

STUDIES ON THE SUBTHERAPEUTIC USE OF DIETARY ANTIBIOTICS AND THE
NUTRITIONAL VALUE OF AEROBICALLY FERMENTED POULTRY
MANURE FOR BROILER CHICKS

BY

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Dedication

This thesis is dedicated to my parents Ishaku Liyakwar Dafwang and Rahila Nadel whose love, prayers, sacrifice, faith and patriotism have been the major driving force for my twenty-three years of formal education

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I would like to express my deep appreciation to Dr. M. L. Sunde and Dr. M. E. Cook for their guidance and support during the course of this study. I am indebted to Professors H. R. Bird, D. J. Pringle and D. M. Schaefer whose guidance and encouragement were indispensable to the success of my studies at the University.

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TABLE OF CONTENTS

	Page
Dedication.....	i
Acknowledgements.....	ii
List of Tables.....	v
List of Figures.....	viii
Abstract.....	1
Introduction.....	4
CHAPTER I. Literature Review.....	6
Persistence of growth promotion by antibiotics.....	6
Antibiotics in laying hen diets.....	15
Hypotheses on the mode of action of antibiotics.....	17
Public health concerns on the subtherapeutic use of antibiotics for animals.....	33
References.....	35
CHAPTER II. Broiler chick growth response to Antibiotics, 1981-1982..	47
Abstract.....	48
Introduction.....	48
Materials and Methods.....	49
Results and Discussion.....	50
References.....	53
CHAPTER III. Bursal, intestinal, and spleen weights and antibody response of chicks fed subtherapeutic levels of dietary antibiotics.....	54
Abstract.....	55
Introduction.....	55
Materials and Methods.....	56
Results and Discussion.....	56
References.....	59
CHAPTER IV. Evaluation of some commercial formulations for the cultivation and enumeration of <u>Clostridium perfringens</u> from chick intestine.....	61
Summary.....	62
Introduction.....	63
Materials and Methods.....	64
Results and Discussion.....	67
References.....	74

CHAPTER V. Effect of antibiotics and water treatment on broiler chick growth, intestinal characteristics and intestinal bacterial populations.....	81
Summary.....	83
Introduction.....	84
Experimental.....	85
Results.....	88
Discussion and Conclusions.....	91
References.....	95
CHAPTER VI. High stocking density as a model for detection of growth promotion by dietary antibiotics.....	106
Abstract.....	108
Introduction.....	109
Materials and Methods.....	110
Results.....	112
Discussion.....	115
References.....	120
CHAPTER VII. Nutritional value of aerobically fermented poultry manure and offal (Fermway) for broiler chicks.....	129
Abstract.....	131
Introduction.....	132
Materials and Methods.....	132
Results and Discussion.....	134
References.....	139
APPENDIX I. Potentiation of skin pigmentation by dietary antibiotics.....	148

LIST OF TABLES

Table.	Page
CHAPTER I.	
1. Date of introduction of animal feed growth promotion/ antibacterial disease prophylaxis drugs.....	9
CHAPTER II.	
1. Composition of basal diets.....	49
2. Effect of antibiotics on body weight and feed conversion of 21-day old broiler chicks.....	50
3. Growth response to antibiotics of chicks from old and young parent stock.....	51
4. Effect of different levels of antibiotics on growth response.....	52
5. Antibiotic usage, basal types, and number of experimental groups (30 birds each) in the experiments reported.....	52
CHAPTER III.	
1. Effect of different basal diets and antibiotics on bursal and intestinal weights of broiler chicks.....	57
2. Effect of graded levels of lincomycin on bursal and intestinal weights of broiler chicks(experiment 4).....	57
3. Effect of antibiotics on bursal, intestinal, and spleen weights and immune response of broiler chicks.....	58
CHAPTER IV.	
1. Colony forming units (log ₁₀) from pure <u>Clostridium perfringens</u> (ATCC 13124) on TSN(tryptose-sulfite-neomycin) and SPS (sulfite-polymyxin-sulfadiazine) agar media at two incubation temperatures.....	76
2. Number of c.f.u.(log ₁₀) from cecal samples and a pure culture of <u>C. perfringens</u> (ATCC 13124) on TSN agar.....	77
3. Colony forming units(log ₁₀) and degree of difficulty for preparation of medium and enumeration of c.f.u. on TSN, SPS, LLA(lecithin-lactose-agar-base) and Clostrisel media.....	78
4. Enumeration of c.f.u.(log ₁₀) on TSN and SPS agar.....	79
5. Colony morphology of bacteria cultivated on TSN and SPS agar media.....	80

CHAPTER V.

1. Performance of broiler chicks fed dietary penicillin or microaid and reared on water troughs with or without regular washing from 0-21 days(Expt. 1).....98
2. Feed efficiency and bursal and intestinal weights of chicks fed diets with or without penicillin and reared on water troughs with or without regular washing(Expt. 2).....101
3. Growth performance and intestinal characteristics of chicks fed dieatary tylan and reared on water troughs with or without regular washing for 15 days(Expt. 3).....102
4. Dirty score (DS) and chemical oxygen demand (COD) of water drank by chicks fed diets with or without tylan and reared on water troughs with or without regular washing.....103
5. Weight gain, feed efficiency and intestinal characteristics of chicks fed dietary penicillin(Expt. 4).....104
6. Growth performance and intestinal fecal bacterial counts of chicks fed diets with or without penicillin and reared on water troughs with or without regular washing(Expt. 5).....105

CHAPTER VI.

1. Space specifications per bird in the three experiments.....122
2. Composition (g/kg) of basal diet.....123
3. Effect of stocking density and dietary penicillin on growth performance of cage reared broiler chicks.....124
4. Effect of dietary penicillin and stocking density on growth (0-35 days), lymphoid tissues and antibody response of broiler chicks(Expt. 2).....125
5. Effect of antibiotics and stocking density on growth, bursal and spleen weights of broiler chicks(Expt. 3).....126
6. Effect of antibiotics and stocking density on fecal moisture and ammonia concentrations.....127
7. Estimated maximum stocking rates and minimum space requirements for chicks reared in battery brooders.....128

CHAPTER VII.

1. Percentage composition of experimental diets (Expt. 2 and 3).....141

2. Amino acid composition(%) of diet, Fermway samples and comparable standards.....	142
3. Weight gain, feed consumption, feed efficiency and organ weights of chicks fed graded levels of Fermway (Expt. 1 and 2).....	144
4. Leg deformity and shank color scores of chicks fed graded levels of Fermway from 0-28 days.....	145
5. Growth performance, shank color scores and organ weights of chicks chicks fed Fermway (Expt. 3).....	146
6. Correllation coefficients between level of dietary Fermway and selected variables.....	147
APPENDIX I.	
1. Effect of dietary antibiotics on growth, shank color scores and organ weights of broiler chicks.....	154

LIST OF FIGURES

Figure	Page
CHAPTER I.	
1. Antibiotic production and percentage utilized for non-medical functions in the U.S.A. from 1951-1978.....	7
2a. Changes in response of chicks fed penicillin from 1950 to 1959. Level of penicillin 4 to 30mg/kg.....	11
2b. Changes in response of chicks fed lincomycin(4ppm), oxytetracycline (50ppm) and penicillin(50ppm) from 1981 to 1984.....	11
CHAPTER V.	
1. Relative weight (%) of chicks fed diets with or without penicillin and reared on water troughs with or without regular washing.....	99

STUDIES ON THE SUBTHERAPEUTIC USE OF DIETARY ANTIBIOTICS
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Istifanus Nandem Ishaku Dafwang

Under the supervision of Professors M.L. Sunde and D.J. Pringle

Over 5,000 chicks were reared on diets with or without subtherapeutic levels of dietary antibiotics. Results show that although there was considerable variation (0-40%) in the magnitude of growth response between experiments, the overall 3 year (1981-1984) cumulative (0-21 days) percentage mean improvements in body weight and feed conversion were: 9.3 (P<.01) and 2.0 (P<.27) for lincomycin; 11.3 (P<.01) and 2.6 (P<.33) for penicillin; 10.4 (P<.01) and 5.3 (P<.01) for oxytetracycline, the three antibiotics that were used most extensively. For two other antibiotics that were used less extensively, the percentage mean responses were 6.0 (P<.11) and 2.6 (P<.46) for bambarmycin and 10.4 (P<.02) and 2.0 (P<.50) for tyran. Differences in growth responses between diets containing the maximum approved levels and diets with lower levels of antibiotics were not significant. There was no loss in effectiveness between 1981 and

1985. The presence or absence of soybean meal or using at least 200% of the NRC (1977) for essential trace nutrients did not alter results.

The bursae of chicks fed antibiotics were significantly heavier ($P < .05$) in some experiments, but this was not accompanied by increased antibody responses to sheep red blood cells. Significant reductions in intestinal weight were observed in antibiotic fed chicks, but effects on spleen weights, intestinal thickness and intestinal lengths were inconsistent.

Growth response to antibiotics was independent of "clean" or "dirty" water consumption. Bacterial populations from ileal and cecal contents cultivated on SPS (sulfite-polymyxin-sulfadiazine) and KF Streptococcus agar were also not affected by water treatment nor dietary antibiotics. Four commercial formulations for the detection and/or enumeration of Clostridium perfringens [SPS; TSN (tryptose-sulfite-neomycin), Clostrisel and lecithin-lactose-agar base] were evaluated and found to be ineffective for the selective cultivation of C. perfringens from the intestine of chicks reared conventionally. The growth depression due to high stocking density ($.017 \text{ m}^2/\text{chick}$) in battery brooders by four weeks old was alleviated by dietary antibiotics. Bursal and thymus weight but not antibody response were depressed by high stocking density. Chick growth on the high stocking density regimen was used to compute a value of $0.208 \text{ cm}^2/\text{g}$ of chick as the estimated minimum space requirement for chicks in battery brooders.

Two samples of aerobically fermented poultry manure and offal (Fermway) were evaluated for use in poultry feed. One of the two samples significantly ($P < .05$) improved weight gain when fed at 8-16%, but decreased skin pigmentation. The amino acid contents of fermway were found to be higher than that of a dried poultry waste standard.

INTRODUCTION

Antibiotics have been defined as chemical substances produced by microorganisms which have the capacity to inhibit or kill other microorganisms at low concentrations. The discovery of penicillin by Fleming in 1928 was an important landmark in medical science and was considered by many to be the answer to the search for the "magic bullet"; a chemical that would destroy infectious organisms without destroying the host. The medical use of antibiotics became widespread during the second world war but their use as growth promotants in the animal industry came later following the discovery by Stokstad and Jukes in 1950 that the inclusion of aureomycin in chick diets promoted growth.

Antibiotics are produced by a wide range of naturally occurring organisms, often as secondary metabolites of the regular metabolic pathways of the microbe. Of the 2500 antimicrobial substances that had been described by 1972 only about 0.3% are reported to be in use for agricultural and medical purposes. They are a very diverse group of low molecular weight compounds. The most well known and used antibiotics are produced by the bacterial genera streptomycetes (e.g. the tetracyclines) and the fungal order Aspergillales (e.g. penicillin).

Worldwide, antibiotics are a critical component of the health care delivery system, where they are often used therapeutically to treat infections or to prevent the spread of infectious organisms. In the United States; close to 50% of all antibiotics produced are utilized for non-medical functions and primarily in agriculture as feed additives for growth stimulation in livestock and poultry. Because of an ever growing concern by a section of the scientific community and some elements in the general public of possible risks that may arise from the continuous subtherapeutic use of these chemicals; there is a critical need for animal scientists to intensify research on the role of these compounds in order to provide the data pool that can be used to justify or nullify their continued usage in the animal industry for non-medical purposes.

The studies reported in this thesis were conducted to meet these objectives:-

1. Accumulate data on the persistence of growth promotion by subtherapeutic levels of dietary antibiotics in an environment that has been subjected to long term use of these antibiotics.
2. Evaluate the influence of dietary and/or environmental variables on the magnitude of the growth response.
3. Use the accumulated data to help explain the mode of action and to develop further studies on the mode of action of antibiotics in growth promotion.

LITERATURE REVIEW

The first report on the growth promoting effects of an antibiotic came from the University of Wisconsin in a paper by Moore et al. (1946) but the results of this report were largely ignored. Between 1947 and 1950 however, the antibiotic chlortetracycline was discovered as a by-product from Streptomyces aureofaciens fermentation residues. The organism was cultivated to produce vitamin B₁₂. Stokstad and Jukes (1950) were the first to demonstrate that adding chlortetracycline to chicks resulted in growth promotion that was over and above the growth that could be obtained on diets that were adequately fortified with all the nutrients then known to be required by chicks. Shortly after this report, the results were confirmed in chicks and in other animals in other laboratories. From then on the use of antibiotics as feed additives for growth promotion and improvements in feed efficiency exploded to the extent that by 1954 it was estimated that up to 245 tons of antibiotics were added to animal feeds (Jukes, 1977). Fig. 1 shows the production of antibiotics and the percentage of that total which went into animal feeds and other non-medical uses in the United States between 1951 and 1978. A list of antibiotics commonly used and the period during which they were introduced is given in Table 1.

Persistence of growth promotion by antibiotics.

Since the growth promotion by dietary antibiotics was produced by

Figure legend

Figure 1. Antibiotic production and percentage utilized for non medical functions in the U.S.A. from 1951-1978.

(Adapted from Van Houwelling 1978).

FIG. 1.

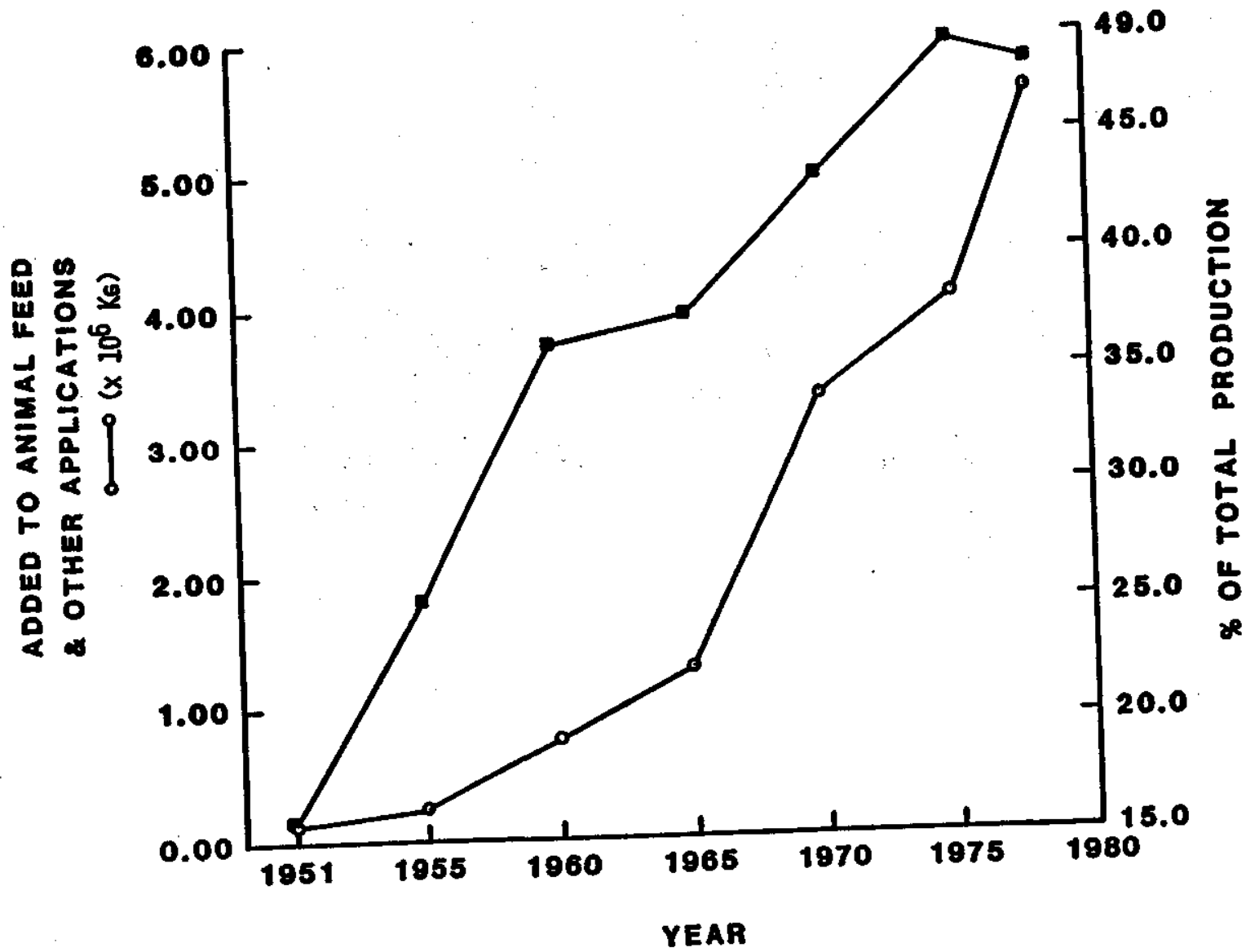


TABLE 1: Date of introduction of animal feed growth promotion/
antibacterial disease prophylaxis drugs (from Braude 1978).

Before 1955	1955-1965	1965-1975
Bacitracin	Nihydrazone	Erythromycin
Chlortetracycline	Oleandomycin	Lincomycin
Penicillin	Tylosin	Racephenicol
Streptomycin		Carbadox
Oxytetracycline		Flavomycin
Neomycin		Virginiamycin
Furazolidone		

chemicals of such a varied nature, researchers were quick to postulate that the effect must be dependent in the changes on gut flora that were induced by the antibiotics. Strong support to this theory came from studies which demonstrated that chicks grown in a germ-free environment did not respond to dietary antibiotics like conventionally reared chicks (Gordon, 1952). "The expectation was that the response of farm animals to antibiotics would be of a transient nature because the emergence of resistant bacterial strains would nullify the effect of continuous feeding of antibiotics to suppress organisms that were deleterious to growth and health" of the animals (Jukes 1984). It was therefore not surprising that reports on the loss of effectiveness of antibiotics in growth promotion began to appear not too long after their introduction to the animal industry. Waibel et al. (1954) reported on "the disappearance of growth response of chicks to dietary antibiotics in an "old" environment". This decrease in growth response was thought to result from an altered microbial population; the harmful bacteria had been eliminated due to continuous use of a certain antibiotic and thus better growth with the control diet resulted. The loss in growth response to antibiotics were also reported by Libby and Schaible (1955), Sherman and Donovan (1958), and Nelson et al. (1963). Some investigators found that the use of a new antibiotic in such an environment restored the growth response (McGinnis et al., 1958; Sherman and Donovan 1958; Sherman et al., 1959; Combs and Bossard 1963). With time however these observations on the loss of effectiveness turned out to be transient trends. Heth

Figure Legends

Figure 2a. Changes in response of chicks fed penicillin from 1950 to 1959. Level of penicillin 4-30mg/kg.(Adapted from Heth and Bird 1962).

Figure 2b. Changes in response of chicks fed lincomycin(4ppm), oxytetracycline (50ppm) and penicillin(50ppm) from 1981 to 1984.(Adapted from Dafwang et al., 1984b).

FIG. 2a.

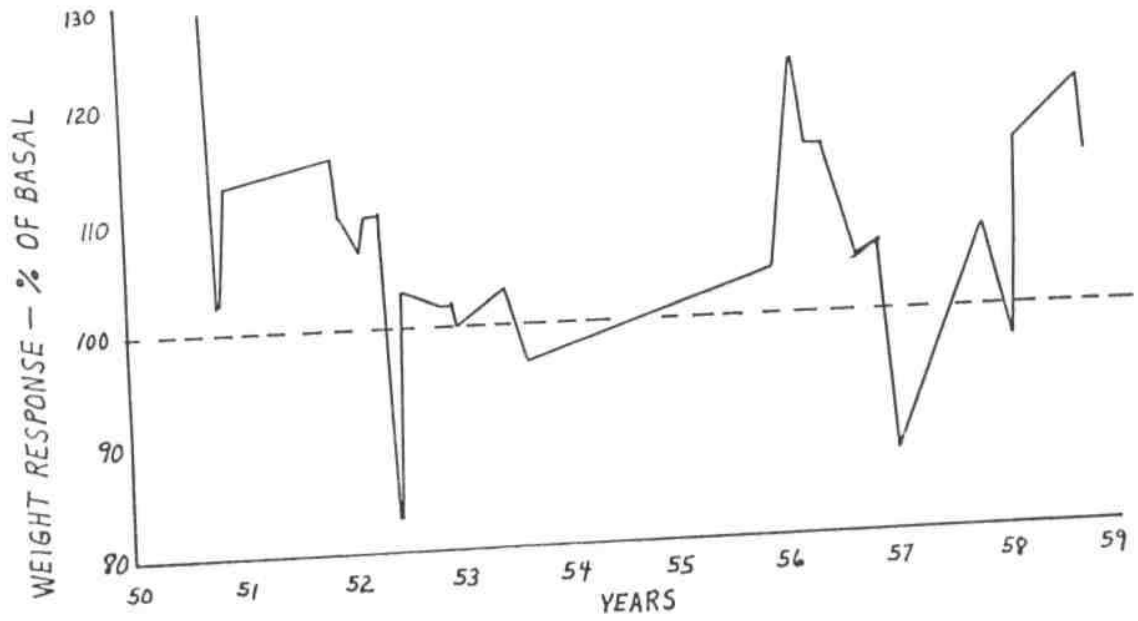
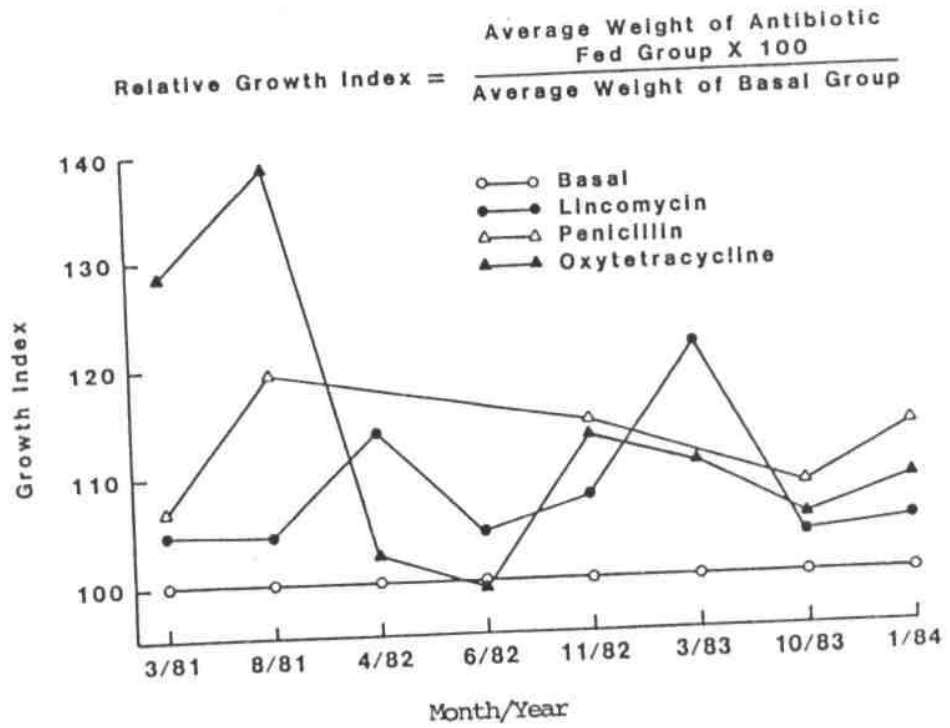


FIG. 2b.



and Bird (1962) summarized the results of experiments from the University of Wisconsin from 1950 to 1961 which showed a mean improvement in chick weight by penicillin of 8.5% in 1950-1953 and 8.8% in 1956-1960. This summary included the data of Waibel et al. (1954) cited earlier. Comparable figures for the tetracyclines were 12.3% in 1950-1953, and 10.2% in 1956 - 1960. More recent data from the same laboratory showed an increase of 11.3% and 10.1% respectively for penicillin and oxytetracycline for the period 1981-1984 (Dafwang et al. 1984b). The growth promotion by penicillin for the period 1950-1960 and 1981-1984 are presented in fig.2. Further evidence to support the persistence of growth promotion by antibiotics in the same environment have been reported by Peter et al. (1966); Marusich et al. (1974) and Griffin (1980). Bird (1968; 1980); summarized data from research on the effectiveness of antibiotics for chick growth promotion from 1951-1968 and from 1968-1980 which showed no consistent trend towards a loss of effectiveness with time. The data came from several laboratories in Europe and America.

The studies cited so far were in the most part conducted with chicks for growth periods prior to 28 days of age. Growth responses to antibiotics at this age average about 10% for body weight and 5% for feed efficiency with very large coefficients of variation. Large coefficients of variation are typical of the growth responses of animals to dietary antibiotics (Rosen 1984). At broiler weights however, mean responses of about 4% have been reported for body weight and 2% for feed conversion (Bird, 1968). March et al. (1972) reported

a mean response of 6% for body weight and feed efficiency which were statistically significant. A mean improvement of 3.5% in body weight and 1% for feed conversion was reported by Foster (1979). The author reported that the improvements in gross margins (excluding the cost of the additives) were not significantly different from controls but were economically so in most cases. Griffin (1979), compared performance of cage reared cockerels on diets with or without nitrovin, penicillin or zinc bacitracin fed individually or in combination for 56 days. The author found a mean response of 8.4% in weight gain and 1.6% for feed conversion for all the groups on the antibiotic diets. In addition the 56 day performance of birds fed the antibiotic diets continuously was better than the performance of birds fed diets in which the antibiotic was either introduced or withdrawn at different ages. This observation is in agreement with the results of other investigators on the value of continuous versus intermittent feeding of dietary antibiotics (Malik and Ichhponani 1970; March et al., 1972 and Marusich et al., 1972). A perusal of recent technical bulletins from some antibiotic manufacturers indicates that an improvement of 2-3% in body weight and 1-2% in feed conversion can be expected from feeding subtherapeutic levels of dietary antibiotics to broilers from day old to market weight in a healthy flock. An added benefit of antibiotic feeding may be an increase in skin pigmentation. Fry et al. (1976)

reported an increase in pigmentation responses in broilers fed flavomycin and 3-nitro-10. Limited studies by Dafwang (1985) demonstrated an increase in shank color scores in three week old chicks fed five different feed additive antibiotics.

Antibiotics in laying hen diets

In contrast to the avalanche of published data on the nutritional value of antibiotics for chicks, information on the value of dietary antibiotics for laying hens is rather scanty especially after 1970. This is rather surprising since many antibiotics approved for chicks are also approved for laying hens for improvements in production (Feed Additive Compendium 1984). Andrews et al. (1964) found that the proportion of laying mashers to which antibiotics had been added increased from 2% in 1950 to 70% by 1951 in feeds that were manufactured in the state of Wisconsin. That was even before the appearance of published evidence on the the value of antibiotics for laying hens. It appears that in general poultry producers assume the benefits of antibiotics that have been reported for chicks are also applicable to laying hens. The published data do not support this view. The first investigations on feeding antibiotics for improved reproductive performance in laying hens produced negative results (Berg et al., 1952; Lillie and Bird 1952; Peterson and Lampman 1952; Waibel et al., 1952 and Brown et al., 1953). Elam et al. (1951) using

purified diets and Reid et al. (1951) obtained improvements in egg production from dietary antibiotics but they used higher levels than those of the other investigators. The first extensive report on the improvement of egg production by antibiotics came from Ryan et al. (1961) using high levels of chlortetracycline (100ppm). Brackett et al. (1960) summarized experiments from several investigators on the subject that indicated many conflicting results even with high levels of antibiotics in the diet. The authors commented that high level antibiotic supplemented diets often demonstrated marked responses only under stressful conditions such as heat stress or the presence of clinical and subclinical infections which often lead to slumps in egg production. Sunde and Guthrie (1962) compared the performance of layers on diets with and without antibiotics as reported in a United States Department of Agriculture publication in 1961 with summaries of sixteen random sample egg tests conducted in the U.S.A. and Canada the previous year. The authors obtained data on the composition of the feeds from thirteen stations of which seven contained low levels of antibiotics. Hens on the antibiotic supplemented diets averaged 220 eggs while the controls averaged 224 eggs. Antibiotics improved feed efficiency from 4.57 to 4.38 and decreased mortality from 12.2% to 11.7%. Recent studies by Carlson et al. (1984) suggest that antibiotics in laying diets were generally more beneficial when

performance was below normal such as toward the end of the laying cycle or when used with oat-soy diets. Earlier Carlson et al. (1975), and Carlson and Nelson (1981) had shown that no subsequent enhancement of egg production resulted from feeding antibiotic supplemented diets to growing pullets. The authors suggested that feeding antibiotic diets to growing pullets may serve only as an insurance factor. It therefore appears that economic considerations must be a critical element in the decision to feed antibiotic diets to pullets and layers, because of the higher levels of antibiotics needed to produce significant responses and the higher cumulative feed consumption of layers.

Hypotheses on the mode of action of antibiotics in growth promotion

Investigations into the mode of action of antibiotics became an active area of research simultaneously with the widespread acceptance of antibiotics as feed additives in the animal industry. In spite of the extensive research by scientists worldwide no hard facts have been produced to explain the phenomenon by which antibiotics act to promote growth in animals. Bird (1969), Hays (1976) and Visek (1978) have cited the following as the most commonly proposed hypotheses:

1. A disease control effect in which microorganisms responsible for clinical and subclinical infections are inhibited.
2. Preventing thickening of the gut wall and thereby promote the efficient utilization of nutrients present in the gut.

3. Inhibiting the production of growth depressing toxins by intestinal bacteria.

4. A nutrient sparing effect by reducing microbial destruction of essential nutrients or by favoring the growth of bacterial species that synthesize nutrients

5. A metabolic effect

A discussion of the evidences that have been produced to substantiate or reject these hypotheses follows:

1. Disease control effect.

Coates et al. (1952) tested the effectiveness of antibiotics in two laboratories; one in which chicks had been present only occasionally ("new" environment), while the other had previously been exposed to a large number of chicks for ten years ("old" environment). Growth response to antibiotics was observed only in the old environment. The penicillin fed chicks in the old environment grew as well as those in the new but the growth of control birds was depressed in the old environment. Similar findings were reported by other investigators (Hill et al., 1953; Lillie et al., 1953). Further observations that supported this concept came from studies which showed that chicks grown in a gnotobiotic environment did not respond to antibiotics (Gordon, 1952). These results were interpreted as implying that there was present in the old environment a subclinical

level of growth depressing infection which was being counteracted by antibiotics. However, no specific infectious agents were identified. Furthermore Hill and Larson (1955), obtained a growth response to chlortetracycline in pigs reared in isolation and in the absence of any recognizable disease. Coates and Harrison (1969) also observed that the response to antibiotics was not always lost in a "clean" environment. In a recent study, Dafwang (1985) revamped this concept by studying the growth response to antibiotics of chicks reared on "clean" versus "dirty" drinkers. A growth depression in chicks reared on the "dirty" drinkers was observed in only one of four experiments. Chick growth response to antibiotics was independent of the conditions of the drinkers even though chicks were reared on the "dirty" drinkers for up to three weeks. It can be concluded from these studies that although subclinical infections could play a role in the mode of action of antibiotics as growth promotants, quantitative and qualitative analysis of such infections are difficult to perform and therefore any discussions about their significance is at best a hypothesis. The observation that even within the same environment, the magnitude of the growth promotion can vary considerably from one experiment to another suggests that the effect on growth is the sum total of the interaction between antibiotics and the internal and external environments of the chick. The accusation by some that antibiotics are being used as a substitute for good management is not

supported by the data reviewed so far. Another important observation is the fact that most of the data on the effects of antibiotics do not show any significant reductions in normal chick mortality rates of chicks grown in a conventional healthy environment.

Closely related to the presence of subclinical infections is the presence of stress factors such as fluctuations in environmental temperature, bird transportation and crowding. Few studies have been reported on the effects of such factors. Freeman et al. (1975) and Freeman and Manning (1976) studied the effects of three kinds of stressors; withdrawal of food for 18 hours; exposure to cold and treatment with adrenocorticotrophic hormone (ACTH) on the response of chicks to antibiotics. These authors reported that the antibiotics did not have any consistent effect on the responses to these stressors and therefore concluded that antibiotics had no stress ameliorating activity. Skamser and Seeger (1958) compared the performance of birds on three stocking densities of 1.0, 0.75 and 0.5 sq ft per bird for broilers reared to 10 weeks old. The results showed that only birds on the control diet lost weight with increasing density, indicating that antibiotics may be alleviating the stress due to crowding. This observation was recently confirmed by Dafwang (1985) using broiler chicks grown in cages.

The use of therapeutic doses of antibiotics to prevent or combat chronic and acute diseases in man and animals is well known and is beyond the scope of this review.

2 and 3. Prevention of gut thickening and inhibition of toxin producing organisms.

The most significant breakthrough in research into the mode of action of antibiotics was the observation by Gordon (1952) that germ-free chicks did not respond to antibiotics and that they had lower intestinal weights than conventionally reared chicks. Gordon demonstrated this effect with penicillin while Pepper et al. (1953) obtained the same result with chlortetracycline. The observation was subsequently confirmed by several other investigators. It is generally accepted that apart from body weight changes and improvements in feed efficiency, the most consistent effect of antibiotics is the reduction in weight of the intestinal tract of chicks fed antibiotics. Hill and his associates (1957) found that the small intestinal weight decreased before an increase in body weight was noted and that lower levels of antibiotics which failed to increase the body weight did decrease the intestinal weight. In later studies, Gordon and Bruckner-Kardoss (1959; 1961) and Abrams et al. (1963) demonstrated that mucosal surface area, mucosal cell replacement rate, the lamina propria and the reticuloendothelial elements were greatly reduced in germ-free animals. More recently, Stutz et al. (1983b) found that the length and percent moisture of the intestine of antibiotic fed chicks were reduced. These authors also reported that there was a negative correlation between antibiotic

level and intestinal weight. Dafwang et al. (1985) and Dafwang (1985) confirmed the weight reduction but found inconsistent effects on length and thickness and no correlation between antibiotic level and intestinal weight.

The similarities in body weight and intestinal weight of germ-free and conventional chicks fed antibiotics has been regarded as evidence to support the hypothesis that antibiotics inhibit the production of toxins or metabolic products which when present in the gut can irritate and cause the gut to thicken, thereby decreasing nutrient absorption and consequently growth depression. Catron et al. (1953) reported an increased rate of glucose absorption in pigs fed rations fortified with antibiotics. Henegan (1963) and Herskovic et al. (1967) reported studies which showed that monosacharides (xylose) and amino acids were more efficiently absorbed by the intestine of gnotobiotic rats and mice. The inhibition of weight gain was found to be associated with decreased feed utilization and malabsorption of fats and carbohydrates but the addition of virginiamycin to the diet resulted in improved intestinal absorption of fats and stimulated growth (Eyssen and De Somer, 1963a, b). In vitro studies with isolated sacs of intestine from germ-free chicks were found to transport glucose and most B vitamins in greater amounts than corresponding preparations from conventional chicks but this result was not confirmed in vivo (Ford and Coates., 1971). Coates et al.

(1981) reported an experiment in which birds whose growth rate was depressed by the presence of either the total gut microflora or a combination of Streptococcus faecium and a fecal filtrate had a thickened wall but uptake of glucose was not impaired. Methionine was retained in the intestinal wall of germ-free chicks in greater amounts (Palmer and Rolls., 1981). Yokota and Coates (1982) however, found that methionine uptake was not altered by the presence of the microflora.

Portal blood ammonia concentrations of germ-free guinea pigs are about 25% of those in conventional animals (Warren and Newton., 1959) and are reduced in conventional animals by oral intake of antibiotics (Stahl., 1963). Since ammonia is a recognized toxin in warm-blooded animals (Visek, 1968, 1972), and in animal environments (Carlie, 1984 and Visek, 1984), it has been suggested that the reduction of bacterial ammonia production may be related to the growth-stimulatory effects of antibiotics. Based on his studies, Visek (1978) believes that ammonia is responsible for the gut thickening and growth depression in animals. He therefore concluded that the mode of action antibiotics can be explained by their ability to suppress ammonia production within the animal's intestinal tract as well as in the fecal output of the animal. Studies by Alvares et al. (1964) indicate that under certain conditions antibiotics reduced urolytic activity and the ammonia concentration in the gut of poultry. Kitai and Arakawa (1979) were able to show that the addition to 1kg of excreta

of 100mg of thiopeptin or zinc bacitracin but not caprylohydroxamic acid significantly decreased ammonia release from the excreta. In contrast however, Heth (1963) was unable to show a consistent reduction in intestinal ammonia from chicks fed antibiotics. He concluded that "dietary antibiotics may cause a transient reduction in intestinal ammonia but additional mechanisms must be involved in bringing about the increase in growth" (Doctoral Dissertation Abstract). More recent studies from the same laboratory (Dafwang, 1985 and Pimentel and Cook, 1985) have given conflicting results. The value of dietary antibiotics in the control of ammonia in animal tissues and animal environments as advocated by Visek (1978, 1984) and Carlie (1984) remains to be established.

4. A nutrient sparing effect

Associated with the thinning of the gut is the hypothesis that antibiotics exert a nutrient sparing effect 1. by increasing the absorption and utilization of essential nutrients, 2. by reducing microbial destruction of the nutrients and 3. by favoring the growth of microorganisms that synthesize essential nutrients. The early literature on the mode of action of antibiotics is replete with reports on the sparing effect of antibiotics for several nutrients, especially the B vitamins. A sparing action for penicillin and/or chlortetracycline was demonstrated for the following nutrients: Thiamin (Waibel et al., 1953); Riboflavin (Biely and March, 1951,

Jukes and Williams, 1953); Niacin (Biely and March (1951); B₁₂ (Stokstad and Jukes 1950); Biotin (Waibel et al., 1952); Vitamin A and carotene (Coates et al., 1952); Vitamin D (Ross and Yacowitz, 1954); Calcium (Gabutten and Shaffner, 1952) and Protein (Machlin et al., 1952, West and Hill, 1955). More recently, Patel and McGinnis (1980) reported on the sparing effect of penicillin for methionine.

It is possible that the nutrient sparing effect mechanism may have been an important function of antibiotics in those days when little was known about the nutrient requirements of animals. However, the fact that antimicrobial agents and indeed other growth promoting chemicals have very widely differing chemical structures and the observation that growth promotion by dietary antibiotics continues to be observed not only on today's well formulated diets but even on diets formulated to provide non-toxic excesses of vitamins and minerals (Dafwang et al., 1984b), does not support the hypothesis of a nutrient sparing effect.

Associated with this hypothesis is the observation that dietary antibiotics can counteract the deleterious effects of some natural feed ingredients especially rye and certain legumes (McAuliffe and McGinnis, 1971, and Goatcher and McGinnis, 1972). Based on these observations, Marusich et al., (1977) proposed the use of a rye-soybean ration as a model for the evaluation of growth promotants in chickens. These authors reported significant growth improvements by seventeen antibacterial agents when used in their model ration.

The only common denominator for all antibiotics is their ability to inhibit in vitro the growth of some microorganisms. It is therefore logical to expect that antibiotics must be exerting their influence by acting on the gut flora. However, attempts to show that antibiotics produce significant changes in total or selective bacterial populations in the gut have proven to be inconclusive. The results were divided between increased, decreased and no effect for several types including Escherichia coli, Lactobacilli, Anaerobes and Enterococci (Jukes, 1955, 1984). Studies with pigs have also produced similar conflicting results (Frolich et al., 1974 and Langlois et al., 1978). Jukes and Williams (1953) thought that the suppression of intestinal clostridia by antibiotics might be responsible for the antibiotic growth effect. These workers therefore fed massive doses of clostridial cultures to chicks but found no growth depressing effects and therefore concluded that the antibiotic growth effect was not due to the suppression of clostridia. Larson and Carpenter (1952) had earlier reached a similar conclusion when they found that the population of C. perfringens in the feces of pigs fluctuated so widely that there was no correlation between dietary antibiotics and fecal populations of this organism. In spite of these reports on the lack of a correlation between dietary antibiotics and intestinal populations of C. perfringens, attempts to re-examine this relationship have continued for the past thirty years because: a) C. perfringens is a

pathogenic bacteria that occurs in the intestines of healthy animals and man. It is known to be involved in the pathogenesis of a number of diseases including gas gangrene derived from wound infections in man, lamb dysentery, enterotoxaemia in lambs, sheep, piglets and cattle, and food poisoning (Buxton and Fraser, 1977); b) It sporadically occurs in populations of 10^2 to 10^4 in the intestine of chicks (Timms, 1968; Barnes et al., 1972; Salanitro et al., 1978); c) It produces toxins (Skjelkvale et al., 1979); d) Its presence in the intestine of gnotobiotic chicks has been associated with growth depression (Lev and Forbes 1959; Cole and Boyd 1967) and with changes in the flavor of chicken meat (Harris et al., 1968); e) When present in large quantities, they can be detected by well defined visual criteria such as gas production, lecithinase production and formation of black colonies on selective media containing sulfite (Angelotti et al., 1962, Hill, 1981); f) It is highly susceptible to many feed additive antibiotics (Stutz and Lawton, 1984b). Lev and Forbes (1959) monocontaminated gnotobiotic chicks with species of E coli, Lactobacillus, Staphylococcus liquefaciens and C. perfringens. They reported that only clostridium species depressed growth. The growth depression was overcome by the administration of penicillin. Similar results were reported by Wostman et al., (1960). A depression in the absorption of some fatty acids was observed by Cole and Boyd (1967) in gnotobiotic chicks contaminated with C. perfringens and Streptococcus

fecalis either singly or in combination. Streptococcus faecium was shown to depress growth in chicks (Fuller et al., 1979 and Fuller and Coates, 1983). The growth depression was counteracted by dietary penicillin resulting also in a decrease in streptococcus counts. C. perfringens and streptococci therefore appear to be good models for studying the interaction between intestinal bacteria and dietary antibiotics. As a result of a series of reports on the effects of dietary antibiotics on intestinal populations of C. perfringens in recent years by Stutz and associates (Stutz et al., 1983a, b and d); Dafwang (1985) attempted to study the relationship between environmental contamination, intestinal and fecal populations of C. perfringens, toxin production, dietary antibiotics and growth of chicks using the techniques of Stutz et al., (1983a). The first part of the study was to test the selectivity of the Tryptose-Sulfite-Neomycin (TSN) medium that these authors used to enumerate C. perfringens from gut contents of chicks. A defined species of C. perfringens was used as control (American Type Culture Collections #13124). Extensive visual and microscopic examination of the cultivated plates demonstrated a sharp contrast in morphology between the bacteria that came from the chicken intestine and the pure culture. Similar observations were made on three other dry media that are commonly marketed as selective media for the detection and/or enumeration of C. perfringens. Dafwang (1985) therefore concluded

that any information on chick gut populations of C. perfringens that is based entirely on the use of selective media without further confirmatory identification to prove that only the desired bacterial species have been enumerated is erroneous and misleading. More recently, Stutz and Lawton (1984a, b) proposed and adopted the use of the iron milk method for enumeration of C. perfringens from chick intestine. The selectivity of such a medium for C. Perfringens cultivation remains to be validated. In order to accurately assess the role of this bacteria in chick growth promotion there is need to develop methods that can accurately and rapidly detect it's presence amidst the myriads of bacteria that are known to occur in the chick intestine. There is also the question of whether C. perfringens with the low population and sporadic ocurrence in the chick's intestine (Barnes et al., 1972) can exert significant effects on growth promotion.

It has been suggested that the chick growth depressing factors contained in raw navy beans, rye and pectin whose adverse effects can be alleviated by dietary antibiotics indicate the involvement of the intestinal microflora (Untawale and McGinnis, 1979; Wagner and Thomas, 1977; Fuller and Coates, 1983). Hewitt et al. (1973) demonstrated that the effects of feeding navy beans was much less severe in germ-free than in conventional chicks. Navy beans have also been shown to induce an increase in the number of bacteria adhering to the

lower part of the small intestine (Untawale and McGinnis, 1976, 1979). The same authors found that rye had a similar effect on the microflora attached to the small intestine. Wagner and Thomas (1977) observed that flora obtained from the ileum of birds fed rye or pectin containing diets produced more butyric acid and gas than than the ileum of birds fed control diets suggesting that clostridia may be responsible. They suggested that the pectin content of rye may be the growth depression factor. Further work by Patel and McGinnis (1980) failed to confirm this suggestion.

It has been reported that the administration of Lactobacillus acidophilus cultures to young chicks produce growth promotion and improved feed efficiency (Tortuero, 1973). It is thought that microbial cultures act by modifying the gastrointestinal environment to favor healthy tissue development (Olentine, 1983). Further research on the role of microbial cultures in growth promotion may offer some explanation on the mode of action of dietary antibiotics.

5. A Metabolic effect

The suggestion that antibiotics may be influencing growth by directly or indirectly exerting some physiological metabolic effects within the tissues of the animal stems from the observation that several mechanisms exist by which antibiotics are known to inhibit the growth of bacteria. Furthermore a comparison of metabolic parameters between germ-free and conventionally reared animals indicates that

basal metabolic rate, iodine uptake by thyroid, cardiac output, blood volume, and arterial blood flow to liver in addition to the intestinal features already discussed are 20 to 50% lower in germ-free animals (Visek, 1978). Parameters that have been shown to be higher in germ-free animals include cecal weight (550%), xylose and amino acid transfer. Urea hydrolysis and bile acid hydrolysis are reported to be non-existent in germ-free animals (Visek, 1978). Since animals fed antibiotics often possess characteristics that are similar to those of germ-free animals, it is implied that the antibiotics act in some way physiologically to produce these metabolic changes (Hays, 1976). However, in view of the fact that at subtherapeutic levels there is rarely any retention of the antibiotics in tissues (Katz et al., 1974), it is doubtful that antibiotics can directly induce metabolic effects that can account for the growth promotion. Furthermore, the growth promotion has been demonstrated by antibiotics that are well absorbed and those that are poorly absorbed. Bunyan et al. (1977) in a study of the properties of fifty-five antimicrobial substances concluded that their growth promoting properties could not be related to the known antimicrobial and absorption characteristics of these substances in animals. It is therefore more likely that antibiotics affect metabolic parameters indirectly through their influence on the gut flora. Because of the low levels of antibiotics that is often observed in the blood of animals fed antibiotics even at very high

levels the use of a potentiation agent such as pterephthalic acid has been suggested for incorporation into the diets of chicks administered therapeutic levels of antibiotics. It is thought that such a chemical would increase the blood levels of the antibiotic and therefore increase it's efficacy for combating infectious organisms in the body (Scott et al., 1982)

In addition to the commonly cited hypotheses Dafwang et al. (1985) hypothesised that contrary to popular belief on the immunosuppressive effects of antibiotics (Naqi et al., 1984), antibiotics fed at subtherapeutic levels may actually be enhancing immunocompetence and therefore promote better growth in chicks. This hypothesis follows the observation that antibiotic growth promotion is most pronounced in young chicks which is also the time during which chicks establish their immunocompetence. To test this hypothesis, Dafwang et al. (1985) examined the bursa and spleen of chicks and also measured the antibody response of the chicks to sheep red blood cells. These authors reported that chicks fed antibiotics tended to have heavier bursa but no consistent differences in antibody responses between antibiotic fed and control birds was observed. The authors concluded that the low levels at which antibiotics are fed to chicks do not result in the enhancement or suppression of the immune response of chicks. The significance of the heavier bursa remains to be explained. The results of Dafwang et al. (1985) is in agreement with

the report of Markham et al. (1955) who fed up to 300ppm of chlortetracycline but found no effect of the antibiotic on the immune response of chicks to vaccination with Newcastle disease vaccine and infectious bronchitis virus. It is also consistent with the observation that dietary antibiotics reduce mortality and improve the body weight gain of bursectomized chicks (Glick et al., 1968).

Public Health Concerns on the Subtherapeutic use of Antibiotics for Animals.

Controversy regarding the continuous use of antibiotics for animal feeding simultaneously with their use for disease control in the public health system began almost at the same time that antibiotics were reported to cause growth promotion in animals. Controversy arose because the occurrence of an increase in the number of resistant bacteria were reported from several laboratories in which antibiotics had been fed to animals for growth promotion. However, after thirty-five years of continuous use and demonstrated effect of use most livestock producers and other advocates of antibiotic use in agriculture have argued against the imposition of a ban on the subtherapeutic use of antibiotics. Conferences, symposia, government established committees, industry and government sponsored research, trade journals and the mass media have produced an avalanche of reports and research data that are too numerous to cover in this review. A few of the important articles published recently on both

sides of the controversy are cited for further reading (Hays, 1976; Levy et al 1976a, b; Fagerberg et al, 1978; Hirsh and Wiger, 1978; Braude, 1978, 1981; Langlois et al, 1978; Solomons, 1978; Van Houweling, 1978; Van Houwelling and Gainer, 1978; Office of Science and Technology Assesment, 1977; National Academy of Sciences, 1980; Council for Agricultural Science and Technology, 1981; Atkinson and Lorian, 1984; Jukes, 1984; Feinman, 1984; Holmberg et al, 1984a and b; Lacey, 1984; Anon, 1985). This review attempts to document data on the limitations of use and the continued effectiveness of antibiotics that have prevailed inspite of a presumed prevalence of resistant bacteria in the environment for the past thirty-five years. The controversies are a challenge to the scientific community to continue active research into the complex nature of the relationships that exist between subtherapeutic use of antibiotics, the gut flora of animals and animal production in order to provide answers that can be used to maintain animal production at levels that will meet human requirements for economically and safely produced animal products. This thesis is an attempt to meet that challenge.

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CHAPTER II

BROILER CHICK GROWTH RESPONSE TO ANTIBIOTICS, 1981-1982

Broiler Chick Growth Response to Antibiotics, 1981-1982¹

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ABSTRACT A study of the growth-promoting effects of penicillin, oxytetracycline, lincomycin, bambamycin, and tylan was made over a 2-year period involving 2030 broiler chicks in 11 experiments. The laboratory used for this study has been subjected to the continuous use of low level dietary antibiotics for over 30 years. Results show that growth promotion by penicillin, oxytetracycline, and lincomycin were still significant ($P < .01$). The effect of tylan was also significant ($P < .05$). The antibiotics tended to promote better growth effects in chicks from young breeder hens. Significant growth improvement by antibiotics was observed in nutritionally adequate diets regardless of the presence or absence of soybean meal and excesses of certain vitamins and minerals. (*Key words:* antibiotics, chicks, lincomycin, oxytetracycline, penicillin, tylan, bambamycin)

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INTRODUCTION

One of the major controversies in the animal industries concerns the use of antibiotics and other antibacterial agents as growth stimulants for animals. Some early studies showed that the continuous feeding of antibiotics to chicks in the same environment resulted in a decrease or even loss of growth response (Waibel *et al.*, 1954; Libby and Schaible, 1955; Sherman *et al.*, 1959). McGinnis *et al.* (1958), Wiese and Peterson (1959), Nelson *et al.* (1963), and Combs and Bossard (1963) observed that new antibiotics, which had not previously been used on the premises, stimulated greater growth than older antibiotics. In contrast, Heth and Bird (1962) summarized the results of experiments conducted at the University of Wisconsin poultry laboratory from 1951 to 1960, which showed that feeding procaine penicillin produced a mean improvement in body weight of 8.5% between 1950 to 1953 and 8.8% between 1956 to 1960. For the tetracyclines, the mean improvement was 12.3% between 1950 to 1953 and 10.2% from 1956 to 1960. Similar results were reported by Peter *et al.* (1966) who observed that chlortetracycline fed over a 10-year period in the same laboratory maintained its effectiveness. Bird (1968) gave a chronological summary of tests with commonly used antibiotics from several laboratories.

The data did not indicate a progressive trend toward loss of effectiveness. Griffin (1980) reported a study between 1971 to 1974 showing that penicillin, zinc bacitracin, and Nitrovin produced consistent improvements in body weight and feed conversion over each annual period. More recently, Bird (1980) summarized the results of experiments on antibiotic growth promoting effects reported by several investigators between 1968 to 1980. The data indicated that feeding penicillin resulted in a median increase in body weight of 11%; the increase was 8 to 10% for the tetracyclines and 4 to 7% for the "new" antibiotics.

The study of dietary factors affecting the growth response to antibiotics has also received considerable attention. Jukes (1977) cited several investigators on the sparing effects of some antibiotics on the requirement for a number of vitamins (thiamine, riboflavin, niacin, pyridoxine, pantothenic acid, folic acid, B₁₂, biotin, and vitamins A and D), calcium, and protein. Patel and McGinnis (1980) reported that penicillin gave a consistent and significant improvement in body weight only when the corn diet was deficient in total sulfur amino acids, indicating that penicillin reduces the methionine requirement for growth. Menge (1973) observed an increase in response to antibiotics with increased soybean meal in the diet. Antibiotics have also been shown to overcome the deleterious effects of rye diets (MacAuliffe and McGinnis, 1971; Marusich *et al.*, 1977). On the interaction with metabolizable energy, Slinger *et al.* (1961) found no effects on dietary metabolizable energy, due to anti-

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biotics, but Nelson *et al.* (1963) reported that some antibiotics increased the metabolizable energy of a practical chick diet. Chlorotetracycline supplementation was found to produce consistent growth response and improved "energy utilization without any effect on the amount of metabolizable energy derived from the diet or on the total intake of feed or energy" (p.1500, Begin, 1971).

The purpose of this study was to observe the effect of antibiotics in chick diets on growth response in an environment that has been exposed to low level feeding of antibiotics over the past 30 years and also to study the effect of other factors that may influence the nature of such responses.

MATERIALS AND METHODS

Chicks and Management. The chicks used in this study were broiler-type chicks hatched

from eggs collected from a breeding flock maintained by the Department of Poultry Science at a site different from the laboratory in which the chick experiments were conducted. All birds were wing-banded and randomly allocated on the basis of equal initial body weights to the different experimental groups. The birds were reared in multiple compartmented battery brooders. Batteries were electrically heated with elevated wire floors. This laboratory has been in continuous use since 1957, but some of the equipment has been in use since before 1950. Throughout this period, there has been low level feeding of one antibiotic or another in the room. A total of 2030 chicks were used in 11 experiments. Each experiment utilized three replicates of 10 birds each per treatment.

Basal Diets. Table 1 shows the composition of the four basal diets used in this study. All

TABLE 1. Composition of basal diets

Ingredients	Basal no. ^{1,2}			
	1	2; 2*	3; 3*; 3**	4
	(%)			
Corn	64.50	50.00	76.00	74.00
Soybean meal (44%)	30.30	38.00
Fish meal	10.00	10.00
Casein	5.00	5.00
Gelatin	5.00	6.00
White grease	...	6.70	...	1.00
Meat and bone meal	3.00	3.10	3.00	3.00
Dicalcium phosphate	1.00	1.00	.50	.50
Limestone	.70	.70
Iodized salt	.25	.25	.25	.25
Methionine	.25	.25	.25	.25
Vitamin-mineral mix ¹	+	+	+	+
Calculated composition				
Crude protein, %	21.3	23.00	23.10	24.00
Metabolizable energy (kcal/kg diet)	2945	3201	3267	3309
Calcium, %	.91	.94	1.03	1.03
Phosphorus, %	.70	.72	.78	.78
Lysine	1.12	1.31	1.37	1.37
Methionine + cystine	.84	.92	.93	.83

¹ Vitamin-mineral mix supplied the following per kilogram of Basals 1, 2, and 3: vitamin A, 7500 IU; vitamin D, 440 IU; vitamin K (MPB), 1.11 mg; riboflavin, 4.58 mg; calcium pantothenate, 14.1 mg; biotin, .09 mg; folic acid, .4 mg; niacin, 54.4 mg; vitamin B₁₂, .01 mg; choline chloride, 1379 mg; manganese, 52.3 mg; zinc, 39.2 mg.

² Basals 2*, 3**, and 4 contained the following per kilogram of diet but the same level of other vitamins and minerals in Basal 1: riboflavin, 7.2 mg; calcium pantothenate, 20 mg; biotin, .31 mg; folic acid, 1.11 mg; choline chloride, 2600 mg; vitamin B₁₂, .02 mg; manganese, 110 mg; and zinc, 80 mg.

³ Basal 3* contained the following per kilogram of diet but the same level of other vitamins and minerals in Basal 1: riboflavin, 6.9 mg; calcium pantothenate, 13 mg; choline chloride, 2461 mg; manganese, 85.2 mg; and zinc, 67.2 mg.

diets were formulated to meet a calorie-protein (metabolizable energy/kg) ratio of between 138 to 140. The levels of vitamins and minerals were formulated to meet the National Research Council (NRC, 1977) standards (Basal 1, 2, 3) or provide excesses of one and a half times the NRC (Basal 3*) or double the NRC (Basals 2*, 3**, and 4) of selected vitamins and minerals. The standard broiler mash is Basal 2. Basal 1 had a lower energy and protein level. In Basal 3, the soybean meal was replaced with casein, gelatin, and fish meal. Basal 4 is similar to Basal 3 except that 1% fat was substituted for part of the corn.

Antibiotics. The antibiotics were purchased commercially or were obtained directly from the manufacturers (Table 2). Penicillin and oxytetracycline have been used in the laboratory since 1950. Lincomycin, tylan, and bambarmycin are relatively new to the laboratory. The levels of antibiotics added to the diets were the maximum levels for which they are approved for inclusion in broiler chick diets for growth promotion and improvement of feed efficiency (*Feed Additive Compendium*, 1982). One trial was to compare the growth response to antibiotics by chicks from young breeder hens (in their 2nd month of egg laying) and chicks from old breeder hens (in their 11th month of egg laying). Two other trials were conducted to evaluate the growth response to different dietary levels of antibiotics.

Data Collection and Analysis. Birds were weighed weekly and feed intake measured. The mean 3-week body weights and feed conversion ratios were pooled from the 11 trials and subjected to the *t* test analysis. For individual experiments, data were subjected to the analysis of variance for detection of significance of differences between means by the least significant difference (Steel and Torrie, 1960).

RESULTS AND DISCUSSION

Table 2 presents a summary of the overall effect of the antibiotics used in this study on growth promotion and feed conversion. The results show that both the old and new antibiotics stimulated growth and improved feed conversion at levels compared to those of earlier investigators (Heth and Bird, 1962; Peter *et al.*, 1966; Bird, 1968, 1980). The mean body weight improvement of 10.4% for penicillin is even higher than the 8.5% reported for 1950 to 1953 by Heth and Bird (1962). Results were

TABLE 2. Effect of antibiotics on body weight and feed conversion of 21-day-old broiler chicks

Antibiotic ¹ and number of comparisons	Mean body weights				Feed conversion			
	Basal	+ Antibiotic	% Improvement in body weight	Significance	Basal	+ Antibiotic	% Improvement	Significance
Penicillin (9)	365.6 ± 53	425.5 ± 64	10.4	0	1.63 ± .08	1.55 ± .11	3.9	.2039
Oxytetracycline (13)	401.6 ± 71	447.9 ± 43	11.5	.0066	1.63 ± .10	1.54 ± .09	5.8	.0038
Lincomycin (19)	432.3 ± 44	475.4 ± 53	10.0	0	1.57 ± .06	1.51 ± .07	4.0	.0044
Bambarmycin (2)	367.5 ± 132	420.1 ± 149	14.3	.1433	1.76 ± .21	1.64 ± .27	7.3	.2048
Tylan (2)	367.5 ± 132	435.7 ± 127	18.5	.0294	1.76 ± .21	1.63 ± .20	8.0	.0489

¹ Antibiotics were purchased commercially from: TH Agric and Nutrition Company, Kansas City, KS for penicillin; American Cyanamid for oxytetracycline; Tuco for lincomycin; American Hoechst for bambarmycin; Elanco for tylan.

similar with oxytetracycline and lincomycin. The 10% improvement with lincomycin is better than the 7% reported for this antibiotic by Marusich *et al.* (1973) and 4 to 7% reported for the "new" antibiotics by Bird (1980). The *t* test analysis of the data from this study shows that the growth stimulation by these three antibiotics was highly significant ($P < .01$). Their effects on feed conversion were not as great but were highly significant for oxytetracycline and lincomycin. Although the improvement by bambarmycin was 14.3 and 7.3% respectively for body weight and feed conversion, this improvement was not statistically significant. A 26% growth improvement had earlier been reported with bambarmycin by Marusich *et al.* (1977). The mean improvements in body weight and feed conversion were 18.5 and 8%, respectively, for the two trials with tylan and were statistically significant ($P < .05$).

A comparison of the data from month to month showed no trend towards a decline in effectiveness of growth stimulation over the 2-year period. However, while no significant growth improvement by antibiotics was observed in the first trial (March, 1981), significant growth improvement was observed in the last experiment (November, 1982) using the same antibiotics.

For trials in which different basal diets were compared, optimum growth response to dietary antibiotics was only observed for chicks fed basal diets that supported maximum growth. This agrees with the observation of other investigators who have reported that antibiotics promote growth only when incorporated into nutritionally adequate diets (Hays, 1976). However, antibiotic supplementation of mar-

ginally adequate diets tended to improve growth performance to levels that were obtained on nutritionally adequate diets without antibiotics. This may have been due to a nutrient sparing effect by antibiotics (Jukes, 1977). However, the observation that antibiotic supplementation of nutritionally adequate diets with excesses of vitamins and minerals still stimulated growth suggests that the antibiotic effect may be more of an increase in nutrient absorption rather than a nutrient sparing effect. It is also possible that antibiotics may have both effects depending on the nature of the diet. The replacement of soybean meal with animal protein depressed growth. Our results are contrary to the results of Menge *et al.* (1973) who found that growth stimulation by antibiotics increased with increasing levels of soybean meal. The data obtained in this study do not support the hypothesis that antibiotics may be promoting growth by the suppression of growth inhibitors in soybean meal.

Results of growth response to antibiotics by chicks from young and old breeder hens are shown in Table 3. The chicks from young hens averaged 38 g while chicks from the older hens averaged 46 g at day-old. Chicks from old hens grew significantly faster than chicks from young hens ($P < .01$). Both antibiotics produced significantly better growth in the two groups of chicks ($P < .01$), but the penicillin (an "old" antibiotic) tended to stimulate greater growth than lincomycin, although such differences were not statistically significant. The body weights of chicks from young hens on antibiotic diets were not statistically different from those of chicks from old hens that were fed only the basal diet, indicating that antibiotics may

TABLE 3. Growth response to antibiotics of chicks from old and young parent stock (21 days)

Treatment ¹ no.	Basal no.	Antibiotic and quantity added	Body weight	Feed conversion
		———— (mg/kg) ————		
1	2	...	441.8 ^{bc}	1.60
2	2	Penicillin	502.5 ^a	1.52
3	2	Lincomycin	487.5 ^{ab}	1.49
4	2	...	377.1 ^d	1.64
5	2	Penicillin	410.4 ^{cd}	1.50
6	2	Lincomycin	400.8 ^{cd}	1.55

a, b, c, d. Means within columns having different superscripts are significantly different ($P < .05$).

¹ Treatments 1 to 3 were chicks from old hens, 4 to 6 were chicks from young hens.

TABLE 4. Effect of different levels of antibiotics on growth response¹

Antibiotic and quantity added		Body weight	Feed conversion
(mg/kg of diet)		(g)	
...	0	318.6 ^c	1.68 ^a
Penicillin	10	362.3 ^{bc}	1.56 ^{ab}
Penicillin	50	398.2 ^{ab}	1.42 ^b
Oxytetracycline	10	426.7 ^{ab}	1.52 ^{ab}
Oxytetracycline	50	460.7 ^a	1.46 ^{ab}
Oxytetracycline	100	436.8 ^{ab}	1.47 ^{ab}
...	0	401.9 ^b	1.59
Lincomycin	2	454.0 ^a	1.59
Lincomycin	4	447.6 ^a	1.54
Lincomycin	6	481.1 ^a	1.54
Lincomycin	8	481.1 ^a	1.57
Lincomycin	10	456.3 ^a	1.43

^{a,b,c} Within each experiment, means having different superscripts are significantly different ($P < .05$).

¹ Basal 2 was used in both experiments.

stimulate enough growth to compensate for the lower initial body weight of the chicks from young breeders. This observation may be significant, because under field conditions, poultrymen often have to raise chicks from young breeder hens. Information on the beneficial effects of antibiotics for such chicks appears to be scanty.

The growth responses to different levels of three antibiotics were also investigated (Table 4). Body weights with 50 ppm penicillin or oxytetracycline from 10 to 100 ppm were similar and significantly better than body weights on the basal diet. Feed conversion was significantly better with 50 ppm penicillin but similar to all other levels of the two antibiotics. A dose versus growth response with lincomycin

at 2, 4, 6, 8, and 10 ppm showed that the response to graded levels of lincomycin was significant ($P < .05$). Regression analysis generated the quadratic equation $Y = 404 + 21.6 \times 1 - 1.61 x^2$. Although the maximum growth stimulation was observed with 6 ppm of lincomycin, the computed predicted body weights show that the magnitude of growth stimulation declines beyond 4 ppm. This regression analysis supports the recommendation of 4 ppm maximum dietary level of lincomycin for growth stimulation (*Feed Additive Compendium*, 1982). For the three antibiotics tested in these two trials, results indicate that growth stimulation with low level antibiotic feeding is similar to that obtained with higher levels. The hypothesis that continuous use of an antibiotic

TABLE 5. Antibiotic usage, basal types, and number of experimental groups (30 birds each) in the experiment reported

Antibiotic	Number of groups, ¹ basal no.							Total number of comparisons
	1	2	2*	3	3*	3**	4	
Penicillin	2	6		1				9
Oxytetracycline	1	7	1		2	1	1	13
Lincomycin	2	12	2	1		2		19
Bambermycin	1	1						2
Tylan	1	1						2

¹ For actual diets see Table 1.

may require higher levels in order to obtain growth promotion effects (Van Houwelling, 1978) was not supported.

The numbers of experimental groups used with the various diets and antibiotics are shown in Table 5. The data presented in this study indicate that in spite of the continuous use of antibiotics in an environment, significant growth-promoting effects can still be obtained. Penicillin and the tetracyclines have been used extensively by the poultry and livestock industry for over 30 years, but the growth-promoting effects observed in this study are comparable or even superior to the effects observed when they were first introduced to the animal industry. Furthermore, the effects of the "old" antibiotics are comparable or superior to that of the "new" antibiotics, although it is known that bacteria resistant to penicillin and the tetracyclines have been present in the broiler chick's environment for over 30 years. Further studies are needed to try and explain this phenomenon to enhance a better understanding of the role of antibiotics in poultry nutrition.

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CHAPTER III

BURSAL, INTESTINAL, AND SPLEEN WEIGHTS AND ANTIBODY RESPONSE OF CHICKS
FED SUBTHERAPEUTIC LEVELS OF DIETARY ANTIBIOTICS

Bursal, Intestinal, and Spleen Weights and Antibody Response of Chicks Fed Subtherapeutic Levels of Dietary Antibiotics

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ABSTRACT Experiments were conducted to study the influence of dietary antibiotics on the bursal, intestinal, and spleen weights and antibody responses of broiler chicks. Results indicate a tendency for chicks fed antibiotics to have heavier bursae but significantly reduced intestinal weights. The antibiotics had no effect on spleen weights and antibody response to sheep red blood cells. It was concluded that the beneficial effects of dietary antibiotics for chicks do not appear to be the result of an enhanced immunocompetence and that the use of antibiotics at subtherapeutic levels does not have an adverse effect on the chicks' humoral immune system.

(*Key words:* antibiotics, bursa, chicks, intestine, antibody response)

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INTRODUCTION

It has been reported that apart from body weight changes and improvements in feed efficiency, the most consistent effect of antibiotics has been a reduction in the weight of the intestinal tract of chicks (Hill *et al.*, 1957). The reduction in intestinal weight of chicks fed antibiotics was first reported by Gordon (1952) who also observed that chicks reared in a germ-free environment had reduced intestinal weights. Further studies by other workers confirmed such observations (Coates *et al.*, 1955; Jukes *et al.*, 1956; Hill *et al.*, 1957). Hill *et al.* (1957) showed that the decrease in intestinal weight occurred before an increase in body weight was noted and that even lower antibiotic levels, which did not increase body weight, caused a decrease in intestinal weights. Gordon and Bruckner-Kardoss (1959) found that in germ-free chickens the amount of lamina propria, lymphoid tissue, and numbers of free reticulo-endothelial cells in the ileal mucosa are reduced concurrently with the reduction in intestinal weight. In a later study (Gordon and Bruckner-Kardoss, 1961) these workers also found that in rats the intestinal surface area was significantly reduced in germ-free animals. More recently, Stutz *et al.* (1983b) reported that feeding zinc bacitracin to chicks significantly reduced the weight, length, and percent moisture of the small intestine. The reduction was more pronounced in the jejunum and ileum. On the basis of these observations, these authors, along with several

others earlier cited, have theorized that antibiotics stimulate growth of animals by eliminating undesirable microorganisms that produce toxins or metabolic products that irritate and increase the thickness of the intestinal wall and, as a result, decrease the absorption of nutrients.

The role of the bursa of Fabricius as an integral component of the avian immune system has been recognized and described (Glick *et al.*, 1956; Glick, 1968, 1979). In studies with bursectomized chicks, Glick *et al.* (1956) were able to show that removal of the bursa resulted in an impairment of the immune response to a variety of antigens. The addition of antibiotics to the diets of the chemically bursectomized chicks reduced mortality and improved body weight gain but did not improve their immunocompetence. However, there appears to be a lack of information on the effect of antibiotics on the normal growth of the bursa and its relationship to the immune response of chicks fed subtherapeutic levels of the antibiotics. Because, in the first 3 weeks of the chick's life, growth response to antibiotics is greatest, rate of growth of the bursa is most rapid, and immunocompetence is established, it seems logical to investigate any relationships that may exist between the three variables. It has been postulated that the feeding or intake of antibiotics does cause immunosuppression (Stevens, 1953; Glick, 1968; Tarnawski and Bartko, 1973). However, little evidence has been published in support. Harmon *et al.* (1973) conducted studies with young pigs that showed that 54-

day-old pigs fed diets containing zinc bacitracin, neomycin, and oxytetracycline produced a higher antibody response to sheep red blood cells (SRBC) than animals fed only the control diet. The higher antibody response was not associated with a response in weight gain or feed efficiency. No increased antibody response was observed with 27-day-old pigs. Tu (1980a,b,c) inoculated mice and pigs that had been subjected to different doses of penicillin before or at inoculation time with live and inactivated swine erysipelas vaccine. The author reported that the antibiotics caused immunosuppression in mice, but results with pigs were inconsistent. The immunosuppression to swine erysipelas vaccine by antibiotics was dose related and was affected by the time differential between antibiotic administration and vaccine inoculation and by whether the vaccine was live or inactivated. If subtherapeutic antibiotic feeding can cause immunosuppression, then the continuous use of antibiotics, which is a routine practice in the animal industry, may result in undesirable consequences in the future.

These studies were conducted to study the relationship between antibiotic feeding, intestinal weight, bursal growth, and humoral immune response of the chick. It is a follow up on an earlier study in which these antibiotics had been shown to produce significant improvements in body weight and feed efficiency (Dafwang *et al.*, 1984).

MATERIALS AND METHODS

The composition of the diets used in these studies has previously been described (Dafwang *et al.*, 1984). The control basal diet contained corn and soybean meal plus adequate levels of vitamins and minerals. The major distinguishing features of the diets used in Experiments 1 and 2 are given in footnotes to Table 1. The management of birds, sources of antibiotics, and other experimental procedures were also described in the earlier report.

The organ weights were taken from the 3-week-old broiler chicks used in the earlier study after an overnight fast. Half of the chicks (15 per group) nearest to the average body weight of each experimental group were slaughtered following carbon dioxide gassing. Overnight fasting prior to killing was necessary to promote gut emptying. The bursae were removed and weighed immediately. The birds were then dissected and the small intestines removed. Even after a 24-hr fast, the intestines

still had to be cleaned of any remaining fecal material by manual squeezing. In Experiments 1 through 4 only the bursae and small intestines were weighed. In Experiments 5 through 7 the spleens were also weighed. The wet bursae were dried in a force draft oven at 100 C for about 24 hr. Drying was stopped when two successive weighings at 2 hr intervals gave similar readings for a randomly selected sample of the bursae being dried. All weights recorded were expressed as milligrams per 100 g of body weight for bursa and spleen and grams per 100 g of body weight for the small intestine.

Hemagglutinating antibodies to SRBC were determined 7 days following a .5 ml intraperitoneal injection of a 5% suspension of thrice washed SRBC into the birds at 2 weeks of age. Hemagglutinating antibody titers were expressed as the \log_2 of the highest dilution of serum that agglutinated an equal volume of .5% SRBC.

All data collected were subjected to analysis of variance and significance of differences between means were determined by the least significant difference (Steel and Torrie, 1960).

RESULTS AND DISCUSSION

The effect of antibiotics on the body weight and feed efficiency of these chicks has previously been reported (Dafwang *et al.*, 1984). The small intestinal weight (g/100 g body weight) of chicks fed lincomycin or oxytetracycline was significantly reduced in Experiments 1, 2, 4, and 5 (Tables 1, 2, and 3). Of the 4 different basal types used, the weights of small intestine of the chicks on the antibiotic diet were all less than those of birds on the equivalent basal without antibiotic (Experiment 2). The results do not indicate any consistent relationship between intestinal weight and basal type. Similarly, it was observed that the decreased intestinal weight did not correlate with the level of lincomycin in the diet (Table 2). All of the 5 antibiotics tested (Experiment 3) resulted in significantly lower intestinal weights. The reduction in intestinal weight observed in this study is in agreement with the results of earlier investigators (Coates *et al.*, 1955; Jukes *et al.*, 1956; Hill *et al.*, 1957; Stutz *et al.*, 1983c). However, the lack of a correlation between the level of dietary antibiotic and intestinal weight is in contrast with the results of Stutz *et al.* (1983c), who reported that the magnitude of reduction in intestinal weight cor-

TABLE 1. Effect of different basal diets and antibiotics on bursal and intestinal weights (\pm SD) of broiler chicks

Experiment no.	Basal type ¹	Antibiotic	Bursa	Small intestine
			(mg/100 g body weight)	(g/100 g body weight)
1	2	...	207 \pm 61 ^b	3.39 \pm .50 ^a
		Oxytetracycline (50)	248 \pm 90 ^{ab}	3.12 \pm .37 ^{ab}
		Lincomycin (4)	295 \pm 117 ^a	3.01 \pm .42 ^{bc}
	2*	...	188 \pm 90 ^b	3.00 \pm .37 ^{bc}
		Oxytetracycline (50)	270 \pm 66 ^{ab}	2.94 \pm .40 ^c
		Lincomycin (4)	261 \pm 79 ^{ab}	3.01 \pm .30 ^{bc}
2	1	...	289 \pm 106 ^{ab}	2.65 \pm .61 ^{ab}
		Lincomycin (4)	337 \pm 88 ^a	2.33 \pm .26 ^{bc}
	2	...	296 \pm 104 ^{ab}	3.01 \pm .78 ^{ab}
		Lincomycin (4)	267 \pm 75 ^{ab}	2.62 \pm .38 ^{ab}
	2*	...	287 \pm 104 ^{ab}	2.73 \pm .77 ^{ab}
		Lincomycin (4)	311 \pm 115 ^{ab}	2.27 \pm .25 ^c
4	...	237 \pm 72 ^b	2.44 \pm .33 ^{bc}	
	Lincomycin (4)	285 \pm 83 ^{ab}	2.16 \pm .25 ^c	
3	2	...	225 \pm 99	3.31 \pm .44 ^a
		Lincomycin (4)	179 \pm 62	2.93 \pm .39 ^b
		Penicillin (50)	230 \pm 81	2.13 \pm .25 ^c
		Oxytetracycline (50)	239 \pm 84	2.28 \pm .45 ^c
		Bambermycin (2)	256 \pm 90	2.53 \pm .31 ^c
		Tylan (50)	223 \pm 91	2.62 \pm .33 ^{bc}

^{a,b,c} For each experiment, means within each column having different superscripts are significantly different ($P < .05$).

¹ Basal types: 1. 21% protein, 2945 metabolizable energy (ME) kcal/kg diet, 100% National Research Council (NRC, 1977) for trace nutrients; 2. 23% protein, 3201 ME kcal/kg diet, 100% NRC for trace nutrients; 2*. 23% protein, 3201 ME kcal/kg diet, 200% NRC for trace nutrients; 4. 24% protein, 3309 ME kcal/kg diet, 200% NRC for trace nutrients. Soybean meal replaced with casein, gelatin, and fishmeal.

² Basal 2 is the control diet used in Experiments 4 to 7. Complete description and composition given in Dafwang *et al.*, 1984.

related well with the dietary zinc bacitracin levels in their diets. The difference between the two results may be due to the type of antibiotic and or diets used; however, in this report type of diet had no effect. The factors that may account for such variability are not known, but the variability is similar to that often observed with the improvement in body weight and feed efficiency between experiments (Bird, 1968, 1980). Several investigators have attempted to identify the factors or metabolites that may be responsible for the decrease in intestinal weight resulting from antibiotic feeding. Herh (1963) hypothesized that antibiotics may exert such an effect by decreasing the level of ammonia in the intestine. After an intensive study, however, he concluded that "dietary antibiotics may cause a

TABLE 2. Effect of graded levels of lincomycin on bursal and intestinal weights (\pm SD) of broiler chicks (Experiment 4)

Level of lincomycin added	Bursa	Small intestine
(ppm)	(mg/100 g body weight)	(g/100 g body weight)
0	248 \pm 66	3.52 \pm .50 ^a
2	302 \pm 100	3.16 \pm .51 ^{ab}
4	294 \pm 84	3.39 \pm .42 ^a
6	307 \pm 102	3.16 \pm .50 ^{ab}
8	336 \pm 115	2.75 \pm .56 ^b
10	285 \pm 127	3.24 \pm .50 ^{ab}

^{a,b} Means within each column having different superscripts are significantly different ($P < .05$).

TABLE 3. *Effect of antibiotics on bursal, intestinal, and spleen weights and immune response (\pm SD) of broiler chicks*

Experiment no.	Antibiotic and quantity added (ppm)	Bursa ¹		Small intestine ²	Spleen ³	Antibody titer ³
		Wet	Dry			
5	...	219 \pm 58	45 \pm 11	2.72 \pm .34 ^a	122 \pm 52	3.33 \pm 1.91 ^b
	Lincomycin (4)	264 \pm 71	58 \pm 30	2.14 \pm .61 ^b	132 \pm 37	5.00 \pm 1.65 ^a
	Oxytetracycline (5)	208 \pm 66	44 \pm 12	2.11 \pm .25 ^b	114 \pm 33	3.86 \pm 1.46 ^{ab}
6	...	217 \pm 55 ^b	39 \pm 10 ^b	2.34 \pm .52 ^a	172 \pm 87	6.27 \pm 3.37
	Lincomycin (4)	282 \pm 83 ^a	53 \pm 15 ^a	1.92 \pm .27 ^b	195 \pm 91	6.07 \pm 2.25
	Virginiamycin (10)	255 \pm 60 ^{ab}	47 \pm 11 ^{ab}	2.24 \pm .49 ^{ab}	148 \pm 45	6.87 \pm 2.85
7	...	278 \pm 82	...	1.99 \pm .22	164 \pm 55	3.67 \pm 1.50
	Lincomycin (4)	253 \pm 74	...	1.99 \pm .29	151 \pm 35	3.13 \pm 1.68
	Virginiamycin (10)	238 \pm 40	...	1.50 \pm .21	148 \pm 54	2.67 \pm 1.50

^{a,b} Means within each experiment having different superscripts are significantly different ($P < .05$).

¹ Milligrams per 100 g of body weight.

² Grams per 100 g of body weight.

³ Sheep red blood cell response expressed as a mean hemagglutinating antibody titer (\log_2).

transient reduction in intestinal ammonia but additional mechanisms must be involved in bringing about the increase in growth" (dissertation abstract.) Other researchers have concentrated on identifying bacteria that may account for growth depression. Monocontamination of gnotobiotic chicks with species of *Clostridium perfringens* (Lev and Forbes, 1959), *Clostridium welchi* and *Streptococcus faecalis* (Cole and Boyd, 1967), and *Streptococcus faecium* (Fuller *et al.*, 1983) have been shown to result in growth depression of gnotobiotic chicks. In all cases the use of specific antibiotics caused a significant reduction in bacterial count and overcame the growth depression by these species of bacteria. Stutz *et al.* (1983a,b,d) demonstrated that the feeding of erythromycin, zinc bacitracin, and thyroprotein significantly reduced the population of *Clostridium perfringens* in the intestine of the chick in addition to growth stimulation and improvement of feed efficiency. These authors concluded that the changes in the intestine are probably indirect and most likely reflect the action of antibiotics on the intestinal microflora. The interaction between the gut microflora, the changes in gut wall thickness, and how they translate into increased utilization of essential nutrients from the diet to produce improvements in body weight and feed efficiency remain to be investigated.

Antibiotic fed chicks had significantly heavier bursae in Experiments 1 and 6 (Tables 1 and 3). In three other experiments the chicks tended to have bigger bursae (Experiments 2, 4, and 5 on Tables 1, 2, and 3) but the differences were not significant in comparison to control birds. In Experiments 3 and 7 (Tables 1 and 3), the bursal weights were similar for both groups of birds. Overall, antibiotic-fed chicks had bursae either numerically or significantly heavier than their respective controls in 18 out of 24 groups. Considering that the weight of the bursa (mg/100 g of body weight) declines as the chick weight increases, bursal weights reported here would have been larger if adjustment had not been made for the bigger size of the antibiotic-fed chicks. The effect on bursal weights was independent of basal type and antibiotic dosage (Tables 1 and 2). Because a functioning bursa is essential for normal immunocompetence, an enlarged bursa may suggest an alteration in antibody production (Yamamoto and Glick, 1982). This was tested in Experiments 5, 6, and 7 (Table 3). In Experiment 5, a higher antibody response to SRBC was ob-

tained in birds fed the diet containing lincomycin. These birds also had numerically heavier bursae than control birds. No such effect was observed in birds fed oxytetracycline in the same experiment. In the two other experiments, however, antibody response and bursal weights were similar for birds on diets with or without antibiotics. Antibiotic feeding also had no effect on spleen weights (Table 3). Although the use of antibiotics to reduce mortality and increase the growth of bursectomized chicks have been demonstrated (Glick, 1968), the possibility that antibiotic feeding may have a beneficial effect on bursal growth, as was observed in this study, has not to our knowledge been previously reported. However, because the study was unable to show a consistent positive correlation overall between antibiotics, bursal weights, and antibody response, it is possible that the increased bursal size observed in most of the experiments may have been due to the presence of some microbial agent or some kind of stress at the time the particular experiments were conducted. How such factors interact with other variables and their relationship to the significant improvements in body weight and feed efficiency (Dafwang *et al.*, 1984) is not known. From the study, however, it can be concluded that feeding antibiotics at subtherapeutic levels does not result in the suppression of antibody response.

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CHAPTER IV

EVALUATION OF SOME COMMERCIAL FORMULATIONS FOR THE
CULTIVATION AND ENUMERATION OF CLOSTRIDIUM PERFRINGENS
FROM CHICK INTESTINE

EVALUATION OF SOME COMMERCIAL FORMULATIONS FOR THE CULTIVATION AND
ENUMERATION OF Clostridium perfringens FROM CHICK INTESTINE¹

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SUMMARY

Four commercial formulations marketed for the detection and/or enumeration of Clostridium perfringens were found to adequately support the growth of a pure culture (ATCC 13124) but enumeration of colony forming units was difficult on Clostrisel and Lecithin-Lactose-Agar base (LLA). Colonies on LLA had typical opalescence zones, a distinctive feature that can aid in presumptive

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identification. Colonies on SPS (sulfite-polymyxin-sulfadiazine) and TSN (tryptose-sulfite-neomycin) agar were morphologically similar but black colonies were observed on TSN incubated at 46° C. Extreme difficulty was encountered in enumerating enteric bacteria from chick intestine on clostrisel because of the pinpoint size of the colony forming units and on LLA because of the opaqueness of the medium. Morphological characteristics of bacteria picked from colonies which formed after these media had been inoculated with chick intestinal contents were in sharp contrast to those of the pure culture of C. perfringens. The numbers of colony forming units were higher than documented levels of C. perfringens in the intestine of chicks. These observations led to the conclusion that the formulations are inadequate for the selective cultivation and enumeration of this bacteria from the intestine of healthy chicks, unless used in combination with further tests for specific identification.

Key Words: Clostridium perfringens, selective media

INTRODUCTION

Involvement of Clostridium perfringens has long been suspected in the mode of action of growth-promoting dietary antibiotics because it is a pathogenic bacteria (4). It often occurs at low concentrations in the chick intestine (2,20), its presence in the intestine of gnotobiotic chicks has been associated with growth

depression (12) and the species is known to be highly susceptible to many antibiotics (17). However, the bacterium is most widely known for its role as a pathogen in food poisoning (9). Although initial studies indicated that the bacteria had no consistent effect on growth (10,11), later studies by Lev and Forbes (12) and Wostmann et al. (21) suggested that the species was associated with a growth depression in chicks that can be alleviated by dietary antibiotics. Prompted by recent reports by Stutz and associates (16,17) on the reduction of C. perfringens populations in chicks fed dietary antibiotics, studies were developed to examine such results. Initial studies in which attempts were made to enumerate C. perfringens using Clostrisel agar showed that there were inherent difficulties associated with the use of this commercial formulation for the detection and enumeration of C. perfringens from chick gut contents. The desire to find more suitable alternatives led to the investigations reported in this study. The TSN agar that had been used by Stutz et al. (16) in their study and two other commercial formulations were tested for their effectiveness in the detection and enumeration of C. perfringens.

MATERIALS AND METHODS

Commercial formulations. Clostrisel, SPS (sulfite-polymyxin-sulfadiazine) and TSN (tryptose-sulfite-neomycin) were purchased from BBL (Cockeysville, MD) and lecithin-lactose agar base (LLA) was obtained from Gibco (Madison, WI). All media were stored and prepared according to manufacturers instructions prior to plating.

Sources of bacteria. A pure culture of C. perfringens (ATCC 13124) was obtained from the Wisconsin State Hygiene Laboratory (Madison, WI) and maintained in meat broth under refrigeration. One day before the start of an experiment, 4 ml of basal medium was inoculated anaerobically with 0.1 ml of the meat broth culture and incubated at 37° C. The composition of the medium per 100 ml was: glucose, 1.0 g (autoclaved separately); K_2HPO_4 0.25 g; yeast extract, 1.0 g; casitone, 1.00 g; $KC_2H_3O_2$, 0.25 g; and 2 ml of 2.5% (w/v) cysteine.HCL.2H₂O solution. The medium was determined in a preliminary study to adequately support the growth of this species. Preparation of the medium and inoculation with pure culture were done anaerobically using the Hungate technique (3). After 12 hr of incubation at 37° C, 0.1 ml of culture were transferred to a second test tube of the medium. This tube was also incubated at 37° C for 4 to 5 hr. A dilution series of this culture was prepared from 0.25 ml taken at the late log phase of bacterial growth. Aerobic and anaerobic dilution techniques were compared in a preliminary experiment to ascertain the effectiveness of aerobic plating techniques for the cultivation of C. perfringens.

In order to maximize the chance of obtaining C. perfringens in the chick intestine, pooled intestinal samples were utilized for plating of chick gut bacteria. For each experiment, five chicks were selected at random from a pool of chicks maintained on practical diets in the University of Wisconsin Poultry Science Laboratory. Chicks were killed by axial dislocation and then about

3 g of contents were aseptically removed from the appropriate intestinal segment of each chick and mixed with a sterile scalpel. One gram of the pooled feces was transferred into a tared 18 x 150 mm test tube containing 9 ml of .1% trypticase (BBL) sterile solution. The mixture was vortexed and further ten-fold serial dilutions up to 10^{-6} were prepared from the initial 10^{-1} dilution. Based upon results of the preliminary experiment, sampling and dilution were done rapidly aerobically to minimize aerobic exposure. Control dilutions of washings from scissors and scalpels used were also plated.

Pour plate methodology. Aliquots (9.5 ml) of the dissolved media were dispensed into 18 x 150 mm test tubes with stainless steel caps (Morton Culture tube closures) prior to or after autoclaving depending on the manufacturer's instructions. Sterile test tubes containing molten agar media were maintained in a water bath at 50° C. At plating time, 0.5 ml of the 10^{-4} to 10^{-6} dilutions was transferred into the 9.5 ml of medium, vortexed for about 20 sec and then poured into sterile 60 x 15 mm petri plates (Falcon, Cockeysville, MD). Dilution series and inoculation of media with bacterial samples were done using a 1000 μ l pipetman (Gilson, Middleton, WI) with disposable tips that had previously been sterilized by autoclaving individually in stoppered 13 x 100 mm glass tubes held in a test tube press. All samples were plated in duplicate. A control plate for each sample was prepared from the 10^{-2} dilution to serve as a check on the effectiveness of the

medium and the incubation conditions. After allowing the plates to cool for 45 min they were inverted and incubated inside a Coy anaerobic chamber (Coy Laboratory Products, Ann Arbor, MI) at 46° C. For temperature comparisons, this chamber was maintained at 37° C and a Dome visible incubator (Colab Laboratories, Inc., Chicago, IL) placed inside the chamber was maintained at 46° C after equilibration with the chamber atmosphere.

Morphological Examination. After incubation, colonies on the plates were examined for type, color, shape and approximate size. Enumeration of colony forming units (cfu) was done on a Quebec colony counter (American Optical Systems). Microscopic examination was done at 1000 x magnification. Four of the investigators were involved in defining the morphological characteristics.

RESULTS AND DISCUSSION

The optimum growth temperature of C. perfringens is reported to be between 43 and 47° C but the species is known to grow well at 37° C (4,13). Results from this study (Table 1) indicate a tendency to have a slightly higher number of cfu (\log_{10}) of pure culture at 46° C after 24 hr incubation than at 37° C (8.29 vs 8.18 on TSN and 8.32 vs 8.00 on SPS). The rate of growth at log phase ($\Delta OD/hr$) was determined in a preliminary study and found to be similar at the two temperatures ($.50 \text{ hr}^{-1}$ at 46° C and $.45 \text{ hr}^{-1}$ at 37° C). The major difference in temperature effects was that black colonies developed only at 46° C on TSN agar.

Mixing the pure culture with intestinal contents or with

autoclaved intestinal contents did not inhibit or stimulate the growth of pure culture (Table 2). This suggests that there are no confounding bacterial growth substances in intestinal contents that could influence the in vitro growth of C. perfringens. However, the growth of the pure culture was more rapid than that of bacteria from intestinal contents. Colonies on plates inoculated with samples of the pure culture could be enumerated by 24 hr of incubation but it took about 60 hr of incubation for cfu to be effectively enumerated from chick gut contents. The slower rate of growth of bacteria from chick gut was responsible for the inability to accurately enumerate cfu on plates that were inoculated with chick gut contents and incubated for less than 60 hr (Tables 2 and 3).

The number of cfu enumerated from the pure culture sample were similar on TSN and Clostrisel agars (7.46 and 7.40) but lower on SPS and LLA agar (6.98 and 6.24, respectively) as shown in Table 3. In other experiments, however, the number of cfu on SPS agar from the same sample was similar or slightly higher than the cfu on TSN agar (Tables 1 and 4). The use of anaerobic dilution to further minimize aerobic exposure did not produce an increase of cfu. On the contrary, the number of cfu from anaerobically diluted sample tended to be lower than the cfu from the conventionally diluted sample (Table 3). This observation was true for all the media and was considered as a validation of the effectiveness of the aerobic plating technique for cultivation of this bacterial species.

Preparation of SPS and Clostrisel involved only the hydration

and sterilization of the dry agar base prior to plating. Both TSN and LLA required not only the hydration and sterilization of dry medium but the addition of a second reagent that had to be prepared and sterilized separately before plating. This additional preparatory step makes the cultivation of C. perfringens on TSN and LLA a more laborious procedure especially so because of the need to maintain sterile conditions while dispensing aliquots of the mixed reagents just before plating. The pinpoint size of cfu on Clostrisel agar and the opaqueness of the LLA medium made it difficult to enumerate cfu on these two media. The lower colony counts on Clostrisel and LLA for most samples tested, as shown in Table 3, are related to these enumeration difficulties. This difficulty was encountered to a greater extent for enumerating cfu from chick gut samples than from pure culture. This was further complicated by the slower rate of growth of the bacteria from chick gut samples. The number of cfu reported for cecal contents or cecal contents and pure culture in Table 3 are, at best, estimates. The differentiation of cfu on plates with mixed samples (cecal content + pure culture) is also an approximation although the presence of black colonies on TSN plates and opalascence zones on LLA made the differentiation more accurate on the two media.

Based on the observations reported in Table 3, the TSN and SPS agar were used to enumerate cfu from three intestinal segments. The results are given in Table 4. Except for cecal contents, the cfu enumerated on SPS agar were higher than those on TSN agar. As may

be expected the cfu on TSN agar increased from the anterior to the posterior part of the intestine (6.36 in the duodenum, 7.29 in ileum and 7.67 in the cecum/g of contents); on SPS agar the cfu were 6.97 in the duodenum, 7.78 in the ileum and 7.63 in the cecum/g of contents. The bacterial colony counts from chick gut contents observed in this study which were in the order of 10^6 to 10^7 /g of contents are much higher than the documented populations of C. perfringens from chick intestine which are in the order of 10^2 to 10^4 /g (2,20).

A major requirement for an effective selective medium is the ability to grow the bacteria in a manner that morphological characteristics of the bacteria can easily be observed and used as a basis for presumptive identification. The sulfite reduction property of C. perfringens was the basis for developing the SPS agar by Angelloti et al. (1). However, it was observed that the medium also supported the growth of other sulfite reducing bacteria. The TSN agar of Marshal et al. (13) was proposed as an improved modification of the SPS agar but the medium was found to inhibit the growth of some C. perfringens strains. In this study black colonies developed on TSN agar at 46° C but not on SPS agar from pure culture samples (Tables 2 and 5). No black colonies were observed on either media from chick gut contents (Table 5). In another study (5) one plate containing black colonies was observed out of 224 SPS plates inoculated with ileal and cecal contents of 32 chicks. Other media that have been developed include the TSC (tryptose-sulfite-

cycloserine) agar of Harmon et al. (8) initially proposed to be used with egg yolk. The use of egg yolk in a medium exploits another distinctive characteristic of C. perfringens which is the production of lecithinase, a toxin that produces a marked opalescence around the colony on egg yolk media or blood agar (1). Colonies from the pure C. perfringens culture developed characteristic zones of lysis on the LLA medium in this study. This characteristic is a distinctive advantage of the media. No such zones were observed on LLA plates inoculated with intestinal contents. There was no visually distinctive characteristic for C. perfringens on clostrisel.

The morphological characteristics of C. perfringens cells are well documented (4,7,9). Among the most distinguishing features are its rod-shaped structure and spore formation. Table 5 is a summary of the observed morphological characteristics of the colony forming units that were enumerated in all experiments. All colonies examined from pure culture plates were rod-shaped, but colonies from plates inoculated with intestinal contents were a mixture of rods and cocci. Plates with mixed samples (pure culture and cecal contents) exhibited the gross and cellular morphological characteristics of both samples. The larger colonies from gut content plates which tended to grow more rapidly than the pinpoint colonies were always cocci. The only Clostridium species known to have cocci (coccibacilli) morphology is C. coccoides reported to have been found in the feces of mice (7). The observation of coccoid bacteria raises serious questions about the selectivity of

clostrisel, SPS, TSN and LLA media for the cultivation of C. perfringens from intestinal contents. Spores were rarely observed in any of these which was not unexpected (4). The difficulty encountered in associating observed cellular morphological characteristics with those that have been defined for C. perfringens led Schaefer and Brotz (14) to label colony forming units on clostrisel agar simply as clostrisel counts. It appears that Stutz et al. (17,18) in their initial studies enumerated all the bacteria growing on TSN agar as C. perfringens. The results of this study do not support such conclusions. Furthermore, Barnes et al. (2) using strict anaerobic techniques have demonstrated that C. perfringens in the chick intestine occurs only occasionally. The sporadic occurrence of C. perfringens at low concentrations of 10^2 to 10^4 /g of intestinal contents have been reported by other investigators (15,20). This is in contrast to the number of cfu of 10^6 to 10^7 reported in this study on TSN and SPS agar. It is concluded that the media evaluated here are not suitable for the selective cultivation of C. perfringens from chick intestine. Additional and extensive nutritional biochemical tests would be needed to confirm that the intestinal bacteria enumerated on these media are C. perfringens.

In view of the continuing interest in the role of this bacteria in chick nutrition, there is need for the development of techniques that can be used to accurately and rapidly identify and enumerate C. perfringens when it is present at low cell concentrations within the

dense population of enteric bacteria in chicks. A recently proposed technique by Erickson and Diebel (6) does offer some promise. Stutz and Lawton (18) adapted this technique and reported that all antibacterial agents that promoted growth on their sucrose diet also resulted in significant reductions in ileal contents of C. perfringens (18,19). Further research is needed to validate the selectivity and effectiveness of this and other media for the accurate and rapid enumeration of C. perfringens from chick intestine.

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Table 1. Colony forming units (cfu in \log_{10}) from pure *C. perfringens* culture (ATCC 13124) on TSN (tryptose-sulfite-neomycin) and SPS (sulfite-polymyxin-sulfadiazine) agars at two incubation temperatures.^{1,2}

Temperature	Medium	
	TSN (n=5)	SPS (n=2)
46°C	8.29 ± 0.03 ³	8.32 ± 0.29
37°C	8.18 ± 0.05	8.00 ± 0.07

¹Incubation for 24 hr.

²C.f.u./0.25 ml of pure culture sampled at late log phase of second transfer inoculum.

³Black colonies.

Table 2. Number of cfu (\log_{10} + SEM) from cecal samples and a pure *C. perfringens* (ATCC 13124) on TSN (tryptose-sulfite-neomycin) agar

Source of bacteria	Colony forming units (n=2)
ATCC 13124 ²	6.68 \pm 0.01
Cecal contents + ATCC 13124	6.40 \pm 0.04 ³
Autoclaved cecal contents + ATCC 13124	6.47 \pm 0.09

¹Incubation at 37°C for 24 hr.

²0.25 ml of pure culture sampled at late log phase of second transfer inoculum.

³"Perfringens-like" colonies enumerated/g. Many pinpoint sized colonies not enumerated.

Table 3. Colony forming units ($\log_{10} \pm$ SE) and degree of difficulty for preparation of medium and enumeration of cfu on TSN, SPS, LLA (lecithin-lactose-agar base) and clostrisel media¹

Source of bacteria	Medium (n=2)			
	TSN	SPS	LLA	Clostrisel
ATCC 13124 ²	7.46 \pm 0.06	6.98 \pm 0.05	6.24 \pm 0.12	7.40 \pm 0.30
ATCC 13124 (anaerobically diluted)	7.06 \pm 0.04	6.83 \pm 0.01	6.06 \pm 0.02	6.43 ³
ATCC 13124 + cecal contents ⁴ "Perfringens-like" colonies ⁵	6.87 \pm 0.02	6.89 \pm 0.00	6.23 \pm 0.12	+++ ⁶
Other colonies	7.26 \pm 0.02	7.28 \pm 0.02	6.90 \pm 0.03	+++
Cecal contents ⁷	6.84 \pm 0.09	7.21 \pm 0.05	6.95 \pm 0.04	6.23 \pm 0.01
Degree of difficulty -for medium preparation ⁸	++	+	++	+
-for bacterial enumeration ⁹	+	+	++	+++

¹Plates were incubated at 46° C for 48 hr.

²See Table 2.

³n=1.

⁴Colony forming units are approximate because there were many pinpoint colonies not enumerable.

⁵Morphology similar to that of cfu from ATCC 13124.

⁶Pinpoint size colonies, not enumerable.

⁷Many pinpoint size colonies not enumerable.

⁸+, one-step preparation; ++, two-step preparation.

⁹+, *C. perfringens* colonies were distinct and easy to enumerate; ++, medium opaque; +++, pinpoint colony size makes it very difficult to accurately enumerate.

Table 4. Enumeration of cfu ($\log_{10} \pm \text{SE}$) on TSN and SPS agar¹

Source of bacteria	Medium	
	TSN	SPS
ATCC 13124 ²	7.24 \pm 0.06	7.41 \pm 0.09
Duodenum ³	6.36 \pm 0.08	6.97 \pm 0.04
Ileum ³	7.29 \pm 0.09	7.78 \pm 0.04
Cecum ³	7.67 \pm 0.05	7.63 \pm 0.11

¹Intestinal samples incubated at 46°C for 66 hr.

²See Table 2.

³cfu/g of contents.

Table 5. Colony morphology of bacteria cultivated on PS and TSN agar media

Source of bacteria	Medium	Gross morphology	Cellular morphology	Gram stain
ATCC 13124 ^a	TSN ^b (46°C)	Black colonies, subsurface with entire or undulate edges and round, 4-8 mm in diameter	Pleomorphic rods, smooth or granular, mainly single but some diploids, 5-75/4 long	positive
ATCC 13124	TSN (37°C)	Same as for 46° C incubation but no black colonies were observed	Pleomorphic rods, smooth or granular, mainly single but some diploids 5-75/4 long.	positive
ATCC 13124	SPS (37°C or 46°C)	Light brown, subsurface colonies with entire or undulate edges and round, 4-8 mm in diameter	Pleomorphic rods, smooth or granular, mainly single but some diploids 5-75/4 long.	positive
Chick gut lumen content ^c	TSN (46°C)	4-8 mm in diameter Predominantly milky white colonies of variable sizes (2 mm diameter). Both surface and subsurface with entire edges.	Mixed cocci and pleomorphic rods (5-18/4 in length). Cocci occurred as single cells or as a few chains (2-8 cells). Rods were predominately short.	positive
Chick gut lumen content ^c	SPS (46°C)	Larger colonies (2 mm diameter) were lenticular and subsurface. Predominantly milky white colonies of variable sizes (2 mm diameter). Both surface and subsurface with entire edges.	Cocci only. Single cells and chains of variable length. Mixed cocci and pleomorphic rods (5-18/4 in length). Cocci occurred as single cells or as a few chains (2-8 cell). Rods were predominately short.	positive
		Larger colonies (2 mm in diameter) were lenticular and subsurface.	Cocci only. Single cells and chains of variable length.	

^aColony morphologies were same for aerobically and anaerobically diluted samples.

^bIn one out of 4 trials, black colonies were not observed on TSN plates.

^cColony morphology was similar for all three segments of the intestine.

CHAPTER V

EFFECTS OF ANTIBIOTICS AND WATER TREATMENT ON BROILER CHICK GROWTH,
INTESTINAL CHARACTERISTICS AND INTESTINAL BACTERIAL POPULATIONS

(to be submitted to the Journal of the Science of Food and Agriculture).

EFFECT OF ANTIOTIOTICS AND WATER TREATMENT ON BROILER
CHICK GROWTH, INTESTINAL CHARACTERISTICS AND
INTESTINAL BACTERIAL POPULATIONS¹

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Summary

Experiments were conducted to evaluate the growth response to antibiotics and Microaid (dried Yucca extract) in broiler chicks reared on "clean" and "dirty" water treatments. The inclusion of Microaid in the diet significantly ($P < 0.05$) depressed growth on "dirty" water and antibody response on "clean" water. The growth response to penicillin was independent of water treatment. The magnitude of growth response was at levels similar to those reported for this antibiotic from the same laboratory in 1950-1960. Bursal weights were significantly increased ($P < 0.05$) and intestinal weights significantly decreased ($P < 0.01$) in chicks fed penicillin. The effects of penicillin and tylan on intestinal thickness and length were inconsistent. Bacterial counts from ileal and cecal contents on SPS and K F Streptococcus agar media were unaffected by penicillin and water treatment.

Keywords: Antibiotics, dirty water, Microaid, chick growth, chick intestine, bursal weight, Clostridium perfringens.

1. Introduction

The mode of action of dietary antibiotics in growth promotion is poorly understood in spite of over thirty years of active research on the subject. Because of the ever growing controversy on the subtherapeutic use of antibiotics in agriculture, there is need to continue this research in order to provide scientific data that can be used as a basis for decisions to deregulate, restrict or ban the use of any antibiotics for non-therapeutic or therapeutic functions.

Coates et al. were among several investigators to report the lack of growth response to antibiotics in a "new" environment.¹⁻³ Antibiotics were also reported to have no growth effect in gnotobiotic chicks.⁴ These results were interpreted as implying that there was present in the "old" environment a subclinical level of growth depressing infection which was counteracted by the antibiotics. Later reports, however, showed a growth response to antibiotics in a "clean" environment.^{5,6} The lack of growth response to antibiotics in gnotobiotic animals and the observation that the only common denominator to all antibiotics is their ability to inhibit some microorganisms in vitro suggests that antibiotics exert their growth promoting effects by acting on the intestinal microflora of the animals. However, attempts to show that dietary antibiotics can produce significant changes in the populations of bacteria in the gut have proven to be inconclusive.⁷ Recent claims by Stutz et al. on the reduction of Clostridium perfringens populations in the ileal contents of chicks fed dietary antibiotics prompted the investigations

reported in this study.⁸ It was hypothesized that the growth response to dietary antibiotics may be aggravated by the use of "dirty" water troughs and that it may be possible to relate such responses in growth to changes in intestinal bacterial populations.

2. Experimental

Day-old broiler chicks (sex not determined) hatched in the university poultry laboratory were randomly allocated to the experimental treatments in battery brooders at the beginning of each experiment. The standard management practices of the laboratory and the composition of the basal diet have previously been described,⁹ except that in this study the meat and bone meal was replaced by fish meal. The sources of antibiotics (incorporated at 50 mg/kg of diet) were also described in the previous report. Microaid (used in experiment 1 at 56 mg/kg) is a natural plant product prepared by drying and pulverizing the stems of Yucca shidigera. The product is reported to produce broiler growth promotion when used as a feed additive¹⁰ and to decrease poultry house litter ammonia when mixed into the litter.¹¹

The water treatment involved washing or no washing of the water troughs from which the chicks drank water. "Clean" water troughs were washed at specific intervals with hot water from a pressure hose and scrubbed down thoroughly with a hard brush. "Dirty" water treated birds were started on "clean" water but the water troughs were not washed throughout the duration of the experiment (15 days for experiment 3 and 21 days for all other experiments). Clean water was

supplied regularly to prevent the troughs from drying out in the course of an experiment. This clean water was tap water that was part of a municipal water supply which contains about 0.1 ppm added chlorine. The distribution of dietary and water treatments as well as the number of chicks per treatment for each of the five experiments are shown in the tables. Chicks were weighed at 3 day intervals in experiments 2 and 3 but at weekly intervals in the other experiments. Feed intake and percent mortality were computed weekly. Water intake in experiments 3 and 4 was determined by taking the difference between water added and discarded at time of washing. Adjustment was made for evaporative losses by measuring the water loss from water troughs that were not part of the experiment but placed in the vicinity of the experimental units.

The antibody response to sheep red blood cells was measured in experiment 1 using blood samples taken from 12 birds per treatment. Bursal and intestinal weights were measured in experiment 2 on 10 birds per treatment. Intestinal thickness and lengths were determined on 8 birds per treatment (experiments 3 and 4). In all cases the chicks were selected at random from all the replicates. The method for measuring antibody response, bursal and intestinal weight had previously been described.¹² Intestinal thickness was measured using a pressure sensitive micrometer (The Dyer Company, Lancaster, PA). Intestinal segments were voided of fecal material prior to thickness determination. Thickness of the intact intestine (double

wall) was measured and then divided by two to give the thickness of intestinal wall.

The chemical oxygen demand (COD) of water samples was determined by AOAC methods.¹³ Water samples for analysis were taken prior to washing of "clean" water troughs. Dirtiness score (extent of water contamination by feeds, feces and other debris) was a subjective estimate of the degree of dirtiness by two of the investigators. The sulfite-polymyxin-sulfadiazide (SPS) agar (BBL Microbiological Systems, Cockeysville, MD) and K F Streptococcus agar (Difco Laboratories, Detroit, MI) which are commercial formulations for the detection and/or enumeration of C. perfringens and fecal streptococci, respectively, were employed to enumerate bacteria from ileal and cecal lumen contents. Prior to culture and enumeration of the bacteria, preliminary evaluations were made to determine appropriate dilutions, temperature and incubation time for cultivation and enumeration of bacteria from chick gut contents (Dafwang et al., unpublished). The effectiveness of an aerobic pour plate technique for the cultivation and enumeration of a pure culture of C. perfringens (ATCC 13124) on SPS and TSN (tryptose-sulfite-neomycin) (BBL) agar media was also tested in the preliminary study. For bacterial enumeration, four chicks were selected randomly from each treatment and killed by axial dislocation. One g of lumen contents from each of the appropriate intestinal segments was aseptically transferred into 9 ml of a 0.1% sterile Trypticase (BBL) solution and tenfold dilutions up to 1×10^{-6} were prepared for each sample. Aliquots of 0.5 ml of the

10^{-4} through 10^{-6} dilutions were aseptically added to the SPS and K.F. Streptococcus agar (9.5 ml each) media previously prepared according to manufacturer's instructions. The mixture was vortexed for about 20 secs and poured into sterile 60 x 15 mm petri plates. All samples were prepared in duplicate. After allowing to cool at ambient conditions for about 45 min., the plates were incubated at 46°C in a COY Anaerobic Chamber (COY Laboratory Products, Ann Arbor, MI) for 66-72 hours. Enumeration was done on dilution plates that contained between 30 and 300 colonies. Representative colonies from enumerated plates were examined under a phase-contrast microscope at 1000 fold magnification. Both the gross and microscopic morphological characteristics were observed and compared to characteristics of the pure C. perfringens culture previously tested to detect similarities that could indicate the presence of C. perfringens on the plates. The results were subjected to statistical analysis.

3. Results

3.1 Broiler performance. In comparison to chicks fed the penicillin diets, growth of chicks fed Microaid was significantly ($P < 0.05$) depressed in experiment 1 (Table 1). Non-significant ($P > 0.05$) growth responses of 0.8% on "clean" water and 9.7% on "dirty" water were observed for penicillin fed chicks in comparison to chicks fed the basal diet in the same experiment. However, the pooled penicillin effect on growth promotion was significant ($P < .05$ in Table 1). In experiments 2, 4 and 5 dietary penicillin significantly ($P < .001$) improved growth (Fig 1. and Tables 5 and 6). This growth

10^{-4} through 10^{-6} dilutions were aseptically added to the SPS and K.F. Streptococcus agar (9.5 ml each) media previously prepared according to manufacturer's instructions. The mixture was vortexed for about 20 secs and poured into sterile 60 x 15 mm petri plates. All samples were prepared in duplicate. After allowing to cool at ambient conditions for about 45 min., the plates were incubated at 46°C in a COY Anaerobic Chamber (COY Laboratory Products, Ann Arbor, MI) for 66-72 hours. Enumeration was done on dilution plates that contained between 30 and 300 colonies. Representative colonies from enumerated plates were examined under a phase-contrast microscope at 1000 fold magnification. Both the gross and microscopic morphological characteristics were observed and compared to characteristics of the pure C. perfringens culture previously tested to detect similarities that could indicate the presence of C. perfringens on the plates. The results were subjected to statistical analysis.

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promotion was independent of water treatment. Penicillin fed chicks on the "dirty" water treatment manifested the best numeric growth in experiments 2 and 5 (Fig 1 and Table 6). No growth response to tyran or water treatment was observed in experiment 3 (Table 3). Cumulative mortality in all experiments was about 2% and was not affected by antibiotics or water treatment.

3.2 Feed efficiency and water intake. In experiment 2, a significant improvement in feed efficiency ($P < .01$) was obtained due to dietary penicillin for 6 and 12 day old chicks (Table 2). In other experiments, numeric improvements in feed efficiency were observed concurrently with significant improvements in weight gain (Tables 1, 5 and 6). A significant improvement in feed efficiency was obtained on the basal and "dirty" water treatment combination compared to that of "clean" water and tyran combination (0.675 vs. 0.611) in experiment 3 (Table 3) but this was not related to an increase in weight gain. Water intake was not affected by tyran or water treatment in the same experiment. A significant increase ($P < .001$) in water intake was observed in experiment 5 (Table 6) for chicks on "dirty" water. Both dirtiness score and chemical oxygen demand numerically increased with time (Table 4) in most cases. This indicates that the degree to which the water was contaminated increased with time. The values for both variables were higher on the "dirty" water treatment from 6-12 days.

3.3 Antibody response, bursal weights and intestinal characteristics. The effect of penicillin and "dirty" water on antibody response was not-significant (Table 1). Antibody response to

sheep red blood cells in Microaid fed chicks was significantly depressed on "clean" water but not "dirty" water (Table 1). Both the lowest and highest titers were obtained on the Microaid treatment. Feeding penicillin significantly increased bursal weight ($P < 0.05$) but decreased intestinal weight ($P < 0.001$) in experiment 2 (Table 2). For all intestinal segments, intestinal wall thickness tended to be reduced in chicks on "clean" water (Table 3). However, the difference was only significant ($P < 0.01$) for the jejunal segment. In contrast chicks on "dirty" water tended to have reduced intestinal lengths. The length of the ileum of chicks on "clean" water was significantly ($P < 0.05$) longer than that of chicks on "dirty" water (Table 3). In experiment 4, penicillin had no effect on intestinal thickness and length (Table 5).

3.4 Bacterial counts. No significant differences were observed in intestinal content bacterial counts on SPS and K F Streptococcus agar (Table 6). The gross morphological characteristics revealed a variety of surface and subsurface colony shapes and sizes. Under the microscope, bacteria picked from colonies growing on both media were predominantly pleomorphic rods and cocci. The larger colonies on SPS agar were predominantly cocci and those picked from the Streptococcus agar were predominantly rods. Black colonies were observed on only one plate. This was a plate prepared from the 10^{-2} dilution of cecal contents of one of the chicks on the penicillin and "dirty" water treatment combination. Because of the high density of bacteria on the 10^{-2} plate, it was impossible to examine or isolate the black

colonies without contamination. Nevertheless, microscopic examination of organisms from such colonies revealed a mixture of rods and cocci.

4. Discussion and Conclusions.

The lack of growth response to Microaid in this study is in contrast to the results of Johnston et al.¹⁰ These investigators reported significant growth promotion in broiler chicks at 28 and 51 days. The observation that antibiotic growth promotion is independent of water cleanliness is contrary to the emphasis on regular cleaning of water troughs as a standard management practice in animal husbandry. This study indicates that under conventional rearing conditions, healthy chicks can tolerate dirty water troughs for extended periods of time without adverse effects on growth, feed efficiency, water intake and survival. That in two of the experiments (Fig 1 and Table 6), the best numeric growth at 21 days was obtained on the penicillin plus "dirty" water treatment combination seems to suggest that there may be a symbiotic effect between microbes in the "dirty" water, the presence of dietary antibiotics and colonization of the chick gut by desirable bacteria. These results do not support the allegations by critics as well as advocates of subtherapeutic antibiotic usage that such use is a substitute for good management practices.¹⁴ It is possible that a certain level of exposure to microbial contamination is beneficial for optimum growth of animals under conventional rearing conditions. Fuller and Coates recently alluded to this principle by stating that "it is perhaps possible to be too hygienic to the point where desirable as well as pathogenic

microorganisms are being removed from the environment."¹⁵ Studies by Nurmi and Rantala demonstrated that chicks increased their resistance to Salmonella infection when they were administered a suspension of adult bird feces.¹⁶

The laboratory facilities in which these studies were conducted have been exposed to continuous use of subtherapeutic levels of dietary penicillin for the past thirty-four years but the magnitude of growth promotion by this antibiotic has persisted at about the same level. The pooled mean improvement in body weight by penicillin in this study which was conducted between 1984-1985 was 11.0% (range of 0.9-18.3%). Earlier reports from the same laboratory indicated mean improvements in body weight of 8.5% in 1950-1953, 8.8% in 1956-1960 and 11.3% in 1981-1984 by the same antibiotic.^{17, 18} The phenomenon by which growth promotion has persisted in this environment is not understood. The possibility that the bacteria which cause growth depression have remained susceptible to antibiotics has been suggested.⁷ Fig. 1 shows that the maximum growth response to penicillin was between days 3-12. Such information is beneficial for the design of experiments to monitor changes in metabolic and other bodily parameters due to dietary antibiotics. It also demonstrates the complex nature of the antibiotic growth response because by this age the amount of antibiotic ingested by the chick is miniscule (4 mg total intake/chick by 6 days) and yet they produce such remarkable results.

The lack of effect on antibody response and increased bursal size by dietary antibiotics has been observed before.¹² A reduction in intestinal weight by antibiotics has always been regarded as an index of decrease in intestinal thickness. The results reported here indicate that a lower intestinal weight does not necessarily mean decreased intestinal thickness (Table 2 vs Tables 3 and 5). The hypothesis has been advanced that a decreased intestinal weight and thinner intestinal wall would result in an increase in intestinal absorption of nutrients with consequent stimulation of growth.⁴ A recent review on the subject, however, indicates that a thinner intestinal wall which is typical in germ free chicks may result in an increase in intestinal absorption in vitro but results of in vivo studies have been inconclusive.¹⁵

The finding that penicillin feeding did not produce a decrease in intestinal bacteria is consistent with the results of other investigators.⁷ Stutz et al.⁸ have advocated that the growth promoting effects of antibiotics are due to the suppression of C. perfringens in the chick gut. These investigators apparently enumerated every bacteria growing on their TSN agar as C. perfringens. The results of this study demonstrate that the selectivity of these media are questionable when used with a mixed population of intestinal bacteria. TSN and SPS agar were examined in the preliminary studies cited earlier and were found to be comparable in their ability to support the growth of bacteria from chick gut that had very contrasting morphological characteristics to those of a pure

culture of C. perfringens cultivated on the same media under the same conditions. It is concluded that these media are not suitable for the enumeration of C. perfringens from among the myriad of bacteria that exist in the chick gut. The recurrent appearance of cocci bacteria on SPS and TSN agars prompted the use of the K.F. Streptococcus agar in this study. It was thought that if the Streptococcus agar was highly selective, the difference in bacterial counts between SPS and K.F. Streptococcus agar could be the population of the rod-shaped bacteria which may be related to Clostridium populations. However, it was found that the Streptococcus agar also had limited selectivity. The possibility that other bacteria present in a given sample can interfere with the detection and identification of C. perfringens has been recognized.¹⁹

C. perfringens has long been a suspect bacterium in the mode of action of antibiotics in growth promotion, and has been the subject of a number of investigations.²⁰⁻²³ Whether C. perfringens in the low populations at which they occur in the chick gut as reported by Timms²⁴ and Barnes et al.²⁵ play a significant role in the mode of growth promotion by antibiotics remains to be validated.

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Table 1. Performance of broiler chicks fed dietary penicillin or Microaid and reared on water troughs with or without regular washing from 0-21 days (experiment 1).^{1,2}

Treatment		Weight gain (g)	Feed Efficiency	Antibody titer ⁴
Wash ³	Feed Additive			
-	-	432 ^{ab}	0.574	9.4 ^{ab}
-	Penicillin	474 ^{ab}	0.611	9.2 ^{ab}
-	Microaid	414 ^b	0.535	10.3 ^a
+	-	494 ^a	0.603	8.5 ^{ab}
+	Penicillin	498 ^a	0.556	9.7 ^{ab}
+	Microaid	452 ^{ab}	0.570	7.8 ^b
Significance of main effects ⁵				
Feed additive		*	NS	NS
Wash		NS	NS	NS

¹ Used 3 replicates of 8 chicks/treatment.

² Within each column means with different superscripts are significantly different $P < .05$.

³ - No washing; +, water troughs washed every 2 days.

⁴ Sheep red blood cell response expressed as a mean hemagglutinating antibody titer (\log_2).

⁵ NS, Not significant; *Significant $P < .05$.

Fig 1. Relative weight gain (%) of chicks fed diets with or without penicillin and reared on water troughs with or without regular cleaning (expt. 2). Daily washing (—△—); 3 day washing (—✱—); No washing (—⊖—); Daily washing + penicillin (—✱—); 3 day washing + penicillin (—); No washing + penicillin (—◇—). Weight gain was expressed to relative that of 3 day interval washing without penicillin.

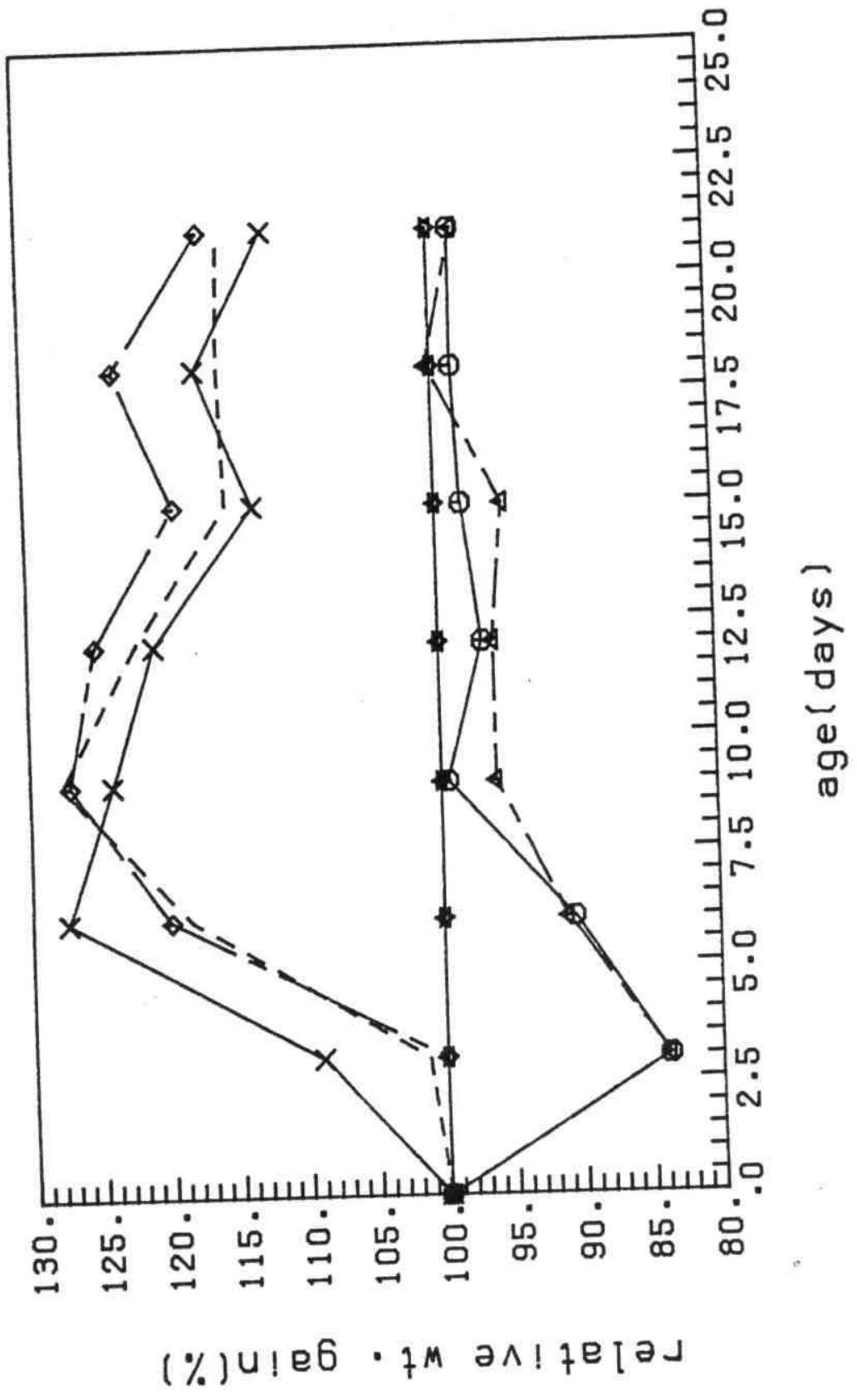


Table 2. Feed efficiency, bursal and intestinal weights of chicks fed diets with or without penicillin and reared on water troughs with or without regular washing (experiment 2).^{1,2,3}

Treatment		Feed Efficiency at age:			Bursal	Intestinal
Wash ⁴	Penicillin	6 days	12 days	21 days	weight ⁵	weight ⁵
1	-	.527 ^{bc}	.558	.568	204 + 14 ^b	2.35 + .28 ^a
3	-	.458 ^c	.537	.567	221 + 21 ^{ab}	2.44 + .12 ^a
-	-	.505 ^{bc}	.578	.600	220 + 18 ^{ab}	2.13 + .10 ^{ab}
1	+	.611 ^{ab}	.644	.578	275 + 33 ^a	1.80 + .06 ^c
3	+	.672 ^a	.659	.606	228 + 19 ^{ab}	1.83 + .08 ^{bc}
-	+	.578 ^{ab}	.668	.631	278 + 22 ^a	1.68 + .02 ^c
Significance of main effects ⁶						
	Penicillin	**	**	NS	*	***
	Wash	NS	NS	NS	NS	NS

¹ Used 4 replicates of 8 chicks per treatment.

² Body weight shown in Fig 1.

³ Within each column means with different superscripts are significantly different ($P < .05$).

⁴ -, No washing, 1, washed everyday; 3 washed every 3 days.

⁵ Bursal and intestinal weights in mg/100 g (\pm SE) of body weight

⁶ NS, not significant ($P < .05$); *, Significant at $P < .05$; **, Significant at $P < .01$; ***, Significant at $P < .001$.

Table 3. Growth performance and intestinal characteristics of chicks fed dietary tylan and reared on water troughs with or without regular washing 0-15 days (Expt 3).^{1,2}

	Treatment				Significance of main effects ⁵	
	"Clean" water ³		"Dirty" water ⁴		Wash	Tylan
	--	+ Tylan	--	+ Tylan		
Weight gain (g) ⁶	201	210	207	197	NS	NS
Feed efficiency ⁶	0.659 ^{ab}	0.611 ^b	0.675 ^a	0.657 ^{ab}	NS	NS
Water Intake (L) ⁶	0.491	0.551	0.535	0.511	NS	NS
-- Intestinal thickness (mm) --						
Duodenum	0.484	0.517	0.552	0.524	NS	NS
Jejunum	0.389 ^{ab}	0.317 ^b	0.420 ^{ab}	0.475 ^a	**	NS
Ileum	0.387	0.377	0.452	0.425	NS	NS
Cecum	0.331	0.344	0.398	0.399	NS	NS
Colon	0.566 ^{ab}	0.466 ^b	0.548 ^{ab}	0.598 ^a	NS	NS
-- Intestinal length (cm) --						
Duodenum	20.7	20.1	19.5	19.3	NS	NS
Jejunum	42.0	46.2	39.2	42.6	NS	NS
Ileum	39.9 ^{ab}	46.6 ^a	36.4 ^b	38.6 ^{ab}	*	NS
Cecum	9.5	9.8	9.0	9.5	NS	NS
Colon	5.4	5.9	5.2	5.6	NS	NS

¹ Used 4 replicates of 8 chicks per treatment.

² Means with different superscripts are significantly different $P < .05$

³ Water troughs washed every 3 days.

⁴ Water troughs not washed for 15 days.

⁵ NS = Not significant $P < .05$; *, Significant at $P < .05$; **, Significant at $P < .01$.

⁶ Cumulative for 15 days.

Table 4. Dirtiness Score (DS)¹ and Chemical Oxygen Demand (COD)² of water drunk by chicks fed diets with or without Tylan and reared on water troughs with or without regular washing (Expt 3).

Duration of experiment (days)		Treatment			
		"Clean" Water ³		"Dirty" Water ³	
		Basal	+ Tylan	Basal	+ Tylan
6	DS	1	1	1.25	1.25
	COD	20.2	18.1	64.8	38.3
9	DS	1.25	1.25	2.25	2.25
	COD	35.3	29.2	53.7	62.8
12	DS	2.25	2.25	3.25	3.25
	COD	164.9	128.1	237.6	172.6
18	DS	1.75	2.75	3.75	3.75
	COD	239.2	200.2	154.6	324.3

¹ Clean = 1; Slightly dirty = 2; dirty = 3; very dirty = 4.

² Chemical Oxygen Demand in mg/L.

³ See Table 3.

Table 5. Weight gain, feed efficiency and intestinal characteristics of chicks fed dietary penicillin (Expt 4).¹

	Basal	+ Penicillin	Significance ²
Wt. gain (g) ³	358	438	***
Feed Efficiency ³	.580	.603	NS
-- Intestinal thickness (mm) --			
Duodenum	.423	.476	NS
Jejunum	.430	.386	NS
Ileum	.434	.352	NS
Cecum	.376	.410	NS
Colon	.484	.552	NS
-- Intestinal length (cm) --			
Duodenum	22.7	22.1	NS
Jejunum	47.7	45.6	NS
Ileum	49.4	47.4	NS
Cecum	11.5	11.7	NS
Colon	6.3	6.4	NS

¹ Used 4 replicates of 8 chicks per treatment.

² NS, Not significant; ***, Significant at $P < .001$.

³ Cumulative for 21 days.

Table 6. Growth performance and intestinal fecal bacterial counts of chicks fed diets with or without penicillin and reared on water troughs with or without regular washing (Expt 5).^{1,2}

	Treatment				S.E.	Significance of main effects ⁵
	"Clean" water ³		"Dirty" water ⁴			
	Basal + Penicillin	Basal + Penicillin	Basal + Penicillin	Basal + Penicillin		
Weight gain (g) ⁶	528 ^b	579 ^a	494 ^b	595 ^a	8.2	***
Feed efficiency ⁶	0.550	0.593	0.560	0.582	0.01	NS
Water Intake (L) ⁶	1.30 ^c	1.42 ^{bc}	1.59 ^{ab}	1.67 ^a	0.03	NS
---- Bacterial counts on SPS agar (\log_{10}/g) ----						
Ileum ⁷	7.2	6.6	7.2	7.1	0.20	NS
Cecum ⁸	8.3	8.0	7.9	8.3	0.07	NS
---- Bacterial counts on KF Streptococcus agar (\log_{10}/g) ----						
Ileum	7.0	6.7	7.4	7.1	0.18	NS
Cecum	7.4	7.0	7.2	8.2	0.18	NS

¹ Used 4 replicates of 10 chicks per treatment.

² Line means with same superscript are significantly different ($P < .05$).

³ Water troughs washed every 3 days.

⁴ Water troughs not washed for 21 days.

⁵ NS, Not significant; * Significant $P < .05$; *** Significant $P < .001$.

⁶ Total for 21 days.

⁷ Ileal contents taken on day 25.

⁸ Ileal contents taken on day 29.

CHAPTER VI

HIGH STOCKING DENSITY AS A MODEL FOR DETECTION
OF GROWTH PROMOTION BY DIETARY ANTIBIOTICS

(to be submitted to British Poultry Science)

HIGH STOCKING DENSITY AS A MODEL FOR DETECTION OF
GROWTH PROMOTION BY ANTIBIOTICS¹

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ABSTRACT

1. The growth depression caused by increasing the stocking density from a space allowance of $.044 \text{ m}^2/\text{chick}$ to $0.017 \text{ m}^2/\text{chick}$ for chicks in battery brooders was ameliorated by subtherapeutic levels of dietary antibiotics in two out of three experiments.
2. The high density regimen resulted in decreased relative weights of the bursa and thymus but no concurrent reduction in antibody response or spleen weights. Feeding dietary antibiotics tended to counteract the decrease in bursal weight due to high density.
3. Feces from birds on high density regimens contained significantly higher moisture.
4. Except for lincomycin on low density, housing fecal ammonia tended to be reduced by the presence of dietary antibiotics. The reduction was significant for dietary oxytetracycline under high density conditions.
5. Optimum growth performance and stocking density effects were used to compute the maximum stocking rate (D value = $0.208 \text{ cm}^2/\text{g}$ of chick) for chicks in battery brooders. The D value was used to compute maximum stocking rates for various classes of chicks.

INTRODUCTION

Although there is an extensive volume of published research on the growth promoting effects of dietary antibiotics under different environmental and/or dietary variables, information on the relationship between stocking density and dietary antibiotics is sparse. This is rather surprising considering that some opponents of the use of antibiotics as feed additives often suggest that antibiotics have been a contributory factor to present day factory farming methods which require growing large numbers of animals in a very limited space (Novick, 1979).

The deleterious effects of high stocking density on the growth of chicks have been reported by several investigators. Such effects include growth depression, increased mortality and increased moisture content of litter. (Hansen and Becker, 1960; Deaton et al. 1968; Bolton et al., 1972; Stanley and Krueger, 1981, and Vo and Fanguy 1982). However, these authors also demonstrated that the higher stocking densities resulted in decreased feed consumption and improved feed efficiency. A reduction in brooding fuel requirements by high stocking density has also been reported (Reece, 1978). The role of high density as a stress factor has been recognized. Vo and Fanguy (1982) reported a decrease in humoral immunity in birds on high density conditions with consequent significant reductions in blood lymphocytes but significant increases in heterophils. Skamser and Seeger (1958) found that antibiotics prevented the growth depression due to high density but Freeman and Manning (1976) studied the effect

of antibiotics on three types of stress factors and reported no consistent effect of the antibiotics on the response of the birds to stressors.

Information on the space requirement for young chicks grown in cages is also scanty. Winter and Funk (1956) recommended 64.5 cm²/chick for the first two weeks and 120 cm²/chick for the third and fourth weeks. North (1984) recommended 155 cm² for the standard Leghorn and 181 cm² for the medium-sized egg breeds for the period 0-5 weeks.

The experiments reported here were conducted to determine the effects of dietary antibiotics and stocking density on growth performance and to provide information on the space requirement for chicks reared in battery brooders.

MATERIALS AND METHODS

The chicks used for this study were Hubbard x Hubbard broilers hatched in the university's facilities. Chicks were reared in five tier brooder battery measuring 1.62 m high, x 1.4 m long and 0.91 m wide (5.3 x 4.58 x 3 ft). Each tier was partitioned into four cells for experimentation. Each cell measured .46 x .813 m. In experiment 1, stocking rates of 8, 12, 16 and 20 birds per cell were utilized. In experiments 2 and 3 a low density stocking rate (10 or 9 chicks per cell respectively) and a high stocking density (20/cell) were compared. Details of space specifications per chick are given in Table 1. At the beginning of each experiment four replicates of the appropriate number of chicks were randomly allocated to each

treatment. Allocation of treatments to each cell was in a manner that ensured equal chick population and distribution of treatments at each battery level.

Composition of the basal diet is given in Table 2. The dietary antibiotics were obtained commercially and the levels incorporated in the diet were the maximum approved levels for incorporating the antibiotics into feed for growth promotion (Feed Additive Compendium, 1984). Feed and tap water were given ad libitum in trough type feeders and waterers.

Individual body weights were measured weekly at which time, mean weights, feed intake, feed conversion and percent mortality were computed for each replicate. The presence of leg deformities was measured by using the subjective leg scoring method of Cook et al. (1984). Bursal and spleen weights and antibody response were determined as described by Dafwang et al. (1985). Thymus weight was determined by opening up the bird's neck after killing by axial dislocation, peeling off the lobes that line the left side of the neck and weighing them immediately. The three lymphoid tissues were taken from twelve birds per treatment. For antibody response assays, blood was sampled from twenty birds per treatment.

Determination of fecal ammonia concentration was by a colorimetric method (Skoog and West, 1976). The assay was done on eight fecal samples per treatment. Two fecal samples (about 10 g each) were taken from the fecal collection pans under each of the four replicate groups of chicks per treatment. The feces had accumulated over a 3 day

period. Colorimetric analyses were conducted on duplicate samples of 1 g each. Another set of 1 g samples in duplicate were dried to constant weight in a conventional force-draft oven at 100°C for dry matter and moisture determinations.

All data collected were subjected to the analysis of variance. The Duncan multiple range test (Steel and Torrie 1976) was used to assess the significance of differences between treatment means.

RESULTS

The growth promotion by penicillin in four-week old broiler chicks under low density conditions (.029 to .044 m²/chick) varied from -1% (875 vs. 885 g in table 4) to 14% (table 3). This level of growth promotion was significant ($P < .05$) in one out of four comparisons in the three experiments (tables 3, 4 and 5). Lincomycin significantly improved weights on the low density treatment (935 vs. 869 g in table 5) but growth promotion by oxytetracycline was only numerically superior to that of the basal (905 vs. 869 g in table 5). In contrast, all the antibiotics tested significantly improved growth under high density conditions (.017-.022 m²/chick). The main effect of feeding penicillin resulted in a significant ($P < .02$) growth response (table 3). The antibiotic main effect was significant ($P < .001$) in experiment 3 (table 5). The magnitude of four week old growth promotion ranged from 6.3% (913 vs. 859 g in table 4) to 11.2% (857 vs. 766 g in table 3) for penicillin. Growth promotion by lincomycin was 11.1% (906 vs. 815 g) and 7.5% for oxytetracycline (876 vs. 815 g) under the high density conditions (table 5). Growth of

chicks fed the basal diet only was significantly depressed by the highest stocking density at 4 weeks of age in experiment 1 and at 5 weeks of age in experiment 2. For all treatments, stocking density effect was significant at $P < .017$ for five week old chicks in experiment 2 (table 4); at $P < .012$ for two week old chicks and at $P < .057$ for four week old chicks in experiment 3 (table 5). A significant ($P < .027$) antibiotic x stocking density interaction on body weight was observed only for two week old chicks in experiment 3 (table 5).

In the absence of antibiotics, there was a tendency for feed conversion to improve with increasing density (correlation of $-.454$ for diets without antibiotics in experiment 1, significant at $P < .077$). The differences in feed conversion due to stocking density (main effect) were significant at $P < .007$ in experiment 2 (table 4); at $P < .001$ for two week old chicks and at $P < .021$ for four week old chicks in experiment 3 (table 5). Dietary antibiotics fed to chicks under low density conditions tended to reduce feed conversion to levels that were similar to those obtained due to high density with or without antibiotics in all three experiments, except for lincomycin in experiment 3 (table 5). The magnitude of improvement in feed conversion was also very variable. The main effect of antibiotic feeding showed significant ($P < .054$) improved feed conversion in two week old chicks and at $P < .044$ for four week olds in experiment 3 (table 5). In the same experiment the interaction between antibiotic and stocking density on feed conversion was significant ($P < .003$ and

$P < .04$ respectively at two and four weeks old). The significant interactions occurred because some of the antibiotics were effective at high density but not at low density.

Stocking density and antibiotics had no consistent effect on leg deformities and mortality rates in experiment 1 (table 3). This was also observed in the other experiments but are not reported. It was observed that the right leg manifested a greater degree of deformity than the left. Such findings have been reported (Cook *et al.* 1984).

Relative bursal weights were significantly reduced by high stocking density [$P < .04$ in experiment 2 (table 4) and $P < .01$ in experiment 3 (table 5)]. Thymus weights were also significantly reduced by high stocking density ($P < .001$ in table 5). However, there was no concurrent reduction in antibody response along with the reduction in bursal and thymus weights (table 5). Antibiotics and stocking density had no effect on spleen weights and antibody response (tables 3 and 5).

High stocking density significantly ($P < .001$) increased fecal moisture content (table 6). The highest values for fecal moisture were from the feces of high density chicks fed the three antibiotics. However, these values were not significantly different from the other treatments except for the fecal moisture of feces from penicillin fed chicks under low density conditions. Except for the lincomycin fed chicks on low density, fecal ammonia was numerically reduced by the presence of dietary antibiotics. A significant ($P < .05$) reduction in the ammonia content of feces from oxytetracycline fed chicks on high

density was observed when fecal ammonia content was expressed per gram of wet feces (table 6).

DISCUSSION

The wide variability and significant improvements in growth promotion and feed conversion by dietary antibiotics have been well documented (Bird, 1968). Standard deviation and coefficients of variation were not affected by the differences in number of chicks (low versus high density) per treatment in this study. The magnitude of growth promotion by antibiotics under high stocking density conditions of 6.3 to 11.2% is similar to the levels of 9 to 11% for three week old chicks under low density conditions in previous studies from the same laboratory (Dafwang et al. 1984). The use of high stocking density did not increase the magnitude of growth promotion but simply provided appropriate conditions under which reproducible growth promotion by antibiotics were detected. The implication is that under similar conditions, the potential growth stimulatory effect of an antibiotic can be demonstrated consistently from one experiment to another. Since high stocking density is an economically attractive management practice in industry, this study provides a model for the evaluation of antibiotics and other growth promotants under conditions similar to those obtained in industry. The use of laboratory diets as models for assaying the effectiveness of growth promotants (Marusich et al., 1977; Stutz et al. 1984) may be ideal for laboratory tests but extending such results to practical type conditions are questionable. Although the experiments in this report lasted four weeks, analysis of

the data collected in these studies (table 5) as well as other unpublished data from this laboratory indicate that the magnitude of growth response to antibiotics is greatest within the first two weeks of age in birds started from day old. Therefore, a refinement for the use of this model in the evaluation of growth promotants would be to use the maximum stocking density for the particular class of chicks per available area and raise the chicks from 0 to 14 days.

Improvements in feed conversion and increases in fecal moisture due to high stocking density observed in this study have been reported by other investigators (Bolton *et al.* 1972, Stanley and Krueger, 1981). The improvement of feed conversion may be due to a reduction in feed wastage or some feed intake restriction that can occur due to limitations of feeder space on the high density treatments. Fecal moisture can be expected to increase with high stocking density due to an increase in the frequency and accumulation of fecal droppings.

Although there was a tendency to have reduced fecal ammonia content by dietary antibiotics in this study, such reduction was significant only for oxytetracycline fed chicks on high density. Kitai and Arakawa (1979) reported a decrease in ammonia production from incubated fresh excreta in the presence of antibiotics that were either added directly to the excreta prior to incubation or added dietarily as feed additives to the chickens that produced the incubated excreta. Visek (1978) suggested that dietary antibiotics promote growth by decreasing intestinal ammonia production and also the fecal ammonia in the environment of the animal. However, Heth

(1963), was unable to demonstrate any consistent reduction in intestinal ammonia by dietary antibiotics in chicks. More recently Pimentel and Cook (1985) obtained conflicting results on the effect of dietary antibiotics on fecal ammonia content. The significance of the reduction in fecal and intestinal ammonia in the mode of action of antibiotics remains to be established.

In order to obtain information on the maximum stocking rates for chicks in battery brooders, growth response on the low and high stocking densities between weeks two to four were compared in all three experiments. In all cases the magnitude of growth promotion by antibiotics tended to decrease with age beyond two weeks. Management conditions became very difficult under high density beyond four weeks because the chicks completely filled up the space and were pushing against the pen partitions. Water troughs and feeders required frequent cleaning and filling. Manure pans were rapidly filled up in addition to the fact that the wire floors were sagging under the weight of the chicks. The four weeks weights were therefore taken as the optimum maximum weights that could be accommodated in the batteries. Based on these deductions, the maximum stocking rates for four classes of chicks (table 7) were computed. To arrive at these figures, 900 g (the maximum mean weights observed for high density chicks fed antibiotics) was taken as the mean body-weight of straight-run four week old broiler chicks. The calculations were as follows:

1. Area of single pen (cell) = 3744 cm².
2. At 20 chicks per cell, total chick weight = 20 x 900 g = 18,000 g.
3. It was assumed that this was the optimum chick weight that can be accommodated in the 3744 cm² of battery brooder cell.
4. If maximum stocking density = D_{max} in g/cm² then

$$D_{\max} = \frac{18,000}{3744} = 4.8 \text{ g/cm}^2$$
5. If the minimum space requirement per g of chick = D value
then D value = $\frac{1}{D_{\max}} = \frac{1}{4.8 \text{ g}} = 0.208 \text{ cm}^2/\text{g}$
6. For an expected body weight/chick of X_g at n weeks old:

Space requirement per chick = D value x X = Y up to n weeks old.

Where D = D value in cm²/g

X = expected mean chick weight in grams

Y = space requirement per chick in cm²

7. To determine the maximum stocking rate per unit area (e. g. 1 m²)

$$\text{No of chicks per m}^2 = \frac{1 \times 10^4 \text{ cm}^2}{Y \text{ cm}^2}$$

These equations are easy to manipulate. Based on the observations in this study, the equations appear to be valid because the choice of chick weight for the computation of D was based not only on growth performance but also on management considerations relating to manure removal and care of feeders and waterers. Whether a D value of 0.208 cm²/g chick can be taken universally remains to be validated. The computations for volume, feeder, and waterers were estimated by taking the available volume, feeder length and waterer length and dividing by the number of chicks as calculated above. The computed values for space requirement are less than those that have been recommended by others. Winter and Funk (1956), recommended 64.5

cm² for 0-2 weeks and 120 cm² for 3-4 weeks. North (1984) recommended 155 cm² for leghorns and 181 cm² for medium-sized breeds, from 0-5 weeks. The basis for those recommendations were not discussed by the authors. Deaton et al. (1971) and Reece (1978) proposed equations designed to determine optimum stocking rates for rearing broilers on litter floors. The equations incorporated male/female ratios or volume requirements that were dependent on measuring the standing height of the birds. These factors were not included directly with the equations proposed in this report because the estimates were based on the use of standard brooder batteries with volume specifications that are considered adequate for the chicks under consideration. Furthermore the male/female ratio is not a critical factor for this age of birds.

The results of this study suggest that under high stocking density growth promotion by dietary antibiotics is consistent. The use of high stocking density as a model for evaluating the effectiveness of antibiotics for growth promotion is suggested. The study proposes a procedure for determining the maximum stocking density and minimum space requirement for chicks in battery brooders.

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TABLE 1. Space specifications per bird in the three experiments.

Expt No.	Cage space m^2 (ft ²)	Volume $m^3 \times 10^{-3}$ (ft ³)	Feeding cm (in)	Drinking cm (in)
1	.044 (.469)	6.7 (.24)	10.2 (4.0)	5.7 (2.25)
1	.029 (.313)	4.5 (.158)	6.8 (2.7)	3.8 (1.5)
1	.022 (.234)	3.4 (.119)	5.1 (2.0)	2.9 (1.13)
1, 2 and 3	.017 (.188)	2.7 (.095)	4.1 (1.6)	2.3 (0.9)
2	.035 (.375)	5.4 (.19)	8.1 (3.2)	4.6 (1.8)
3	.039 (.417)	6.0 (.211)	9.0 (3.6)	5.1 (2)

TABLE 2. Composition (g/kg) of basal diet

Ground maize	498
Soybean meal (44%)	385
White grease	65
Fishmeal	25
Dicalcium phosphate	14
Limestone	8
Iodized salt	2.5
Methionine	2.5
Vitamin-mineral mix ¹	+

¹ Vitamin mineral mix added to supply the following per kg of diet: vitamin A, 7500 I.U., vitamin D, 440 I.U.; vitamin K (MPB), 1.11 mg; riboflavin, 7.2 mg; calcium pantothenate, 20 mg; biotin, .31 mg; folic acid, 1.11 mg; niacin, 54.4 mg; vitamin B₁₂, .02 mg; choline chloride, 2600 mg; manganese, 110 mg; and zinc 80 mg.

TABLE 3. Effect of stocking density and dietary penicillin on 4 week growth performance of cage reared broiler chicks (Expt. 1)¹.

Cage floor space (m ²)	Penicillin (mg/kg)	Body Weight (g)	% Increase	Feed Conversion	Leg Score ²		% Mortality
					Right	Left	
.044	0	827 ^{bc}	-	1.85	1.78 ^{ab}	1.52	3.1
.044	50	862 ^{ab}	4.2	1.73	2.13 ^a	1.76	9.4
.029	0	784 ^d	-	1.78	1.49 ^b	1.34	4.1
.029	50	894 ^a	14.0	1.68	1.76 ^{ab}	1.60	2.1
.022	0	790 ^{cd}	-	1.77	2.16 ^a	1.76	3.1
.022	50	873 ^{ab}	10.5	1.76	1.92 ^{ab}	1.81	1.6
.017	0	766 ^d	-	1.69	1.75 ^{ab}	1.70	3.75
.017	50	857 ^{ab}	11.9	1.64	1.86 ^{ab}	1.53	2.5

Significance of main effects (P <)³

Antibiotic	.001	NS	NS	NS	NS
Stocking density	NS	NS	NS	NS	NS

¹ Means with differing suffixes are significantly different. (P < 0.05).

² 1 = No deformity; 2 = slight deformity; 3 = moderate deformity; 4 = severe deformity; 5 = completely crippled.

³ NS, Not significant at P < .05.

TABLE 4. Effect of dietary penicillin and stocking density on growth (0-35 days), lymphoid tissues and antibody response of broiler chicks (expt. 2)¹.

Penicillin (mg/kg)	Cage Space (m ²)	Body Weight (g) on day			Feed Conversion	Tissue weight (mg/100g) ²		Antibody Titer ³	
		21	28	35		Bursa	spleen thymus		
0	.035	558 ^b	885 ^{ab}	1182 ^a	2.10 ^a	263 ± 24 ^a	155 ± 14	266 ± 22 ^a	6.7 ± .57
50	.035	574 ^{ab}	875 ^{ab}	1188 ^a	1.98 ^{ab}	246 ± 17 ^a	147 ± 12	243 ± 14 ^{ab}	6.4 ± .60
0	.017	563 ^b	859 ^b	1103 ^b	1.82 ^b	197 ± 22 ^b	139 ± 10	192 ± 14 ^b	6.7 ± .59
50	.017	603 ^a	913 ^a	1165 ^a	1.84 ^b	227 ± 16 ^{ab}	151 ± 11	190 ± 22 ^b	6.4 ± .55

Significance of main effects (P < .05)⁴.

Antibiotic .002 .018 .024 .007
 Stocking density NS NS .017 .007

NS NS
 NS NS

1 Means bearing different suffixes differ significantly (P < .05).

2 Means ± SE.

3 Sheep red blood cell response expressed as a mean hemagglutinating antibody titer (log₂).

4 NS, not significant at P < .05.

TABLE 5. Effect of antibiotics and stocking density on growth, bursal and spleen weights of broiler chicks. (Expt 3)¹.

Antibiotic	Cage Space (m ²)	Body weight (g)				x Increase	Feed Conversion		x Increase	Bursal wt ²	Spleen wt ²
		week 2	week 4	week 2	week 4		% Improvement	week 4			
—	.035	289 ^{cd}	869 ^{bc}	—	1.45 ^{ab}	—	1.86 ^a	—	266 ± 26	144 ± 6.5	
Penicillin	.035	312 ^{bc}	894 ^{ab}	2.9	1.34 ^{bc}	8.2	1.68 ^b	10.7	275 ± 18	140 ± 12	
Oxytetracycline	.035	311 ^{bc}	905 ^{ab}	4.1	1.34 ^{bc}	8.2	1.69 ^b	10.1	286 ± 24	128 ± 9	
Lincomycin	.035	300 ^{cd}	935 ^a	7.4	1.61 ^a	-11.0	1.86 ^a	0	276 ± 24	139 ± 8	
—	.017	287 ^d	815 ^c	—	1.23 ^{cd}	—	1.76 ^{ab}	—	219 ± 14	129 ± 12	
Penicillin	.017	338 ^a	898 ^{ab}	10.2	1.07 ^d	15.0	1.61 ^b	9.3	226 ± 25	129 ± 11	
Oxytetracycline	.017	315 ^{abc}	876 ^b	7.5	1.33 ^{bc}	-8.1	1.75 ^{ab}	0	238 ± 20	148 ± 19	
Lincomycin	.017	325 ^{ab}	906 ^{ab}	11.2	1.10 ^d	11.2	1.60 ^b	10	263 ± 13	120 ± 8	
Significance of main effects (P<) ³ .											
Antibiotic (Ab)		.001	.001		.054		.044		NS	NS	
Stocking density (sd)		.012	.057		.001		.021		.001	NS	
Ab x sd		.027	NS		.003		.040		NS	NS	

¹ Means with different suffixes are significantly different (P<0.05).

² Weight in mg/100 g of body weight, means ± SE.

³ NS, Not significant at P<.05.

TABLE 6. Effect of antibiotics and stocking density on fecal moisture and ammonia concentrations (Expt. 3)¹.

Antibiotic	Cage space (m ²)	% Moisture	Ammonia (mg/100 g) ²	
			dry feces	wet feces
--	.039	74.2 ^{abc}	50.0 ± 5.0	12.9 ± 1.3 ^{ab}
Penicillin	.039	71.0 ^c	37.9 ± 6.5	10.3 ± 1.6 ^{ab}
Oxytetracycline	.039	72.3 ^{bc}	34.7 ± 5.0	9.7 ± 1.4 ^{ab}
Lincomycin	.039	73.6 ^{abc}	51.0 ± 11.8	13.2 ± 2.9 ^{ab}
--	.017	73.6 ^{abc}	62.4 ± 14.1	16.7 ± 4.3 ^a
Penicillin	.017	77.1 ^{ab}	49.8 ± 15.1	10.8 ± 2.6 ^{ab}
Oxytetracycline	.017	77.8 ^a	39.4 ± 5.0	8.8 ± 1.2 ^b
Lincomycin	.017	77.9 ^a	43.1 ± 9.0	9.4 ± 1.8 ^{ab}

Significance of main effects (P <)³

Antibiotic	NS	NS	NS
Stocking density	.001	NS	NS

¹ Means with different suffixes are significantly different.

² Means ± SE

³ NS, not significant at P < .05

TABLE 7. Estimated maximum stocking rates and minimum space requirements per chick for chicks in battery brooders.

Brooding period	Leghorn ¹	Medium-sized egg-breeds ¹	Broilers	
			N.R.C. 1984 ²	Expt. 3 ³
0-7 days				
Predicted weight (g)	90	114	125	150
Space (cm ²)	19	24	26	31
Chicks/m ²	526	417	385	323
Volume (cm ³)	297	376	412	495
Waterer space (cm)	0.2	0.3	0.3	0.4
Feeder space (cm)	0.4	0.5	0.6	0.7
0-14 days				
Predicted weight (g)	145	191	310	350
Space (cm ²)	30	40	65	73
Chicks/m ²	333	250	154	135
Volume (cm ³)	478	630	1023	1155
Waterer space (cm)	0.4	0.5	0.8	0.9
Feeder space (cm)	0.7	0.9	1.4	1.6
0-21 days				
Predicted weight (g)	205	282	540	630
Space (cm ²)	43	59	112	131
Chicks/m ²	233	169	89	76
Volume (cm ³)	676	930	1780	2079
Waterer space (cm)	0.5	0.7	1.4	1.6
Feeder space (cm)	0.9	1.3	2.4	2.8
0-28 days				
Predicted weight (g)	273	373	825	900
Space (cm ²)	57	78	172	187
Chicks/m ²	175	128	58	53
Volume (cm ³)	901	1231	2722	2969
Waterer space (cm)	0.7	1.0	2.1	2.3
Feeder space (cm)	1.2	1.7	3.7	4.0

¹ Predicted weights taken from North (1984).

² Predicted weights taken from NRC (1984).

³ Predicted weights are mean weights of heaviest replicates on high density regimen in Expt. 3.

CHAPTER VII

NUTRITIONAL VALUE OF FERMWAY FOR BROILER CHICKS

(Prepared for Submission to Poultry Science)

**Nutritional Value of Aerobically Fermented
Poultry Manure and Offal (Fermway) for Broiler Chicks¹**

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ABSTRACT

The nutritional value of two samples of an aerobically fermented poultry product (Fermway) for broiler chicks were evaluated. Incorporation of Fermway up to 16% in the diet had no adverse effect on growth. A significant ($P < .05$) growth stimulation was obtained on one sample at 8-16% of the diet. Feed efficiency, liver, heart or gizzard weights, or leg deformity scores were not affected by dietary Fermway. The significant growth stimulation ($P < .01$ and $P < .06$) and increases in bursal weight suggested that Fermway may have an unidentified growth factor. Increasing the dietary Fermway levels significantly ($P < .07$ and $P < .01$) decreased skin pigmentation.

Keywords: chicks, growth, manure, fermented poultry offal

INTRODUCTION

Poultry house waste management has become increasingly important in the face of mounting opposition to environmental pollution by the public in general and environmental protection activists in particular. This has stimulated research in finding means by which poultry wastes can be utilized for agricultural production. Several uses have been proposed and tested including the use of poultry manure for feeding poultry (Sunde, 1975; North, 1984). Previous studies have demonstrated some value for dried poultry manure in the diet of chicks and laying hens (Biely et al., 1972; El Boushy and Vink, 1977; Dluymet et al., 1979). However, manure drying can be costly and impractical (Naber, 1984). Naber (1984) described an aerobically fermented poultry manure product and demonstrated its dietary value for chicks and laying hens. The author reported that the incorporation of 5% of this product into the diet of chicks had no adverse effect on growth. Similarly, up to 20% was utilized in laying diets without adverse effects on egg production although there was a tendency to have poorer feed efficiency and significant reduction in yolk color. The purpose of this study was to evaluate the nutritional value of two samples of the aerobically fermented poultry product and offal product (Fermway 1 and 2) in the diet of broiler chicks.

MATERIALS AND METHODS

Experiment 1 was conducted as one of two collaborative experiments to run concurrently at two stations using the same chick source and feeds. Straight-run Ross x Arbor Acres broiler chicks were

supplied by A.G. Coop. (Arcadia, WI), manufacturers of the aerobically fermented litter product, while the feeds were formulated by Wills and co-workers (1983) of NutriVet Company (Rockford, Ohio). Fermway was made by composting broiler house litter with offal from the processing plant combined to make a product with about 50% moisture. The diets were formulated to contain 0, 4, 8, 12 and 16% of Fermway 1. Both chicks and diets were supplied to the two stations on the same day. Four replicates of 10 chicks each were randomly allocated to each of the five test diets. Experiment 2 was conducted with the extra chicks from Experiment 1. Two replicates of 10 birds each were randomly allocated to two dietary treatments formulated locally to contain 0 and 8% of the same Fermway 1 sample. For Experiment 3, a second sample of the test product (Fermway 2) was obtained and incorporated into three diets at 4, 8 and 12%. Two diets without Fermway but with or without an antibiotic (tylan at 50 ppm) were formulated to serve as positive and negative controls. As in Experiment 1, four replicates of 10 chicks each were assigned to each of the dietary treatments. The chicks for Experiment 3 were hatched from eggs produced by the University farm. The composition of the experimental diets are given in Table 1.

The amino acid composition of the control diet (0% Fermway + tylan), 8% Fermway 2 diet and the two Fermway samples were analyzed using the Beckman 6300 Amino Acid Analyzer (Table 2).

All chicks were reared in battery brooders from 0-21 days old. Feed and water were supplied ad libitum. Individual chick weights,

feed intake and efficiency were determined weekly. For determination of organ weights in Experiment 1, eight chicks were randomly selected from half of the experimental units after an overnight fast and sacrificed for organ removal. The chicks were sacrificed by CO₂ gassing, the necessary organs were removed and weighed immediately. The remaining half of the experimental units were retained for one more week at which time leg deformity and shank color scores were measured. Leg deformity was scored by the method of Cook et al. (1984) while shank color was measured by the Heiman-Carver color wheel. Organ weights and/or shank color in Experiments 2 and 3 were measured on 3-week-old chicks.

All data collected in Experiments 1-3 were subjected to the analysis of variance and the significance of differences between means computed by the least significant difference (Steel and Torrie, 1976).

RESULTS AND DISCUSSION

The incorporation of up to 16% Fermway had no adverse effect on growth (Tables 3 and 4). On the contrary, growth on diets containing 8-16% Fermway were significantly better ($P < .05$) than the growth on 0 and 4% Fermway diets in Experiment 1. Data from the collaborative study were not available for inclusion in this report, but a summary of the data provided by Wills, et al. (1983) and Danko and Naber (1984) indicated that growth on the 8% Fermway diet was significantly better than on all the other treatments. However, the weight gains from that experiment were 20% lower than the figures in Table 3. Feed efficiency was similar in the two studies. The significant growth

stimulation by Fermway levels of 8-16% were accompanied by non-significant improvements in feed efficiency and feed cost (Table 3). In Experiment 3 (Table 4), no growth response to Fermway was observed. Increasing levels of Fermway resulted in a non-significant decrease in feed efficiency in Experiment 3, but the feed efficiency on these diets were numerically better than that obtained on the basal diet without antibiotic (Table 4). Liver and heart weights along with leg deformity scores were unaffected by Fermway. The significant increase in gizzard weights in chicks fed Fermway in Experiment 1 was not repeated in Experiment 2.

Tables 3, 4 and 5 indicated that inclusion of Fermway had no effect on intestinal weight. However, bursal weights were significantly increased when 16 or 8% Fermway were included in the diets in experiments 1 and 2, respectively. Chicks fed the basal diet without antibiotic showed significant increases in intestinal weight and a non-significant increase in bursal weight in experiment 3 (Table 4). The growth stimulation and the increase in bursal weight by Fermway suggests that Fermway may have some unidentified growth factor.

The significant growth response to the antibiotic tylan obtained in Experiment 3 is consistent with earlier reports on the continued effectiveness of long-term feeding of dietary antibiotics (Dafwang et al., 1984)

The lack of detrimental effects on growth by Fermway is in agreement with the results reported by Naber (1984). A major consideration that may limit the use of Fermway in chick and laying

diets is its effect on skin pigmentation and egg yolk color which may significantly affect consumer acceptability of poultry products from birds reared on Fermway diets. Shank color score was significantly depressed by dietary Fermway levels of as low as 4% by four weeks of age (Table 6). By three weeks the reduction in color score was not significant at $P < .05$ but was highly correlated to dietary Fermway levels (Tables 4 and 5). The loss of color has also been reported in egg yolk (Naber, 1984). Similar effects have been reported to result from the use of dried poultry waste (Oluyemi, et al., 1979). A numerically higher shank color score was obtained on the basal diet with antibiotic and without Fermway. Some unpublished data from this laboratory lends support to the potentiation effect of dietary antibiotics on skin pigmentation.

Wide variability in the chemical composition of Fermway has been reported. In similarity with dried poultry manure, the composition of the product is affected by age of the chickens from which the manure was collected, duration of fecal accumulation and extent of feather and litter contamination. An additional source of variation in Fermway composition is the source of carbon and quality of offal added prior to fermentation. The report of Naber (1984) shows that of six Fermway samples analyzed, crude protein was 12-31%; 1430-2510 estimated metabolizable energy (kcal/kg); 2-7.2% calcium; and 0.6-1.8% phosphorous. The amino acid composition of the two Fermway samples used in this study is given in Table 2. The results indicate that, although the amino acid content varied between the two samples,

both had much higher levels of amino acids than dried poultry manure. This suggests that the inclusion of offal and the fermentation process may improve the protein quality of poultry manure. Even though the analyzed values for methionine were thought to be lower than expected, the reported values were 2.2-3.4 times higher than the methionine content of dried poultry manure. The amino acid values were, however, lower than those of hydrolyzed feather meal and poultry by-product and hydrolyzed feather meal. Comparison of the amino acid composition of the control diet and 8% Fermway diet shows that in most cases there was a tendency to have higher levels of indispensable amino acids on the Fermway diet. This indicates that the partial substitution of corn and soybean meal with Fermway did not have a negative effect on the amino acid balance of the diets. The possibility that the amino acids in Fermway may have low availability was not investigated. This could be significant because of the heat generated during the fermentation process. El Boushy and Roodbeen (1984) demonstrated that the average availability of amino acids in dried poultry manure was only 57% (range of 47-66%) compared to fish meal with 68% (range of 56-78%) and soybean meal with 93% (range of 90-95%). Fermway, like dried poultry waste, contains a high level of non-protein nitrogen as indicated by the high levels of ammonia (Table 2).

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TABLE 1. Percentage composition of experimental diets
(Experiments 2 and 3)

<u>Ingredients</u>				
Corn	50.00	48.00	46.00	44.00
Ferroway	--	4.00	8.00	12.00
Soybean meal (44%)	38.50	36.40	34.30	32.20
White grease	6.30	6.40	6.50	6.60
Fish meal	2.50	2.50	2.50	2.50
Dicalcium phosphate	1.40	1.40	1.40	1.40
Limestone	0.80	0.80	0.80	0.80
Iodized salt	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25
Vitamin-mineral premix ¹	+	+	+	+
Calculated analysis				
Crude protein (%)	22.87	22.81	22.75	22.69
Metabolizable energy (kcal/kg)	3188	3185	3182	3179

¹Vitamin-mineral mix supplied the following per kg of diet:

Vitamin A, 7,500 I.U.; vitamin D, 440 I.U.; vitamin K (MPB),
1.11 mg; riboflavin, 7.2 mg; calcium pantothenate, 20 mg; biotin,
.31 mg; folic acid, 1.11 mg; choline chloride, 2,600 mg;
vitamin B₁₂, .02 mg; manganese, 110 mg; and zinc, 80 mg.

TABLE 2. Amino acid composition (%) of diet, Ferway samples and comparable standards

Amino Acid	Basal		NRC 1984 ²	Ferway		Michigan DPM ³	Feather Meal ³	By-product	
	Basal	+8% Ferway ¹		1	2			Meal + Feather Meal ⁴	
Arginine	3.90	3.77	1.44	0.55	1.67	0.47	5.42	3.9	3.9
Glycine	1.95	2.18	1.50 ⁶	2.36	2.18	0.82	6.31	5.9	5.9
Serine	2.50	2.62	---	1.37	2.15	0.52	9.26	---	---
Histidine	1.76	2.03	0.35	2.50	1.57	0.20	0.34	0.71	0.71
Isoleucine	1.65	1.71	0.80	0.91	1.01	0.50	3.26	2.9	2.9
Leucine	3.85	3.85	1.35	1.54	2.01	0.80	6.72	5.2	5.2
Lysine	2.41	2.29	2.41	0.70	0.67	0.49	1.67	2.4	2.4
Methionine	0.27 ⁵	0.36 ⁵	0.50	0.20 ⁵	0.31 ⁵	0.09	0.42	1.22	1.22
Cystine	0.37 ⁵	0.70 ⁵	0.93 ⁷	0.59 ⁵	0.82 ⁵	1.09	4.0	2.24	2.24
Phenylalanine	1.96	1.91	0.72	0.59	0.93	0.45	3.26	3.2	3.2
Tyrosine	1.53	1.62	1.34 ⁸	0.62	0.84	0.26	6.31	2.2	2.2
Threonine	1.82	1.84	0.72	0.93	1.22	0.50	3.43	2.9	2.9
Tryptophan	---	---	0.23	---	---	0.53	0.50	0.5	0.5
Valine	1.53	1.62	0.82	1.48	1.49	0.62	5.57	3.8	3.8
Aspartate	4.92	4.78	---	1.85	2.27	1.06	---	---	---
Glutamine	8.18	7.84	---	2.71	3.35	1.54	---	---	---
Proline	2.68	2.80	---	1.96	2.30	---	---	---	---
Alanine	2.36	2.40	---	2.83	1.76	1.06	---	---	---
NH ₃ ¹⁰	0.59	0.70	---	1.65	1.14	1.69 ¹¹	---	---	---

(continued)

(continued -- footnotes for Table 2)

- ¹ Ferriway sample 2.
- ² Nutrient requirements of broilers, 0-3 weeks MRC (1984).
- ³ Michigan dried poultry waste, cited by Biely et al. (1972).
- ⁴ By-product meal + feather meal composition taken from Scott et al. (1982).
- ⁵ Methionine and cystine levels suspected lower than expected probably due to loss by oxidation.
Calculated methionine content of Basal diet was 0.645%.
- ⁶ Glycine + serine.
- ⁷ Methionine + cystine.
- ⁸ Phenylalanine + tyrosine.
- ⁹ Tryptophan considered destroyed during the analytical process.
- ¹⁰ NH₃ - ammonia + other non-amino acid nitrogen.
- ¹¹ From Biely et al. (1972).

TABLE 3. Weight gain, feed consumption, feed efficiency and organ weights of chicks fed graded levels of fermway (Experiments 1 and 2)¹

Diet No.	% Fermway	Weight Gain (g)	Feed Consumption (g)	Feed Efficiency	Feed Cost ²	Organ Weights g/100g Body Weight				
						Liver ²	Heart	Gizzard	Small Intestine Bursa	
Experiment 1										
1	0	452 ^b	758 ^{ab}	0.597	0.281	3.31	.509 ^{ab}	2.03 ^b	1.59	.327 ^b
2	4	446 ^b	750 ^b	0.596	0.281	3.34	.563 ^a	2.34 ^{ab}	1.54	.357 ^{ab}
3	8	506 ^a	825 ^a	0.614	0.270	3.22	.451 ^b	2.28 ^{ab}	1.50	.395 ^{ab}
4	12	509 ^a	818 ^{ab}	0.625	0.263	3.26	.502 ^{ab}	2.44 ^a	1.54	.450 ^{ab}
5	16	496 ^a	821 ^{ab}	0.604	0.269	3.23	.480 ^{ab}	2.38 ^a	1.52	.476 ^a
Experiment 2										
6	0	497	720	0.644	--	3.31	.545	2.51	1.88	.283 ^b
8	8	514	797	0.692	--	3.38	.520	2.43	2.10	.379 ^a

¹ Column means with different superscripts are significantly different ($P < .05$).

² Feed cost (\$/kg wt gain) = cost of feed/kg x kg of feed consumed per kg of weight gain

TABLE 4. Growth performance, shank color scores, and organ weights (\pm sd) of chicks fed Ferway (Experiment 3)¹

% Ferway	Weight					Shank Color		Small Intestinal	
	Gain (g)	Feed Efficiency	Score	Weight (g/100g)	Bursa (g/100g)	Score	Weight (g/100g)	Bursa (g/100g)	
0	375 \pm 69 ^b	.563 \pm .03	5.22 \pm 3.09	2.60 \pm .72 ^a	.210 \pm .08				
0 ²	428 \pm 93 ^a	.614 \pm .04	6.06 \pm 2.77	2.04 \pm .20 ^b	.229 \pm .10				
4 ²	435 \pm 79 ^a	.581 \pm .05	5.65 \pm 2.30	1.96 \pm .23 ^b	.263 \pm .08				
8 ²	436 \pm 64 ^a	.569 \pm .03	5.47 \pm 2.20	1.89 \pm .21 ^b	.262 \pm .08				
12 ²	426 \pm 92 ^{ab}	.588 \pm .04	5.03 \pm 2.22	2.14 \pm .48 ^{ab}	.287 \pm .14				

¹ Column means with different superscripts are significantly different ($P < .05$).

² + 50 ppm tyran.

TABLE 5. Correlation coefficients (r^2) between level of dietary Ferriway and selected variables

Variable	Experiment 1		Experiment 3	
	r^2	Significance (P < .)	r^2	Significance (P < .)
Weight gain	.617	.116	.017	.871
Feed efficiency	.307	.333	.373	.389
Intestinal weight	.442	.221	.076	.724
Bursal weight	.988	.001	.879	.063
Shank color	.711	.073	.978	.011

TABLE 6. Leg deformity and shank color scores
 (\pm sd) of chicks fed graded levels of
 Ferroway from 0-28 days¹ (Experiment 1)

<u>% Ferroway</u>	<u>Leg Deformity Score²</u>	<u>Shank Color Score</u>
0	1.20 \pm 1.28	8.70 \pm 3.61 ^a
4	1.55 \pm 0.89	5.85 \pm 1.23 ^b
8	0.75 \pm 0.72	6.30 \pm 3.00 ^b
12	1.10 \pm 1.02	5.60 \pm 0.94 ^b
16	0.85 \pm 0.99	5.05 \pm 1.05 ^b

¹Column means with different superscripts are significantly different ($P < .05$).

²Leg deformity scores; 0 = normal; 1 = slight deformity; 2 = moderate deformity; 3 = severe deformity; 4 = completely crippled.

APPENDIX I

POTENTIATION OF SKIN PIGMENTATION BY
DIETARY ANTIBIOTICS

ABSTRACT

Broiler chicks fed dietary flavomycin and penicillin had significantly ($P < .05$) higher shank pigmentation at three weeks old. Numerically superior shank pigmentation was observed in chicks fed dietary lincomycin, oxytetracycline and tylan in comparison to the shank pigmentation scores of chicks on the basal diet. Body weight, feed conversion, and antibody response were not significantly affected by antibiotics. Dietary antibiotics tended to result in increased bursal weights but decreased spleen and intestinal weights. The increase in bursal weight and reduction in spleen weights was significant for penicillin while the reduction in intestinal weights were significant for flavomycin, oxytetracycline, penicillin and tylan.

INTRODUCTION

Improvements in growth promotion and feed efficiency by dietary antibiotics have been documented for the past thirty-four years but information on their effects on skin pigmentation is very scanty. Fry et al. 1976a, 1976b) reported increased pigmentation responses in broilers fed diets containing flavomycin, 3-Nitro and coccidiostats. These authors attributed such increases in pigmentation to the overall improvements in growth and feed efficiency which in their opinion would be accompanied by the improved utilization of xanthophyll absorption and utilization. The experiment reported here was prompted by the observation that broiler chicks fed a control diet containing tylan had numerically higher shank color scores in studies on the nutritional value of a fermented product containing broiler litter plus broiler offal for broiler chicks (Dafwang 1985). As part of a continuing series of studies on the effect of antibiotics on chick growth, the shanks of chicks fed dietary antibiotics were scored for intensity of yellow pigmentation along with the measurement of variables that have been known to be affected by the presence of antibiotics in the diet in one of the experiments.

MATERIALS AND METHODS

Straight-run broiler chicks hatched from the university's poultry research laboratory were randomly allocated to six dietary treatments in lots of three replicates of ten chicks per treatment. The chicks were reared in battery brooders. The composition of the basal diet,

sources of antibiotics, and the methods for measurement of organ weights and antibody response had previously been described (Dafwang et al. 1985).

At three weeks old both shanks of each chicks were scored for intensity of yellow pigmentation by the Heiman-Carver color wheel. The three week body weights, feed conversion, organ weights, antibody titers and shank color score were subjected to the analysis of variance. Significant differences between means were detected by the Duncan's multiple range test (Steel and Torrie 1976).

RESULTS AND DISCUSSION

Except for flavomycin, all the antibiotics tested produced numeric but non-significant growth responses of 3.5-8.3% (Table 1). The growth responses are typical of the known variations in growth responses but were lower than the 9-11% to dietary antibiotics reported by Dafwang et al. 1984. Non-significant numeric improvements in feed efficiency were reported in the previous study but in this experiment feed efficiency was poorer on the antibiotic diets. The tendency for chicks fed antibiotic diets to have heavier bursa but reduced intestinal weights has been reported (Dafwang et al. 1985). Lower increase in bursal weight and reduction in spleen weight was also observed in penicillin fed chicks. Spleen weights were reduced on antibiotic diets in this study. Inconsistent effects had been seen previously. Antibody response was not affected. The significant reductions in intestinal weight as observed in this study by dietary

antibiotics have long been known and is the basis for several hypotheses on the mode of action of antibiotics in growth promotion.

The observation that significant differences ($P < .05$) in shank color scores was observed in chicks as young as three weeks old has not previously been reported. Penicillin and flavomycin significantly increased pigmentation of the shanks and all of the other three antibiotics produced chicks with higher pigmentation scores than the basal diet. Early studies in which dietary penicillin was shown to produce increased levels of liver Vit A and serum carotenoids made no mention of an increase in skin pigmentation (Jukes, 1955). Contrary to the conclusions of Fry *et al.* (1976) who attributed the increased pigmentation to the overall increase in weight gain and feed efficiency, the increased pigmentation observed in this study was independent of weight and feed efficiency responses. If such increases in skin pigmentation can be related to increases in xanthophyll and carotenoid absorption and utilization, this would provide a useful model for studying the effect of dietary antibiotics on nutrient absorption and utilization.

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TABLE 1. Effect of dietary antibiotics on growth, shank color scores and organ weights of broiler chicks.¹

Antibiotic	Body weight (g)	Feed conversion	Shank color Scores	Bursal wt mg/100g body wt	Spleen wt body wt	Intestinal wt g/100 g body wt	Antibody titers
Basal	458	1.30	5.6 ^b	162 ^b	138 ^a	2.04 ^a	3.8
Lincomycin (4 ppm)	474	1.56	7.0 ^{ab}	184 ^{ab}	123 ^{ab}	1.94 ^{ab}	4.2
Oxytetracycline (50 ppm)	483	1.39	6.9 ^{ab}	189 ^{ab}	123 ^{ab}	1.76 ^{bc}	3.1
Penicillin (50 ppm)	496	1.41	7.4 ^a	216 ^a	101 ^b	1.47 ^d	4.9
Flavomycin (2 ppm)	459	1.39	7.3 ^a	190 ^{ab}	123 ^{ab}	1.66 ^{cd}	3.9
Tylan (50 ppm)	483	1.37	7.1 ^{ab}	178 ^{ab}	131 ^{ab}	1.67 ^{cd}	3.5

¹ Column means with different superscripts are significantly different ($P < .05$).

² Maximum dilution of serum from chicks inoculated w/ sheep red blood cells that will agglutinate an equal volume of .5% SRBC.

TITLE OF THESIS Studies on the subtherapeutic use of dietary anti-
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manure for broiler chicks.

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