

Effect of Feed Antibiotics on the Performance and Intestinal Microflora of Weanling Pigs in China**

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ABSTRACT : Ninety-six crossbred (Large White × Landrace × Duroc) pigs, weaned at 35 days of age, were assigned to four dietary treatments in order to investigate the effects of oral antibiotics on the performance and the intestinal microflora of weanling pigs. Pigs were fed either a basal diet, without antibiotics, or the basal diet plus either 50 ppm acetylspiramycin, 50 ppm olaquinox, or 100 ppm bacitracin zinc. The pigs were housed eight per pen with three pens per treatment in an environmentally controlled nursery. Ten days after weaning, three pigs from each treatment were slaughtered and intestinal pH, microflora, and volatile fatty acid concentration were determined. At the end of the 4 week trial, the remaining pigs were weighed and feed consumption was measured. Average daily gains for pigs fed acetylspiramycin, olaquinox, bacitracin zinc and the control diet were 0.43, 0.40, 0.37, and 0.34 kg per day ($p=0.001$), respectively. Antibiotic addition did not modify feed intake, but acetylspiramycin improved feed conversion ($p=0.003$). In comparison with the control, acetylspiramycin significantly increased *Bifidobacteria* numbers in the jejunum ($p=0.082$) and ileum ($p=0.014$) and decreased total bacterial counts throughout the intestine ($p<0.01$ except for the ileum where $p=0.079$). Acetate production was significantly lower in the cecum ($p=0.028$) and colon ($p=0.079$) of pigs fed acetylspiramycin. In addition to increasing numbers of *Bifidobacteria* in the jejunum ($p=0.082$) and ileum ($p=0.014$), olaquinox increased *Lactobacillus* in the jejunum ($p=0.004$) and decreased *E. coli* in the colon ($p=0.022$). Bacitracin zinc increased *Lactobacillus* numbers in the jejunum ($p=0.004$) and *Bifidobacterium* concentrations in the jejunum ($p=0.082$) and ileum ($p=0.014$). (*Asian-Aus. J. Anim. Sci.* 2000. Vol. 13, No. 11 : 1554-1560)

Key Words : Antibiotics, Pigs, Intestinal Microflora, Performance

INTRODUCTION

The Chinese swine industry is undergoing a dramatic change with increasing numbers of pigs being raised in large, commercial-type operations and a lower percentage being raised in the traditional, small, and backyard operations (Zhang, 1992; Simpson et al., 1994). As pig production becomes more industrialized, the risk of major economic loss due to disease is likely to increase (Alexander and Harris, 1992).

Antibiotics were first used in swine diets over 50 years ago (Carpenter, 1950; Jukes et al., 1950; Luecke et al., 1950) and widely utilized in most intensive swine operations (Dunlop et al., 1998; Dewey et al., 1999). They are used at subtherapeutic levels to promote growth, increase the efficiency of feed

utilization and prevent disease (Wallace, 1970). The efficacy of antibiotics has been well documented (Hays and Muir, 1979; Wheelhouse and Groves, 1985; Moore et al., 1986).

Despite of the widespread use of antibiotics, there are still some disputes about their mode of actions (Wallace, 1970; Visek, 1978; Thomke and Elwinger, 1998). It has been postulated that antibiotics improve growth performance either by a nutrient sparing effect, or by a disease control effect (Wallace, 1970). More recent studies suggested that antibiotics as growth promoters alleviate challenges to the immune system through their gut flora regulating effects (Thomke and Elwinger, 1998).

There are differences in the growth responses of pigs by kinds of antibiotics. Therefore, the following experiment was conducted to compare the effectiveness of three antibiotics commonly used by swine producers in China and to monitor their ability to alter the gastrointestinal flora of weanling pigs.

MATERIALS AND METHODS

Experimental design and animals

Ninety-six, 35 day-old crossbred (Large White × Landrace × Duroc) pigs, weighing an average of 8.3 ± 0.6 kg, were divided into four groups on the basis of sex and weight. They were fed a basal diet without any antibiotic inclusion, or one of three similar diets

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** This research was sponsored by a China National Grant. Additional funds for manuscript preparation were provided by the Canadian International Development Agency.

Received March 8, 2000; Accepted May 16, 2000

supplemented with either 50 mg/kg acetylspiramycin (Northern China Medicine Factory, Baoding, Hebei Province, China), 50 mg/kg olaquinox (Guang Ji Pharmaceutical Company, Wuxue City, Hubei Province, China) or 100 mg/kg bacitracin zinc (Shenzhou Tongde Veterinary Medicine Factory, Shenzhou City, Hebei Province, China). Basal diet was formulated to provide 18.0% crude protein and contained sufficient vitamins and minerals to meet or exceed NRC (1998) requirements (table 1) and all diets were provided by mash forms.

During the 28 days growth trial, all pigs were housed in an environmentally controlled building (temperature maintained at 25-28°C) with 1.8×2.4 m wired-floored pens equipped with self feeders. Three pens, containing four gilts and four castrates, were assigned to each treatment. Pigs were permitted *ad libitum* access to feed and water throughout the experiment. Weight gain and feed intake were recorded and used to calculate feed conversion at the completion of the trial. The incidence of diarrhea, which was expressed as the sum of pigs suffered from diarrhea each day, among the pigs was observed and recorded for first 14 days of the trial.

On day 10 of the trial, 12 non-fasted pigs (3 pigs per

treatment) were randomly selected and slaughtered by electrocution followed by exsanguination. The viscera were immediately exposed via a midline incision and the stomach, duodenum, jejunum, ileum, cecum and colon were aseptically isolated, doubly ligated and removed. Digesta was collected by gently massaging the intestine and into 50 ml Biomex beakers.

The pH of the different parts of the gastrointestinal tract was determined using a Cole Palmer bench top pH meter (Third Analytical Equipment Factory, Shanghai, China). Volatile fatty acids in digesta obtained from the cecum and colon were tested as fatty acid methyl esters on a gas chromatograph (Hewlett Packard GC-6890).

Analytical methods

The total number of bacteria in each digesta sample was counted with a hemocytometer (Sixth Analytical Equipment Factory, Beijing, China) after dilution with 20% formalin. Digesta was diluted with an anaerobic diluent and cultured on differential media for enumeration of *Bifidobacteria*, *E. coli* and *Lactobacilli*. *Bifidobacteria* were cultured on de Man-Rogosa-Sharpe agar+5% NNT solution, a medium selective for *Bifidobacterium* (Alm et al., 1989), in anaerobic Hungate roll tubes. *E. coli* were cultivated on MacConkey agar (Beijing Haidian Microbiological Culture Factory, Beijing, China) and *Lactobacillus* were cultured on selective Lactobacilli agar (Difco Laboratories, Detroit, Michigan) in 5% CO₂. All tubes and plates were incubated at 37°C for 24 to 48 hours and the number of colonies counted on each plate or tube.

Statistical analysis

One way analysis of variance was performed by the GLM procedures of SAS (1989). Where appropriate, differences among treatment means were compared using Duncan's multi-comparison test (Duncan, 1955).

RESULTS

As compared to control, acetylspiramycin improved ($p=0.001$) the growth rate of weanling pigs (table 2). The growth rate of pigs fed olaquinox and bacitracin zinc were intermediate to those of pigs fed the control and the acetylspiramycin treatments but the magnitude of these differences did not reach statistical significance. Feed intake was unaffected by dietary treatments while the feed conversion of pigs fed acetylspiramycin was significantly ($p=0.003$) improved in comparison with the control group. The incidence of diarrhea was numerically lower for pigs fed antibiotics than the control group among antibiotics-fed groups, pigs fed acetylspiramycin and olaquinox

Table 1. Ingredients and chemical composition of a basal diet for weanling pigs

Ingredients (%)	Control
Corn	61.80
Soybean meal	23.50
Fish meal	5.00
Whey powder	5.00
Soybean oil	0.50
Dicalcium phosphate	1.74
Limestone	0.77
Salt	0.30
Lysine-HCl (78%)	0.34
Vitamin-mineral premix ¹	1.00
Antibiotics ²	+
Chemical composition (% as fed)	
Crude protein	18.01
Calcium	0.95
Phosphorus	0.75
Lysine	1.25
Methionine+cystine	0.68
Digestible energy (kcal/kg)	3,338

¹ Provided per kilogram of complete diet: vitamin A, 5,510 IU; vitamin D₃, 1,102 IU; menadione, 4.4 mg; vitamin B₁₂, 0.02 mg; riboflavin, 4.4 mg; niacin, 22 mg; d-pantothenic acid, 17.6 mg; choline, 220 mg; folic acid, 0.33 mg; thiamin, 0.55 mg; pyridoxine, 1.1 mg; d-biotin, 0.04 mg; Zn, 110 mg; Mn, 55 mg; Fe, 110 mg; Cu, 22 mg; I, 0.28 mg; Se, 0.3 mg.

² Antibiotics were added for treatment groups.

Table 2. Effects of antibiotics on the growth performance and incidence of diarrhea in weanling pigs¹

	Control	Acetylspiramycin	Olaquinox	Bacitracin Zinc	SEM ²	p value
Average daily gain (kg)	0.34 ^b	0.43 ^a	0.40 ^{ab}	0.37 ^{ab}	0.034	0.001
Average daily feed intake (kg)	0.68	0.67	0.68	0.68	0.017	0.684
Feed conversion	1.97 ^b	1.57 ^a	1.72 ^{ab}	1.85 ^b	0.09	0.003
Incidence of diarrhea ⁴	17	8	9	12	nd	nd

¹ Means with different superscripts differ at the p value indicated.

² Standard error of the mean.

³ nd=not determined.

⁴ The incidence of diarrhea was expressed as the sum of pigs suffered from diarrhea each day.

tended to produce a greater reduction in the incidence of diarrhea than those fed zinc bacitracin.

The effects of the various antibiotics on the pH in different sections of the intestinal tract are shown in table 3. The pH in the duodenum of pigs fed olaquinox was significantly ($p=0.086$) lower and the pH in the ileum of pigs fed the acetylspiramycin was significantly ($p=0.056$) higher than that of pigs fed any of the other diets.

Bifidobacteria counts in the jejunum ($p=0.082$) and ileum ($p=0.014$) were significantly increased by the addition of the antibiotics as compared to control (table 4). However, there was no effect of antibiotics on the numbers of bifidobacteria in the cecum or colon. In jejunum, lactobacilli counts were higher ($p=0.004$) for pigs fed olaquinox and zinc bacitracin than for pigs fed acetylspiramycin or the control diet. However, lactobacilli were lower in the cecum for pigs fed acetylspiramycin than for pigs fed zinc bacitracin. Lactobacilli counts were unaffected by antibiotic treatments in ileum, colon and rectum.

In comparison with the control group, zinc bacitracin reduced *E. coli* counts in the ileum ($p=0.007$), cecum ($p=0.091$), colon ($p=0.022$) and rectum ($p=0.066$; table 4). Olaquinox reduced *E. coli* counts in the colon ($p=0.022$) but not in other parts of the gastrointestinal tract. Acetylspiramycin had no effect on *E. coli* numbers in any section of the gastrointestinal tract.

Acetylspiramycin was effective in reducing total bacteria counts (table 4) in the jejunum ($p=0.0003$),

ileum ($p=0.079$), cecum ($p=0.0001$), colon ($p=0.001$) and rectum ($p=0.003$). Total bacteria count was greater ($p=0.0001$) in the cecum of pigs fed bacitracin zinc as compared to the control group.

As compared to the control group, the concentration of acetate in the cecum of pigs fed acetylspiramycin decreased while it was increased in pigs fed olaquinox (table 5). The acetate concentration in the colon of pigs over control group was decreased by the addition of acetylspiramycin or olaquinox ($p=0.079$).

DISCUSSION

Acetylspiramycin is a macrolide antibiotic produced by *Streptomyces ambofaciens* and is chemically related to erythromycin, tylosin and spectinomycin (Kunesh, 1981). It has a fairly broad spectrum of antimicrobial effects but is most effective against gram-positive bacteria (Nielsen and Gyrd-Hansen, 1998) while some gram-negative bacteria are also inhibited (Weinstein, 1975). Acetylspiramycin is well absorbed from the intestinal tract after oral administration and has been shown to be effective in the control of proliferative enteritis (Tsinas et al., 1998) and *Mycoplasma pneumoniae* (Weinstein, 1975; Nielsen and Gyrd-Hansen, 1998).

Under the conditions of this experiment, acetylspiramycin was the only antibiotic to improve both growth rate and feed conversion. Hays and Speer (1960) reported that acetylspiramycin was superior to

Table 3. Effect of antibiotics on the pH in different sections of the gastrointestinal tract of weanling pigs¹

	Acetylspiramycin	Olaquinox	Bacitracin Zinc	Control	SEM ²	p value
Pylorus	2.32	3.97	3.32	3.30	0.761	0.145
Fundus	4.12	3.02	3.00	4.48	1.211	0.367
Duodenum	6.40 ^a	5.06 ^b	5.84 ^a	5.89 ^a	0.54	0.086
Jejunum	6.77	6.24	6.45	6.52	0.43	0.972
Ileum	7.05	6.20 ^b	6.44 ^b	6.58 ^b	0.313	0.056
Cecum	5.99	5.75	5.60	5.84	0.311	0.536
Colon	6.31	6.21	6.04	6.22	0.189	0.435

¹ Means with different superscripts differ at the p value indicated.

² Standard error of the mean.

Table 4. Effect of various feed antibiotics on Lactobacilli and Bifidobacteria concentration in the intestine of weanling pigs (CFU log₁₀/g)¹

	Acetylspiramycin	Olaquinox	Bacitracin Zinc	Control	SEM ²	p value
<i>Lactobacilli</i>						
Jejunum	6.18 ^a	7.44 ^b	7.67 ^b	6.75 ^a	0.36	0.004
Ileum	7.15	7.92	7.68	7.40	0.37	0.137
Cecum	7.43 ^a	8.12 ^{ab}	8.43 ^b	7.98 ^{ab}	0.43	0.109
Colon	8.55	8.55	8.68	8.57	0.13	0.593
Rectum	8.29	8.25	8.66	8.50	0.25	0.238
<i>Bifidobacteria</i>						
Jejunum	7.50 ^a	7.91 ^a	7.67 ^a	6.95 ^b	0.23	0.082
Ileum	8.60 ^a	8.75 ^a	9.13 ^a	7.62 ^b	0.26	0.014
Cecum	9.20	9.32	9.17	9.11	0.18	0.126
Colon	9.09	9.33	9.30	9.13	0.17	0.442
Rectum	9.19 ^a	9.16 ^a	9.68 ^b	9.20 ^a	0.16	0.056
<i>E. coli</i>						
Jejunum	5.82	5.59	4.81	5.69	0.44	0.131
Ileum	7.83 ^a	6.93 ^a	5.92 ^b	7.32 ^a	0.44	0.007
Cecum	6.73 ^{ab}	6.63 ^{ab}	5.52 ^a	7.21 ^b	0.71	0.091
Colon	6.87 ^{ab}	5.98 ^b	4.98 ^c	7.50 ^a	0.80	0.022
Rectum	6.98 ^a	5.68 ^{ab}	4.86 ^b	7.20 ^a	1.01	0.066
<i>Total bacteria</i>						
Jejunum	8.86 ^a	9.68 ^b	9.59 ^b	9.37 ^b	0.13	0.0003
Ileum	8.86 ^a	9.60 ^b	9.73 ^b	9.65 ^b	0.34	0.079
Cecum	9.64 ^a	10.2 ^b	10.5 ^c	10.2 ^b	0.10	0.0001
Colon	9.92 ^a	10.6 ^b	10.6 ^b	10.7 ^b	0.16	0.001
Rectum	9.92 ^a	10.7 ^b	10.6 ^b	10.7 ^b	0.19	0.003

¹ Means with different superscripts differ at the p value indicated.² Standard error of the mean.**Table 5.** Effect of various feed antibiotics on volatile fatty acid concentrations in the cecum and colon of weanling pigs (mmol/l)¹

	Acetylspiramycin	Olaquinox	Bacitracin Zinc	Control	SEM ²	p value
<i>Cecum volatile fatty acids</i>						
Acetate	67.8 ^a	89.0 ^c	77.6 ^b	77.3 ^b	6.68	0.028
Propionate	47.2	35.6	50.1	39.9	7.73	0.151
Butyrate	17.7	29.7	20.1	26.7	8.50	0.324
Total	132.8	154.3	147.8	143.9	13.2	0.307
<i>Colon volatile fatty acids</i>						
Acetate	69.8 ^a	7.01 ^a	78.9 ^{ab}	85.2 ^b	7.82	0.079
Propionate	36.2	32.6	34.3	39.6	10.96	0.849
Butyrate	16.6	26.1	18.8	29.0	8.64	0.262
Total	122.5	128.8	131.9	153.8	19.71	0.229

¹ Means with different superscripts differ at the p value indicated.² Standard error of the mean.

chlortetracycline as a growth promoter for swine. They reported on the results of two experiments with one experiment showing improvements in performance of a greater magnitude and one showing improvements of a lesser magnitude than that observed in the present study.

Acetylspiramycin was superior to other antibiotics to reduce total bacterial numbers in all parts of the

gastrointestinal tract. A reduction in total bacteria counts could account for the improvement in animal performance since it is well established that micro-organisms compete with the host for available nutrients (Wallace, 1970). In addition, it has been suggested that a reduction in intestinal bacteria may result in a thinner intestinal wall and thus facilitate nutrient absorption (Wallace, 1970).

Lactobacilli numbers were not reduced by acetylspiramycin in the present trial. It is not in agreement with the result of Decuypere (1973), who demonstrated a decline in enteric lactobacilli counts due to feeding spiramycin. The failure of acetylspiramycin to reduce *E. coli* numbers is consistent with its antibacterial spectrum (Weinstein, 1975).

Bacitracin is a polypeptide antibiotic produced by *Bacillus subtilis* and is effective against gram-positive bacteria, but most gram-negative organisms are resistant to bacitracin. Bacitracin, in the form of zinc bacitracin or bacitracin methylene disalicylate, is a very popular growth promoter for using in growing-finishing pigs in North America (Dewey et al., 1998) and for treatment and prevention of *Clostridial enteritis*.

In the present experiment, zinc bacitracin was the least effective of three antibiotics in improving the performance of weanling pigs. This finding is consistent with that of Hays and Muir (1979) who reported that bacitracin ranked eighth out of eleven antibiotics in its ability to improve the growth performance of weaner pigs. The failure of bacitracin to improve animal performance was likely due to its inability to reduce total bacteria counts in the intestine.

Although total bacterial counts were not reduced by bacitracin, *E. coli* counts were low in most parts of the intestine. This result is not easy to explain, since the *Enterobacteriaceae* including *E. coli*, are resistant to bacitracin (Weinstein, 1975). A possible explanation is that this is an effect mediated by the action of bacitracin on intestinal bacteria other than *E. coli*, with the net result that other bacteria, that compete with *E. coli*, are increased in number at the expense of *E. coli* counts.

One of the mechanisms by which normal intestinal microorganisms prevent attachment and colonization by enteropathogens has been proposed to be related to saturation of the adhesion receptors in the epithelium by normal microflora thus preventing attachment by pathogenic organisms (Stavric and Kornegay, 1995). Bacitracin increased the bifidobacteria counts in the jejunum and ileum and it has been suggested that bifidobacteria may attach preferentially to the intestinal epithelia and block attachment by harmful microflora such as *E. coli* (Meng et al., 1998).

Olaquinox is a chemical antimicrobial of the quinoxaline group, closely related to methylacetyl-quinoxaline and carbadox (Linton et al., 1988). The quinoxaline antibiotics are effective against a broad spectrum of gram-negative bacteria (Asplund et al., 1998). Quinoxaline antibiotics are commonly used in swine production for growth promotion and treatment of enteric diseases such as colibacillosis and swine dysentery (Williams and Shively, 1978).

The performance of pigs fed olaquinox was

inferior to that of pigs fed acetylspiramycin. This finding is consistent with the findings of Asplund et al. (1998) who reported that olaquinox was less effective than spiramycin in the control of the enteropathogen *Yersinia enterocolitica*.

Based on its antimicrobial spectrum, it might have been expected that olaquinox would have had the greatest effect on *E. coli* numbers and these in fact were significantly lower than the control group when measured in the colon. However, it is surprising that the bacitracin zinc treatment reduced *E. coli* counts to an even greater extent than did olaquinox. This suggests that competitive exclusion may be as important a mechanism in controlling bacterial growth as are the direct effects of the antibiotics.

The interactions among the bacteria inhabiting the intestinal tract are highly complex, with both competitive and beneficial relationships observed among bacteria. Muralidhara et al. (1977) reported that *Lactobacillus acidophilus* inclusion in the diet of young pigs decreased the number of *E. coli* in the digestive tract. Ibrahim (1994) reported that *Bifidobacteria* suppress the growth of *E. coli*. On the other hand, Hillman (1998) demonstrated that the growth of *Bifidobacterium* and *Lactobacillus* are stimulated when co-cultured with *E. coli*. Since only three genera of more than 50 bacterial species present in the intestinal tract (MacFarlane and MacFarlane, 1997) were cultured in this study, it is evident that many of these interactive effects still remain to be elucidated.

Volatile fatty acids in digesta arise mainly from microbial fermentation of carbohydrates (Haberer et al., 1999). In the pig, the main sites for the production and absorption of volatile fatty acids are the cecum and colon (Elsden et al., 1946). Therefore, the concentration of volatile fatty acids at these sites can be used as an indication of microbial activity. In the present study, the level of volatile fatty acids, in both the cecum and the colon, directly reflected the total bacterial counts at these two sites with the treatments with the highest total bacteria counts generally having the highest volatile fatty acid concentration and the treatments with the lowest bacteria counts having the lowest volatile fatty acid concentration.

Haberer et al. (1999) recently reported that propionate was the predominant volatile fatty acid in the intestine of the pig. However, the results of the present study do not support their findings but rather agree with the findings of Friend et al. (1963) who reported that acetate was the predominant volatile fatty acid in the pig intestine.

In conclusion, the overall results of this study show that antibiotics are generally effective in improving weanling pig performance. Not all antibiotics are equally effective. Therefore antibiotics

should be utilized by their efficacy. This study supports the concept that the growth promoting effects of antibiotics are modulated by their effects on the intestinal microflora.

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