Immuno-Modulation and Anti-Inflammatory Effects of Antibiotic Growth Promoters Versus Probiotics in the Intestinal Tract

Anita Menconi, Lisa R. Bielke, Billy M. Hargis, and Guillermo Tellez*

Department of Poultry Science, University of Arkansas, Fayetteville, AR, USA.

Email for Correspondence: gtellez@uark.edu

ABSTRACT

Antibiotics as growth promoters (AGP) in the feed of different animal species have been used for many years to improve feed efficiency and growth. The mechanisms of how antibiotics can promote growth are not well known; nevertheless several studies have focused on interactions between antibiotics and intestinal microflora. However, many scientists have suggested that there may be other modes of action of some antibiotics, including the capacity of these antibiotics to produce immunomodulatory and anti-inflammatory effects. It has been demonstrated that antibiotics are able to inhibit one or more functions of inflammatory cells and pro-inflammatory cytokine production. Consequently, AGP may prevent immunologic stress that is associated with metabolic changes, allowing more energy to be available for muscle development thus, improving growth. Although the use of AGP in animal production has advantages, its extensive use has been suggested to have contributed to the emergence of antimicrobial resistance in zoonotic pathogens, resulting in a ban by the European Union with suggestive restrictions by other countries. The withdrawal of AGP from livestock production has led to an increase in cost, a decrease in production, and an increase in disease incidence, forcing food animal producers to find alternatives including probiotics, which have been used to enhance intestinal health. The major functional effect of probiotics may be the balance of both gastrointestinal microflora and immune-stimulation, particularly by maintaining the equilibrium of pro-inflammatory and anti-inflammatory cytokines. This review describes the immuno-modulation and anti-inflammatory effects of antibiotic growth promoters versus probiotics in the intestinal tract.

Keywords: Antibiotic growth promoter, probiotic, enteric health, inflammation

INTRODUCTION

Antibiotics are considered growth promoters when administered at non-therapeutic (low) concentrations in the feed of food animals to stimulate growth and improve feed efficiency (Costa et al., 2011; Lin et al., 2013). Antibiotics growth promoters (AGP) in the feed of different animal species has been used for more than 60 years in the United States, as well as in other countries (Dibner and Richards, 2005; Costa et al., 2011), and their effect has been related to feed efficiency improvement and indirect growth improvement (Dibner and Richards, 2005).

The mechanisms of how antibiotics can promote growth are still unclear, with the most common hypotheses being:

1. improvement of nutrient absorption by thinning the intestinal wall and villi and reducing intestinal size, which could be caused by a loss of mucosal cell proliferation due to the lack of short chain fatty acids in the lumen, which is provided through microbial fermentation;
2. limiting nutrient use by bacteria;
3. decreasing the number of bacteria and bacterial toxins; and
4. reducing the incidence of subclinical infections by decreasing the metabolic cost of the immune system (Gaskins et al., 2002; Butaye et al., 2003; Dibner and Richards, 2005; Niewold, 2007; Costa et al., 2011).

Many studies regarding the mode of action of AGP have focused on interactions between antibiotics and the intestinal microflora, as reviewed extensively by Dibner and Richards (2005). However, several scientists indicate that the main activity of several growth promoting antibiotics could be due to an anti-inflammatory effect rather than antimicrobial activity per se, and the microflora changes would be a consequence of the intestinal changes (Niewold, 2007; Buret, 2010; Costa et al., 2011).

Although the use of AGP has been a common practice of modern animal production (Butaye et al., 2003), its extensive use has been suggested to contribute to the emergence of antimicrobial resistance in zoonotic pathogens (Costa et al., 2011; Lin et al., 2013). As a result, the European Union employed a ban on the administration of all AGP to livestock on January 1, 2006 (Anadon et al., 2006). Moreover, restrictions to the use of AGP in the United States has been anticipated as well (Dibner and Richards, 2005; Costa et al., 2011). Consequently, such a ban has led to an increase in the costs of animal production, a decrease in livestock production (Costa et al., 2011), and an increase in the incidence of some animal diseases (Dibner and Richards, 2005), forcing animal producers to find alternatives such as enzymes, organic acids, probiotics, prebiotics, essential oils, and immune-stimulants (Huyghebaert et al., 2011). Regulation of immune functions in the intestine is associated with establishment of the microflora, which has led to the introduction of therapeutic interventions with the use of cultures of beneficial live microorganisms known as probiotics (Isolauri et al., 2001). Moreover, due to their characteristics and mode of action, probiotics have been extensively studied as an alternative for AGP in animal production (Chaucheyras-Durand and Durand, 2010).

Probiotics are commonly known as beneficial supplements to humans and animals (Applegate et al., 2010; Soccol et al., 2010). They have been used for many years to enhance intestinal health and treat intestinal disorders (Patterson and Burkholder, 2003; Nicholson, 2002; Aureli et al., 2010) and their efficiency is reported in a multitude of studies, as reviewed by Laudanno et al. (2006), Chaucheyras-Durand and Durand(2010), Vila et al. (2010), and Ringel et al. (2012). Additionally, probiotic features such as metabolic activity of specific strains, survivability and persistence in the gastrointestinal tract, and concentration administered are important for probiotic optimal efficacy (Chaucheyras-Durand and Durand, 2010; Huyghebaert et al., 2011).

Several mechanisms of action of probiotics have been proposed, including competition for receptor sites and nutrients, production of antimicrobial substances such as bacteriocins, hydrogen peroxide, and volatile fatty acids (Patterson and Burkholder, 2003; Vandeplas et al., 2010). Also, probiotics have been described to cause a decrease in intestinal pH by the production of organic acids, which in turn would create favorable conditions for both transient and resident microflora (Chaucheyras-Durand and Durand, 2010) and production of nutrients and growth factors, stimulating intestinal microflora (Delcenserie et al., 2008). Among the mechanisms proposed for probiotic functions, modulation of innate and acquired immune systems has received great attention (Kabir, 2009; Jijon et al., 2004; Ng et al., 2009; Flore et al., 2010; Dicks and Botes, 2010; Soccol et al., 2010). Moreover, it has been described that specific probiotic strains show anti-inflammatory properties, which has led to the research and discovery of new mechanisms of action of selected probiotic strains, such as promoting a balance between local pro-inflammatory and anti-inflammatory cytokines (Isolauri et al., 2002; Pagnini et al., 2010).

### Mode of action of growth promoter antibiotics on intestinal inflammation

The fact that most AGP are administered at doses that are less than the minimum inhibitory concentration for most bacteria disputes the hypothesis of bacterial inhibition as proposed mechanism of action of AGP (Niewold, 2007). Furthermore, the hypothesis of microflora modulation by AGP could also be contested, since AGP have similar effect on multiple animal species that contain different intestinal microflora (Niewold, 2007). On the other hand, even though the subtherapeutic doses of AGP are not able to inhibit bacteria, AGP are able to cause a change in the microflora composition by affecting the intestinal morphology and immunity, thus leading to growth promoting effects in the animal host (Huyghebaert et al., 2011; Lin et al., 2013). According to Niewold (2007), changes in immune responses can cause modulation of the microflora composition. Therefore, there has been a rise of acceptance of the hypothesis that AGP effects involve anti-inflammatory and immune-modulatory responses by the host (Costa et al., 2011).

Important features of animal production such as growth, feed utilization, reproduction, and milk and egg production are affected directly or indirectly in the presence of an inflammatory reaction, since animals depend on energy and proteins to develop and maintain immune functions, which can be costly during uncontrolled inflammation (Buret, 2010). It has been proposed that the use of AGP such as streptomycin, penicillin, and tetracycline might prevent immunologic stress, which is associated to metabolic changes, consequently allowing the energy to be available for muscle development, improving growth performance (Roura et al., 1992; Costa et al., 2011). Niewold (2007) presented a table in which it was
pointed out that AGP commonly used in poultry production have high intracellular accumulation levels and inhibit phagocytic function, supporting the hypothesis that AGP have more effect on the host than enteric microbial populations. According to Costa et al. (2011), AGP could reduce immunologic stress in the host, since the intestinal mucosa is in continuous interaction with the microflora, and consequently in a state of “physiologic inflammation”. In a study, Costa et al. (2011) used chlortetracycline, a common AGP for mammalian livestock, in mice to test an ‘immunomodulation hypothesis’ regarding the mode of action of AGP. The authors showed up-regulation of interleukin 22 transcripts, a cytokine that initiates innate immune responses against bacterial pathogens, at the peak stage of Citrobacter rodentium induced intestinal infection, and down-regulation of tumor necrosis factor-α (TNF-α), interleukin-1B (IL-1B), and IL-17A transcripts, all of which are cytokines involved in inflammation, and innate and adaptive immunity, at the late stage of the intestinal infection, when compared to control mice. Overall, Costa et al. (2011) indicated that the immunomodulatory action of AGP is consistent with an increase in body weight gain, since the immunomodulation provides a catabolic benefit to the host.

Niewold (2007) stated that antibiotics are able to accumulate in phagocytic inflammatory cells improving the intracellular killing of bacteria and partially inhibiting the innate immune response. As a result, treated animals would have a decrease in pro-inflammatory cytokines (Buret, 2010), which in turn would save catabolic energy facilitating its growth. As elucidated by Buret (2010), another contribution of the AGP in reducing inflammation could be explained by an induction of neutrophil apoptosis by some types of antibiotics such as macrolides, which has also been described to reduce pro-inflammatory cytokines of both innate and acquired immune systems (Steel et al., 2012). Antibiotics have showed many anti-inflammatory and immunomodulatory properties such as suppression of bacterial virulence factors, accumulation in inflammatory cells, downregulation of the expression of the transmembrane receptor integrin, which participates in immune cell adhesion at infection sites, and inhibition of T-cell maturation and proliferation, and neutrophil migration (Rubin and Tamaoki, 2005).

Mode of action of probiotic products on intestinal inflammation

A major functional effect attributed to the consumption of probiotics is balance of both gastrointestinal microflora and immune-stimulation (Parvez et al., 2006; Parracho et al., 2007; Hammes and Hertel, 2002). Several human and animal studies have showed clear evidence that specific strains of probiotics are able to stimulate the innate immune system in many ways (Alvarez-Olmos and Oberhelman, 2001; Reveneau et al., 2002; Jouani et al., 2012; Farnell et al., 2006) as well as to increase humoral immunity (Parvez et al., 2006; Joint, 2001; Arvola et al., 1999; Kalliomaki et al., 2001; Ouwehand et al., 2002). Moreover, the effects of many probiotics are related to immune regulation, mostly by maintaining the equilibrium of pro-inflammatory and anti-inflammarory cytokines (Borchers et al., 2009; Braat et al., 2004). Intestinal inflammation can be related to an imbalance betweenintestinal microflora and immune system (Isolauri et al., 2001; Isolauri et al., 2002). The most described pro-inflammatory cytokines involved in intestinal inflammation are IL-1, IL-8, TNFα, and interferon gamma (IFNγ; Dinarello, 2000). On the other hand, some of the most common anti-inflammatory cytokines are interleukin 10 (IL-10) and TGF-β (Opal and Depalo, 2000). It has been proposed that the anti-inflammatory mechanisms of probiotic bacteria are induced by an improvement in barrier function, synthesis of antimicrobials, and a modulation of both microflora and mucosal immune system which, in general, occurs by decreasing the production of pro-inflammatory cytokines and increasing production of anti-inflammatory cytokines (Ewaschuk and Dieleman, 2006).

According to Isolauri et al. (2001, 2002), probiotic bacteria are able to equilibrate local pro-inflammatory and anti-inflammatory cytokines. These beneficial microorganisms have shown a reduction in lymphocyte proliferation and cytokine production by T-cells, and also a reduction in intestinal inflammatory responses through the stimulation of secretory IgA, which protects the mucosal surface by non-activation of inflammatory responses (Isolauri et al., 2001). In a study on the influence of lactic acid bacteria on the intestinal mucosa of mice, Galdeano and Perdigon (2004) reported an increase in the number of IL-10 cells and a stimulation of IgA production after treatment with a viable Lactobacillus casei probiotic strain. There have also been descriptions of the capability of probiotic bacteria to increase production of anti-inflammatory cytokines such as IL-10 and TGF-β (Isolauri et al., 2002). Moreover, probiotics have been shown to decrease the secretion of inflammatory cytokines by increasing the degradation of antigens in the intestine (Isolauri et al., 2002).

Okada et al. (2009) described a downregulation of mRNA expression of IL-1 β and TNF-α, which are released by macrophages during intestinal inflammation, by probiotic strains of Bifidobacterium species. Furthermore, the authors reported that the commensal Enterococcus faecalis,bacterium that has been described to play a role in inflammatory bowel disease (IBD) in both animals and humans, stimulated macrophages to produce IL-12, a cytokine that facilitates the differentiation of CD4+ T helper cells through the activation of IFN-γ production (Okada et al., 2009). Yan et al. (2011) reported that a soluble protein derived from the probiotic strain of Lactobacillus rhamnosus was able to minimize...
the effects of dextran sodium sulfate (DSS) induced colitis in mice by reducing intestinal epithelial apoptosis, through the activation of epidermal growth factor receptor.

Interestingly, genetically modified probiotic bacteria engineered to produce anti-inflammatory cytokines could also play a role in controlling intestinal inflammation. Steidler et al. (2000) showed a reduction in induced DSS colitis of mice treated with a strain of Lactococcus lactis genetically engineered to secret IL-10. The stimulation of the release of IL-10 by a mixture of probiotic strains (Bifidobacterium longum, Bifidobacterium infantis, Bifidobacterium breve, Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus delbrueckii subsp. bulgaricus, Lactobacillus plantarum, and Streptococcus salivarius subsp. thermophilus) known as VSL#3 was also described by Drakes et al. (2004) and Hart et al. (2004), in an in vitro model using dendritic cell cultures. Moreover, the probiotic combination VSL#3 was tested in an in vivo experiment with mice and showed a stimulation of epithelial innate immunity (Pagnini et al., 2010). In addition, an increase in IL-10 has been described in mice fed Lactobacillus delbrueckii subspecies bulgaricus and Lactobacillus casei (Ghosh et al., 2004).

In a DSS or trinitrobenzenesulfonic acid induced colitis model in mice, Bacillus polyfermenticus demonstrated a reduction in the expression of inflammatory molecules such as chemokine (C-X-C motif) ligand 1, intercellular adhesion molecule, and TNF-α. The same strain also increased the expression of IL-10, thus decreasing colon inflammation (Im et al., 2009). In an in vitro experiment using human peripheral blood mononuclear cells, a probiotic combination of Bacillus mesentericus, Clostridium butyricum, and Enterococcus faecalis showed a decrease in TNF-α levels and an increase in IL-10 levels, increasing the number of T regulatory cells (Hua et al., 2010). Also, using human peripheral blood mononuclear cells, Imaoka et al. (2008) observed an increase in the production of IL-10, and an inhibition of IL-8 (cytokine associated with inflammation in ulcerative colitis) secretion by a probiotic composed by Bifidobacterium umbilicus and Bifidobacterium breve. Furthermore, in a study conducted with IBD and healthy human patients, Shadnoush et al. (2013) showed an increase in serum levels of the cytokines IL-6 and IL-10, and a decrease in serum levels of IL-1β and TNF-α in IBD patients treated with a probiotic yogurt containing Bifidobacterium and Lactobacillus.

**DISCUSSION AND CONCLUSIONS**

In summary, the mechanisms of immunomodulation and anti-inflammatory effects of probiotics and subtherapeutic doses of in-feed antibiotics in animal production are not entirely known. It has been demonstrated that antibiotics are able to inhibit one or more functions of inflammatory cells such as chemotaxis, reactive oxygen species production, and pro-inflammatory cytokine production. Inhibiting pro-inflammatory cytokine production would cause a decrease in acute phase response, which is linked to a high uptake of catabolic energy (Niewold, 2007) This seems especially likely when one considers the fact that AGP are administered at levels below the minimum inhibitory concentration for bacteria, thus suggesting their mode of action is not one that directly affects bacteria. Besides their well-known effects, probiotic bacteria may act to stabilize intestinal inflammation by balancing intestinal microflora, maintaining mucosal barrier, and modulating and improving the intestinal mucosal immune system, especially by preserving the balance of pro-inflammatory and anti-inflammatory cytokines and production of intestinal IgA (Isolauri et al., 2002; Hua et al., 2010; Ashraf and Shah, 2013; Zagato et al., 2014).

Government and consumer pressures for the withdrawal of AGP from livestock and poultry production has caused concerns about potential cost increase of meat production related to decreases in production parameters such as body weight and feed conversion, and increase in bacterial disease incidence. Antibiotics are unique compounds and there is a low probability that another compound or mixture could completely replace their use as growth promoters in animal production, having the same exact mechanism of action and reproducible effect. However, research demonstrates that both antibiotics and probiotics have effects on intestinal microflora modulation and similar impact on reducing intestinal inflammation through the down-regulation of pro-inflammatory cytokines (Niewold, 2007; Costa et al., 2011; Steel et al., 2012; Ewaschuk and Dieleman, 2006; Okada et al., 2009; Hua et al., 2010; Shadnoush et al., 2013).

This suggests that while the mechanism of action may not be the same for AGP and probiotics, effects on animals and GIT are similar and probiotics have potential to serve as alternatives for AGP in animal production. Although the immune stimulation by probiotic bacteria is strain specific and varies greatly depending among single strains and combinations (Ashraf and Shah, 2013), the use of probiotics in animal production has a potential to mimic the effects of AGP or mitigate the losses caused by their withdrawal, and be an important tool in raising meat animals under antibiotic free programs.

**REFERENCES**
