

EFFECT OF ANTIBIOTICS AND WATER QUALITY ON THE GROWTH, INTESTINAL CHARACTERISTICS AND BACTERIAL POPULATIONS OF BROILERS CHICKS

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Received 12 July 1994; Accepted 28 March, 1996

ABSTRACT

Experiments were conducted to study the effect of subtherapeutic levels of dietary antibiotic supplementation on the growth, intestinal characteristics and bacterial populations of chicks reared on "dirty" versus "clean" water. Results showed that growth response to penicillin was independent of water treatment and the magnitude of growth response ranged from 0.9 to 18.3% levels. Bursal weights were significantly increased ($P < 0.05$) and intestinal weights significantly decreased ($P < 0.01$) in chicks fed penicillin. The effect of antibiotic on intestinal thickness and length were inconsistent while bacterial counts on Sulfite - Polymyxin-Sulfadiazine (SPS) and K.F. streptococcus agar media were unaffected by penicillin and water treatments.

Key Words: Antibiotics, Water Quality, Chicks, Poultry, Bacteria.

INTRODUCTION

Concern about the public health implications of the use of dietary antibiotics in animal production simultaneously with their use for disease control in animals and man have have subjects of considerable interest. Several investigations have been conducted and committees set up in different countries over the past four decades to assess the relationship between the use of dietary antibiotics, occurrence of resistant bacterial strains and antibiotics, occurrence of resistant bacterial strains and antibiotic residues in animal products. (Swan (1969)); National Academy of Sciences (1980); Holmberg, Wells

and Cohen (1984) and Oyekunle and Olubi (1992). A common assumption by critics of antibiotic use in animal production is that they tend to be used as a substitute for "bad management" (Novick, 1979).

Coates *et al.* (1952) among others reported the lack of growth response to antibiotics in a "new" environment. Antibiotics were also reported to have no growth effect in gnotobiotic chicks (Visek, 1978). Later reports, however, showed a growth response to antibiotics in a "clean" environment (Hill and Larson, 1955 and Coates and Harrison, 1969). 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 the growth of some microorganisms *in vitro* suggest 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 change in the populations of bacteria in the gut have proven to be inconclusive (Jukes, 1984). Claims by Stutz *et al.* (1983) on the reduction of *Clostridium Perfringens* (*C. Perfringens*) populations in the ileal contents of chicks fed dietary antibiotics promoted the investigations reported in this study. 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 bacterial populations of the chick intestine.

MATERIALS AND METHODS

Day-old broiler chicks hatched in the University of Wisconsin 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 corn-soy basal diet (13386 KJ ME/kg and 23% protein) have previously been described (Dafwang *et al.*, 1984), except that in this study the meat and bone meal was replaced by fish meal. The sources of antibiotics (incorporated at 50mg/kg of diet) were also described in the previous report.

Water quality was adjusted by washing or not washing the water troughs. "Clean" water troughs were washed at specific intervals with hot water from a pressure hose and scrubbed 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 2 and 21 days for all other experiments). Clean water with 0.1ppm Cl supplied regularly to prevent troughs from drying out in the course of an experiment. Four lots of eight chicks were randomly allocated to each treatment in experiment 1-3 while four lots of ten chicks were utilized per treatment in experiment 4.

Chicks were weighed at 3 days intervals in experiments 1 and 2 but at weekly intervals in the other experiments. Feed intake and mortality were computed weekly. Water intake in experiment 2 and 3 was determined by taking the difference between water added and discarded at time of washing. Adjustment was made for evaporate losses by measuring the water loss from water troughs that were not part of the experiments but placed in the vicinity of the experimental units.

Bursal and intestinal weights were measured (Dafwang *et al.* 1985) in the experiment 1 from 10 birds per treatment. Intestinal thickness and lengths were determined on 8 birds per treatment (experiments 2 and 3). In all cases the chicks were selected at random from all the

replicates.

Intestinal segments were voided of lumen contents prior to thickness determination. Thickness of the intact intestine (double wall) was measured using a pressure sensitive micrometer (The Byer Company, Lancaster, PA and then averaged to give the thickness of intestinal wall.

Chemical oxygen demand (COD) of water samples was determined by AOAC (1980) methods. Water troughs were sampled for analysis at regular intervals. Dirtiness score (extent of water contamination by feeds, faeces and other debris) was a subjective estimate of the degree of dirtiness by two of the investigators. The sulfite-polymyxin-sulfadiazine (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 faecal streptococci, respectively, were employed to enumerate bacteria from ileal and caecal lumen contents. Prior to culture and enumeration of the bacteria, preliminary evaluations were made in our laboratory to determine appropriate dilutions, temperature and incubation time for cultivation and enumeration of bacteria from chick gut contents. Effectiveness of anaerobic pour plate technique for the cultivation and enumeration to a pure culture of *C. perfringens* (ATCC 13124) on SPS and TSN (tryptose-sulfite-neomycin) (BBL) agar media was also confirmed in the preliminary study (Dafwang *et al.*, 1987).

For bacterial enumeration, four chicks were selected randomly from each treatment and killed by axial dislocation. One gram of lumen contents from each of the appropriate intestine segments was aseptically transferred into 9 ml of a 0.1% sterile Trypticases (BBL) solution and tenfold dilutions up to 1×10^{-6} were prepared for each sample (in duplicates). Aliquots of 0.5 ml of the 10^{-4} through 10^{-6} dilutions were aseptically added to the SPS and K.F. Streptococcus agar (9.5ml each) media previously prepared according to the manufacturers' instructions. The mixture was stirred for about 20 sec, poured into sterile 60

TABLE 1. FEED EFFICIENCY, BURSAL AND INTESTINAL WEIGHTS OF CHICKS REARED ON WATER TROUGHS WITH OR WITHOUT REGULAR WASHING AND FED DIETS WITH OR WITHOUT PENICILLIN (EXPERIMENT 1) 1,2

Treat,emt		Feed Efficiency at age:			Bursal	Intestinal
Wash ³	Penicillin	6 days	12 days	21 days	Weight ⁴	Weight ⁴
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 ^b	2.13 ± .10 ^{ab}
1	+	.611 ^{ab}	.544	.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 ⁵						
	Penicillin	**	**	NS	*	***
	Wash	NS	NS	NS	NS	NS

¹Relative weight gain shown in fig. 1

² Within each column means with different superscripts are significantly different (P.05)

³- No washing: 1, washed everyday: 3, washed every 3 days.

⁴. Bursal and intestinal weight in mg/100 g (±SE) of body weight

⁵NS, not significant (P > .05); *, Significant at P < .05; **, Significant at P < .01; ***, Significant at P < .001)

TABLE 2. GROWTH PERFORMANCE AND INTESTINAL CHARACTERISTICS OF CHICKS REARED ON WATER TROUGHS WITH OR WITHOUT REGULAR WASHING FOR 15 DAYS AND FED A BASAL DIET WITH OR WITHOUT TYLAN (EXP 2).

	"Clean" Water ²		Treatment "Dirty" Water ³		SE	Significance of main Effects ⁴		
	Basal	Tylan	Basal	Tylan		Water	Tylan	WxT
Weight gain (g) ⁵	201	210	207	197	5.07	NS	NS	NS
Feed efficiency ⁵	0.659 ^{ab}	0.611 ^b	0.675 ^a	0.657 ^{ab}	0.03	NS	NS	NS
Water Intake (e) ⁵	0.491	0.551	0.535	0.511	0.02	NS	NS	NS
--Intestinal wall thickness (mm)--								
Duodenum	0.484	0.517	0.552	0.524	0.02	NS	NS	NS
Jejunum	0.389 ^{ab}	0.317 ^b	0.420 ^{at}	0.475 ^a	0.06	**	NS	NS
Ileum	0.387	0.377	0.452	0.425	0.03	NS	NS	NS
Cecum	0.331	0.344	0.398	0.399	0.03	NS	NS	NS
Colon	0.566 ^{ab}	0.466 ^b	0.548 ^{ab}	0.598 ^a	0.05	NS	NS	NS
--Intestinal length (cm)--								
Duodenum	20.7	20.1	19.5	19.3	0.55	NS	NS	NS
Jejunum	42.2	46.2	39.2	42.6	2.49	NS	NS	NS
Ileum	39.9 ^{ab}	46.6 ^a	36.4 ^b	38.6 ^{ab}	3.81	*	NS	NS
Cecum	9.5	9.8	9.0	9.5	0.29	NS	NS	NS
Colon	5.4	5.9	5.2	5.6	0.26	NS	NS	NS

¹Means within a row with different superscripts are significantly different P < .05

²Water troughs washed every 3 days

³Water troughs not washed for 15 days

⁴NS = Not significant at P > .05; *,

Significant at P < .05; **, Significant at P < .01

⁵Cumulative for 15 days

x 15mm petri plates, allowed to cool at ambient conditions for about 45 min then 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 colonial and microscope morphological characteristics were observed and compared

with those of the pure *C. perfringens* culture previously tested to detect similarities that could indicate the presence of *C. perfringens* on the plates. Data were subjected to statistical analysis and significant differences between treatment means were determined by Duncan's multiple range test (Steel and Torrie, 1976).

RESULTS

In experiment 1, dietary penicillin significantly ($P < 0.01$) improved growth (Fig. 1). A significant improvement in feed efficiency ($P < 0.01$) was obtained for 6 and 12 day old chicks (Table 1) due to dietary penicillin. Chicks fed the penicillin diets exhibited significantly increased bursal size ($P < 0.05$) but decreased intestinal weights ($P < 0.001$). In all cases the penicillin effect was independent of water treatment.

No growth response to tylosin (tylosine) or water treatment was observed although a significant improvement in feed efficiency was obtained on the "dirty" water and basal diet treatment combination (experiment 2 (Table 2). Water intake was not affected by water or antibiotic treatment. Intestinal wall thickness for all the segments tended to be reduced in chicks on "clean" water. 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. Both dirtiness score and chemical oxygen demand numerically increased with time (Table 3) in most cases, indicating that the extent of water contamination increased with time. The values for both variables were higher for the "dirty" water treatment from 6 to 12 days. Results obtained in experiment 3 showed that in contrast to tylosin in experiment 2, dietary penicillin significantly improved growth, confirming the results observed in experiment 1. The significant improvement in weight gain was accompanied by numeric improvement in feed efficiency (Table 4). However, penicillin had no effect on intestinal wall thickness and

intestinal length.

In experiment 4 as in previous experiments, dietary penicillin significantly improved weight gain (Table 5). Penicillin fed chicks on "dirty" water treatment exhibited the best numeric growth. A significant increase ($P < 0.001$) in water intake was observed for chicks on the "dirty" water treatment. No significant differences were observed in intestinal content bacterial counts on SPS and KF Streptococcus agar media (Table 5). Examination of the plates 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. Coccoidal cells should not have been present if the SPS medium was stringently selective for clostridia which are characteristically rod-shaped. Larger colonies on SPS agar were composed of predominantly cocci and cells picked from colonies on the Streptococcus agar were predominantly rods.

Black colonies were observed on only one SPS agar plate. Microscopic examination of organisms from such colonies revealed a mixture of rods and cocci. Cumulative mortality in all experiments was about 2% and was not affected by antibiotics or water treatment.

DISCUSSION

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 practices 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. In two of the experiments (Fig. 1 and Table 2), the best numeric growth at 21 days was obtained on the penicillin plus "dirty" water treatment combination which seems to suggest that there may be a symbiotic effect between microbes in the "dirty" water and the presence of dietary antibiotics on colonization of the chick gut by de-

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TABLE 3. DIRTINESS SCORE (DS)¹ AND CHEMICAL OXYGEN DEMAND (COD)² OF WATER DRUNK BY CHICKS REARED ON WATER TROUGHS WITH OR WITHOUT REGULAR WASHING AND FED DIETS WITH OR WITHOUT TYLAN (EXPERIMENT 2)

Duration of experiment	(days)	"Clean" Water ³		Treatment "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	234.3

¹Clear = 1; slightly dirty = 3; very dirty = 4

²Chemical oxygen demand in mg/l,

³See Table 5 for definition of water trough management.

TABLE 4. WEIGHT GAIN, FEED EFFICIENCY AND INTESTINAL CHARACTERISTICS OF CHICKS FED A BASAL DIET WITH OR WITHOUT PENICILLIN (EXPERIMENT 3).

	Diet		Penicillin	Significance ¹
	Basal			
Wt. gain (g) ²	358		438	***
Feed Efficiency ²	.580		.603	NS
--Intestinal wall thickness (mm)--				
Duodenum	.423		.476	NS
Jejunum	.430		.386	NS
Ileum	.434		.352	NS
Ileum	.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

¹NS, Not significant; ***, Significant at P < .001

²Cumulative for 21 days

irable bacteria. These results do not support the allegation by critics (Novick, 1979) as well as advocates of subtherapeutic antibiotic usage that such use is a substitute for good management practices. 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 (1983) 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". Studies by Nurmi and Rantala (1973) demonstrated that chicks increased their resistance to salmonella infection when they were administered a suspension of adult bird faeces. The laboratory facilities in which these studies were conducted have been

TABLE 5. GROWTH PERFORMANCE AND BACTERIAL COUNTS IN INTESTINAL CONTENTS OF CHICKS REARED ON WATER TROUGHS WITH OR WITHOUT REGULAR WASHING AND FED DIETS WITH OR WITHOUT PENICILLIN (EXP 4)1

	Treatment				S.E.	Significance of main effects ⁴		
	"Clean" Water ²		"Dirty" Water ³			Penicillin	Water	P x W
	Basal	Penicillin	Basal	Penicillin				
Weight gain(g) ⁵	528b	579a	494b	595a	8.2	***	NS	NS
Feed efficiency ⁵	0.550	0.593	0.560	0.582	0.01	NS	NS	NS
Water Intake(l) ⁵	1.30 ^c	1.42 ^{bc}	1.59 ^{ab}	1.67 ^a	0.03	NS	***	NS
---Bacterial counts on SPS agar (log10/g)---								
Ileum ⁶	7.2	6.6	7.2	7.1	0.20	NS	NS	NS
Cecum ⁷	8.3	8.0	7.9	8.3	0.07	NS	NS	NS
---Bacterial counts on KF Streptococcus agar (log/g)---								
Ileum	7.0	6.7	7.4	7.1	0.18	NS	NS	NS
Cecum	7.4	7.6	7.2	8.2	0.18	NS	NS	NS

¹Means within a row with different superscript are significantly different (P < 0.05)

²Water troughs washed every 3 days

³Water troughs not washed for 21 days

⁴NS, Not significant; * Significant P < 0.05;

*** Significant P < 0.001\

⁵Total for 21 days

⁶Ileal contents taken on day 25

⁷Ileal contents taken on day 29.

FIGURE 1

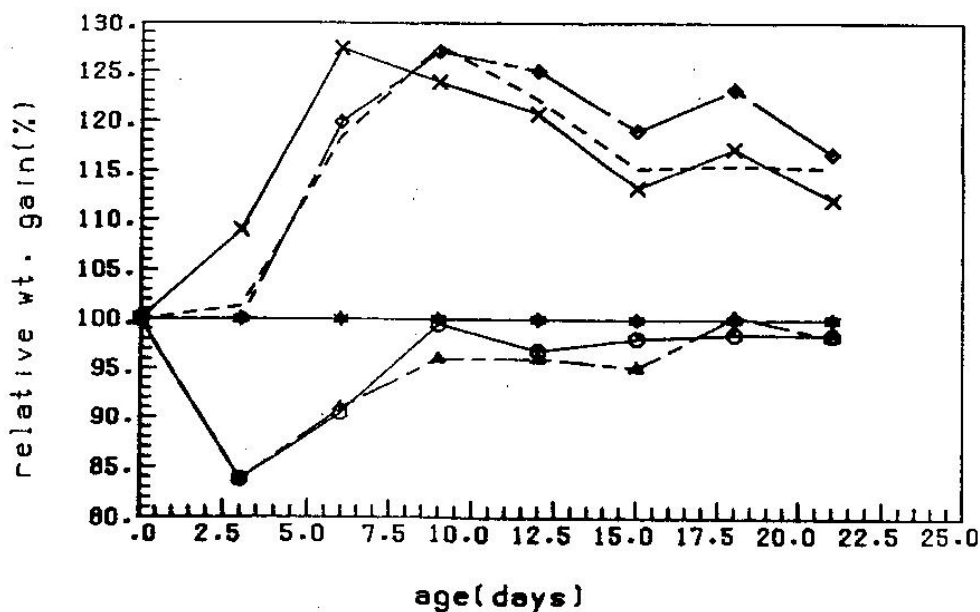


Fig.1
Relative weight gain (%) of chicks fed diets with or without penicillin and reared on water troughs with or without regular cleaning (exp.2).

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

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exposed to continuous use of subtherapeutic levels of dietary penicillin since 1950 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 was 11.0% (range of 0.9-18.3%). Earlier reports from the same laboratory indicated mean improvement in body weight of 8.5% in 1950-1953, 8.8% in 1956-1960 and 11.3% in 1981-1984 by the same antibiotic (Heth and Bird, 1962 and Dafwang *et al.*, 1984). 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 by Jukes (1984). Figure 1 shows that the maximum growth response to penicillin was between days 6 to 12. Such information is beneficial for the design of experiments to monitor changes in metabolic and other parameters due to dietary antibiotics. The increased bursal size due to dietary antibiotics has been observed before by Dafwang *et al.* (1985). A reduction in intestinal weight by antibiotics has always been regarded as an index of decrease in intestinal thickness (Visek, 1978; Fuller and Coates, 1983). This observation was confirmed in this study.

The finding that penicillin feeding did not produce a decrease in intestinal bacteria is consistent with the results of other investigators Jukes (1984). Stutz *et al.* (1983) reported the growth promoting effects of antibiotics was due to the suppression of *C. perfringens* in the chick gut. These investigators apparently enumerated every bacterium growing on their TSN agar as *C. perfringens*. The results of this study demonstrate that the selectivity of those media are questionable when used with a mixed population of intestinal bacterial. TSN and SPS agar media were examined in preliminary studies in our laboratory 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. The recurrent appearance of coccoidal bacteria on SPS agar prompted the use of the Kf. 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 populations of the rodshaped 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 by Hill (1981). *C. perfringens* has long been a suspect species in the mode of action of antibiotics in growth promotion, and has been the subject of a number of investigations (Jukes and Williams, 1953, Lev and Forbes, 1959, and Westmann *et al.* 1960). Whether *C. perfringens* in the low populations at which it occurs in the chick gut as reported by Timms (1968) and Barnes *et al.* (1972) plays a significant role in the mode of growth promotion by antibiotics remains to be validated.

ACKNOWLEDGMENT

The technical assistance of Mr. Philip Bortz, Department of Meat Animal Science, University of Wisconsin, in developing the technique employed for bacterial cultivation and enumeration is highly appreciated.

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