

## INVITED REVIEW

# The Nonantibiotic Anti-Inflammatory Effect of Antimicrobial Growth Promoters, the Real Mode of Action? A Hypothesis

T. A. Niewold

*Nutrition and Health, Department of Biosystems, Katholieke Universiteit Leuven,  
Kasteelpark Arenberg 30, 3001 Heverlee, Belgium*

**ABSTRACT** Societal concern and government regulations increasingly press for restricting the use of antibiotics as antimicrobial growth promoters (AGP). The search for alternatives is on, hampered by a lack of knowledge about the exact mechanism of AGP. Feed additives, such as AGP and alternatives, interact with the intestine. In the intestine, feed components, microbiota, and the mucosa interact in a very complex and dynamic way. Various mechanisms for AGP have been proposed, invariably based on the direct antibiotic influence on the microbial composition of the intestines. In the literature on antibiotics, however, the direct effects of antibiotics on host cells, in particular inflammatory cells, have been described.

**Key words:** antimicrobial growth promoter, inflammation, inhibition, catabolism, growth permitting

2007 Poultry Science 86:605–609

## INTRODUCTION

Antimicrobial growth promoters (AGP) as feed additives have proved to be effective in improving growth and feed efficiency. Antimicrobial growth promoters are antibiotics added to feed in low, subtherapeutic amounts. Increasing restrictions on the use of AGP have prompted a search for the development of alternatives to AGP. A variety of replacements for AGP have been proposed and tested: pre-, pro-, and synbiotics; enzymes; (organic) acids; and herbs and herbal extracts. None of the alternatives tested have been as efficient as AGP, and in general have given variable results, whereas those obtained with AGP have been consistent and reproducible (Gaskins et al., 2002; Niewold, 2006; Page, 2006). The search for effective alternatives to AGP is hampered by a lack of knowledge about the mechanisms of AGP-mediated growth enhancement. At least 4 major mechanisms have been proposed to explain AGP-mediated growth enhancement (Gaskins et al., 2002; Dibner and Richards, 2005; Page, 2006): 1) AGP inhibit endemic subclinical infection, thus reducing the metabolic costs of the (innate) immune sys-

tem; 2) they reduce growth-depressing metabolites (such as ammonia and bile degradation products) produced by microbes; 3) they reduce microbial use of nutrients; and 4) they enhance the uptake and use of nutrients, because the intestinal wall in AGP-fed animals is thinner. All these points share the common hypothesis that the intestinal microflora depress animal growth, either directly or indirectly, and that the mechanism of AGP is based on its antibiotic properties. Usually, the absence of a growth-promoting effect of AGP in germ-free animals and the depression of growth upon inoculation of the latter with bacteria are seen as the strongest arguments for this hypothesis. It is also the reason why AGP is sometimes referred to as growth permitting rather than growth promoting.

## WHY AN ANTIBIOTIC MECHANISM FOR AGP IS UNLIKELY

All 4 mechanisms proposed show various weaknesses, and it is at least remarkable that alternative mechanisms, such as a direct effect on the host, are hardly ever considered. In this review, the merits of the 4 proposed mechanisms are discussed, and an alternative, more plausible mechanism is proposed.

*1. Antimicrobial growth promoters inhibit endemic subclinical infection, thus reducing the metabolic costs*

©2007 Poultry Science Association Inc.

Received October 9, 2006.

Accepted January 8, 2007.

<sup>1</sup>Correspondence: theo.niewold@biw.kuleuven.be

of the (innate) immune system. Antimicrobial growth promoters are given in subtherapeutic doses. These are lower than the minimum inhibitory concentration (MIC) for pathogens. Furthermore, chronic (sub-MIC) use of antibiotics is known to induce antibiotic resistance in pathogens and other bacteria (Aarestrup et al., 2001; Teuber, 2001). It is thus unclear how the inhibition is presumed to work.

2. *They reduce growth-depressing metabolites (such as ammonia and bile degradation products) produced by microbes, and*

3. *They reduce microbial use of nutrients.* Both arguments are based on the assumption that certain bacterial populations can be influenced selectively by the antibiotic action of AGP. Whereas this is true for antibiotics in concentrations higher than the MIC, it remains unclear how this can be achieved by low (sub-MIC) concentrations, because it is equally unclear for pathogens (see preceding discussion). Concerning the growth-depressing metabolites, Gaskins et al. (2002) remarked that "it is curious that a class of organisms that appears to depress growth (*Lactobacillus* and *Enterococcus*) are also often used as probiotic organisms for promoting growth in livestock" (p. 33). Furthermore, the majority of the intestinal microbiota are unknown (Xu and Gordon, 2003). Therefore, the overwhelming majority of publications thus far have dealt with the culturable (small) proportion of the microbiota, which leaves statements about nutrient use by the microbiota on weak underpinnings.

4. *They enhance uptake and use of nutrients, because the intestinal wall in AGP-fed animals is thinner.* Two separate elements can be distinguished in this argument. Concerning the uptake of nutrients, the apparent assumption is that there is an inverse relationship between mucosal thickness and uptake. In the literature, no data are available on whether AGP use actually enlarges the total mucosal surface. It is also a simplified concept of absorptive capacity for uptake, which is determined by the total mucosal surface area at the villus tips (Pappenheimer, 1998) rather than by the mucosal thickness. Furthermore, uptake is also influenced by the differentiation state of the epithelial cells, which can have different consequences for the uptake of different nutrients (Zhang et al., 1998). The second element, increased nutrient use by a thicker and larger intestine, is logical, because increased organ weight is associated with a larger contribution to body energy expenditure (Pond et al., 1988). Furthermore, a thinner intestinal wall is not necessarily a consequence of a direct effect of AGP on the microflora, as discussed in the next paragraph.

Even when assuming that AGP could have an antibiotic effect at sub-MIC concentrations, additional points can be made that cast further doubt on the theory of a direct effect of AGP on the microbiota. 1) Antimicrobial growth promoters have a similar effect in various production animals. These animals (e.g., poultry and pigs) differ considerably in the composition of intestinal microbiota. Furthermore, major shifts in microbiotic composition are seen during growth and development (Lu et al., 2003). These facts are hard to reconcile with a direct antibiotic effect

of AGP, and suggest instead a common, basic mechanism. 2) Antimicrobial growth promoters form a family of widely varying chemical classes and have different antimicrobial spectra of activity (e.g., predominantly gram positive or gram negative). In other words, despite the fact that different antibiotics influence different bacterial populations, similar effects are obtained. This is at least remarkable if the microbiota are the target. 3) Not all antibiotics have growth-promoting activity, whereas they should all influence the microbiota according to the microflora-management theory. One would at least expect antibiotics with a similar spectrum of activity to act similar to AGP. However, as cited by Page (2006), earlier authors concluded "there appears to be no obvious explanation for the great variation in growth-promoting activity between the different classes of antimicrobial substances studied" (p. 22). 4) Chronic use of low concentrations of antibiotics is known to induce resistance against antibiotics in most bacteria (Aarestrup et al., 2001; Teuber, 2001). It is thus less clear why (selective) shifts in microbiota composition still occur. 5) Alternatives to AGP that are known or purported to affect the microbiotic composition (e.g., probiotics) differ from AGP in that the effect is much less predictable and is highly variable. A possible explanation for the lack of success of these alternatives probably lies in the fact that the intestines are essentially a very complex and dynamic ecosystem (Xu and Gordon, 2003), and it is unclear how the composition of the microbiota can be manipulated toward a desired one. First, as mentioned, the composition is largely unknown, and second, what the desired composition should look like is unknown. Progress in this area can only be expected using more advanced molecular genomic techniques (Lu et al., 2003; Niewold et al., 2005; Niewold, 2006).

In any case, the differences between AGP and alternatives suggest a different target, and suggest that the target of AGP is most likely not the microbiota. Hence, it is worthwhile to take other possible nonantibiotic alternative mechanisms for AGP into consideration, and a very plausible one is available from the existing literature.

## **MOST ANTIBIOTICS HAVE A NONANTIBIOTIC ANTI-INFLAMMATORY EFFECT**

It is well established that many antibiotics have physiological side effects, most of which are specific to the chemical class of the compound. However, what the antibiotics have in common is that they accumulate in inflammatory cells (van den Broek, 1989; Labro, 1998, 2000). Most accumulated antibiotics enhance the intracellular killing of bacteria, and they can inhibit (parts) of the innate immune response. Scientists involved in immunological research are familiar with the inhibitory effects of antimicrobial compounds on phagocytic cells (macrophages and polymorphonucleocytes; van den Broek, 1989; Schoevers et al., 1999; Labro, 1998, 2000). This possible mechanism is absent from those commonly listed for AGP. Possible effects on the host are dismissed by saying that some

**Table 1.** Intraphagocytic accumulation of antibiotics that can lead to inhibition of function,<sup>1</sup> and relationship with use as antimicrobial growth promoters (AGP)

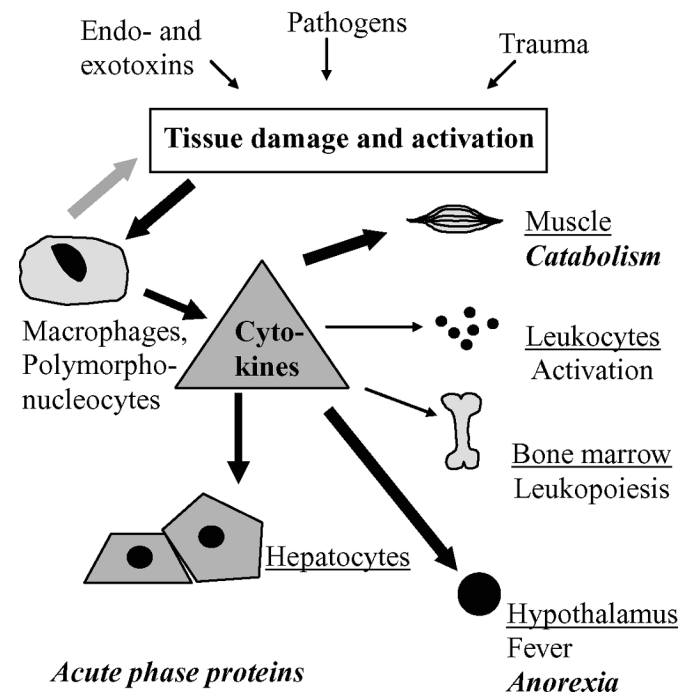
Antibiotic (class)	Intracellular accumulation (C:E ratio <sup>2</sup> )	Phagocyte function inhibition	AGP use, past or present
Chloramphenicol	4	No	No
$\beta$ -Lactams	<1	No/some/limited	No/some/limited
Cyclines	2	Yes	Yes
Quinolones	5	No	No
Macrolides	10–100	Yes	Yes
Streptogramin (peptide)	40	Yes	Yes

<sup>1</sup>Labro, 1998, 2000.<sup>2</sup>C:E ratio = the cellular:extracellular concentration.

AGP are nonabsorbable (e.g., Dibner and Richards, 2005) and that concentrations are too low, and hence are unlikely to cause effects beyond the intestinal lumen. Concerning the argument of nonabsorbability, the existence of recommended preslaughter withholding times for at least some AGP suggests otherwise (Feed Additive Compendium, 2006). Furthermore, nonabsorbability may be true, to a certain extent, in the healthy intestine, but episodes of enhanced intestinal permeability are not uncommon in production animals (Niewold et al., 2000). Moreover, one of the consequences of (intestinal) inflammation is increased macromolecular intestinal permeability (MacDonald and Monteleone, 2005), which certainly would enhance local penetration of (low molecular weight) antibiotics. Extracellular concentrations of AGP are too low for an antimicrobial effect. Phagocytic cells can accumulate antibiotics, in some cases 10- to 100-fold the ambient concentration. The relevant effect of this accumulation of many antibiotics in phagocytic inflammatory cells would be attenuation of the inflammatory response. As a consequence, the levels of proinflammatory cytokines would be lower than those in untreated animals, which would result in a lower catabolic stimulus. This is also consistent with a growth-permitting rather than a growth-promoting effect.

Antibiotic compounds can essentially be divided into 3 groups based on their interaction with inflammatory cells, namely, 1) nonaccumulating, 2) accumulating without inhibition of function, and 3) accumulating with inhibition of function. For the purpose of this investigation, the literature was searched for (classes of) antibiotics for which data were available on both antibiotic accumulation (as reviewed by van den Broek, 1989; Labro, 1998, 2000) and (past or present) use as AGP (as reviewed by Dibner and Richards, 2005; Page, 2006). In the case of  $\beta$ -lactams, data on both phagocyte inhibition and effectiveness as AGP are conflicting and inconsistent. Furthermore, in general, peptide antibiotics do not significantly alter phagocyte function. Streptogramin is a notable exception. Concerning bacitracin, an AGP used extensively in the United States, data are scarce on possible effects on phagocytes. van den Broek (1989) described an inhibitory effect of bacitracin on phagocytosis. Of 2 recent *in vitro* studies, one showed a possible proinflammatory role for bacitracin (Higuchi et al., 2004), whereas the other sug-

gested an anti-inflammatory effect (Alloza and Vandenberg, 2005). In the other classes of antibiotics, however, there appeared to be a good relationship between inhibition of inflammatory function and use as AGP (Table 1). It is suggested that this relationship is more than coincidental. Antibiotics have been shown to inhibit one or more of several different functions of inflammatory cells, chemotaxis, the production of reactive oxygen species, and proinflammatory cytokine production. In the context of growth, the latter effect is most important for the following reasons. Upon release of these cytokines, an acute phase response occurs, which has an overall catabolic effect. In addition to a shift in hepatic protein production toward acute phase proteins, catabolism of muscle tissue occurs and, furthermore, a loss of appetite (Gruys et al.



**Figure 1.** Schematic representation of the inflammatory response (modified after Jacobsen, 2003). Inflammatory cells are activated by tissue damage, causing production and excretion of proinflammatory cytokines. Circulating cytokines induce responses in different tissues. A reduction of growth results mainly from 3 responses (indicated in boldface italics). If unchecked, the inflammatory response itself can lead to further tissue damage (gray arrow), perpetuating catabolism.

2006; Figure 1). The acute phase response is a process clearly associated with the greatest physiological expenses (Humphrey and Klasing, 2003). Because of the magnitude of this effect, one would expect measurable effects from inhibitors. This would explain the consistent effect of AGP as compared with the varying effects (if any) found using alternatives with known effects on the microbiota.

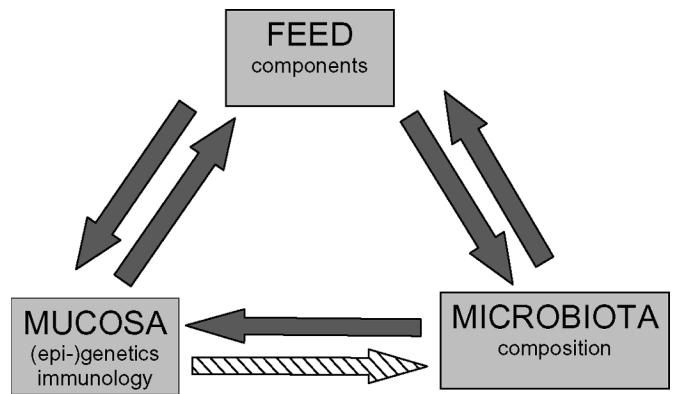
The intestines have been described as an organ in a state of constant controlled inflammation (Biancone et al., 2002). It is imperative for organisms to contain (intestinal) inflammation, and to that end, important physiological mechanisms are in place (Tracey, 2002; Metz and Tracey, 2005). Problems that do occur are usually the consequence of too strong, rather than too weak, a response. This is certainly true for polymorphonucleocytes and macrophages (Tracey, 2002; Metz and Tracey, 2005). It is suggested that the use of AGP lowers the level of inflammation. Intestinal inflammation usually leads to accumulation of inflammatory cells in the mucosa, leading to a thicker intestinal wall. The thinner intestinal wall observed using AGP is consistent with reduced inflammation because of the reduced influx and accumulation of inflammatory cells (Larsson et al., 2006).

Based on the preceding discussion, one can expect the largest effect of AGP in less optimal conditions. This is indeed consistent with the observation that effects are greatest when under the greatest infectious pressure, such as occurs at certain ages, in certain husbandry conditions, and in certain regions (Page, 2006). It also explains why the effect of AGP is absent from germ-free animals, because no bacterial challenge exists in that situation.

Earlier, the observed changes in microflora in response to AGP were considered a logical consequence of a direct effect of AGP on the microflora. However, it has become clear that the host itself has a large influence on the composition of the microbiota. The intestine is best described as a complex and dynamic ecosystem (Xu and Gordon, 2003). Intestinal microbial metabolism constitutes an important biochemical activity in the body, with important consequences for health and disease (Reid et al., 2003). The epithelial cells lining the intestines are influenced by the intestinal content (food and microbiota) in terms of differentiation and functionality. However, inborn (epi-)genetic factors and the immune system (and other components of the intestinal mucosa) are essential in the maintenance of equilibrium with commensals and in the defense against pathogens (Diekgraefe et al., 2000; Hooper and Gordon, 2001; Figure 2). The different microbial compositions when using AGP are, in this view, a consequence of an altered immune status rather than of a direct effect on the microbiota.

## INFLAMMATION MANAGEMENT RATHER THAN MICROFLORA MANAGEMENT

One can conclude that most arguments point toward an anti-inflammatory role for AGP, which reduces wasting energy and spares energy for production. Labro (2000)



**Figure 2.** Schematic representation of the complex and dynamic mutual interactions between the 3 main components of the intestinal ecosystem. The shaded arrow shows the often underestimated influence of the mucosa on microbial composition in the intestine.

describes this side effect of antibiotics, of reducing adverse effects while maintaining the beneficial ones, in human medicine as “the non-antibiotic effect of antimicrobial compounds” (p. 639). There is great interest in these direct anti-inflammatory properties in human medicine, such as for macrolides in pulmonary inflammatory conditions (Hoyt and Robbins, 2001). It is suggested that AGP work similarly in another mucosal system, the intestines, by managing inflammation.

## CONCLUSIONS

Based on the preceding discussion, effective alternatives for AGP should be found among nonantibiotic compounds with similar properties. They should accumulate in inflammatory cells and inhibit the inflammatory response. A search can be performed using relatively simple and inexpensive established laboratory techniques, with isolated cells or cell lines. Finally, the nonantibiotic anti-inflammatory mechanism of AGP is the first theory that, in a coherent way, explains the observations without the apparent contradictions and inconsistencies associated with the microflora-management theory.

## REFERENCES

- Aarestrup, F. M., A. M. Seyfarth, H. D. Emborg, K. Pedersen, R. S. Hendriksen, and F. Bager. 2001. Effect of abolishment of the use of antimicrobial agents for growth promotion on occurrence of antimicrobial resistance in fecal enterococci from food animals in Denmark. *Antimicrob. Agents Chemother.* 45:2054–2059.
- Alloza, I., and K. Vandebroek. 2005. The metalloproteinase inhibitor bacitracin inhibits interleukin-12  $\alpha\beta$  and  $\beta 2$  secretion. *J. Pharm. Pharmacol.* 57:213–218.
- Biancone, L., I. Monteleone, G. Del Vecchio Blanco, P. Vavassori, and F. Pallone. 2002. Resident bacterial flora and immune system. *Dig. Liver Dis.* 34:S37–S43.
- Dibner, J. J., and J. D. Richards. 2005. Antibiotic growth promoters in agriculture: History and mode of action. *Poult. Sci.* 84:634–643.
- Diekgraefe, B. K., W. F. Stenson, J. R. Korzenik, P. E. Swanson, and C. A. Harrington. 2000. Analysis of mucosal gene expres-

- sion in inflammatory bowel disease by parallel oligonucleotide arrays. *Physiol. Genomics* 4:1–11.
- Feed Additive Compendium. 2006. Miller Publishing Company, Minnetonka, MN.
- Gaskins, H. R., C. T. Collier, and D. B. Anderson. 2002. Antibiotics as growth promotants: Mode of action. *Anim. Biotechnol.* 13:29–42.
- Gruys, E., M. J. M. Toussaint, T. A. Niewold, S. J. Koopmans, E. van Dijk, and R. H. Meleen. 2006. Monitoring health by values of acute phase proteins. *Acta Histochem.* 108:229–232.
- Higuchi, T., Y. Watanabe, and I. Waga. 2004. Protein disulfide isomerase suppresses the transcriptional activity of NF- $\kappa$ B. *Biochem. Biophys. Res. Commun.* 318:46–52.
- Hooper, L. V., and J. L. Gordon. 2001. Commensal host-bacterial relationships in the gut. *Science* 292:1115–1118.
- Hoyt, J. C., and R. A. Robbins. 2001. Macrolide antibiotics and pulmonary inflammation. *FEMS Microbiol. Lett.* 205:1–7.
- Humphrey, B. D., and K. C. Klasing. 2003. Modulation of nutrient metabolism and homeostasis by the immune system. Pages 137–144 in *Proc. 14th Eur. Symp. Poult. Nutr.*, Lillhammer, Germany.
- Jacobsen, S. 2003. The bovine acute phase response to endotoxin and Gram-negative bacteria. PhD thesis. Royal Veterinary and Agricultural University, Copenhagen, Denmark.
- Labro, M. T. 1998. Antibacterial agents-phagocytes: New concepts for old in immunomodulation. *Int. J. Antimicrob. Agents* 10:11–21.
- Labro, M. T. 2000. Interference of antibacterial agents with phagocyte function: Immunomodulation or immuno-fairy tales? *Clin. Microbiol. Rev.* 13:615–650.
- Larsson, A. E., S. Melgar, E. Rehnström, E. Michaëlsson, L. Svensson, P. Hockings, and L. E. Olsson. 2006. Magnetic resonance imaging of experimental mouse colitis and association with inflammatory activity. *Inflamm. Bowel Dis.* 12:478–485.
- Lu, J., U. Idris, B. Harmon, C. Hofacre, J. J. Maurer, and M. D. Lee. 2003. Diversity and succession of the intestinal bacterial community of the maturing broiler chicken. *Appl. Environ. Microbiol.* 69:6816–6824.
- MacDonald, T. T., and G. Monteleone. 2005. Immunity, inflammation, and allergy in the gut. *Science* 307:1920–1925.
- Metz, C. N., and K. J. Tracey. 2005. It takes nerve to dampen inflammation. *Nat. Immunol.* 6:756–757.
- Niewold, T. A., G. J. van Essen, M. J. A. Nabuurs, N. Stockhofe-Zurwieden, and J. van der Meulen. 2000. A review of porcine pathophysiology: A different approach to disease. *Vet. Q.* 22:209–212.
- Niewold, T. A., H. H. D. Kerstens, J. van der Meulen, M. A. Smits, and M. M. Hulst. 2005. Development of a porcine small intestinal cDNA microarray: Characterization, and functional analysis of the response to enterotoxigenic *E. coli*. *Vet. Immunol. Immunopathol.* 105:317–329.
- Niewold, T. A. 2006. Intestinal genomics for evaluation of alternatives for AGP, current situation and perspectives. Pages 361–368 in *Antimicrobial Growth Promoters: Where Do We Go from Here?* D. Barug, J. de Jong, A. K. Kies, and M. Verstegen, ed. Wageningen Academic Publishers, Wageningen, the Netherlands.
- Page, S. W. 2006. Current use of antimicrobial growth promoters in food animals: The benefits. Pages 19–51 in *Antimicrobial Growth Promoters: Where Do We Go from Here?* D. Barug, J. de Jong, A. K. Kies, and M. Verstegen, ed. Wageningen Academic Publishers, Wageningen, the Netherlands.
- Pappenheimer, J. R. 1998. Scaling of dimensions of small intestines in non-ruminant eutherian mammals and its significance for absorptive mechanisms. *Comp. Biochem. Physiol.* A Mol. Integr. Physiol. 121:45–58.
- Pond, W. G., H. G. Jung, and V. H. Varel. 1988. Effect of dietary fiber on young adult genetically lean, obese and contemporary pigs: Body weight, carcass measurements, organ weights and digesta content. *J. Anim. Sci.* 66:699–706.
- Reid, G., M. E. Sanders, H. R. Gaskins, G. R. Gibson, A. Mercenier, R. Rastall, M. Roberfroid, I. Rowland, C. Cherbut, and T. R. Klaenhammer. 2003. New scientific paradigms for probiotics and prebiotics. *J. Clin. Gastroenterol.* 7:105–118.
- Schoevers, E. J., L. A. M. G. van Leengoed, J. H. M. Verheyden, and T. A. Niewold. 1999. Effects of enrofloxacin on porcine phagocytic function. *Antimicrob. Agents Chemother.* 43:2138–2143.
- Teuber, M. 2001. Veterinary use and antibiotic resistance. *Curr. Opin. Microbiol.* 4:493–499.
- Tracey, K. J. 2002. The inflammatory reflex. *Nature* 420:853–859.
- van den Broek, P. J. 1989. Antimicrobial drugs, microorganisms, and phagocytes. *Rev. Infect. Dis.* 11:213–245.
- Xu, J., and J. I. Gordon. 2003. Honor thy symbionts. *Proc. Natl. Acad. Sci. USA* 100:10452–10459.
- Zhang, H., C. Malo, C. R. Boyle, and R. K. Buddington. 1998. Diet influences development of the pig (*Sus scrofa*) intestine during the first 6 hours after birth. *J. Nutr.* 128:1302–1310.