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ANTIBIOTICS AS GROWTH PROMOTANTS: MODE OF ACTION

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ABSTRACT

Recent concerns about the use of growth-promoting antibiotics in pig diets have renewed interest in the immunologic and growth-regulating functions of the gastrointestinal (GI) tract. The numerically dense and metabolically active microbiota of the pig GI tract represents a key focal point for such questions. The intestinal microbiota is viewed typically as a beneficial entity for the host. Intestinal bacteria provide both nutritional and defensive functions for their host. However, the host animal invests substantially in defensive efforts to first sequester gut microbes away from the epithelial surface, and second to quickly mount immune responses against those organisms that breach epithelial defenses. The impact of host responses to gut bacteria and their metabolic activities require special consideration when viewed in the context of pig production in which efficiency of animal growth is a primary objective. Here, we summarize the working hypothesis that antibiotics improve the efficiency of animal growth via their inhibition of the normal microbiota, leading to increased nutrient utilization and a reduction in the maintenance costs of the GI system. In addition, novel molecular ecology techniques are described that can serve as tools to uncover the relationship between intestinal microbiology and growth efficiency.

INTRODUCTION

Numerous physiological, nutritional, and metabolic responses to feed-grade antibiotics have been reported, as summarized in Table 1.[1] From a production

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Table 1. Summary of Reported Physiological, Nutritional and Metabolic Effects of Growth Promoting Antibiotics[1]

Physiological	Nutritional	Metabolic
Increase		
Nutrient absorption	Energy retention	Liver protein synthesis
Feed intake	Nitrogen retention	Gut alkaline phosphatase
	Vitamin absorption	
	Trace element absorption	
	Fatty acid absorption	
	Glucose absorption	
	Calcium absorption	
	Plasma nutrients	
Decrease		
Food transit time	Gut energy loss	Amonia production
Gut wall diameter	Vitamin synthesis	Toxic amine production
Gut wall length		Aromatic phenols
Gut wall weight		Bile degradation products
Fecal moisture		Fatty acid oxidation
Mucosal cell turnover		Fecal fat excretion
		Gut microbial urease

standpoint, feed antibiotics have been consistently shown to improve pig weight gain and feed efficiency as summarized in Fig. 1. The data demonstrate that growth and feed efficiency are improved to a greater extent in young pigs than in older animals. Also, the enhancement of body weight gain is greater than the improvement in feed efficiency, indicating that feed intake is increased in animals receiving antibiotic-supplemented feed. However, antibiotics increase gain and feed efficiency even at constant feed intake,[2] consistent with a direct effect on

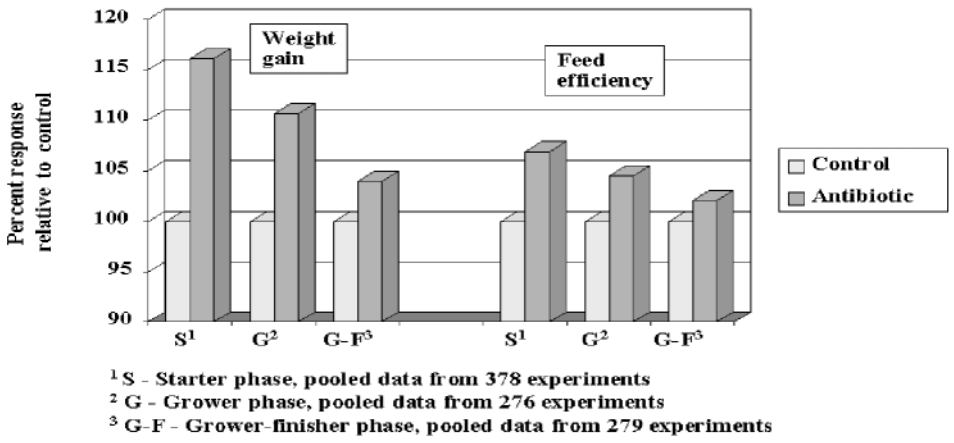

Figure 1. Effect of growth-promoting antibiotics on weight gain and feed efficiency in swine.[53]

growth which is independent of feed intake. These growth responses are associated with improved nitrogen metabolism, including an increase in apparent nitrogen digestibility (3.0%), increased nitrogen retention (5.8%), and reduced nitrogen excretion (10%) in pigs fed tylosin (10 ppm to 50 ppm; Table 2). Growth-promoting antibiotics also benefit protein metabolism irrespective of protein concentration in the diet.[3] Mechanisms by which antibiotics enhance growth should thus be consistent with their demonstrated effects on growth, feed efficiency, nitrogen metabolism, and with the relatively greater responses observed in younger animals.

Proposed Mechanisms for Antibiotic-Mediated Growth Enhancement

At least four mechanisms have been proposed as explanations of antibiotic mediated growth enhancement: (1) inhibition of sub-clinical infections, (2) reduction of growth-depressing microbial metabolites, (3) reduction of microbial use of nutrients, and (4) enhanced uptake and use of nutrients through the thinner intestinal wall associated with antibiotic-fed animals.[4–6] These share the common postulate that intestinal bacteria, whether commensal or pathogenic, depress animal growth, either directly or indirectly, through their metabolic activities. Perhaps the best support of this hypothesis is the observation that oral antibiotics do not enhance the growth of germfree animals,[7] while inoculating germfree animals with GI bacteria depresses growth.[8] A clear difference between germfree and conventional animals is a thinner wall of the small intestine, with a reduction in connective tissue and lymphoid elements.[8] Microscopic evaluation of germfree intestine reveals a more regular and slender villus structure, with a thinner lamina propria. Further, the rate of renewal of epithelial cells is slower in germfree animals,[8] which may have a beneficial effect on basal energy expenditure and the efficiency of nutrient utilization. These observations are consistent with the view of

Table 2. Effect of Antibiotics on Nitrogen Metabolism in Swine[1]

Parameter	Control	Tylosin	Relative Percent Improvement
Apparent nitrogen digestibility (% of nitrogen intake)	82.4	84.9	3.0%
Nitrogen retention (g N retained/day)	22.6	23.9	5.8%
Relative nitrogen excretion (g N excreted/g N retained)	1.01	0.91	10.0%
Nitrogen utilization efficiency (N retained as % of N intake)	54.5	57.0	4.6%

[1]See Ref. 54.

Reeds and coworkers[9] that in rapidly growing young animals, the GI tract and skeletal muscle draw from the same limited supply of nutrients and, in effect, compete for nutrients.

The Intestinal Microbiota is Competitive with the Host in the Small Intestine but Cooperative in the Large Intestine

Most of the attention given the pig intestinal microbiota has focused on the large intestine. Indeed, the large intestine (cecum and colon) is a major site of microbial colonization because of slow digesta turnover, and it is characterized by large numbers of bacteria (10^{10}–10^{11} per g or mL of content), low redox potential, and relatively high short chain fatty acid (SCFA) concentrations. The composition of the hindgut microbiota is both diverse and stable. Several hundred anaerobic bacterial species and strains appear to coexist without one or a few becoming dominant.[10–18] Microbial activity in the cecum and colon appears to benefit the host,[19] with estimates up to 5–20% of the pig's total energy being provided by fermentation end-products.[20] However, the small intestine is the principal site of nutrient and energy absorption, and thus is the region in which bacterial activity is likely to have the greatest influence on the efficiency of growth (Fig. 2).[6,21]

In the proximal small intestine, the rate of digesta flow and thus the rate of bacterial washout, exceeds the maximal growth rates of most bacterial species. Accordingly, this intestinal region is colonized typically by bacteria that adhere to the mucus layer or epithelial cell surface. Acid-tolerant lactobacilli and

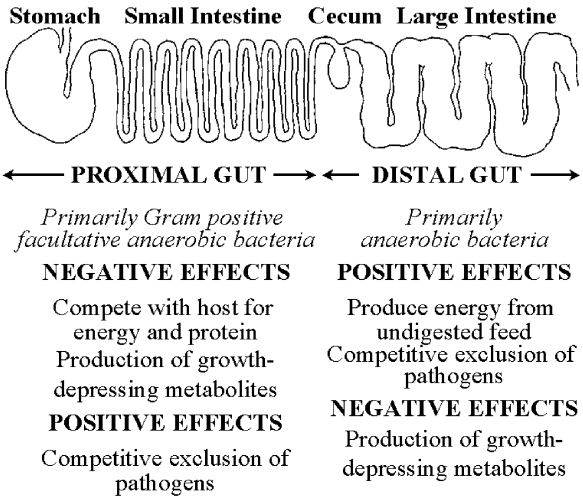

Figure 2. Diagram showing the positive and negative effects of microbiota in the proximal and distal gut.[18]

streptococci are thought to predominate in the upper small intestine.[10] The distal small intestine (ileum) maintains a more diverse microbiota and higher bacterial numbers (108 per g or mL of contents) than the upper intestine, and is considered a transition zone preceding the large intestine. In a study of microbial gas production in various gastrointestinal regions, Jensen and Jørgensen[22] reported that the highest H2 concentrations and production rates were found in the distal small intestine in pigs. This indication for significant microbial activity in the pig ileum was substantiated in their study by dense populations of culturable anaerobic bacteria and the detection of high ATP concentrations.

Culture-based studies have shown that small intestinal bacteria tend to compete with the host for energy and amino acids.[19] For example, as much as 6% of the net energy in pig diets can be lost due to bacterial utilization of glucose in the small intestine.[23] Bacterial use of glucose to produce lactic acid reduces the energy available to the host epithelium.[24] Lactic acid also enhances peristalsis, thus increasing the rate of nutrient transit through the intestine.[24] Amino acids, which are also degraded by small intestinal bacteria, are made unavailable to the pig and produce toxic metabolites such as amines, ammonia, phenols and indoles.[25] Microbial deconjugation and dehydroxylation of bile acids impair lipid absorption and produces toxic degradation products.[26] Mucolytic activities of intestinal bacteria are likely key for intestinal colonization but compromise the mucosal barrier and indirectly affect the efficiency of growth via stimulation of additional mucus production.[27]

It is interesting to note that although different types of bacteria may generate one or more of the metabolites mentioned, the Gram-positive facultative anaerobes, which are oxygen-tolerant and predominant in the small intestine, often contribute more than one toxic catabolite.[21] Further, the small intestinal microbiota consists predominantly of Gram-positive bacteria,[28] and most growth-promoting antibiotics target Gram-positive organisms (Table 3). These observations are consistent with involvement of small intestinal Gram-positive bacteria in growth depression.

It is curious that a class of organisms that appear to depress growth, namely Gram-positive facultative anaerobes including strains of Lactobacillus and Enterococcus, are also often used as probiotic organisms for enhancing health and promoting growth in livestock.[29] The growth-promoting effect of probiotics in livestock is less consistent than that observed with antibiotic supplementation.[29] Supplementation of animals and humans with certain probiotic bacteria has been shown to provide protection against intestinal, diarrhea-producing pathogens.[30] Therefore, probiotics may promote growth under situations in which certain pathogens are present; however, these same organisms in a cleaner facility may suppress growth via the mechanisms discussed above.

The combined potential of microbial activities to negatively impact intestinal functions clearly supports the hypothesis that certain bacterial populations commonly inhabiting the pig small intestine, though not necessarily pathogenic, cause a depression in growth which is reversed when the responsible organism(s)

Table 3. Growth-Promoting Antibiotics and Their Antibacterial Mode of Action[1]

Class	Trade Name	Generic Name	Antimicrobial		Orally Absorbed[2]
			Spectrum	Mechanism of Action	
Diterpene	Tiamulin	tiamulin	$Gram^+$	Protein synthesis inhibition	Yes
Glycopeptide	Avotan	avoparcin	$Gram^+$	Cell wall synthesisinhibition	No
Lincosaminides	Lincomix	lincomycin	$Gram^+$	Protein synthesis inhibition	Yes
Macrolide	Tylan	tylosin	$Gram^+$	Protein synthesis inhibition	Yes
	Spira 200	spiramycin			
Oligosaccharide	Maxus	avilamycin	$Gram^+$	Protein synthesis inhibition	No
β-lactam	Penicillin	penicillin	$Gram^+$	Cell wall synthesis inhibition	Yes
Peptides	Bacitracin	bacitracin	$Gram^+$	Cell wall synthesis inhibition	No
	Zn Bacitra	bactitractin			
Streptogramin	Stafac	virginiamycin	$Gram^+$	Protein synthesis inhibition	Yes
Phosphoglycolipid	Flavomycin	bambermycin	$Gram^+$	Cell wall synthesis inhibition	No
Polyether	Salocin	salinomycin	$Gram^+$	Membrane alterations	No
	Monteban	nerasin			
Quinoxalines	Mecadox	carbadox	Broad	DNA synthesis inhibition	Yes
	Bayonox	olaquindox			
Sulfonamides	Sulfamethazine	sulfamethazine	Broad	Metabolic inhibition	Yes
	Sulfa thizole	sulfathiazole			
Tetracycline	Aureomycin	chlortetracycline	Broad	Protein synthesis inhibition	Yes
	Terramycin	oxytetracyline			

[1]See Ref. 1, 55, 56.
[2]Commonly accepted property. "No" does not necessarily mean 0% absorption nor is "Yes" equal to 100% absorption.

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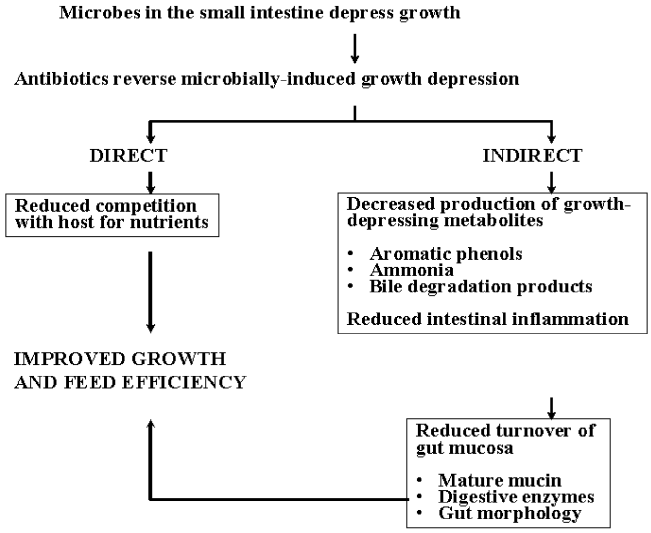

Figure 3. Diagram of the proposed effects of antibiotics mediated through their effects on small intestinal microflora.[6]

are metabolically inhibited or eliminated by feed-grade antibiotics (Fig. 3). However, the basic questions relating to the effects of microbial production of toxic catabolites in the pig intestine remain mostly unanswered, including the taxonomy, ecology, and metabolic properties of target bacteria. Because of the profound bias introduced by cultivation-based techniques, these questions will be defined most efficiently through the use of novel molecular ecology techniques.

Novel Methods for Intestinal Microbial Ecology Studies

Several limitations are associated with cultivation-based microbiological techniques, particularly for surveying the intestinal ecosystem.[31] In addition to being time- and labor-intensive, the use of selective media for different types of bacteria imposes an a priori bias on the types of bacteria that can be enumerated. Further, only 20–40% of bacterial species from the mammalian GI tract can be cultured and identified using current cultivation techniques. In other words, 60–80% of intestinal bacterial species may be overlooked.[31–33] In view of this, we have defined the utility and limitations of a cultivation-independent technique that uses the phylogenetic information contained in 16S rDNA to objectively study the intestinal microbiota.[34–36]

Denaturing gradient gel electrophoresis (DGGE) is a polymerase chain reaction (PCR)-based technique in which DNA from a mixed sample is amplified using conserved 16S rDNA bacteria-domain primers.[37] Although all PCR products are of approximately equal size, when electrophoresed on a poly-

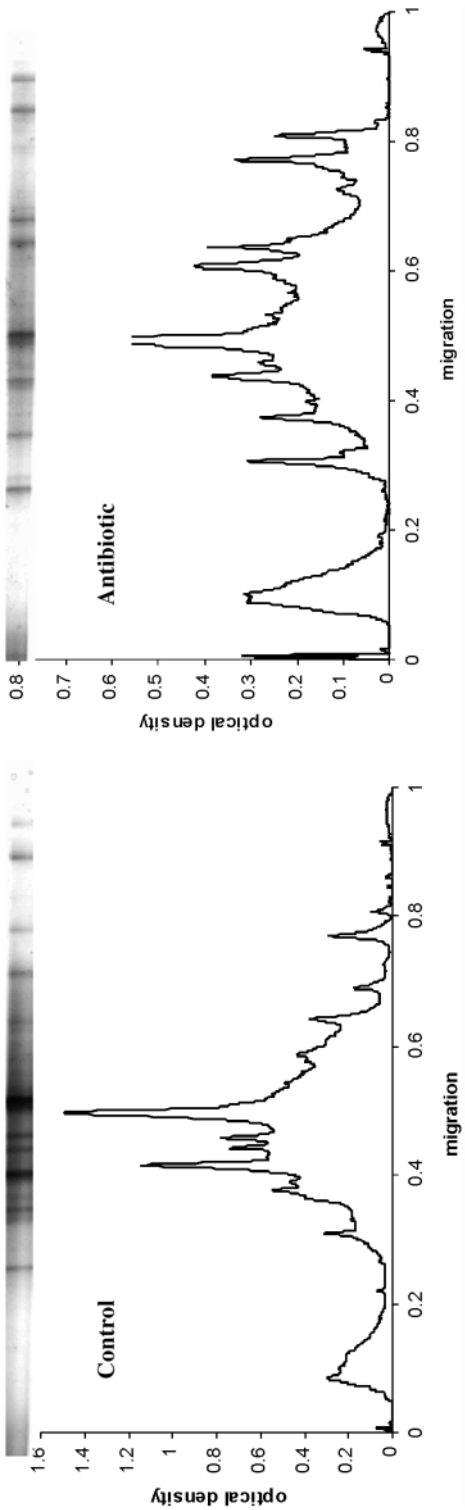

Figure 4. Representative linear plots of polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) bands from ileal samples of ileal-cannulated pigs fed control or antibiotic. A visual representation of the method used to determine the effects of antibiotics on PCR-DGGE profiles. Plots are read from the left, with the left-most area corresponding to the origin of the lane; numbers along the x-axis represent relative band migration distance in the gel. The peaks represent individual band positions within the gel; the area underneath the peak corresponds to the intensity of the peak.

acrylamide gel containing an increasing gradient of DNA denaturants, individual amplicons cease to migrate as the double-stranded products denature according to their G+C content.[37] Thus, this approach allows separation of individual sequences based on 16S rDNA G+C content, corresponding to the different microbial species within the sample. Banding patterns from mixed samples are compared to evaluate the relative similarity of microbial communities from different habitats or treatments. Further, following electrophoresis, individual bands can be excised from the gel for sequencing and phylogenetic identification. This allows the characterization of complex microbial populations independent of bacterial cultivation.

Effects of Antibiotics on the Normal Microbiota

We have recently used 16S rDNA PCR-DGGE to examine the effects of feed grade antibiotics on the intestinal microbiota in ileal-cannulated pigs. Two antibiotic regimes were compared with a standard nonmedicated diet: (1) continuous administration of a single antibiotic and (2) an antibiotic rotation sequence in which a different antibiotic was used each week. Newly weaned pigs were fed control (no antibiotic) or medicated diets for five weeks. Ileal microbiota profiles were compared by DGGE-PCR analysis as demonstrated in the preliminary data in Fig. 4 (Collier et al., unpublished observations). Similar gels were generated from all pigs. Through the use of statistical measures of community diversity, we observed that the ileal microbiota is more homogenous in antibiotic-treated pigs. This finding reflects the fact that bacterial community profiles generally varied among individual animals and did so to a greater extent in the control pigs that were weaned to a non-medicated diet. Other molecular-based ecology studies, by us and others, in mice, pigs, and humans also demonstrate that, although the intestinal bacterial community within a single individual is relatively stable over time, bacterial populations from different individuals vary significantly.[34,38–40] Individual variation in bacterial community profiles is consistent with the possibility that genetically encoded chemical epitopes expressed in mucus may dictate which bacteria colonize specific intestinal segments. This has significant implications for intestinal health issues and may also partly explain individual differences in the realization of growth potential in livestock species.

The inclusion of antibiotics also eliminated particular bacterial groups as evidenced by the disappearance of certain 16S rDNA PCR bands on the DGGE gels (Fig. 4). Individual bands that were eliminated by antibiotics were excised from DGGE gels and cloned and sequenced to identify the bacteria affected. Thus far, six *Lactobacillus* and two *Streptococcus* species have been identified through this process as being selectively depleted in antibiotic-treated pigs. Interestingly, lactobacilli are thought to be one of the main contributors to microbial bile acid biotransformation in the small intestine.[41] Indeed, Feighner and Dashkevicz[42,43] proposed that an important mechanism of growth-promoting antibiotics is the

inhibition of microbial bile acid biotransformation in the intestine. Microbial deconjugation and dehydroxylation of bile impairs lipid absorption by the host animal[44,45] and produce toxic degradation products that can impair growth.[46] Bile acids are not deconjugated in the germfree intestine, demonstrating the important role of commensal bacteria in this process.[47] The particular contributions of the lactobacilli to bile acid biotransformation in the intestine is demonstrated by evidence that ileal bile salt hydrolase activity in conventional mice is reduced 86% by the elimination of lactobacilli from the microbiota, and by greater than 98% when both lactobacilli and enterococci are eliminated.[41] Using chicks, Eyssen and DeSomer[48] first suggested that bile acid transformation products might be responsible for the growth depression caused by intestinal bacteria. Additional evidence from chick studies showed that bile acid deconjugation by intestinal bacteria causes growth depression that is reversible by antibiotic supplementation.[49] Further, Feighner and Dashkevicz[42,43] have shown an inverse relationship between the level of cholyltaurine hydrolase activity in the small intestine and growth rate in broiler chickens fed antibiotics. Antibiotic-treated pigs also demonstrated decreased concentrations of lithocholic acid and corresponding increases in average daily gain and feed efficiency, consistent with work reported in poultry.[50] While additional studies are required to verify the contribution of bile biotransformation to growth depression, these data clearly demonstrate the utility of molecular ecology to study the effects of antibiotics on the intestinal microbiota.

CONCLUSIONS

It is proposed that antibiotic modification of the small intestinal microbiota of swine permits more efficient intestinal and therefore whole animal growth. Recent advances in swine growth research have been directed primarily at affecting growth, feed efficiency, and body composition by altering the metabolism of muscle and fat.[51,52] An understanding of the inter-relationship of intestinal physiology, microbiology, and immunology to swine growth will become increasingly important to critically evaluate the impact of commensal bacteria on animal growth. In that regard, it is particularly exciting that molecular techniques are now available that allow a better understanding of how the intestinal microbial profile is changed with the use of antibiotics. These advances will allow the development of novel technologies, management systems, and modified nutrition to optimize intestinal health and animal growth.

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