EFFECTS OF BIOPLUS 2B AND LEVUCELL SB ON WEANLING PIG GROWTH PERFORMANCE AND FECAL SHEDDING IN RESPONSE TO ORAL CHALLENGE WITH *SALMONELLA* SEROVAR TYPHIMURIUM

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Summary

Eighty-five pigs (initially 12.9 lb and 15 ±1 d of age) were used in two 28-d trials to determine the effects of the probiotics BioPlus 2B (a bacillus-based product from Chr. Hansen BioSystems), a source of *Bacillus subtilis* and *Bacillus licheniformis*, and Levucell SB (an active dry yeast product from Lallemand Animal Nutrition), a yeast (*Saccharomyces cerevisiae*) product that is a source of mannanoligosaccharides on growth and performance of *Salmonella enterica* serovar Typhimurium shedding in a young growing pig model.

Pigs were fed one of five dietary treatments: 1) A control diet containing no probiotics or antibiotics; 2) the control diet with carbadox (50 g/ton); 3) the control diet with BioPlus 2B (0.05% of the diet); 4) the control diet with Levucell SB (100 g/ton); 5) the control diet with Bioplus 2B (0.05% of the diet) and Levucell SB (100 g/ton). Diets did not contain growth promoting levels of zinc oxide or copper sulfate.

Significant differences in the two trials were seen, with the second trial having 0.1 lb/d greater growth and .18 lb/d greater feed intake than the first trial. In trial 1, pigs fed the control diet and Bioplus 2B had greater gains and feed intakes ($P<0.05$ and $P<0.02$ respectively) than those fed carbadox. In trial two, pigs fed carbadox had better performance than those fed the control and combination diets, with the BioPlus 2B and Levucell SB treatments having intermediate growth and feed intake. Fecal shedding rates of *salmonella* were not different ($P>0.10$) between diets. Results indicate that within an environment where enteric disease may be active, BioPlus 2B and Levucell SB may provide growth enhancement over a diet devoid of antimicrobials.

(Key Words: Weanling Pigs, Disease Challenge, *Salmonella*, BioPlus 2B, and *Saccharomyces cerevisiae*)

Introduction

With greater consumer concerns over antibiotic resistance, there has been increased pressure to remove and find alternatives to antibiotics in livestock diets Alternatives to antimicrobials that may hold promise include probiotic feed additives. Supplementation of swine diets with probiotics to alter the intestinal microbial population and to promote gut health has shown variable responses in growth. Possible reasons for variation among trials may be due to various levels and species of enteric organisms within production sys-

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tems. Weanling pigs were fed the probiotics Bioplus 2B, a bacillus-based product, and Levucell SB, an active yeast culture, in order to determine the growth responses and bacterial shedding rates in the presence of an orally administered enteric pathogenic challenge.

Materials and Methods

The Kansas State University Institutional Animal Care and Use Committee approved the experimental protocol used in this study. Eighty-five weanling pigs (initially 12.9 lb) were blocked by initial weight and allotted to one of five dietary treatments in two 28 d trials. Two pigs were assigned to a pen, with a total of eight or nine replicates per treatment. All pigs were housed in two environmentally controlled rooms. One feeder and one nipple-waterer were provided in each pen to allow ad libitum access to feed and water. All pigs were fed a common diet for the first 7 d after weaning. The pigs were switched to the experimental dietary treatments for 14 d before inoculation. The basal diet contained no added antimicrobials, while the positive control diet contained carbadox (50 g/ton). The three test diets contained a bacillus-based product (BioPlus 2B, Chr. Hansen BioSystems; 0.05% of the diet), an active dry yeast product (Levucell SB, Lallemand Animal Nutrition; 100 g/ton), and a combination of the two products. The experimental diets were fed in meal form (Table 1).

Prior to challenge, fecal samples were taken to insure that all animals were not shedding salmonellae organisms. On d 14, pigs were inoculated with *Salmonella enterica* serovar Typhimurium (2.36×10^6 and 1.22×10^6 CFU in trials 1 and 2, respectively). This dose was used to closely mimic a chronic, but containable gut pathogen load. Rectal temperatures and feed intakes were determined daily for the first week after inoculation. Pigs were weighed and ADFI, ADG, and F/G were determined on days 0, 7, 14, 21, and 28 of the experimental period. Fecal samples were collected on d 21 and 28 days to be cultured for semi-quantitative counts of salmonellae.

Table 1. Diet Composition[ab] (As-fed)

Ingredients	Control
Corn	50.74
Soybean meal	27.94
Spray dried whey	10.00
Select menhaden fishmeal	4.50
Soy oil	3.00
Monocalcium phosphate, 21% P	1.20
Limestone	0.68
Salt	0.35
Vitamin premix	0.25
Trace mineral premix	0.15
Lysine HCl	0.15
DL-Methionine	0.05
Cornstarch[b]	1.00
Total	100.00%
Calculated analysis	
Lysine, %	1.39%
ME, kcal/kg	3388
Calcium, %	0.90
Phosphorous, %	0.79

[a]Diets did not contain growth promoting levels of zinc oxide or copper sulfate.
[b]Test ingredients, carbadox (50 g/ton), BioPlus 2B (0.05% of the diet), and Levucell SB (100 g/ton) replaced cornstarch to provide the additional dietary treatment.

A semi-quantitative method for evaluating *Salmonella* shedding was developed for the experiment. *Salmonella* growth was classified into one of four categories; 1) confluent growth over the entire agar plate (score 3); 2) any number of wells with growth on the agar plate (score 2); 3) only growth that was obtained on enrichment (score 1); 4) or no growth detected (score 0). The shedding and colonization scores were analyzed using the non-parametric Kruskal-Wallis Test. On d 27 and 28 of the study pigs were euthanized and fecal samples from the colon were collected and tested for the presence of culturable *Salmonella*.

Data were analyzed in a randomized complete block design replicated over time using the Mixed model procedures of SAS. All means presented are least-square means.

Results and Discussion

Both trials are reported separately due to significant trial differences (P<0.05) for all variables except feed efficiency, and diet x trial interactions (P<0.05) for all variables except feed efficiency and daily rectal temperatures.

Growth Performance

Trial 1. No differences in ADG or ADFI were seen in the two weeks prior to challenge (Figure 1, left panels). In the two weeks following challenge the pigs fed the control diet or Bioplus 2B diet had greater ADG than pigs fed the antibiotic or Levucell diets (P<0.01). Pigs fed the diet containing the combination of Bioplus 2B and Levucell had decreased ADG compared to the control pigs (P>0.03), but not with the pigs fed the diet containing Levucell (P<0.06). Greater feed intakes were observed for the pigs fed the control diet and diet containing Bioplus 2B compared to the pigs fed carbadox, Levucell, or the combination treatment (P<0.03). For the overall trial, pigs fed the control and Bioplus 2B diets had greater ADG and ADFI than the pigs fed carbadox, with the pigs fed Levucell and the combination being intermediate in ADG (P<0.03) and ADFI (P<0.02). No differences were observed in feed efficiency.

Growth performance in the first trial was surprising in that pigs fed carbadox gained more slowly than control pigs. No obvious reasons for this effect could be identified. Initially it was hypothesized that the carbadox and control diets may have been accidentally switched at the feed mill. However, upon analysis of the diets at a commercial laboratory, it was determined that the carbadox diet did indeed contain the antimicrobial at the appropriate level and the negative control contained no antimicrobials. At present we don't have an explanation for the failure of carbadox to improve growth performance compared with control pigs. Pigs fed the Bioplus 2B diets performed similarly to the pigs fed the control diet, with the Levucell and combination fed pigs performing intermediate in response to enteric challenge. The combination of the two probiotics showed no additive benefit before or after enteric challenge.

Trial 2. Pigs fed carbadox had greater ADG (P<0.05) and ADFI (P<0.04) when compared to the pigs fed the control, Bioplus 2B, and combination diets prior to challenge (Figure 1, right panels). Pigs fed the diet containing Levucell had intermediate ADG (P<0.07) and ADFI (P<0.04) in comparison to carbadox and control, Bioplus 2B, or the combination diets. In the 2 weeks following bacterial challenge pigs fed the diet containing carbadox had greater ADG than pigs fed the control diet (P<0.01), with the Bioplus 2B, Levucell, and combination fed pigs intermediate in growth (P<0.40). Following challenge, carbadox-fed pigs had greater feed intakes than the control or combination-fed pigs (P< 0.01). The Bioplus 2B and Levucell-fed pigs were also higher (P<0.05) in feed intake than the control. Overall, carbadox-fed pigs had greater growth than the control (P<0.01) and combination diets (P<0.05), with pigs fed Bioplus 2B (P<0.09) and Levucell having intermediate performance (P<0.12). Overall in trial 2, the carbadox fed pigs had greater (P<0.02) feed intake than the control or combination pigs. The Bioplus 2B and Levucell fed pigs were intermediate in intake. There were no differences in feed efficiency.

The increase in feed intake and growth when feeding carbadox both before and after enteric challenge is consistent with several previous research trials in our laboratory. Bioplus 2B and Levucell SB pigs were intermediate in growth and feed intake in comparison to the control and carbadox-treated pigs.

Although neither Levucell nor Bioplus 2B treatments matched carbadox in growth response, both did numerically, though not significantly, better than the control fed pigs. The combination of Bioplus 2B and Levucell showed no additive benefit before or after challenge.

Fecal Salmonella Shedding Scores

Fecal *Salmonella* were cultured and measured 7 and 14 d following challenge with *Salmonella* serotype Typhimurium (corresponds to d 21 and 28 of trials). Pen shedding scores did not differ between treatments and were low and variable between days and trials, generally ranging between scores of 0 and 1 among treatments.

Initially it was hypothesized that fecal scores would be lowest for carbadox treated pigs, with the Bioplus 2B and Levucell being intermediate. Our hypothesis was based on the ability of antimicrobials to inhibit the growth of pathogenic organisms within the gut. Throughout both trials, fecal scores were typically low for presence of *Salmonella* for days 21 and 28 of the trials. No obvious treatment differences were observed.

Summary

Growth performance and feed intake were not typical in trial 1 for the control and antibiotic treatments in comparison to previous research conducted within our laboratory. Reasons for the underperformance of the antimicrobial regimen are uncertain in the presence of an enteric pathogen. However, trial 2 showed all treatments having improved performance over the control diet. Trial 2 also showed the expected growth enhancement properties of carbadox in the presence of enteric disease. Although not significant, Bioplus 2B and Levucell showed improvement over the control with about a 10% improvement in growth and 7% improvement in feed intake. The combination treatment showed only a 7% improvement in growth over the control in trial 2. These, diets containing Bioplus 2B or Levucell SB in a production environment may provide improved growth enhancement over diets with no antimicrobials in the presence of an enteric pathogen.

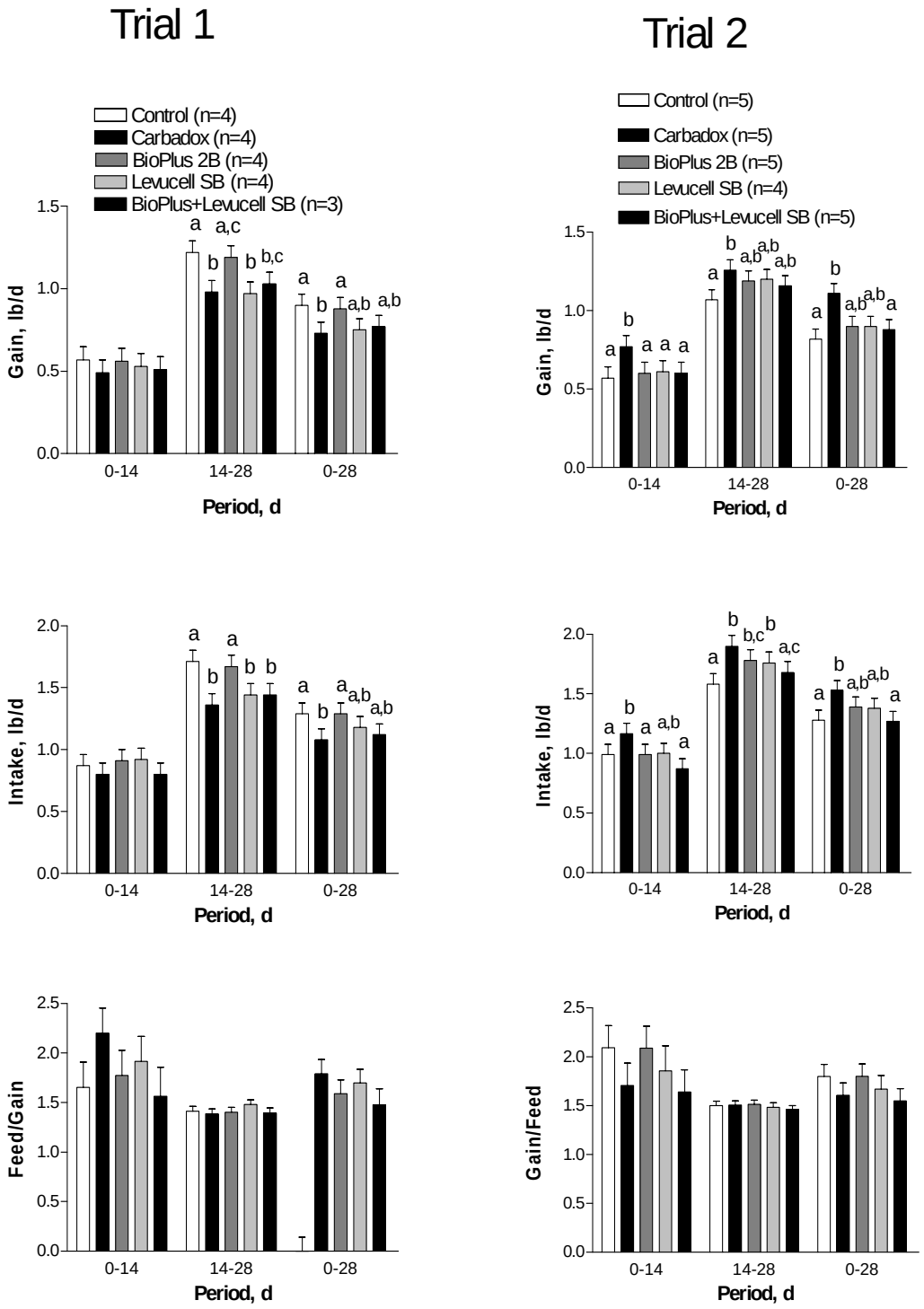

Figure 1. Effect of BioPlus 2B and Levucell SB on growth performance of weaned pigs prior to and after oral challenge with 106 *Salmonella* Serovar Typhimurium on d 14 in both trials. The number of pens per treatment is given in parenthesis in the figure legends. Within period bars with different superscripts differ (P< 0.05).