Evaluation of *Bacillus* Cultures (88/18) and Antibiotic Supplementation Administered in the Diets of Nursery Pigs on Growth Performance and Potential Immunological Mode of Action

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### Story in Brief

A total of 288 pigs were weaned to a wean-to-finish facility and blocked by BW to determine the effect of a *Bacillus*-based direct-fed microbial (DFM) and antibiotic (AGP) supplementation on the blood cytokine profiles. Treatments were arranged as a 2 x 2 factorial with 2 levels of AGP and 2 levels of DFM. Peripheral blood mononuclear cells (PBMC) were isolated from 4 pigs/treatment on d 20 and d 42 postweaning (PW) and cytokine production was elaborated by stimulating with lipopolysaccharide (LPS) for tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) concentrations and with concanavalin A for interleukin-4 (IL-4) concentrations. Plasma samples were obtained to measure circulating concentrations of TNF-α. Monocyte IL-1β concentrations from pigs fed DFM were decreased (P < 0.05) from d 20 to d 42 PW, whereas IL-1β concentrations from pigs not fed DFM were similar across the 2 days (DFM x day interaction, P < 0.05). Interleukin-4 concentrations from PBMC from pigs fed DFM were similar across the 2 days PW, whereas PBMC IL-4 concentrations from pigs not fed DFM decreased (P = 0.05) from d 20 to d 42 (DFM x day interaction, P < 0.05). Plasma TNF-α concentrations decreased (P < 0.05) from d 20 to d 42 PW in pigs fed AGP, but remained similar across the 2 days in pigs not fed AGP (AGP x day interaction, P = 0.10). No differences were detected in unstimulated PBMC TNF-α concentrations on d 20 PW amongst treatments; however, PBMC TNF-α concentrations from pigs fed AGP were lower (P < 0.05) on d 42 than from pigs fed the control diet, whereas TNF-α concentrations from pigs fed DFM or DFM+AGP did not differ from control or AGP supplemented pigs (DFM x AGP x day interaction, P = 0.09). These data indicate that both DFM and AGP supplementation alters the cytokine profile of nursery pigs by decreasing the production of pro-inflammatory cytokines (IL-1β and TNF-α) at the end of the nursery phase.

### Introduction

The mechanisms by which growth-promoting levels of antibiotics improve pig performance have not been completely elucidated. One hypothesis is that alterations in the intestinal microbial population may decrease the production of negative growth factors such as the inflammatory cytokines, which have been documented to decrease feed intake and negatively alter metabolic growth processes (Spurlock, 1997). The gastrointestinal tract of animals is the site of complex interactions between the host immune system and various dietary factors and their breakdown products, as well as microorganisms, parasites, and exogenous toxins (Gaskins, 2001). Studies on colonization of the intestinal tract of gnotobiotic animals with either defined enteric bacteria or incompletely defined normal gut microflora revealed that the microbial population drives gut immune system development (Cebra, 1999). A direct-fed microbial containing *Bacillus* strains has been shown to decrease feed intake and improve feed efficiency (Spurlock, 1997). The mechanisms by which growth-promoting levels of antibiotics improve pig performance have not been completely elucidated. One hypothesis is that alterations in the intestinal microbial population may decrease the production of negative growth factors such as the inflammatory cytokines, which have been documented to decrease feed intake and negatively alter metabolic growth processes (Spurlock, 1997). The gastrointestinal tract of animals is the site of complex interactions between the host immune system and various dietary factors and their breakdown products, as well as microorganisms, parasites, and exogenous toxins (Gaskins, 2001). Studies on colonization of the intestinal tract of gnotobiotic animals with either defined enteric bacteria or incompletely defined normal gut microflora revealed that the microbial population drives gut immune system development (Cebra, 1999). A direct-fed microbial containing *Bacillus* strains has been shown to decrease feed intake and improve feed efficiency (Spurlock, 1997).

### Experimental Procedures

**Animals and Housing.** Approximately 288 pigs from 32 litters were weaned and transported to the University of Arkansas Wean-to-Finish Facility (~ 2 miles). Pigs were blocked by initial body weight, penned in groups of 7 to 8 pigs/pen, and housed in 36 slatted pens (1.5 m x 3.0 m) equipped with a radiant heater, 2-hole feeder, and wean-to-finish cup waterers.

**Treatment Allocation.** One of four dietary treatments was randomly assigned to each pen within block and administered during Phase 1 (d 0 to 8 post-weaning), Phase 2 (d 8 to 21 post-weaning), and Phase 3 (d 21 to 43 post-weaning) of the nursery period. Treatments were arranged in a 2 x 2 factorial design, with 2 levels of antibiotic supplementation (0 g and 50 g Carbadox/ton of feed during Phase 1 and Phase 2; 0 g and 400 g oxytetracycline/ton of feed during Phase 3) and 2 levels of supplementation with a *Bacillus*-based direct-fed microbial (0% and 0.25% MicroSource 88/18 to provide 7.5 x 10^5 cfu/g of feed). Pigs allotted to treatments containing *Bacillus* cultures were separated from those fed diets devoid of *Bacillus* to eliminate any exposure of the pigs to the feces that might cross-contaminate pens not administered *Bacillus*.

**Sampling.** A 15 mL blood sample was collected and mixed with EDTA (ethylenediaminetetraacetic acid) for the isolation of peripheral blood mononuclear cells (PBMC) on d 20 and d 42 post-weaning. Cytokine elaboration of lymphocytes was induced by the addition of concanavalin A (ConA) to determine interleukin (IL-4) concentrations. Monocyte-derived macrophages were enriched from PBMC by adhesion to glass surfaces. Monocyte-derived...
macrophages were then primed with interferon-γ and stimulated with lipopolysaccharide (LPS) to determine tumor necrosis factor (TNF-α) and IL-1β production. A 5 mL blood sample was collected and mixed with EDTA for the determination of plasma TNF-α concentrations.

Statistical Analysis. Data were analyzed as a randomized complete block design with pen as the experimental unit. Analysis of variance was performed using the GLM procedure of SAS (SAS Institute, Inc., Cary, N.C.). The model included the effects of antibiotic addition, Bacillus supplementation, day, and appropriate interactions.

Results and Discussion

Bacillus supplementation to pigs during the nursery phase of production did not influence (P > 0.18) production of TNF-α, IL-1β, or IL-4 from unstimulated PBMC (Table 1). Plasma TNF-α concentrations were also unaffected (P = 0.67) by Bacillus supplementation to pigs during the nursery period (Table 1). However, pigs fed Bacillus cultures had a higher (P < 0.05) production of IL-1β from LPS stimulated PBMC on d 20 after weaning compared to d 42 after weaning, while IL-1β production was similar (P > 0.10) on d 20 and d 42 after weaning in pigs fed diets devoid of Bacillus cultures (Bacillus x day interaction, P = 0.05; Fig. 1). Furthermore, the production of IL-4 from ConA stimulated PBMC of pigs supplemented with Bacillus cultures was similar (P > 0.10) on d 20 and 42 after weaning, but pigs fed diets devoid of Bacillus cultures had a higher (P < 0.05) production of IL-4 from ConA stimulated PBMC on d 20 after weaning compared to production on d 42 after weaning (Bacillus x day interaction, P = 0.05; Fig. 2). Pigs fed antibiotics had lower (P < 0.05) plasma TNF-α concentrations on d 42 after weaning when compared to d 20 after weaning and compared to pigs fed diets devoid of antibiotics on d 20 and 42 after weaning (Antibiotic x day interaction, P = 0.10; Fig. 3). Moreover, pigs fed diets containing Bacillus cultures and antibiotics in combination on d 20 after weaning had a lower (P < 0.05) production of TNF-α from unstimulated PBMC when compared to d 42 after weaning and compared to pigs fed the negative control or antibiotics-only diets on d 42 after weaning (Bacillus x Antibiotic x day interaction, P = 0.09; Fig. 4).

The improvements in growth performance due to antibiotic supplementation observed in the current study and others (Gustafson and Bowen, 1997) may be due to decreased concentrations of plasma TNF-α by the end of the nursery period. Tumor necrosis factor-α is a pro-inflammatory cytokine that is released by macrophages during inflammation to activate cellular and humoral components of the immune system. This pro-inflammatory cytokine and others such as IL-1β and IL-6 are responsible for redistributing the body’s nutrients from tissue growth to support immune function (Wan et al., 1989). Therefore, the reduction in plasma TNF-α due to antibiotic supplementation may allow the piglet to utilization energy for growth rather than for immune system development/activation.

Supplementation of Bacillus cultures has been reported to improve growth performance in weaning pigs (Yang et al., 2003); however, results have been variable. Administration of Bacillus cultures has resulted in improved feed intake in swine when evaluated in field trials on commercial swine farms and in university research experiments. At d 42 after weaning, pigs fed the diet with Bacillus cultures and antibiotics in combination had a higher concentration of TNF-α from unstimulated PBMC than at d 20 after weaning. The negative effects seen in feed efficiency and increased production of TNF-α from unstimulated PBMC due to Bacillus and antibiotic supplementation to pigs during Phase 3 may be due to the use oxytetracycline during this phase of production. A study conducted by Agtech Products, Inc. reported that oxytetracycline inhibits the growth of Bacillus spores in vitro. Therefore, the use of oxytetracycline during Phase 3 may have inhibited the growth or killed the Bacillus organisms, thereby eliciting an inflammatory immune response and negatively affecting the pig’s growth response.

The results of this study indicate that care should be taken when combining direct-fed microbial products with antibiotics that may either mask their effect or be a detriment to performance due to the inflammatory responses that may be generated from the killing of the organisms by antibiotics. Although the concentrations of inflammatory cytokines measured earlier in the nursery phase in response to Bacillus supplementation were higher, at the end of the nursery period pigs supplemented with Bacillus cultures had reduced TNF-α and IL-1β concentrations from stimulated PBMC when compared to pigs fed diets devoid of Bacillus. These anti-inflammatory responses may be a result of the immune system responding to an initial inflammatory response from feeding Bacillus, such that the cytokine profile of these pigs develops toward a more anti-inflammatory profile as a feedback control response to inflammation. In this respect, the initial inflammation from Bacillus supplementation that may result in decreased performance guides the immune system development toward anti-inflammatory responses that promote growth and efficiency.

Literature Cited

### Table 1. Effects of pigs fed diets with two levels of antibiotic supplementation (0 g and 50 g Carbadox/ton of feed during Phase 1 and Phase 2; 0 g and 400 g oxytetracycline/ton of feed during Phase 3), and two levels of Bacillus supplementation (0% and 0.25% 88/18) during Phase 1, Phase 2, and Phase 3 on cytokine elaboration responses from peripheral blood mononuclear cells cultures on days 20 and 42 after weaning.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Bacillus at d 42 after weaning</th>
<th>Antibiotics at d 42 after weaning</th>
<th>Days Post-weaning</th>
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<tbody>
<tr>
<td></td>
<td>-</td>
<td>+</td>
<td>SE</td>
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<tr>
<td><strong>TNF-α (pg/mL)</strong></td>
<td></td>
<td></td>
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<tr>
<td>Plasma*</td>
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<td>3,735</td>
<td>1095-1140</td>
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<td><strong>IL-1β (pg/mL)</strong></td>
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<tr>
<td><strong>IL-4 (pg/mL)</strong></td>
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<tr>
<td>Stimulated**</td>
<td>328</td>
<td>267</td>
<td>60</td>
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</table>

* Values represent the concentration of tumor necrosis factor-α (TNF-α) or interleukin-1 (IL-1β) in wells stimulated with lipopolysaccharide (LPS) minus the concentration of TNF-α or IL-1β in the unstimulated wells.

** Values represent the cytokine concentration in wells stimulated with concanavalin A minus the cytokine concentration in the unstimulated wells.

* Antibiotic x day interaction, P = 0.10 (see Fig. 3).

** Bacillus x day interaction, P ≤ 0.05 (see Fig. 1 & 2).

*** Antibiotic x Bacillus x day interaction, P = 0.09 (see Fig. 4).
Fig. 1. Monocyte IL-1β elaboration responses from LPS stimulated cell cultures of peripheral blood mononuclear cells isolated from pigs fed diets with or without *Bacillus* cultures during the nursery phase of production (*Bacillus* x day interaction, P = 0.05). a,b Bars with differing letters represent means that differ (P < 0.05).

Fig. 2. Lymphocyte IL-4 elaboration responses from Con A stimulated cell cultures of peripheral blood mononuclear cells isolated from pigs fed diets with or without *Bacillus* cultures during the nursery phase of production (*Bacillus* x day interaction, P = 0.05). a,b,c Bars with differing letters represent means that differ (P < 0.05).
Fig. 3. Plasma TNF-α concentrations from pigs fed diets with or without antibiotics during the nursery phase of production (Antibiotic x day interaction, P = 0.10). a,b Bars with differing letters represent means that differ (P < 0.05).

Fig. 4. Monocyte TNF-α elaboration responses from unstimulated cell cultures of peripheral blood mononuclear cells isolated from pigs fed diets containing *Bacillus* cultures and/or antibiotics during the nursery phase of production (*Bacillus* x Antibiotic x day interaction, P = 0.09). a,b,c Bars with differing letters represent means that are differ (P < 0.05).