.4	63.8	83.0	87.2	87.2	87.2	87.2
Total (171)	1.2	1.8	2.3	5.8	11.1	21.6	24.0	34.5	60.8	63.2	80.7	83.0	83.0

TABLE 34. Minimum inhibitory concentrations of zinc bacitracin of identified species from the turkey intestinal tract

Species (no. strains)	average MIC in µg/ml (range of values)
Gram-negatives	
B. fragilis (2)	>512 (512 - >512)
B. hypermegas (6)	53 (32-64)
B. uniformis (2)	384 (256-512)
E. coli (7)	>512 (64->512)
Fusobacterium sp (1)	128
B. fragilis ATCC 25285 (MIC reference strain)	>512
B. thetaiotaomicron ATCC 29741 (MIC reference strain)	512 (256->512)
Gram-positives	
L. acidophilus A3 (25)	155 (1->512)
L. acidophilus B2 (1)	16
Lactobacillus sp 1 (14)	2 (0.125-16)
Lactobacillus sp 2 (26)	16 (0.125-128)
L. fermentum (5)	10 (2-16)
L. plantarum (3)	215 (4->512)
L. salivarius subsp. *salivarius* (10)	122 (1-256)
E. tortuosum (3)	128
Eubacterium sp. (17)	110 (0.125-512)
S. bovis (2)	320 (128->512)
S. intermedius (1)	64
S. morbillorum (1)	32
Anaerobic cocci (5)	78 (0.125-256)
C. perfringens ATCC 13124 (MIC reference strain)	0.625 (0.25-1.0)
C. perfringens (1)	16

A major problem in such experiments is the determination of bacitracin concentration in the feces. The Association of Official Analytical Chemists (Horwitz, 1975) recommends a pyridine extraction method for assaying bacitracin in feeds. Ragheb (1979) conducted a collaborative study and reported that there was excessive inter-laboratory variation in results with this method. The same method was used by Webb and Fontenot (1975) to determine the concentration of zinc bacitracin in the feces of broiler chickens. They found the same amount of anti-bacterial activity in birds not fed bacitracin as in those that were; i.e., the pyridine extraction method was not sufficiently selective for bacitracin. Because the antibiotic-treated birds showed the same amount (or less) of anti-bacterial activity in their feces as the controls, this might indicate that the original bacitracin in the diet had been destroyed.

Bare et al. (1965) studied the concentration of zinc bacitracin in the lower ileum and ceca of chickens. In two-week-old birds they found 36.5 and 40.6 µg of zinc bacitracin per gram wet weight of contents in the lower ileum and ceca, respectively, but the original diet they used contained only 11 µg/g of feed. The antibiotic concentration in the ileum was significantly lower than in the ceca. Bacitracin was assayed by filtering a 1:50 dilution of feces and then directly testing its antimicrobial activity. However, Bare et al. did not report a control of non-antibiotic treated birds and, based on the results of Webb and Fontenot, it's quite possible that the activity they measured was not entirely due to bacitracin. Scudi et al. assayed

bacitracin using methods similar to those of Bare et al. They reported that feces from untreated dogs had no anti-bacterial activity.

Lower Ileum - Experiment 2

Many researchers have reported differences in intestinal floras that were claimed to be due to antibiotic feed supplements. The hallmark of most of these studies has been their lack of reproducibility. Therefore, before initiating more detailed experiments with the strains already isolated, it was thought essential to repeat one of the previous studies. The lower ileum was chosen to be re-analyzed since there seemed to be more differences associated with antibiotic treatment than in the duodenum.

Methods. The feed trial design was the same as the one used in the first lower ileum study except that each diet was fed to six pens of males and six of females (432 birds total). The same sampling procedures were used as with the first ileum study. Colonies were randomly picked as in the previous studies except that they were streaked directly to BHIA roll tubes instead of being placed in broth.

Results and discussion. The effects of diet, sex, and antibiotic treatment at two weeks of age are shown in Table 35. The male poults on a 26% protein diet showed the largest growth response (10.2%, $p < 0.01$). The mean weights of the control and antibiotic treated groups were 176.5 and 194.5 grams, respectively. The four control birds sampled weighed 172, 173, 177, and 181 grams. The treated birds weighed 193, 193, 195, and 197 grams.

TABLE 35. Effects of protein and antibiotic concentration on body weights of two-week-old turkey poults[1]

Sex	Protein concentration			
	30%		26%	
	Control	Antibiotic[2]	Control	Antibiotic
Males	210.2	203.0	176.1	191.4
	187.9	190.4	179.9	188.9
	177.7	187.2	179.6	186.8
	160.4	206.8	172.8	201.1
	172.4	210.2	173.2	183.8
	197.7	183.8	177.4	214.8
Mean	184.4^{abc}	196.9^{a}	176.5^{cd}	194.5^{ab}
Females	187.9	179.0	167.3	188.6
	182.4	187.9	163.0	163.9
	180.5	169.6	162.0	152.5
	175.1	191.0	181.5	165.2
	181.2	190.3	164.9	181.0
	173.1	175.1	161.6	183.7
Mean	180.0^{cd}	182.2^{bc}	166.7^{d}	172.5^{cd}

[1]Average pen weight (in grams) of nine birds. Means not followed by the same superscript are significantly ($p < 0.05$) different.

[2]Zinc bacitracin, 55 ppm.

Total cultural and microscopic counts are in Table 36. Microscopic counts were not affected by antibiotic treatment, but cultural counts declined ($p < 0.25$) and the percent recovery was significantly lower ($p < 0.025$).

The species isolated are listed in Table 37. Antibiotic treatment was associated with a decrease in Lactobacillus sp. 6 ($p < 0.1$) and Eubacterium sp. 2 ($p < 0.25$), and an increase in Lactobacillus sp. 4a ($p < 0.05$). In agreement with the first lower ileum study, there was a nonsignificant decrease in L. acidophilus A3 and an increase in Lactobacillus species 2. When the data from both lower ileum experiments were pooled, the difference in counts of L. acidophilus A3 and Lactobacillus species 2 approached significance ($p < 0.25$ and $p < 0.1$, respectively). The incidence of Eubacterium species and E. tortuosum seem to be independent of antibiotic treatment since they increased in the first ileum study and decreased in the second one.

Other lactobacilli isolated were, in order of incidence, L. salivarius subsp. salivarius, L. plantarum, and L. fermentum. Eubacterium species were frequently isolated from the lower ileum but no single species predominated. With the exception of Eubacterium sp. 2, most groups were composed of a single strain. The only other species that occurred frequently in the lower ileum were B. hypermegas and E. coli, which were present in about half of the birds sampled. B. hypermegas is a common isolate from poultry ceca. In the ileum its incidence varied from less than 2% up to 53% of the cultivable flora. E. coli also showed a large variation in numbers ranging from less than

TABLE 36. Effect of antibiotic treatment and media on recovery of bacteria from the lower ileum of two-week-old turkey poults.

Treatment	DMCC[a]	BHIA		RGCAP-30	
		Count[b]	% Recovery	Count	% Recovery
Control	3.62	1.44	39.7	1.40	38.6
	18.7	14.0	75.2	15.3	81.8
	9.91	2.80	28.3	2.46	24.8
	6.07	4.72	77.7	4.78	78.7
Mean	9.58	5.74	55.2	5.98	56.0
Antibiotic[c]	5.03	0.41	8.2	0.31	6.2
	5.13	1.27	24.9	0.95	18.6
	23.8	2.22	9.3	1.36	5.7
	24.6	1.99	8.1	2.31	9.3
Mean	14.6	1.47	12.6	1.23	10.0

[a]Direct microscopic clump count. Microscopic and cultural counts are times 10^8 bacteria per gram wet wt of contents.

[b]Mean values of three replicates incubated at 41°C for five days.

[c]Zinc bacitracin, 55 ppm.

TABLE 37. The predominant species from the lower ileum of two-week-old poults - Second Study

Species	Control				Total	Antibiotic-treated birds				Total
	1	2	3	4		1	2	3	4	
L. acidophilus A3	-[a]	11	14	12	37	-	-	-	18	18
L. acidophilus B2	-	2	-	-	2	-	-	1	-	1
Lactobacillus sp. 1	2	-	6	-	8	6	2	1	1	10
Lactobacillus sp. 2	24	3	17	-	44	19	31	8	6	64[b]
Lactobacillus sp. 4a	1	-	-	-	1	5	9	3	4	21[b]
Lactobacillus sp. 4b	1	1	-	-	2	2	-	1	3	6
Lactobacillus sp. 4c	7	1	2	-	10	4	-	-	-	4
Lactobacillus sp. 5	7	-	-	-	7	10	-	35	-	45[c]
Lactobacillus sp. 6	4	2	-	5	11	-	-	-	-	0
L. salivarius subsp. salivarius	-	4	3	3	10	1	-	-	5	6
L. plantarum	3	2	-	-	5	-	-	-	-	0
E. tortuosum	-	1	-	1	2	-	-	-	-	0
Eubacterium sp. 1	-	-	-	2	2	-	-	-	-	0[d]
Eubacterium sp. 2	-	4	8	14	26	-	-	-	-	0
Eubacterium other	-	4	-	1	5	-	-	-	1	1
Peptostreptococcus sp.	-	1	-	-	1	-	-	-	-	0
Bif. animalis	-	1	-	-	1	-	-	-	-	0
Gemmiger sp.	-	4	-	-	4	-	-	-	-	0
E. coli	4	-	2	-	6	-	6	-	11	17
B. hypermegas	-	9	-	16	25	-	1	-	-	1
Total isolates	53	50	52	54	209	47	49	49	49	194

[a] See legend for Table 26

[b] $p < 0.05$

[c] $p < 0.1$

[d] $p < 0.25$

2% to 35% of the flora. This large variation agrees with the results obtained with selective media in earlier experiments.

The phenotypic tests that differentiate the predominant turkey intestinal lactobacilli are listed in Table 38. With the exception of species 3, all of the Lactobacillus species are more similar to the L. acidophilus group than to any other named species. The unnamed Lactobacillus species are primarily separated from each other on the basis of lactose and esculin fermentation. The elusive nature of lactose fermentation was discussed earlier. The lactobacillus species were clearly distinct from the L. acidophilus groups on the basis of cellular morphology, electrophoretic patterns, and gelatin liquefaction. The last character is unusual for the described lactobacilli.

The biochemical characters associated with the species which shifted due to antibiotic treatment were glycogen, pyruvate, and trehalose fermentation, and gelatin liquefaction. The nutritional significance of these characters to the host, if any, is unknown although poultry are not able to digest trehalose.

Effect of Antibiotics on Total Counts

Although microscopic counts were not affected in the second lower ileum study, cultural counts were lower with antibiotic treatment ($p < 0.25$, Table 36). Even more dramatic was the marked reduction in recovery rate for the antibiotic-treated birds ($p < 0.025$). These observations prompted a re-examination of the recovery data from previous experiments.

TABLE 38. Differential characters for turkey intestinal lactobacilli[a,b]

Species	Fermentation of:						Gelatin liquefaction	Hydrolysis of:		Milk curd	NH_3 from:		Morphology
	Esc	Gly	Lactose	Melib	Pyr	Treh		Starch	Esc		PY	arginine	
L. acidophilus A3	+	+	+	+	+	−	−	+	ND	+	−	−	r
L. acidophilus B2	+	−	+	+	−	−	−	−	ND	+	−	−	r
Lactobacillus sp. 1	+	−	+	+	$-^{+}$	+	$+^{-}$	v	v	v	v	−	cb
Lactobacillus sp. 2	+	−	−	+	−	+	+	−	v	−	$+^{-}$	CT	cb
Lactobacillus sp. 3	−	−	−	−	+	−	−	−	−	−	+	CT	r
Lactobacillus sp. 4a	−	−	+	+	−	+	$+^{-}$	+	−	+	−	$-^{+}$	cb
Lactobacillus sp. 4b	−	−	+	+	v	+	+	−	−	+	−	v	cb
Lactobacillus sp. 4c	−	−	+	+	−	+	+	v	+	+	+	CT	cb
Lactobacillus sp. 5	−	−	−	+	−	+	+	−	v	−	v	+	cb
Lactobacillus sp. 6	−	−	−	−	−	+	$+^{-}$	+	v	−	$-^{w}$	+	cb

[a] Abbreviations stand for esculin, glycogen, melibiose, pyruvate, and trehalose; r = rod, cb = coccobacillus; ND = not done; CT = can't tell (since control was positive for NH_3). Superscripts = less usual reaction.

[b] Lactobacillus sp. 3 fermented only fructose, glucose, raffinose, and sucrose. All other strains fermented cellobiose, fructose, galactose, glucose, maltose, mannose, raffinose, salicin, sucrose, and hydrolyzed esculin (in PY broth). All were negative for adonitol, arabinose, dulcitol, erythritol, gluconate, glycerol, inositol, inulin, mannitol, rhamnose, sorbitol, sorbose, indole, meat digestion, H_2S, growth in 6.5% NaCl, hemolysis, acetylmethylcarbinol, hippurate hydrolysis, conversion of threonine to propionate, hydrogen production, and catalase. Reactions were variable for nitrate reduction, gas from glucose, reduction of nitrate, neutral red and resazurin, growth in bile, and fermentation of amygdalin, melezitose, ribose, and xylose. Esculin hydrolysis was tested on bile-esculin agar.

As discussed earlier in the selective media studies, zinc bacitracin treatment had no significant effect on total or microscopic counts in the crop, gizzard, upper ileum, or ceca. There was evidence for an effect on the duodenal and lower ileum floras. The results for the selective media and the flora studies are summarized in Tables 39 and 40.

There was no significant difference in cultural or microscopic counts of the duodenum or lower ileum when the results of the different feed trials were averaged. What the pooled results did reveal was a significantly ($p < 0.05$) lower percent recovery of duodenal bacteria from antibiotic-treated birds. Recovery from the lower ileum was also lower with antibiotics ($p < 0.1$). These results suggested that zinc bacitracin was decreasing the viability of the intestinal bacteria without actually lysing the cells. Thus, there were no significant differences in microscopic counts, but the recovery rate declined. The cultivable counts showed a nonsignificant decrease, probably because the small sample size was inadequate to compensate for the large amount of bird to bird variation. There was no evidence that the decline in percent recovery might have been due to a shift in the bacterial populations because: i) the species isolated in the second lower ileum experiment (where the recovery rate was very low) were similar to those isolated in the first lower ileum experiment; and ii) all of the predominant morphotypes observed microscopically were represented by the isolates that were characterized.

Data from the selective media studies clearly showed a steady increase in bacterial numbers from the proventriculus to the ceca

TABLE 39. Effect of antibiotic treatment on the total counts of bacteria in the duodenum of turkey poults

Experiment	Cultivable flora[a]		DMCC[a]		% Recovery	
	C	Ab[b]	C	Ab	C	Ab
1	1.29	2.63	1.55	6.72[c]	87.2	39.3[c]
2	5.18	2.11[d]	7.04	3.07[d]	75.1	60.9
3	5.99	2.25	11.1	6.52	60.2	38.9
Mean	4.16	2.33	6.55	5.44	80.8	46.4[e]

[a]Cultivable flora and DMCC (direct microscopic clump count) are times 10^8 bacteria per gram wet weight of contents. They are the average values for four controls and four antibiotic-treated poults (two to three weeks old).

[b]C = controls, Ab = antibiotic-fed (zinc bacitracin, 55 ppm).

[c]$p < 0.1$

[d]$p < 0.25$

[e]$p < 0.05$

TABLE 40. Effect of antibiotic treatment on the total counts of bacteria in the lower ileum of turkey poults

Experiment	Cultivable flora[a]		DMCC[a]		% Recovery	
	C	Ab[b]	C	Ab	C	Ab
1	3.27	3.54	3.64	5.14	79.6	47.3
2	3.56	0.39[c]	4.46	1.38[d]	78.5	45.9[e]
3	3.89	3.84	3.58	4.20	98.3	100.4
4	0.60	0.12[e]	0.96	1.46	56.0	10.0[d]
Mean	2.83	1.99	3.16	3.03	78.1	50.9[f]

[a]Cultivable flora and DMCC (direct microscopic clump count) are times 10^9 bacteria per gram wet weight of contents. They are the average values for four controls and four antibiotic-treated poults (two to three weeks old).

[b]C = controls, Ab = antibiotic-fed (zinc bacitracin, 55 ppm).

[c]$p < 0.1$

[d]$p < 0.025$

[e]$p < 0.25$

[f]$p < 0.1$

(Table 41). Because the data in Tables 39 and 40 indicated a decrease in viable counts in the small intestine, the changes in numbers vs. sites were examined. These are represented in Table 42 as the proportions of total counts at different areas of the tract. The duodenum:gizzard ratio was higher in the controls ($p < 0.05$). This was due to a depression in the total cultivable flora in the duodenum by bacitracin because there was no difference in bacterial numbers in the gizzard. The upper ileum:duodenum and lower ileum:duodenum ratios also were higher in the controls. The most striking difference was the lower ileum:gizzard ratio which was much higher in the controls ($p < 0.005$). In other words, as the bacteria moved from the gizzard through the small intestine to the lower ileum, they increased in numbers more in the controls than in the treated birds. Counts from the gizzard to the lower ileum increased 12.5 to 20X in the controls, but only 0 to 3.4X in the antibiotic-fed birds.

On the basis of the above data, it seemed possible that a principle mechanism of action of antibiotics was the suppression of the increases in bacterial populations that normally occur as the ingesta moves down the intestinal tract. The evidence for a decrease in viability or metabolic activity was further supported by data that showed that antibiotic treatment was associated with a significant decrease in the lactic acid concentration in the lower ileum ($p < 0.005$; Table 43).

An important point, though, is whether the suppression of bacterial growth in the small intestine is a general mechanism of action of antibiotics or is restricted to zinc bacitracin. Therefore,

TABLE 41. Total cultivable flora at different sites of the turkey intestinal tract

Site	Controls	Antibiotic-fed[a]
Crop	4.29×10^9 (7)[b]	3.39×10^9 (5)
Proventriculus	4.41×10^6 (1)	1.36×10^7 (3)
Gizzard	2.42×10^8 (4)	2.54×10^8 (4)
Duodenum	4.16×10^8 (12)	2.33×10^8 (12)
Upper ileum	6.53×10^8 (4)	4.23×10^8 (4)
Lower ileum	2.83×10^9 (16)	1.99×10^9 (16)
Ceca	9.48×10^{10} (4)	6.73×10^{10} (4)

[a]Zinc bacitracin, 55 ppm.

[b]Average number of bacteria per gram wet weight of intestinal contents. In parenthesis are the number of two to thre-week-old poults that were sampled.

TABLE 42. Ratios of cultivable flora at different sites in the turkey intestinal tract.

Sites (no. of birds)[a]	Control	Antibiotic-fed[b]
Duodenum:gizzard (4)	2.0	1.1[c]
Upper ileum:duodenum (4)	9.9	1.3
Lower:upper ileum (4)	14.1	36.0
Lower ileum:duodenum (8)	33.7	19.4
Lower ileum:gizzard (4)	15.9	2.1[d]
Ceca:lower ileum (4)	166.8	184.4

[a] Three-week-old turkey poults.

[b] Zinc bacitracin, 55 ppm.

[c] $p < 0.05$

[d] $p < 0.005$

TABLE 43. Effect of antibiotic treatment on lactic acid concentration (μmole/g wet weight contents) in the lower ileum of turkey poults

Experiment[a]	Controls	Antibiotic-fed[b]	Difference due to treatment[c]
1	153.1	133.4	-
2	329.8	39.4	$p < 0.025$
3	110.0	68.4	-
4	293.8	63.4	$p < 0.025$
Mean	221.7	76.2	$p < 0.005$

[a]Each experiment was a separate feed trial. The lactic acid concentrations are the mean values of four control and four antibiotic-fed birds.

[b]Zinc bacitracin, 55 ppm.

[c]One-way analysis of variance.

a feed trial was set up with diets containing either no antibiotics, 22 ppm chlortetracycline, or 22 ppm procaine penicillin. Weight and feed consumption data are in Appendix VI. Total counts from the duodenum and the lower ileum were made at two weeks. The data are summarized in Table 44. Penicillin had the same effect on the small intestinal flora that bacitracin did. There was a decrease in percent recovery in the duodenum and lower ileum ($p < 0.1$) and a decrease in the ileum: duodenum ratio ($p < 0.25$) indicating a suppression of bacterial growth. Penicillin also caused a significant ($p < 0.05$) decrease in the lactic acid concentration in the lower ileum. CTC depressed cultivable flora counts and percent recovery in the duodenum ($p < 0.25$) but had no effect on the ileal flora or lactic acid concentration.

Penicillin and bacitracin have similar antimicrobial spectra and appear to stimulate the growth of poultry by the same mechanism of action. Tetracycline's effect on the bacterial flora, however, appears to occur farther up the tract. The fact of CTC in the poultry intestinal tract has not been well studied and it may be that it is absorbed or inactivated before reaching the ileum.

SUMMARY AND CONCLUSIONS

Growth stimulation of animals by antibiotics is mediated by effects on the intestinal flora, but the site(s) of action of this effect is unknown. Therefore various selective media were used to sample different sites in the intestinal tract. No reproducible

TABLE 44. Effect of antibiotic[a] treatment on total numbers of small intestinal bacteria in two-week-old poults

Treatment[b]	Duodenum			Lower ileum			Ileum:duodenum[c]
	DMCC	cultivable flora	% Recovery	DMCC	cultivable flora	% Recovery	
Control	8.76	8.69	143.4	9.32	9.27	96.7	7.6
Penicillin	8.99	8.46	53.7[d]	8.96	8.69[d]	45.5[d]	2.1[e]
CTC	8.60	8.40[e]	73.6[e]	9.40	9.16	74.2	5.5

[a]Average values for six poults. Microscopic and cultivable flora counts are $\log_{10}$ bacteria/g wet weight of intestinal contents.

[b]Penicillin was in the form of procaine penicillin (22 ppm) and CTC = chlortetracycline (22 ppm).

[c]Ratio of cultivable flora.

[d]$p < 0.1$ for the treatment compared to the control

[e]$p < 0.25$ for the treatment compared to the control

differences in bacterial flora associated with antibiotic treatment occurred at any site.

The duodenum and lower ileum were the sites selected to be extensively characterized microbiologically because of the evidence that antibiotic treatment affects the nutrition and feed efficiency of the host. As far as specific alterations of the intestinal flora, no significant effect of antibiotic treatment on the composition of the duodenal flora was observed. In the lower ileum, antibiotic treatment was associated with a decrease in L. acidophilus A3 and Lactobacillus sp. 6 and an increase in Lactobacillus sp. 2 and 4. Because of the many phenotypic similarities between Lactobacillus sp. 1, 2, 4, 5, and 6, DNA homology experiments would be necessary to confirm their actual degree of similarity. Even if all of the groups are genetically distinct, their correlation with antibiotic treatment might be fortuitous and have nothing to do with an increase in the growth of the host. In order to test this possibility, strains of Lactobacillus sp. 2 and 4a, both positively correlated with antibiotic treatment, were chosen to be fed to young poults to see if any growth response occurred. A preliminary feed trial provided no evidence that either of these species stimulated growth when one-day-old birds were colonized with the strains.

Although there were no major differences in the bacterial species present in the small intestine, antibiotic treatment did cause a significant decrease in the recovery rate of bacteria from the duodenum and lower ileum. Comparison of viable counts at different sites in the tract revealed that the intestinal populations increased 12.5 to 20-fold from the gizzard to lower ileum in the controls but only 0 to

3.4 times in the antibiotic-fed birds. Additional evidence for the decrease in viability or metabolic activity by antibiotic treatment was the significantly lower concentrations of lactic acid in the lower ileum of antibiotic-fed birds. Penicillin had the same effect on the percent recovery of the small intestinal flora that bacitracin did. Also, the lower ileum:duodenum ratio of cultivable flora counts decreased with penicillin treatment, as did the lactic acid concentration in the lower ileum.

The proposed mechanism of action of antibiotics in growth stimulation of poultry is a general, nonspecific depression of bacterial growth in the small intestines.

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Appendix I. Composition of Isolation Media

Commercial media were prepared according to the manufacturers instructions. Media for isolation of anaerobes were pre-reduced and anaerobically sterilized according to the procedures described in the VPI Anaerobe Laboratory Manual (Holdeman et al., 1977). Formulas for stock solutions of hemin, resazurin, salts solution, and vitamin K_1 are contained in the above manual.

Brain heart infusion agar (BHIA)*

Brain heart infusion broth (dehydrated)	37 g
Yeast extract	5 g
Cysteine-HCl•H_2O	0.5 g
Agar	25 g
Hemin solution	10.0 ml
Vitamin K_1 solution	0.2 ml
Resazurin solution	4.0 ml
Distilled water	1000 ml

*Cysteine and resazurin were omitted when BHIA was used for isolation of facultative bacteria.

Littman Oxgall agar (Difco)

Peptone	10 g
Glucose	10
Oxgall	15
Crystal violet	0.01
Agar	20
Distilled water	1000 ml

Autoclave, cool to 50°C and add 30 µg streptomycin per ml of medium (0.03 g/l). Streptomycin sulfate (Sigma) was used.

M98-5

Trypticase	2 g
Glucose	0.3
Cellobiose	0.3
Maltose	0.3
Hemin	0.002
Na_2CO_3	4
$Na_2S.9H_2O$	0.25
Cysteine-HCl.H_2O	0.25
Rumen fluid	400 ml
Glycerol	0.3 ml
S2 mineral solution	50 ml
Resazurin solution	0.4 ml
Distilled H_2O	550 ml
Agar	20

S2 mineral solution

KH_2PO_4	0.82 g
NaCl	18.12
$MgSO_4.7H_2O$	1.82
$CaCl_2.2H_2O$	0.59
$(NH_4)_2SO_4$	2.91
$MnCl_2.4H_2O$	0.004
$CoCl_2.6H_2O$	0.0004
Distilled water	1000 ml

MacConkey agar (Difco)

Peptone	17 g
Proteose peptone	3
Lactose	10
Bile salts no. 3	1.5
NaCl	5
Neutral red	0.03
Crystal violet	0.001
Agar	13.5
Distilled water	1000 ml

Final pH 7.1

M-Enterococcus agar (BBL)

Yeast extract	5 g
Trypticase peptone	15
Glucose	2
Phytone peptone	5
Potassium phosphate	4
Sodium azide	0.4
Tetrazolium chloride	0.1
Agar	15*
Distilled water	1000 ml

Mix thoroughly; heat with agitation until dissolved, then autoclave. Cool to 50°C and add 0.5 ml of sterile Tween 80 and 2.0 ml of a sterile (autoclaved) 10% aqueous solution of sodium carbonate.

Colonies of fecal streptococci are pink to maroon.

*The medium supplied by BBL contains only 1% agar.

Rogosa SL agar (Difco)

Trypticase peptone	10 g
Yeast extract	5
KH_2PO_4	6
Ammonium citrate	2
Glucose	20
Sorbitan monooleate	1
Sodium acetate hydrate	25
$MgSO_4.7H_2O$	0.575
$MnSO_4.2H_2O$	0.12
$FeSO_4.7H_2O$	0.034
Agar	15
Distilled water	1000 ml

Mix, heat to dissolve agar, and boil for one minute. Add 1.32 ml of acetic acid. Do not autoclave. Final pH 5.5 $\pm$ 0.1.

Rumen fluid (30%) -glucose-cellobiose agar (RGCA-30)

Glucose	0.248 g
Cellobiose	0.248
Maltose	0.248
Soluble starch	0.5
Yeast extract	0.5
Agar	20
$(NH_4)_2SO_4$	1.0
Cysteine-$HCl \cdot H_2O$	0.5
Resazurin solution	4.0 ml
Salts solution	500 ml
Distilled water	200 ml
Rumen fluid	300 ml
Hemin solution	10.0 ml
Vitamin K_1 solution	0.2 ml

Final pH 6.8-7.0.

RGCA-10

Same as RGCA-30 except with only 10% rumen fluid.

RGCAP-30

Same as RGCA-30 plus 0.05% peptone.

RGCAP-10

Same as RGCA-10 plus 0.05% peptone.

SS agar (Difco)

Beef extract	5 g
Proteose peptone	5
Lactose	10
Bile salts no. 3	8.5
Sodium citrate	8.5
Sodium thiosulfate	8.5
Ferric citrate	1
Agar	13.5
Brilliant green	0.00033
Neutral red	0.025

Staphylococci Medium no. 110 (Difco)

Yeast extract	2.5 g
Tryptone	10
Gelatin	30
Lactose	2
D-mannitol	10
NaCl	75
Dipotassium phosphate	5
Agar	15
Distilled water	1000 ml

Sulfite-cycloserine agar (Hauschild and Hilsheimer, 1974)

Tryptose	15 g
Soytone	5
Yeast extract	5
Ferric ammonium citrate	1
Sodium bisulfite (meta)	1
Agar	20
Distilled water	960 ml

Adjust to pH 7.6 and autoclave. Add 40 ml of a 1% filter-sterilized solution of D-cycloserine to give a final concentration of 400 µg/ml. Count the black colonies at 24 hours.

Wilkins-Chalgren agar (BBL)

Trypticase	10 g
Gelysate	10
Yeast extract	5
Glucose	1
Pyruvate	1
Arginine	1
NaCl	5
Hemin	0.005
Vitamin K_1	0.0005
Agar	15
Distilled H_2O	1000 ml

Final pH 7.0-7.2

APPENDIX II. Comparison of Recovery Media.

A. Effects of medium and incubation time on recovery of three-week-old turkey cecal bacteria incubated at 37°C

Medium	Days of incubation					
	2		5		14	
	Count[a]	% Recovery	Count	% Recovery	Count	% Recovery
RGCAP-10	3.73	92.3	3.87	95.9	3.93	97.3
	3.93	58.9	4.30	64.3	4.45	66.6
	2.09	32.9	3.10	48.8	3.03	47.7
	5.95	81.4	6.06	82.8	6.26	85.6
Mean	3.92	66.4	4.33	73.0	4.42	74.3
RGCAP-30	ND[b]	ND	3.97	98.4	3.94	97.5
	4.55	68.1	4.32	64.7	4.58	68.6
	2.45	38.5	2.95	46.3	2.96	46.6
	7.69	105.1	7.51	102.7	7.97	109.0
Mean	4.90	70.6	4.69	78.0	4.86	80.4
RGCA-30	3.23	79.9	3.46	85.7	3.56	88.3
	3.87	57.9	3.61	54.0	3.85	57.6
	2.50	39.3	3.06	48.1	3.15	49.5
	7.48	102.3	7.48	102.3	7.79	106.5
Mean	4.27	70.0	4.40	72.5	4.59	75.5
RGCA-10	4.10	101.5	4.08	101.2	4.30	106.7
	3.57	53.5	3.76	56.3	4.06	60.7
	1.96	30.9	2.93	46.0	3.15	49.5
	6.59	90.1	7.28	99.6	7.21	98.5
Mean	4.06	69.0	4.51	75.8	4.68	78.9
M98-5	ND	ND	3.44	85.2	3.37	83.5
	3.84	57.5	4.16	62.3	3.89	58.3
	2.39	37.6	3.08	48.4	3.15	49.5
	6.28	85.8	6.28	85.8	6.05	82.7
Mean	4.17	60.3	4.24	70.4	4.12	68.5

Appendix II. A. Continued.

Medium	Days of incubation					
	2		5		14	
	Count[a]	% Recovery	Count	% Recovery	Count	% Recovery
BHIA	ND	ND	2.54	63.0	2.43	60.2
	2.66	39.8	3.01	45.0	3.03	45.4
	1.34	21.1	2.06	32.4	1.90	29.9
	5.29	72.3	5.88	80.3	5.67	77.6
Mean	3.10	44.4	3.37	55.2	3.26	53.2

[a]Mean colony counts (of five replicates) from four turkeys x 10^{11} bacteria per gram dry wt of cecal contents.

[b]Not done.

Appendix II. B. Effects of medium and incubation time on recovery of three-week-old turkey cecal bacteria incubated at 41°C.

Medium	Days of incubation					
	2		5		14	
	Count[a]	% Recovery	Count	% Recovery	Count	% Recovery
RGCAP-10	4.25	105.2	4.45	110.3	4.59	113.7
	4.00	59.8	4.04	60.5	4.40	65.9
	3.08	48.4	3.80	59.8	3.94	62.0
	8.49	116.0	9.20	125.8	8.93	122.1
Mean	4.96	82.4	5.37	89.1	5.46	90.9
RGCAP-30	ND[b]	ND	4.50	111.6	4.57	113.3
	4.16	62.3	4.24	63.5	4.57	68.4
	3.56	55.9	4.04	63.5	3.91	61.5
	6.34	86.6	7.16	97.8	7.13	97.5
Mean	4.69	68.3	4.98	84.1	5.04	85.2
RGCA-30	3.88	96.3	3.85	95.4	3.85	95.4
	3.63	54.4	4.28	64.0	4.40	65.9
	3.54	55.7	4.45	69.9	3.77	59.4
	6.50	88.9	7.20	98.4	7.24	98.9
Mean	4.39	73.8	4.94	81.9	4.82	79.9

Appendix II. B. Continued.

Medium	Days of incubation					
	2		5		14	
	Count[a]	% Recovery	Count	% Recovery	Count	% Recovery
RGCA-10	3.88	96.1	3.85	95.4	3.82	94.7
	3.76	56.3	4.23	63.4	4.55	68.2
	3.63	57.1	4.19	65.9	4.26	67.0
	6.42	87.8	6.89	94.2	6.52	89.1
Mean	4.42	74.3	4.79	79.7	4.79	79.8
M98-5	ND	ND	3.40	84.2	3.48	86.2
	3.54	53.1	4.11	61.5	4.62	69.2
	3.87	60.9	3.83	60.3	4.13	65.0
	6.95	95.0	6.93	94.7	7.12	97.3
Mean	4.79	69.7	4.57	75.2	4.84	79.4
BHIA	ND	ND	3.23	80.0	3.15	78.0
	2.73	40.9	2.92	43.8	3.29	49.2
	1.57	24.8	2.26	35.6	2.13	33.5
	4.96	67.8	5.16	70.6	4.91	67.1
Mean	3.09	44.5	3.39	57.5	3.37	56.9

[a]Mean colony counts (of five replicates) from four turkeys x 10^{11} bacteria per gram dry wt of cecal contents.

[b]Not done.

Appendix II. C. Effects of media and incubation temperatures on recovery of cecal bacteria from six-week-old turkeys.

Medium	37°		41°		Rank	
	Count[a]	% Recovery	Count	% Recovery	37°	41°
M98-5	4.53	156.8	5.47	189.1	1	1
	3.74	124.1	3.59	119.2	1	2
	4.73	164.2	5.29	183.6	1	1
	ND[b]	-	3.13	69.1	-	5
	ND	-	4.30	144.4	-	1
Mean	4.33	148.4	4.36	141.0	1.0	2.0
RGCAP-30	3.56	123.3	5.26	182.0	3	2
	2.82	93.7	3.44	114.1	5	3
	3.65	126.7	3.48	120.7	5	5
	ND	-	4.31	95.1	-	1
	ND	-	3.55	119.4	-	4
Mean	3.34	114.6	4.01	126.3	4.3	3.0
RGCAP-10	3.54	122.3	5.00	173.2	5	3
	3.40	113.0	3.21	106.7	2	5
	3.86	134.0	3.72	129.0	2	4
	ND	-	3.74	82.7	-	3
	ND	-	3.88	130.4	-	2
Mean	3.60	123.1	3.91	124.3	3.0	3.4
RGCA-30	3.56	123.3	4.33	149.9	3	5
	3.12	103.6	2.92	97.1	3	6
	3.66	127.1	4.61	160.2	4	2
	ND	-	3.87	85.4	-	2
	ND	-	3.76	126.1	-	3
Mean	3.45	118.0	3.90	123.7	3.3	3.6
RGCA-10	3.21	111.2	4.80	166.1	6	4
	2.67	88.8	3.25	107.9	6	4
	3.71	128.9	4.45	154.4	3	3
	ND	-	3.23	71.4	-	4
	ND	-	3.52	118.2	-	5
Mean	3.20	109.6	3.85	123.6	5.0	4.0

Appendix II. C. Continued.

Medium	37°		41°		Rank	
	Count[a]	% Recovery	Count	% Recovery	37°	41°
BHIA	3.82	132.2	4.04	139.7	2	6
	2.99	99.2	3.65	121.1	4	1
	3.05	106.0	3.45	119.8	6	6
	ND	-	2.20	48.5	-	6
	ND	-	1.72	57.8	-	6
Mean	3.29	112.5	3.01	97.4	4.0	5.0

[a]Mean colony counts (of five replicates) times 10^{11} bacteria per gram dry wt. of cecal contents. Samples were incubated for five days.

[b]Not done.

Appendix II. D. Rank order of media based on percent recovery. Effects of media, incubation time, and temperature using three-week-old poults.

Medium	Days of incubation					
	2		5		14	
	37°	41°	37°	41°	37°	41°
RGCAP-10	2	1	3	2	3	1
	2	2	2	5	2	4
	4	5	1	5	4	3
	5	1	5	1	4	1
Mean	3.25	2.25	2.75	3.25	3.25	2.25
RGCAP-30	ND[b]	ND	2	1	2	2
	1	1	1	2	1	2
	2	3	4	3	5	4
	1	5	1	3	1	3
Mean	1.33	3.0	2.0	2.25	2.25	2.75
RGCA-30	3	2	4	3	4	3
	3	4	4	1	5	4
	1	4	3	1	1	5
	2	3	2	2	2	2
Mean	2.25	3.25	3.25	1.75	3.0	3.5
RGCA-10	1	3	1	3	1	4
	5	3	5	3	3	3
	5	2	5	2	1	1
	3	4	3	5	3	4
Mean	3.5	3.0	3.5	3.25	2.0	3.0
M98-5	ND	ND	5	5	5	5
	4	5	3	4	4	1
	3	1	2	4	1	2
	4	2	4	4	5	5
Mean	3.67	2.67	3.5	4.25	3.75	3.25
BHIA	ND	ND	6	6	6	6
	6	6	6	6	6	6
	6	6	6	6	6	6
	6	6	6	6	6	6
Mean	6.0	6.0	6.0	6.0	6.0	6.0

[a]Not done.

APPENDIX III. First Selective Media Experiment. A. Weight and feed efficiency diet.[a]

Age/Sex	26% Protein				28% Protein				30% Protein			
	Weight		F.E.		Weight		F.E.		Weight		F.E.	
	C	Ab	C	Ab	C	Ab	C	Ab	C	Ab	C	Ab
Week 1												
Males	99.1	117.4	0.59	0.75	101.6	104.9	0.56	0.74	87.7	122.6	0.55	0.86
	98.6	121.1	0.66	0.70	108.9	109.8	0.64	0.68	111.1	120.2	0.70	0.80
	102.6	100.6	0.65	0.62	112.9	109.0	0.67	0.70	121.8	117.0	0.80	0.72
	106.8	105.8	0.60	0.62	110.9	100.7	0.70	0.60	102.4	117.8	0.66	0.68
Females	105.5	102.5	0.58	0.66	94.3	105.0	0.54	0.67	93.6	93.6	0.54	0.55
	102.6	93.3	0.60	0.57	94.2	111.8	0.64	0.68	90.6	103.5	0.61	0.67
	101.0	99.0	0.61	0.59	110.6	102.8	0.73	0.63	102.7	111.9	0.64	0.79
	98.7	104.5	0.64	0.62	95.4	94.9	0.64	0.59	111.4	112.7	0.69	0.76
Week 2												
Males	188.4	200.7	0.64	0.56	197.3	202.4	0.59	0.61	168.6	239.1	0.63	0.68
	182.2	221.8	0.60	0.61	204.1	208.8	0.62	0.62	212.3	216.4	0.62	0.59
	196.6	195.5	0.62	0.66	213.1	195.3	0.63	0.59	228.7	228.7	0.61	0.64
	211.5	191.4	0.59	0.60	207.0	197.8	0.65	0.62	204.5	234.5	0.69	0.67
Females	197.6	198.0	0.57	0.61	167.7	202.7	0.58	0.59	183.5	188.0	0.59	0.66
	193.5	175.3	0.54	0.61	178.9	217.8	0.61	0.64	168.1	201.6	0.58	0.65
	200.8	161.0	0.60	0.55	207.6	192.2	0.59	0.58	198.1	213.6	0.64	0.63
	192.2	200.7	0.62	0.59	175.3	189.1	0.59	0.67	220.8	212.2	0.64	0.63

[a]Weight data are the cumulative average pen weight (in grams) of ten poults. Feed efficiency (F.E. = weight gained/feed consumed) data were calculated on a weekly basis. C = controls and Ab = antibiotic-fed (zinc bacitracin, 55 ppm).

Appendix IIIA. continued

Age/Sex	26% Protein				28% Protein				30% Protein			
	Weight		F.E.		Weight		F.E.		Weight		F.E.	
	C	Ab	C	Ab	C	Ab	C	Ab	C	Ab	C	Ab
Week 3												
Males	323.1	327.0	0.62	0.50	335.2	341.2	0.58	0.60	285.1	421.4	0.46	0.64
	304.9	368.3	0.56	0.60	328.8	354.8	0.53	0.62	348.4	379.1	0.70	0.60
	291.9	281.2	0.44	0.42	298.6	305.1	0.38	0.50	342.5	337.9	0.52	0.45
	355.4	297.3	0.57	0.51	344.2	336.9	0.59	0.58	334.8	365.8	0.66	0.52
Females	316.3	330.4	0.55	0.58	291.3	354.5	0.56	0.60	306.4	317.7	0.65	0.56
	293.9	261.2	0.43	0.46	264.6	317.9	0.43	0.43	272.4	314.0	0.57	0.50
	326.8	279.9	0.55	0.62	350.2	326.3	0.59	0.58	329.1	382.0	0.68	0.65
	327.1	333.6	0.58	0.54	295.9	326.2	0.56	0.59	376.9	350.8	0.71	0.59
Week 4												
Males	536.7	537.3	0.59	0.56	549.0	547.6	0.53	0.55	464.4	667.1	0.57	0.58
	482.5	525.4	0.54	0.46	525.0	596.1	0.55	0.60	548.8	593.4	0.55	0.54
	478.4	512.0	0.57	0.65	519.4	489.7	0.62	0.54	562.1	617.0	0.59	0.69
	571.8	503.9	0.54	0.54	569.0	514.2	0.58	0.49	551.2	601.9	0.57	0.57
Females	500.0	526.2	0.54	0.56	482.4	558.5	0.52	0.54	508.0	521.0	0.57	0.51
	512.9	456.3	0.70	0.62	476.9	551.7	0.63	0.61	465.6	544.3	0.58	0.66
	502.2	487.7	0.50	0.57	541.3	563.9	0.53	0.64	527.9	570.1	0.54	0.52
	513.1	544.6	0.53	0.55	461.9	537.4	0.52	0.56	574.7	572.5	0.53	0.58

Appendix IIIB. Total contents and percent dry weight in the gastrointestinal tract of individual two-week-old poults. First selective media study

Area of tract	Control bird no.				Antibiotic-fed bird no.			
	1	2	3	4	1	2	3	4
	Total Contents (g wet weight)							
Crop	1.69	0.78	4.88	3.65	ND	1.84	5.86	4.57
Duodenum	0.86	0.87	0.63	0.7	0.63	1.12	0.62	1.68
Upper Ileum	1.25	1.39	1.58	1.51	3.35	2.48	2.74	1.77
Lower Ileum	0.73	1.02	1.97	2.42	2.34	2.01	2.13	2.06
Ceca	3.06	0.65	0.74	1.74	0.85	2.26	0.65	1.31
	% Dry weight							
Crop	40.0	28.2	35.7	39.8	28.6	47.9	31.5	54.4
Duodenum	15.9	21.6	20.4	33.3	13.6	10.2	25.0	16.5
Upper Ileum	20.0	18.2	14.6	19.4	15.4	14.1	21.5	20.5
Lower Ileum	13.8	12.5	17.2	15.8	13.8	15.4	18.8	16.9
Ceca	14.7	22.4	22.0	9.2	14.0	36.4	19.6	18.5

Appendix IIIC. Effect of zinc bacitracin on bacterial counts of turkey intestinal bacteria using selective media.

Flora	Control bird no.				Antibiotic-fed bird no.			
	1	2	3	4	1	2	3	4
				Crop				
DMCC	9.21[a]	9.32	9.99	9.46	9.81	9.95	9.49	9.74
Total anaerobes	9.58	9.48	9.87	9.45	9.52	9.76	9.60	8.81
% Recovery	232.9	142.4	74.9	96.9	50.4	64.3	127.5	11.9
Lactobacilli	ND[b]	8.72	10.17	8.28	ND	9.03	9.50	8.51
Sulfite-reducers	1.94	0[c]	1.85	0	0	0	0	2.53
Total facultatives	7.61	8.08	8.08	8.53	7.37	8.36	7.41	8.71
MacConkey's	5.05	4.69	4.80	4.86	4.43	5.89	2.48	7.27
SS agar	4.94	4.21	2.97	4.55	3.64	5.78	1.43	6.24
Total staphylococci	5.75	5.15	5.60	6.03	5.17	5.66	5.31	6.40
S. aureus	0	2.21	4.31	2.51	0	4.74	4.73	4.61
Streptococci	7.35	7.02	7.65	6.83	5.70	6.60	4.85	7.46
Detection limit	1.64	1.90	1.37	1.43	2.53	1.93	1.43	1.53
				Duodenum				
DMCC	8.41	7.24	8.42	7.92	9.10	8.75	8.82	8.33
Total anaerobes	8.51	7.46	7.90	7.93	8.65	7.78	8.66	7.95
% Recovery	126.7	169.0	29.8	102.8	35.6	10.8	69.3	41.4
Lactobacilli	ND	6.71	8.30	7.64	ND	7.56	8.06	7.68
Sulfite-reducers	4.31	0	0	0	0	0	0	0
Total facultatives	6.29	6.01	4.88	5.71	7.71	7.46	7.03	6.02
MacConkey's	5.47	<2.93	<3.20	3.76	<3.20	4.71	<3.23	5.75
SS agar	2.18	0	0	0	0	4.14	0	2.47
Total staphylococci	2.35	1.93	1.20	2.76	3.53	3.79	3.74	2.39
S. aureus	0	0	0	0	0	0	3.41	0
Streptococci	5.03	4.57	4.16	4.31	4.79	5.01	4.09	4.67
Detection limit	1.87	1.93	1.20	2.28	2.20	1.70	2.23	1.69

[a] All counts are $\log_{10}$ of bacteria per gram wet wt of contents.

[b] Not done.

[c] None detected (see detection limit).

Appendix IIIC. continued

Flora	Control bird no.				Antibiotic-fed bird no.			
	1	2	3	4	1	2	3	4
				Upper Ileum				
DMCC	8.96	8.41	8.48	9.04	9.14	8.64	9.25	8.20
Total anaerobes	8.93	8.09	8.24	9.17	8.88	7.91	8.90	7.76
% Recovery	92.6	46.9	58.2	134.9	54.6	18.9	45.4	36.5
Lactobacilli	ND	6.80	5.30	8.43	ND	7.85	8.78	6.77
Sulfite-reducers	1.69	0	0	1.61	0	0	0	0
Total facultatives	7.85	6.32	3.71	7.73	7.22	7.18	7.48	5.93
MacConkey's	6.66	0	0	4.52	0	6.53	4.77	6.20
SS agar	>6.47	0	0	2.96	1.85	5.85	2.69	3.87
Total staphylococci	6.06	2.70	2.19	4.82	3.87	3.69	3.68	4.23
S. aureus	0	0	1.71	2.09	0	3.31	3.54	3.90
Streptococci	6.48	5.53	3.78	5.52	5.40	5.31	5.03	4.96
Detection limit	1.69	1.79	1.71	1.61	1.54	1.70	1.69	1.72
				Lower Ileum				
DMCC	8.96	8.68	10.07	9.16	9.26	10.09	9.34	9.64
Total anaerobes	8.69	8.35	10.02	9.27	8.44	10.09	9.17	8.22
% Recovery	53.8	47.0	89.4	128.2	15.0	102.0	68.4	3.8
Lactobacilli	ND	7.63	9.37	8.56	ND	8.99	8.89	7.61
Sulfite-reducers	4.96	3.00	0	4.29	6.20	4.97	2.26	5.34
Total facultatives	7.35	7.48	9.11	8.75	7.62	9.44	8.23	8.33
MacConkey's	6.77	5.15	5.75	6.97	5.94	9.52	5.52	8.33
SS agar	>6.63	3.24	5.10	5.11	5.66	>6.47	2.26	6.23
Total staphylococci	6.37	4.76	5.58	6.35	6.40	6.95	5.42	6.87
S. aureus	0	0	3.58	2.44	0	0	2.48	3.34
Streptococci	6.75	5.57	5.74	8.24	7.82	7.66	5.95	7.51
Detection limit	1.85	1.91	1.78	1.74	1.54	1.69	1.77	1.66

Appendix IIIC. continued

Flora	Control bird no.				Antibiotic-fed bird no.			
	1	2	3	4	1	2	3	4
				Ceca				
DMCC	10.66	11.28	11.09	10.60	10.43	10.50	10.92	11.04
Total anaerobes	11.01	11.26	10.23	10.88	10.58	10.91	10.68	11.01
% Recovery	226.5	95.6	13.9	190.8	140.2	258.0	58.3	92.3
Lactobacilli	ND	10.51	9.93	8.71	ND	8.08	8.82	8.64
Sulfite-reducers	6.94	5.73	3.97	6.43	4.94	4.74	4.28	4.54
Total facultatives	7.76	8.48	9.32	8.37	8.46	8.31	8.81	9.42
MacConkey's	6.81	7.65	6.47	8.16	6.25	8.90	8.19	9.61
SS agar	5.69	7.30	5.78	6.75	5.11	6.70	6.31	>6.68
Total staphylococci	5.39	4.58	5.03	5.87	4.29	5.08	4.99	5.48
S. aureus	0	2.58	3.31	2.47	0	1.96	3.74	3.38
Streptococci	6.24	5.88	6.28	8.26	6.63	5.80	5.35	7.05
Detection limit	1.46	2.28	2.10	1.86	2.04	1.65	2.20	1.90

APPENDIX IV. Short chain fatty acid (SCFS) and analysis of gastrointestinal contents of individual turkey poults. A. First selective media.

SCFA	Control bird no.				Antibiotic-fed bird no.			
	1	2	3	4	1	2	3	4
	Crop							
Acetic	129.0[a]	72.2	87.3	65.9	NP[b]	130.0	92.8	92.5
Formic	32.9	-	-	-		-	-	19.5
Lactic	431.7	77.0	303.5	140.0		356.2	129.5	219.9
Succinic	68.4	15.6	39.1	17.5		54.6	23.2	12.8
Total VFA	161.9	72.2	87.3	65.9		130.0	92.8	112.0
Total SCFA	662.0	164.8	429.9	223.4		540.8	245.5	344.7
Limit[c]	.13	.24	.07	.08	1.01	.26	.08	.10
	Duodenum							
Acetic	148.1	NP	NP	-	NP	7.6	NP	NP
Formic	172.8			5.7		-		
Lactic	134.6			-		-		
Succinic	65.1			-		-		
Total VFA	320.9			5.7		7.6		
Total SCFA	520.6			5.7		7.6		
Limit	.22	.26	.48	0.57	.48	.15	.51	.15
	Upper Ileum							
Acetic	129.2	7.5	4.7	28.5	38.8	72.3	32.7	17.6
Propionic	11.9	-	-	-	-	48.2	-	-
Lactic	353.4	-	-	242.8	223.1	30.1	111.4	-
Succinic	78.0	-	-	5.6	25.7	34.6	8.2	-
Total VFA	141.1	7.5	4.7	28.5	38.8	120.5	32.7	17.6
Total SCFA	572.5	7.5	4.7	276.9	313.2	185.2	152.3	17.6
Limit	.15	.19	.15	.12	.10	.15	.15	.16

[a] µmoles/g wet wt of contents.

[b] No products detected.

[c] Lower detection limit.

Appendix IVA. continued

SCFA	Control bird no.				Antibiotic-fed bird no.			
	1	2	3	4	1	2	3	4
	Lower Ileum							
Acetic	163.0	27.2	86.0	47.9	113.1	245.0	44.9	128.9
Formic	94.4	-	-	21.5	59.1	-	-	131.7
Propionic	40.7	-	32.9	-	12.4	87.6	-	-
Butyric	-	-	16.5	-	24.9	13.4	-	-
Isobutyric	12.9	-	-	-	-	-	-	-
Lactic	130.8	145.8	89.7	246.1	184.7	129.2	154.6	65.1
Succinic	62.2	-	26.5	6.6	38.9	43.8	14.4	18.0
Total VFA	311.0	27.2	135.4	69.4	209.5	346.0	44.9	260.6
Total SCFA	504.0	173.0	251.6	322.1	433.1	519.0	213.9	343.7
Limit 0.1	.22	.25	.18	.17	.10	.15	.18	.14
	Ceca							
Acetic	199.7	355.1	292.8	122.7	417.2	214.7	202.9	336.9
Propionic	110.4	171.8	131.0	59.2	251.7	120.4	120.8	180.8
Butyric	48.2	74.4	77.1	35.1	76.2	57.4	48.3	66.0
Isovaleric	4.4	-	7.7	-	-	-	-	12.0
Valeric	4.4	-	19.3	-	9.9	9.6	-	-
Lactic	-	-	-	-	-	-	-	-
Succinic	6.6	-	-	-	132.5	5.5	-	-
Formic	-	-	-	-	-	42.4	-	-
Total VFA	367.1	601.3	527.9	217.0	755.0	402.1	372.0	595.7
Total SCFA	373.7	601.3	527.9	217.0	887.5	450.0	372.0	595.7
Limit 0.1	.09	.19	.38	.22	.33	.14	.48	.24

Appendix IVB. Second selective media study.

SCFA	Control bird no.				Antibiotic-fed bird no.			
	1	2	3	4	1	2	3	4
	Crop							
Acetic	476.0	113.8	29.6	NC	NC	86.4	NC	NC
Formic	-	-	-			55.7		
Lactic	337.2	-	505.0			647.6		
Succinic	-	17.7	8.6			26.0		
Total VFA	476.0	113.8	29.6			142.1		
Total SCFA	813.2	131.5	543.2			815.7		
Limit 0.1	2.05	.53	.09			.13		
	Gizzard							
Acetic	16.3	15.6	NP	51.0	NP	13.9	NP	23.1
Lactic	7.0	-		-		40.5		-
Succinic	1.8	-		-		-		-
Total VFA	16.3	15.6		51.0		13.9		23.1
Total SCFA	25.1	15.6		51.0		54.4		23.1
Limit 0.1	.09	.09	.09	.09		.09		.08
	Duodenum							
Acetic	265.0	174.2	NP	4.5	21.8	134.2	NP	7.7
Formic	194.5	65.3		-	-	-		-
Propionic	43.5	22.8		-	-	-		-
Butyric	-	-		-	7.7	-		-
Lactic	-	-		-	24.9	-		-
Succinic		49.4		-	11.0	32.6		-
Total VFA	503.0	262.3		4.5	29.5	134.2		7.7
Total SCFA	601.5	311.7		4.5	65.4	166.8		7.1
Limit 0.1	.41	.33		.11	.31	.33		.15

Appendix IVB. continued

SCFA	Control bird no.				Antibiotic-fed bird no.			
	1	2	3	4	1	2	3	4
	Lower Ileum							
Acetic	163.3	264.6	30.3	54.1	212.8	1089.7	NP	44.9
Formic	208.3	71.5	30.2	-	352.0	-		-
Propionic	-	-	-	-	-	180.9		-
Lactic	380.3	494.0	86.1	358.7	157.6	-		-
Succinic	22.9	25.7	6.6	-	34.9	415.8		4.0
Total VFA	371.6	318.1	60.5	54.1	564.8	1270.6		44.9
Total SCFA	774.8	837.8	153.2	412.8	757.3	1686.4		48.9
Limit 0.1	.14	.65	.14	.15	.20	6.5		.12
	Ceca							
Acetic	1709.0	83.4	119.9	2.9	1028.5	1418.6	211.5	207.6
Formic	-	54.5	48.4	3.6	-	-	-	26.0
Propionic	106.0	-	6.6	-	-	53.7	-	17.2
Butyric	463.6	23.6	35.3	-	179.3	240.8	73.7	70.6
Valeric	-	-	-	-	-	23.8	-	-
Lactic	-	363.0	-	220.7	-	-	-	37.1
Succinic	120.6	3.8	12.5	-	95.5	132.2	-	11.8
Fumaric	-	-	-	-	-	-	-	16.0
Total VFA	2278.6	161.5	210.2	6.4	1207.8	1736.9	285.2	321.4
Total SCFA	2399.2	528.3	222.7	227.1	1303.3	1869.1	285.2	386.3
Limit 0.1	1.16	.07	.20	.09	.57	1.18	.92	.97

Appendix IVC. Third selective media study.

Duodenum

SCFA	1	2	3	4	5	6
Control						
Acetic	19.4	3.8	NP	NP	NP	NP
Lactic	139.0	-				
Procaine penicillin						
Acetic	42.4	NP	9.5	11.4	13.7	4.3
Lactic	247.1		-	-	-	17.7
Chlortetracycline						
Acetic	NP	NP	7.5	NP	6.4	NP
Lactic			-		80.6	

Lower ileum

SCFA						
Control						
Acetic	17.5	102.7	53.1	162.4	29.0	52.0
Formic	-	126.3	-	100.6	-	-
Butyric	-	11.4	-	32.8	8.5	8.1
Lactic	269.0	151.0	-	152.3	105.6	182.3
Succinic	-	3.0	-	-	-	-
Procaine penicillin						
Acetic	50.3	40.3	12.1	NP	54.4	50.7
Formic	-	-	-		24.2	-
Lactic	266.0	-	-		-	-
Succinic	21.6	-	-		4.0	-
Chlortetracycline						
Acetic	NP	40.3	32.9	56.3	31.7	79.3
Formic		-	-	14.4	-	47.2
Lactic		87.7	-	290.4	364.4	-
Succinic		-	-	7.7	-	4.0
Zinc bacitracin						
Acetic	43.3	35.2	42.4	56.4		
Lactic	201.5	-	72.0	-		
Succinic	-	-	-	5.2		

Appendix IVC continued

Ceca

SCFA	Control bird no.				Zinc bacitracin bird no.			
	1	2	3	4	1	2	3	4
Acetic	11.0	541.8	816.7	285.3	846.7	487.5	216.5	129.2
Propionic	-	17.8	16.4	8.1	32.8	2.5	107.1	136.5
Butyric	-	91.7	144.8	94.4	268.0	140.3	8.2	23.6
Valeric	-	-	-	14.1	-	-	-	-
Lactic	93.4	-	-	-	-	-	-	36.4
Succinic	-	-	-	-	-	-	70.5	10.0

Appendix IVD. Duodenal flora study.
Chromatographic analyses of duodenal contents

SCFA	Control bird no.				Antibiotic-fed bird no.			
	1	2	3	4	1	2	3	4
Acetic	11.1	NP	NP	4.8	NP	NP	NP	NP
Lactic	68.7			18.4				
Succinic	-			2.1				

Appendix IVE. Lower ileum. First experiment.

SCFA	Control bird no.				Antibiotic-fed bird no.			
	1	2	3	4	1	2	3	4
Acetic	33.1	37.2	106.2	43.5	18.1	99.4	NP	35.9
Formic	-	-	141.8	44.9	-	9.9		-
Propionic	.5	-	-	8.9	15.0	11.7		-
Isobutyric	-	-	-	-	-	.13		-
Butyric	-	-	11.5	-	-	10.9		-
Lactic	283.2	372.5	387.8	131.6	-	72.5		182.0
Succinic	22.7	19.9	20.4	5.7	-	33.5		-
Total VFA	33.6	37.2	259.5	97.3	33.1	132.0		35.9
Total SCFA	339.5	429.6	667.7	234.6	33.1	238.0		217.9
Limit	.13	.09	.13	.11	.17	.13	.19	.32

APPENDIX V. Second Selective Media Study.

A. Total contents and % dry weight at different sites of the intestinal tract.

Site	Control bird no.				Antibiotic-fed bird no.			
	1	2	3	4	1	2	3	4
	Total Contents (g wet weight)							
Crop	0.08	0.34	4.67	NC	NC	0.99	NC	NC
Proventriculus	NC	NC	0.05	NC	NC	0.23	0.02	0.05
Gizzard	4.22	4.69	3.84	2.17	3.98	5.79	3.27	4.05
Duodenum	0.66	0.62	0.97	1.90	1.43	0.85	0.24	1.41
Lower Ileum	1.62	0.38	1.98	2.62	0.66	0.33	1.53	2.03
Ceca	0.52	3.01	1.04	1.64	0.98	0.80	0.70	3.61
	% Dry weight							
Crop	20.0	22.8	49.2	NC	NC	44.4	NC	NC
Proventriculus	NC	NC	7.7	NC	NC	46.5	25.0	5.0
Gizzard	60.1	65.1	30.2	29.2	58.3	68.6	43.6	27.9
Duodenum	21.5	14.0	16.4	10.0	21.7	20.9	5.8	14.1
Lower Ileum	23.1	17.9	19.2	10.1	17.4	19.6	13.3	12.1
Ceca	21.0	37.0	8.8	4.5	16.7	14.9	11.1	9.6

Appendix VB. Colony counts from the second selective media study.

Flora	Control bird no. 1	2	3	4	Mean	Antibiotic-fed bird no. 1	2	3	4	Mean
					Crop					
DMCC	9.46[a]	9.07	9.71	NC[b]	9.49	NC	9.22	NC	NC	
Cultivable flora	9.59	9.27	9.86		9.64		9.53			
% Recovery	136.4	158.1	141.1		145.2		203.0			
% Dry wt.	20.00	22.81	49.18		30.7		44.44			
Total staphylococci	6.81	5.21	5.59		6.37		6.28			
S. aureus	0	0	4.12		<3.70		2.79			
Total streptococci	8.38	8.77	9.00		8.78		8.57			
"*S. faecalis*"	8.16	8.77	6.63		8.39		8.57			
Detection limit	3.13	2.55	1.78				1.94			
					Proventriculus					
DMCC	NC	NC	8.77	NC		NC	7.09	8.51	7.72	8.11
Cultivable flora			6.64				6.77	7.53	6.13	7.13
% Recovery			0.76				48.0	10.3	2.59	20.3
% Dry wt.			7.69				46.5	25.0	5.0	25.5

[a] All counts are $\log_{10}$ of bacteria per gram wet wt of contents.

[b] No contents.

Appendix VB. continued

Flora	Control bird no. 1	2	3	4	Mean	Antibiotic-fed bird no. 1	2	3	4	Mean
					Gizzard					
DMCC	8.68	8.68	7.98	8.62	8.57	8.90	8.55	7.95	8.41	8.57
Cultivable flora	8.51	8.56	7.85	8.32	8.38	8.78	8.23	7.94	8.20	8.40
% Recovery	67.4	75.9	73.6	49.3	66.6	76.2	51.0	97.8	61.0	71.5
% Dry wt.	60.1	65.1	30.2	29.2	46.2	58.3	68.6	43.6	27.9	49.6
					Duodenum					
DMCC	8.89	9.13	7.93	8.78	8.85	8.49	8.59	8.06	8.61	8.49
Cultivable flora	8.78	8.87	7.66	8.83	8.71	8.31	8.29	7.48	8.62	8.32
% Recovery	78.2	55.1	53.9	113.0	75.1	66.1	49.6	26.4	101.5	60.9
% Dry wt.	21.5	14.0	16.4	10.0	15.6	21.7	20.9	5.8	14.1	15.7
Total staphylococci	2.43	2.65	0	2.87	<2.60	0	4.76	2.86	0	<4.17
S. aureus	0	0	0	0	<2.25	0	0	0	0	<2.49
Total facultatives	5.59	7.71	6.20	5.19	7.13	4.01	6.25	4.77	3.31	5.66
Detection limit	2.43	2.34	2.17	1.87		2.30	2.34	2.86	2.00	

Appendix VB. continued

Flora	Control bird no. 1	2	3	4	Mean	Antibiotic-fed bird no. 1	2	3	4	Mean
					Lower Ileum					
DMCC	9.57	9.80	9.40	9.73	9.65	8.83	9.46	8.91	8.98	9.13
Cultivable flora	9.61	9.74	9.15	9.52	9.55	8.79	8.23	8.39	8.73	8.60
% Recovery	109.7	87.4	55.5	61.5	78.5	91.1	5.8	30.5	56.3	45.9
% Dry wt.	23.1	17.9	19.2	10.2	17.6	17.4	19.6	13.3	12.1	15.6
Sulfite-reducers/1 day	3.91	0	2.81	0	<3.37	0	0	2.47	3.22	<3.20
Sulfite-reducers/3 day	3.81	3.75	5.00	2.30	4.45	0	0	3.27	5.41	<4.82
Coliforms	8.49	9.33	8.78	8.20	8.91	4.91	8.09	5.13	7.53	7.59
Total streptococci	6.00	7.65	7.41	6.94	7.30	4.76	6.01	6.53	3.82	6.05
"S. faecalis"	5.39	7.61	7.41	6.92	7.27	4.76	6.01	5.59	3.76	5.57
Detection limit	1.95	2.63	1.96	1.99		2.13	3.63	1.98	1.91	
					Ceca					
DMCC	11.49	11.16	9.73	9.77	11.06	11.18	11.71	10.69	10.69	11.28
% Dry wt.	21.0	37.0	8.8	4.5	17.8	16.7	14.9	11.1	9.6	13.1
Lactobacilli	10.59	9.80	8.61	10.49	10.28	9.59	10.45	7.99	8.16	9.91
Sulfite-reducers/1 day	0	0	0	0	<2.40	0	0	0	0	<2.65
Sulfite-reducers/3 day	7.79	6.20	7.42	4.76	7.35	6.65	5.85	8.20	6.20	7.61
Total streptococci	6.14	7.35	6.86	7.91	7.45	5.78	6.92	6.05	4.47	6.40
"S. faecalis"	5.93	7.18	6.86	7.86	7.38	4.73	6.92	4.63	4.46	6.32
Lactate	<4.89	<4.68	<4.11	<3.78	<4.56	5.18	<4.89	8.83	8.36	<8.35
Detection limit	2.89	1.68	2.11	1.78		2.58	2.89	2.78	1.64	

APPENDIX VI. Third Selective Media Experiment. A. Weight and feed efficiency data.[a]

Age/Diet	Control			Penicillin[b]			Chlortetracycline[b]			Zinc bacitracin[b]		
	Weight	Cons.	F.E.	Weight	Cons.	F.E.	Weight	Cons.	F.E.	Weight	Cons.	F.E.
Week 1												
Standard	104.0	77.9	0.61	124.8	92.3	0.75	95.0	60.5	0.63	104.9	78.9	0.63
	102.3	79.0	0.60	112.6	79.1	0.73	104.3	73.0	0.65	113.1	85.1	0.68
	101.3	72.4	0.64	119.6	81.9	0.76	112.6	79.0	0.73	103.0	72.9	0.67
Mean	102.5		0.62	119.0		0.75	104.0		0.67	107.0		0.66
Crude	95.7	72.6	0.60	112.5	77.0	0.73	105.6	77.0	0.65	107.0	77.3	0.66
	104.7	71.5	0.68	112.7	76.3	0.75	106.7	78.9	0.66	99.7	72.8	0.59
	94.1	67.8	0.57	113.2	78.0	0.74	103.7	77.8	0.62	101.8	74.2	0.63
Mean	98.2		0.62	112.8		0.74	105.3		0.64	102.8		0.63
Week 2												
Standard	191.1	173.1	0.50	258.0	212.9	0.63	191.7	166.5	0.58	218.0	187.6	0.60
	208.6	179.6	0.59	233.8	191.1	0.63	210.4	177.6	0.60	227.4	206.1	0.55
	188.3	150.4	0.58	246.5	205.9	0.62	238.4	214.7	0.59	199.1	173.4	0.55
Mean	196.0		0.56	246.1		0.63	213.5		0.59	214.8		0.57
Crude	159.6	127.3	0.50	221.1	179.4	0.61	192.0	178.0	0.49	195.0	161.0	0.55
	185.2	151.6	0.53	222.6	173.8	0.63	191.9	164.6	0.52	170.8	142.4	0.50
	155.1	127.1	0.48	219.3	167.1	0.63	189.1	165.1	0.52	181.2	156.0	0.51
Mean	166.6		0.50	221.0		0.62	191.0		0.51	182.3		0.52

[a] See legend for Appendix IIIA. Feed consumption data (cons.) was calculated on a weekly basis.

[b] Procaine penicillin and CTC were added to a basal 26% protein diet at a level of 22 ppm and zinc bacitracin at 55 ppm.

Appendix VIA. continued

Age/Diet	Control			Pen			CTC			Zbac		
	Weight	Cons.	F.E.	Weight	Cons.	F.E.	Weight	Cons.	F.E.	Weight	Cons.	F.E.
Week 3												
Standard	333.6	256.8	0.56	465.2	325.0	0.64	331.7	274.4	0.51	373.6	273.2	0.57
	351.4	257.2	0.56	429.0	307.5	0.63	345.3	237.3	0.57	392.1	290.2	0.57
	326.7	254.1	0.54	450.1	327.7	0.62	410.9	324.3	0.53	357.7	273.7	0.58
Mean	337.2		0.55	448.1		0.63	362.6		0.54	374.4		0.57
Crude	243.6	173.3	0.48	393.2	271.0	0.64	287.0	216.5	0.44	314.2	243.9	0.49
	279.9	198.3	0.48	391.0	281.5	0.60	319.5	252.7	0.50	277.2	207.5	0.51
	226.2	168.7	0.42	402.1	289.9	0.63	290.8	229.6	0.44	285.2	208.0	0.50
Mean	249.9		0.46	395.4		0.62	299.1		0.46	292.2		0.50
Week 4												
Standard	577.7	449.0	0.54	745.0	537.1	0.52	582.0	454.0	0.55	608.2	458.3	0.51
	542.1	413.0	0.46	723.9	509.5	0.58	595.5	417.3	0.60	656.5	509.6	0.52
	567.3	427.1	0.56	758.2	558.7	0.55	663.0	509.3	0.50	621.3	482.3	0.55
Mean	562.4		0.52	742.4		0.55	613.5		0.55	628.7		0.53
Crude	384.3	291.4	0.48	619.6	412.6	0.55	426.2	331.9	0.42	490.0	362.3	0.49
	415.4	327.1	0.41	620.8	458.1	0.50	488.1	394.7	0.43	440.6	330.8	0.49
	346.7	265.1	0.45	616.4	460.7	0.47	439.7	366.2	0.41	454.9	348.1	0.49
Mean	382.1		0.45	618.9		0.51	451.3		0.42	461.8		0.49

APPENDIX VII. Duodenal Flora Experiment.
A. Weight and feed efficiency data.[a]

Age/Sex	Protein concentration in diet											
	26%				28%				30%			
	Weight		F.E.		Weight		F.E.		Weight		F.E.	
	C	Ab	C	Ab	C	Ab	C	Ab	C	Ab	C	Ab
Week 1												
Males	78.6	83.4	0.48	0.55	76.3	77.8	0.49	0.56	72.1	83.7	0.54	0.58
	83.0	78.3	0.68	0.53	75.7	86.1	0.53	0.62	76.3	80.3	0.51	0.56
	83.6	71.1	0.58	0.50	73.6	67.4	0.47	0.44	75.6	79.1	0.49	0.56
	72.2	83.6	0.44	0.58	85.0	80.3	0.60	0.52	82.3	86.8	0.57	0.65
Females	67.4	79.8	0.45	0.54	84.0	72.3	0.56	0.54	76.5	79.0	0.51	0.53
	71.5	76.3	0.46	0.51	77.3	67.0	0.55	0.29	65.6	72.9	0.37	0.47
	71.2	81.2	0.53	0.57	78.8	73.1	0.55	0.48	70.6	72.7	0.45	0.43
	72.7	84.4	0.51	0.53	69.4	73.5	0.48	0.46	65.3	76.8	0.39	0.48
Week 2												
Males	153.3	161.9	0.60	0.58	139.2	141.9	0.55	0.54	137.1	166.1	0.60	0.62
	145.9	148.0	0.55	0.61	128.3	153.6	0.47	0.54	138.2	151.3	0.55	0.57
	157.5	136.1	0.57	0.60	137.8	139.4	0.59	0.62	138.2	155.0	0.55	0.60
	131.2	165.3	0.52	0.62	161.2	148.6	0.58	0.56	160.3	156.5	0.60	0.53
Females	122.0	152.1	0.56	0.61	131.5	137.5	0.43	0.61	144.1	132.6	0.58	0.55
	137.0	145.3	0.64	0.56	128.9	135.4	0.45	0.71	123.7	143.9	0.55	0.63
	133.0	157.6	0.62	0.62	131.3	116.7	0.57	0.45	123.0	123.7	0.52	0.50
	131.9	160.9	0.55	0.58	129.1	141.5	0.55	0.61	131.7	140.6	0.67	0.53

[a] See legend for Appendix IIIA.

Appendix VIIA. continued

Age/Sex	Protein concentration in diet											
	26%				28%				30%			
	Weight		F.E.		Weight		F.E.		Weight		F.E.	
	C	Ab	C	Ab	C	Ab	C	Ab	C	Ab	C	Ab
Week 3												
Male	248.9	250.9	0.54	0.47	240.3	241.1	0.57	0.54	222.3	299.7	0.52	0.65
	234.0	245.7	0.51	0.77	227.0	272.2	0.56	0.61	224.8	245.2	0.53	0.53
	250.6	225.0	0.48	0.54	242.2	237.0	0.61	0.56	228.7	268.6	0.51	0.58
	222.4	255.5	0.53	0.50	260.4	281.1	0.53	0.66	284.8	278.5	0.57	0.57
Female	186.9	228.0	0.32	0.47	231.2	210.6	0.55	0.47	232.6	210.2	0.52	0.58
	206.8	204.8	0.45	0.40	215.5	191.7	0.55	0.42	188.6	221.4	0.45	0.49
	190.3	229.8	0.42	0.46	198.4	209.8	0.46	0.57	205.8	216.1	0.52	0.60
	194.7	249.6	0.44	0.48	216.3	205.0	0.54	0.43	198.4	245.6	0.46	0.56
Week 4												
Male	413.2	414.3	0.52	0.56	416.4	437.4	0.61	0.61	390.3	484.8	0.63	0.58
	376.4	405.7	0.55	0.59	408.6	344.3	0.59	0.30	377.9	415.3	0.55	0.57
	411.7	402.2	0.55	0.64	408.9	428.0	0.56	0.62	395.1	439.1	0.56	0.58
	358.9	368.0	0.50	0.43	429.1	489.8	0.57	0.63	454.4	421.5	0.53	0.51
Female	307.3	354.8	0.58	0.53	390.8	331.0	0.56	0.52	383.8	329.4	0.57	0.51
	355.2	345.1	0.54	0.56	373.2	326.4	0.59	0.57	323.0	349.3	0.59	0.55
	328.1	380.8	0.59	0.56	262.9	347.7	0.32	0.59	364.0	377.1	0.59	0.76
	332.2	413.6	0.59	0.56	329.6	303.8	0.49	0.47	346.9	433.6	0.59	0.46

Appendix VIIB. Effect of antibiotic treatment on weight and moisture content of turkey duodenal digesta.[a]

Treatment	Total contents (grams)	% Dry wt.
Control	1.09	20.49
	0.20	2.56
	0.70	15.42
	2.00	6.80
Mean	1.00	11.32
Antibiotic	0.50	18.53
	0.50	15.54
	0.55	16.02
	0.38	16.16
Mean	0.48	16.56

[a]Zinc bacitracin, 55 ppm.

APPENDIX VIII. First Lower Ileum Flora Study.
A. Weight and feed efficiency data.[a]

Age/Sex	Protein concentration in diet							
	26%				30%			
	Weight		F.E.		Weight		F.E.	
	C	Ab	C	Ab	C	Ab	C	Ab
Week 1								
Males	67.9	79.5	0.45	0.55	80.2	84.5	0.54	0.66
	75.3	73.5	0.50	0.56	82.9	85.8	0.81	0.66
	76.2	79.7	0.56	0.56	74.8	86.6	0.53	0.63
Females	72.1	74.9	0.49	0.50	73.8	71.5	0.62	0.49
	67.5	62.5	0.43	0.25	68.9	75.0	0.50	0.49
	74.7	74.4	0.47	0.43	68.1	72.5	0.44	0.48
Week 2								
Males	105.5	126.0	0.46	0.50	125.7	130.2	0.46	0.44
	124.3	122.5	0.48	0.50	124.7	150.2	0.40	0.55
	124.5	116.2	0.47	0.42	124.2	144.1	0.54	0.54
Females	106.0	118.9	0.40	0.44	113.0	119.5	0.44	0.53
	111.8	108.0	0.52	0.50	106.8	122.0	0.42	0.52
	119.6	122.8	0.43	0.49	95.2	122.3	0.33	0.51
Week 3								
Males	184.9	222.5	0.55	0.56	241.2	246.7	0.57	0.59
	211.4	204.2	0.52	0.53	221.9	273.5	0.55	0.60
	218.9	210.2	0.54	0.52	227.7	237.0	0.58	0.54
Females	179.7	246.7	0.55	0.48	196.4	203.7	0.53	0.53
	157.0	273.5	0.36	0.47	182.7	202.1	0.51	0.53
	195.2	237.0	0.50	0.50	181.4	197.6	0.49	0.50

[a]See legend for Appendix IIIA.

Appendix VIIIB. Effect of antibiotic treatment on weight and moisture content in the lower ileum of two-week-old turkey poults.[a]

Treatment	Total contents (grams)	% Dry wt.
Control	1.61	12.26
	2.17	15.65
	1.93	9.40
	1.88	8.08
mean	1.90	11.35
Antibiotic	1.03	5.65
	1.89	13.16
	0.77	14.29
	0.48	15.79
mean	1.04	12.22

[a]Zinc bacitracin, 55 ppm.

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EFFECTS OF SUBTHERAPEUTIC DOSES OF ANTIBIOTICS ON POULTRY INTESTINAL BACTERIA

by

Roger W. Kelley

(ABSTRACT)

Supplementation of the diet with low concentrations of antibiotics stimulates the growth of poultry by affecting the intestinal flora. The bacterial flora of the small intestine of turkey poults was extensively analyzed in an attempt to correlate changes in populations with growth response. Lactobacillus species comprised almost 100% of the duodenal flora of two-week-old poults but there was no difference in species associated with antibiotic (zinc bacitracin, 55 ppm) treatment. The ileal flora also was predominantly lactobacilli (average 75% of the flora). The most common lactobacilli from the turkey intestinal tract were several previously undescribed Lactobacillus species followed by L. acidophilus, L. salivarius subsp. salivarius, L. fermentum, and L. plantarum. Antibiotic treatment resulted in a shift in the proportions of several of the unnamed Lactobacillus sp. Preliminary feed trials using two strains of lactobacilli that belonged to species that increased in numbers with antibiotic treatment did not stimulate growth when one-day-old birds were colonized with the strains.

A probable explanation for the increase in growth is the effect of antibiotic treatment on the multiplication of bacteria in the small

intestine. As the digesta move from the gizzard to lower ileum an average 16-fold increase in bacteria occurs in untreated birds. In antibiotic-treated birds the increase was only 2-fold. This inhibition of growth is not due strictly to cell lysis because there are no significant differences in microscopic counts, but the viable counts do decrease. As a corollary there is significantly less lactic acid in the lower ileum of antibiotic-fed birds. Antibiotics did not affect total microscopic or viable counts in the crop or ceca. The above experiments were all done with zinc bacitracin; however, the inhibition of bacterial multiplication was also observed with procaine penicillin.

The conclusion from my data is that zinc bacitracin, and probably procaine penicillin, stimulate the growth of turkey poults by a general suppression of the small intestinal flora rather than by an effect on any individual bacterial species.