

Title: Understanding the relationship between immune response, intestinal microbial ecology and growth performance in nursery pigs fed diets with, or without in-feed antibiotics or a combination of beta-glucan and vitamin C. **NPB # 06-103**

Investigator: Marcos H. Rostagno

Institution: USDA,ARS, Livestock Behavior Research Unit, West Lafayette, IN

Date Submitted: 03/13/2009

II. Industry Summary

Weaning and the subsequent nursery phase represent a time of gastrointestinal and immune instability for the pig. This research project was developed to understand the effect(s) of two commonly used in-feed antibiotics (carbadox and tylosin), and a dietary combination of beta-glucan and vitamin C on the immune system and intestinal bacteria of nursery pigs. Two hundred and sixteen crossbred weanling pigs were used in a 4-week experiment. Pigs were weaned at 21 days of age, and housed in a total of 24 pens (9 pigs/pen). Diets were typical commercial nursery diets and experimental treatments included; 1) control diet with no antibiotic added; 2) in-feed antibiotic A (carbadox); 3) in-feed antibiotic B (tylosin); 4) in-feed beta-glucan and Vitamin C supplementation. Growth performance (i.e., weight gain and weight gain to feed intake ratio) were determined weekly throughout the experiment. Fecal samples were collected from each pen on a weekly basis to monitor common intestinal bacteria (*Escherichia coli*, coliforms, and *Lactobacilli*). At weaning, 2 weeks post-weaning (35 days of age), and 4 weeks post-weaning (49 days of age), 1 pig per pen (6 pigs/treatment) was randomly selected for euthanasia and collection of intestinal samples. Intestinal contents were collected for microbiological analysis. Blood was collected to determine the red blood cell (RBC) percentage and total white blood cell (WBC) percentage, as well as a quantitative analysis of different types of white blood cells (neutrophils, lymphocytes, and monocytes). Mesenteric lymph nodes and lung tissue (approximately 3 cm²) were also collected, and the relative abundance of an anti-microbial compound (PR-39), bacterial recognition molecules (TLR 2, 4), and the inflammatory molecule IL-1 and its competitor, IL-1 receptor antagonist, were determined. Growth performance for weeks 1, 2, 3, and 4 p.w., as well as for the collective 4 week period (i.e., overall), did not differ between males and females nor for pigs receiving different treatments. Intestinal bacterial populations did not differ between males and females nor for pigs that received different treatments and were euthanized at weaning and 2 weeks post-weaning. However, at week 4 post-weaning ileal *Lactobacilli* counts were less in piglets fed BGA compared to those fed the control diet, carbadox, or tylosin. Bacteria found in the feces were not affected by gender or by any of the four treatments in weeks 1, 2, and 3 post-weaning. However, at week 4 p.w., males fed BGA had an increase in fecal coliform counts compared to females fed tylosin. Analysis of the MLN showed an elevation of PR-39, TLR 2, TLR 4, IL-1, and IL-1Ra expression at week 2 p.w., which returned to levels detected at weaning by week 4 post-weaning. Neutrophil

These research results were submitted in fulfillment of checkoff-funded research projects. This report is published directly as submitted by the project's principal investigator. This report has not been peer-reviewed.

For more information contact:

National Pork Board • PO Box 9114 • Des Moines, IA 50306 USA • 800-456-7675 • Fax: 515-223-2646 • pork.org

count and total WBC count were greatest in BGA treated pigs, especially in relation to carbadox treated pigs, at 2 weeks post-weaning. Analysis of the lung tissue revealed that pigs fed the diet without antibiotics (i.e., control) had either an increasing expression of PR-39, TLR 2, TLR 4, IL-1, and IL-1Ra from week 2 to week 4 p.w., or the expression of each remained constant. The expression for the tylosin and BGA treated pigs was similar for IL-1 and PR-39 in the lung tissues. Although the use of tylosin in nursery diets did not improve growth performance, it was most favorable to the pig's immune system by protecting the pig from an increase in intestinal pathogens and insuring the health of the animals. Administration of beta-glucan and vitamin C may be an attractive alternative to antibiotics, but additional research needs to be conducted.

III. Scientific Abstract

Weaning and the subsequent nursery phase represent a time of gastrointestinal and immune instability for the pig. This research project was developed to understand the effect(s) of two commonly used in-feed antibiotics (carbadox and tylosin), and a dietary combination of beta-glucan and vitamin C on the immune system and intestinal bacteria of nursery pigs. Two hundred and sixteen crossbred weanling pigs were used in a 4-week experiment. Pigs were weaned at 21 days of age, and housed in a total of 24 pens (9 pigs/pen). Diets were typical commercial nursery diets and experimental treatments included; 1) control diet with no antibiotic added; 2) in-feed antibiotic A (carbadox); 3) in-feed antibiotic B (tylosin); 4) in-feed beta-glucan and Vitamin C supplementation. Growth performance (i.e., weight gain and weight gain to feed intake ratio) were determined weekly throughout the experiment. Fecal samples were collected from each pen on a weekly basis to monitor common intestinal bacteria (*Escherichia coli*, coliforms, and *Lactobacilli*). At weaning, 2 weeks post-weaning (35 days of age), and 4 weeks post-weaning (49 days of age), 1 pig per pen (6 pigs/treatment) was randomly selected for euthanasia and collection of intestinal samples. Intestinal contents were collected for microbiological analysis. Blood was collected to determine the red blood cell (RBC) percentage and total white blood cell (WBC) percentage, as well as a quantitative analysis of different types of white blood cells (neutrophils, lymphocytes, and monocytes). Mesenteric lymph nodes and lung tissue (approximately 3 cm²) were also collected, and the relative abundance of an anti-microbial compound (PR-39), bacterial recognition molecules (TLR 2, 4), and the inflammatory molecule IL-1 and its competitor, IL-1 receptor antagonist, were determined. Growth performance for weeks 1, 2, 3, and 4 p.w., as well as for the collective 4 week period (i.e., overall), did not differ between males and females nor for pigs receiving different treatments. Intestinal bacterial populations did not differ between males and females nor for pigs that received different treatments and were euthanized at weaning and 2 weeks post-weaning. However, at week 4 post-weaning ileal *Lactobacilli* counts were less in piglets fed BGA compared to those fed the control diet, carbadox, or tylosin ($p < 0.05$). Bacteria found in the feces were not affected by gender or by any of the four treatments in weeks 1, 2, and 3 post-weaning. However, at week 4 p.w., males fed BGA had an increase in fecal coliform counts compared to females fed tylosin ($p < 0.05$). Analysis of the MLN showed an elevation of PR-39, TLR 2, TLR 4, IL-1, and IL-1Ra expression at week 2 p.w., which returned to levels detected at weaning by week 4 post-weaning. Neutrophil count and total WBC count were greatest in BGA treated pigs, especially in relation to carbadox treated pigs, at 2 weeks post-weaning ($p < 0.05$). Analysis of the lung tissue revealed that pigs fed the diet without antibiotics (i.e., control) had either an increasing expression of PR-39, TLR 2, TLR 4, IL-1, and IL-1Ra from week 2 to week 4 p.w., or the expression of each remained constant. The expression for the tylosin and BGA treated pigs was similar for IL-1 and PR-39 in the lung tissues. Although the use of tylosin in nursery diets did not improve growth performance, it was most favorable to the pig's immune system by protecting the pig from an increase in intestinal pathogens and insuring the health of the animals. Administration of beta-glucan and vitamin C may be an attractive alternative to antibiotics, but additional research needs to be conducted.

IV. Introduction

Weaning and the subsequent nursery phase represent a time of gastrointestinal and immune instability for the pig. The combination of unstable gastrointestinal pH and changes in substrate availability in the gastrointestinal tract (GIT), provide the perfect environment for opportunistic, pathogenic bacteria colonization of the GIT causing a decline in health of the pig. To prevent colonization of the GIT by detrimental pathogenic microorganisms, subtherapeutic concentrations of antibiotics are routinely fed to nursery pigs. Improvements in growth performance from antibiotic use have been extensively reported (Zimmerman, 1986; Dewey et al., 1999; Cromwell, 2002; Dritz et al., 2002). However, in-feed antibiotics are coming under increasing public scrutiny as a result of concerns over bacterial antibiotic resistance. Therefore, the animal production industry is under increasing pressure to find alternatives to antibiotics.

This research project was developed to understand the effect(s) of two commonly used in-feed antibiotics (carbadox and tylosin), and a dietary combination of beta-glucan and vitamin C on the immunological status and intestinal microbial ecology of nursery pigs. By understanding the mode of action and effects of these commonly used in-feed antibiotics; more efficient alternatives can be developed and implemented. This research project has the unique characteristic of comparatively evaluating treatments with different approaches. The use of antibiotics is expected to have a direct impact on the intestinal microbial ecology, and consequently on immune responses from the host, whereas the combination of beta-glucan and vitamin C is expected to affect the immune status of the host, and potentially, the overall health and growth of the pigs. Based on the results obtained from this research, a better understanding of the relationship between immunological status, intestinal microbial ecology and growth performance, in the presence of in-feed antibiotics is expected.

V. Project Objectives:

The objectives of the proposed research are; **1)** To determine the effect of in-feed antibiotics on the immunological status and intestinal microbial ecology in nursery pigs; and **2)** to determine the effect of dietary beta-glucan and vitamin C on the immunological status, intestinal microbial ecology and growth performance in nursery pigs.

VI. Materials and Methods:

Study Design - Two hundred and sixteen crossbred weanling pigs were used in a 4-week experiment. Pigs were weaned at approximately 21 days of age, blocked by weight, gender and ancestry, and randomly assigned to treatments. Pigs were housed in a total of 24 pens (9 pigs/pen). Diets were typical commercial nursery diets (based on corn and soybean meal) and experimental treatments included:

- 1) Negative control with no antibiotic added (Treatment 1);
- 2) Treatment 1 + in-feed antibiotic A (Carbadox);
- 3) Treatment 1 + in-feed antibiotic B (Tylosin); and
- 4) Treatment 1 + Beta-Glucan and Vitamin C supplementation.

Sampling and Sample Processing - Growth performance (i.e., weight gain and feed conversion) was determined weekly throughout the experiment. Fecal samples were collected from each pen on a weekly basis to monitor the longitudinal pattern of *Escherichia coli*, coliforms, and *Lactobacilli* shedding from the groups subjected to the different treatments. At weaning (21 days of age), 2 weeks post-weaning (35 days of age), and 4 weeks post-weaning (49 days of age), 1 pig per pen (6 pigs/treatment) was randomly selected for euthanasia and necropsy. During necropsies conducted at the indicated time points (i.e., day 0; week 2; and week 4), ileal and cecal contents were collected for microbiological analysis, including; enumeration of *Escherichia coli*, coliforms and *Lactobacilli* populations, based on plating of 10-fold serial dilutions on selective media (3M

Petrifilm EC, and Rogosa agar, respectively). Blood was collected into one 10-ml acid citrate dextrose (ACD) tube and one 5-mL EDTA tube, then placed on ice for transport to the laboratory. The EDTA blood was used to determine hematocrit percentage, granulocyte, peripheral blood mononuclear cell, and total white blood cell counts using a Hemavet. Tissue samples were collected from the mesenteric lymph nodes, and lung (approximately 3 cm²). These samples were washed immediately with sterile Hank's Balanced Salt Solution (HBSS) and placed on ice for transport to the laboratory, where RNA was extracted using RNEasy mini kits (Qiagen). Quality of RNA was checked by spectrophotometry. Following reverse transcription, real-time PCR was used to amplify and quantify the genes of interest. The relative abundance of anti-microbial peptide (PR-39), toll-like receptors 2 and 4, and the cytokine IL-1 and IL-1 receptor antagonist were determined using real-time PCR. The threshold was determined for each gene and relative total RNA quantified. A standard curve was created to approximate the concentration of the gene of interest. Then data was presented as a ratio of the gene of interest to the reference gene 18S.

Statistical Analysis - *Escherichia coli*, coliform, and *Lactobacilli* enumeration data were transformed (from CFU counts to log₁₀ per gram of sample) before statistical analysis. The ANOVA was used for the comparison between treatments. Immunologic data was analyzed using GLM procedures of SAS (SAS Institute Inc., Cary, NC.). Data was checked for normality and homogeneity of variance, and then transformed when necessary. Covariance structures were tested and the Bayesian criterion used to select the best fit of the data. Growth performance data was analyzed using Mixed models procedures of SAS as randomized complete block design with repeated measures over time on each experimental unit (individual pens), with replicate as random variable. Statistical inferences of significance were based $p < 0.05$, whereas trends or tendencies toward significance were based on $p < 0.10$.

VII. Results

Growth Performance. There was no significant evidence for difference in body weight (BW) at weeks 1, 2, 3, and 4 post-weaning (p.w.) between genders and treatments. However, there was a trend toward significance for the gender by treatment interaction at week 4 p.w. ($p = 0.0701$). Barrows fed carbadox tended to have a greater BW than gilts fed carbadox and barrows fed control diet. Gilts fed the control diet had a tendency toward a larger BW than did barrows fed control diet or tylosin and gilts fed carbadox. Also, there was a tendency for barrows fed control diet to have a lower BW than gilts fed tylosin or beta-glucan and vitamin C (BGA). Barrows tended to be heavier than gilts sacrificed at week 4 p.w. ($p = 0.0989$).

Feed intake for weeks 1, 2, and 4 p.w. were not affected by gender or treatment. However, the interaction of gender and treatment for week 3 and 4 combined ($p = 0.0536$) as well as week 4 only ($p = 0.0514$), tended to be significant. Barrows fed control diet had a tendency to have less feed intake than did barrows fed carbadox, and gilts fed BGA or the control diet for weeks 3 and 4 combined, as well as week 4 alone. There was also a tendency toward greater feed intake for barrows fed carbadox than for barrows fed tylosin and gilts fed carbadox (at week 4 post-weaning) or tylosin for weeks 3 and 4 combined. Data for week 2 p.w. were missing due to an inability to accurately measure the feed intake for each pen. Growth performance for weeks 1, 2, 3, and 4 p.w., as well as for the collective 4 week period (i.e., overall), was not affected by treatment or gender. Gilts started showing a tendency to greater average daily gain than barrows at week 4 p.w. ($p = 0.0793$).

Neither gender nor treatment affected feed conversion at weeks 1, 3, and 4 post-weaning. Gilts tended to have better feed conversion (i.e. gain more body weight per feed intake) than did barrows for week 3 p.w. ($p = 0.0989$). Feed conversion data from weeks 3 and 4 combined were not affected by gender or treatment.

Microbial Ecology. At weaning, gilts tended to have higher cecal *Lactobacilli* counts than barrows ($p = 0.0841$) at weaning. Pigs sacrificed 2 weeks p.w. expressed a trend toward significance for the gender by treatment interaction in cecal *E. coli* counts ($p = 0.0731$). Gilts fed carbadox tended to have lower counts than did barrows fed carbadox and gilts fed tylosin of the control diet. There was also a tendency for gilts fed BGA

to have lower *E. coli* counts than gilts fed tylosin, barrows fed carbadox, and barrows fed BGA. The intestinal microbial ecology was affected by treatments for those pigs sacrificed at week 4 post-weaning. Ileal *Lactobacilli* counts were lower in pigs fed BGA compared to those fed the control diet, carbadox, or tylosin ($p < 0.05$) (Figure 1). There were no other significant differences in bacterial counts for gender or treatment. However, there was a trend toward significance of treatment for cecal *E. coli* counts, in which pigs fed tylosin expressed higher *E. coli* counts than those fed the control diet, carbadox, or BGA ($p = 0.0787$).

Microbial shedding (i.e., fecal count) was not affected by gender or by treatment in weeks 1, 2, and 3 post-weaning. However, barrows tended to shed more *Lactobacilli* than gilts at week 1 p.w. ($p = 0.0931$). Also, there was a trend toward significance for the gender by treatment interaction in *Lactobacilli* at week 2 p.w. ($p = 0.0608$). Gilts fed the control diet tended to have higher counts than did gilts fed carbadox and barrows fed tylosin, BGA, or control diet. Also, gilts fed tylosin tended to have greater counts than did gilts fed carbadox and barrows fed the control diet. At week 4 p.w., there was a gender by treatment interaction in coliform shedding ($p < 0.05$). This interaction expressed a difference between gilts and barrows across the tylosin treated pigs and the BGA treated pigs, at which barrows fed BGA had an increase in coliform shedding compared to gilts fed tylosin ($p < 0.05$) (Figure 2). There was no difference in gilts across treatments or barrows across treatments. Also, barrows tended to shed more *Lactobacilli* than gilts ($p = 0.0989$).

Immunological status. There were no differences in neutrophil, lymphocyte, and monocyte percentages between gender and treatments at weaning. Neutrophil count and total WBC count were affected by treatment at week 2 post-weaning. BGA treated pigs expressed a higher neutrophil count than did carbadox treated pigs. Total WBC count was also greater in BGA treated pigs versus control treated and carbadox treated pigs ($p < 0.05$). There was a tendency for BGA treated pigs to have a greater monocyte count than pigs fed carbadox ($p = 0.1002$). Gilts tended to have a higher HCT value than barrows ($p = 0.0566$). Data at week 4 p.w. was missing due to an inability to accurately measure the blood cells (Table 1).

Lung TLR2 (a Gram-positive recognition molecule), began with greater expression at weaning for the piglets assigned to the carbadox or tylosin treatments ($p < 0.05$). By week 2 p.w., all treatments were equally expressing TLR2 genes. At week 4 p.w., the control pigs continued to increase expression of TLR2, which left greater expression than for the carbadox and tylosin treated pigs ($p < 0.05$).

Expression of TLR4 (a Gram-negative recognition molecule) in lungs of pigs to be treated with carbadox and tylosin also began at greater values than the other two treatments ($p < 0.05$). The control treated pigs increased TLR4 expression through weeks 2 and 4 p.w., making their expression greater than BGA (week 2) and carbadox pigs (weeks 2 and 4) ($p < 0.05$).

Interleukin-1 (a communication molecule) expression in the lung tissue of pigs to be treated with carbadox was greater than that from pigs to be treated with BGA and the control diet at weaning ($p < 0.05$). By week 2 p.w., IL-1 expression in lung tissues increased in all pigs, with all treatments having greater values than the controls ($p < 0.05$). At week 4 p.w., the 3 treatment groups decreased to near weaning values, while the control pig IL-1 expression remained constant, but not different than the treatments.

Interleukin-1 receptor antagonist expression did not differ among the treatments throughout the study. All treatments increased expression at week 2 p.w., which returned to near weaning values by week 4 post-weaning (Figure 3).

Lung tissue expression of an antimicrobial peptide, PR-39, also began with the pigs to receive carbadox having higher expression than the BGA and control pig groups at weaning ($p < 0.05$). The carbadox treated pigs did not change expression levels at week 2 p.w., but the other 3 treatments all increased expression. The tylosin treated pigs had more PR-39 expression in lung tissues at week 2 than the control and carbadox treated pigs ($p < 0.05$). The BGA treated pigs were only different in expression of PR-39 compared to the control treated pigs ($p < 0.05$) (Figure 4).

The mesenteric lymph nodes (MLN) had a different profile of expression. Only the TLR2 expression differed among treatments at weaning. The pigs of the control group had greater expression than did the tylosin treated pigs ($p < 0.05$). By week 2 p.w., the BGA, control and tylosin treatments had greater TLR2 expression in MLN compared to the carbadox treated pigs ($p < 0.05$). By week 4 p.w., all but the control pigs had TLR2

expression in MLN near weaning values ($p < 0.05$). Throughout the study, the carbadox treated pigs did not change TLR2 expression. The control pigs began with higher expression which dropped over the course of the 4-week experiment (Figure 5).

The expression of TLR4 in MLN began with all treatments at similar levels. In pigs fed carbadox, this expression decreased slightly at week 2 p.w., whereas it increased for the other 3 treatments creating a significant difference between carbadox and the other treatments ($p < 0.05$). By week 4 p.w., the control pig's expression of TLR4 did not change, but the BGA and tylosin dropped to expression levels similar to the carbadox treated pigs ($p < 0.05$). Overall, the carbadox and control treated pigs only changed expression of TLR4 slightly, while the BGA and tylosin expression profile increased at week 2 p.w. compared to weaning and week 4 post-weaning.

Expression of interleukin-1 and its receptor antagonist (IL-1Ra) in MLN was not different among treatments at weaning or week 4 post-weaning. At week 2 p.w., the pigs treated with tylosin, BGA, and the control pigs increased IL-1 expression in the MLN ($p < 0.05$). The carbadox treated pigs did not change over the 4 week experiment (Figure 5).

The antimicrobial peptide, PR-39, had similar expression in all pigs at weaning in MLN. Carbadox treated pigs did not change expression of PR-39 at week 2 or 4 post-weaning. The other 3 treatments increased expression of PR-39 at week 2 post-weaning. The BGA treated pigs had expression of PR-39 return to near weaning expression by week 4 post-weaning ($p < 0.05$). The tylosin treated pigs had PR-39 expression that was greater than that of BGA at week 4 post-weaning (Figure 4).

VIII. Discussion

Each pen was balanced by body weight, as results expressed no bias in body weight between gender or between treatment groups at weaning. This balance was seen throughout the 4 week study, representing no gender or treatment having a greater impact on the weight of the pigs. The balance in body weight was supported by the feed intake data. The feed intake of pigs for week 1, 3, and 4 p.w. was not affected by gender or treatment. The average weekly and daily body weight gain was similar across treatments and gender. Thus, no treatment group or gender expressed a better growth to feed ratio.

Ileal and cecal *Escherichia coli*, coliforms, and *Lactobacilli* were not altered by treatment, nor did they differ between genders, for the first 2 weeks post-weaning. At 4 weeks p.w., ileal *Lactobacilli* counts were less in pigs treated with beta-glucan and vitamin C compared to the other 3 treatments, even though ileal *Lactobacilli* counts had decreased from week 2 p.w. across all treatments. This decrease suggests that BGA was not as effective in maintaining ileal *Lactobacilli* counts as carbadox, tylosin, and even the control diet.

Similar to the intestinal microbial ecology, there were no differences in fecal shedding of coliforms, *Escherichia coli* and *Lactobacilli* until 4 weeks post-weaning. At 4 weeks p.w., coliform counts were greater for barrows given beta-glucan and vitamin C compared to gilts given tylosin. This observation suggests that beta-glucan and vitamin C were not as effective in controlling or suppressing the coliform population of the pigs as the other 3 treatments were.

Neutrophil count and total WBC count were greatest in BGA treated pigs, particularly in relation to carbadox treated pigs, at 2 weeks post-weaning. Thus, we hypothesize that BGA was the least efficient in suppressing the invasion of pathogenic bacteria, which ultimately led to a greater immune response in the pig.

The profile expression of the MLN was similar for the BGA and tylosin treated pigs, and to a lesser extent the control pigs. Overall there was an elevation of expression at week 2 p.w., which returned to near baseline expression by week 4 post-weaning. Carbadox appeared to change very little over the 4 week experimental period. The lung expression profile was much more complex, with the control pigs increasing expression from week 2 to week 4 p.w. The expression for the tylosin and BGA treated pigs was similar for IL-1 and PR-39 in the lung tissues.

BGA appears to be as effective at protecting the host from pathogens as the antibiotic tylosin. Both treatments have been shown to modulate the immune system. Consumption of carbadox on the other hand resulted in immune suppression.

Treatments were shown to have some limited effects on the immune system and intestinal microbial ecology of the pigs, but no treatment seemed to affect the growth of the pigs. According to the immunological analysis, administration of BGA and tylosin would be considered the most beneficial to the pig's immune system. The microbial analysis suggests that BGA was not as effective as tylosin in suppressing potential intestinal pathogen colonization. Thus, we can conclude under the conditions of this study that although the use of tylosin in nursery diets did not improve growth performance, it was most favorable to the pig's immune system by protecting the pig from potential pathogens colonizing the gastrointestinal tract and insuring the health of the pig. Administration of beta-glucan and vitamin C may be an efficient alternative to antibiotics but more research needs to be conducted.

Figure 1. log₁₀ CFU of *Lactobacilli* per gram of intestinal ileal contents 4 weeks post-weaning.

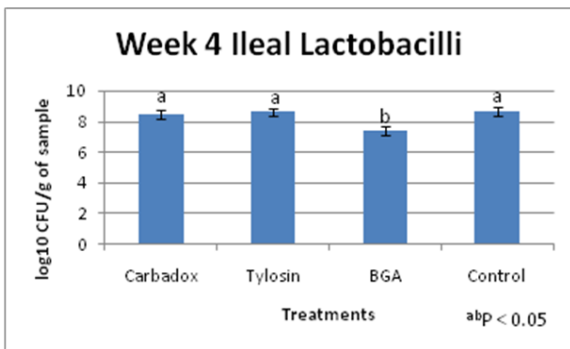


Figure 2. log₁₀ CFU of coliforms per gram of fecal sample 4 weeks post-weaning

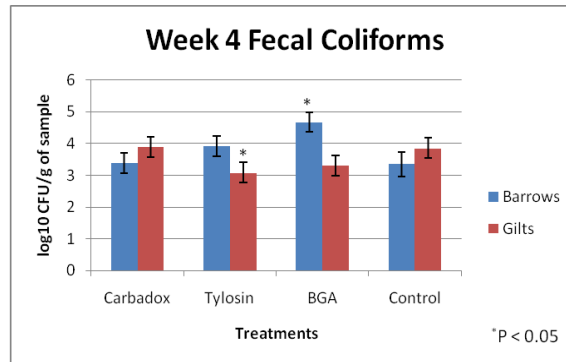


Table 1. Blood cell counts (x 10⁴/mL) and percentages.

Blood Cell Counts and Percentages									
Weeks	Trt	HCT	Counts (x 10 ⁴ /mL)				Percentages		
			WBC	Lymph.	Mono.	PMN	Lymph.	Mono.	PMN
1	Carbadox	65.02	7.12	27.87
	Tylosin	64.38	4.82	30.80
	BGA	62.37	10.00	27.63
	Control	65.98	7.22	26.80
2	Carbadox	25.88	^b 15.26	6.33	0.43	^b 8.29	41.70	2.78	54.06
	Tylosin	26.27	^{ab} 17.80	6.12	0.52	^{ab} 10.93	34.78	2.90	61.08
	BGA	26.73	^a 28.21	11.33	0.94	^a 15.23	37.67	3.32	56.58
	Control	26.48	^b 15.91	6.07	0.54	^{ab} 9.125	38.26	3.49	57.18
4	Carbadox
	Tylosin
	BGA
	Control

^{a,b}p < 0.05

Figure 3. Relative abundance of expression of toll-like receptors 2 and 4 and acute phase cytokine IL-1 and its receptor antagonist in lung tissues during a post-weaning 4-wk period.

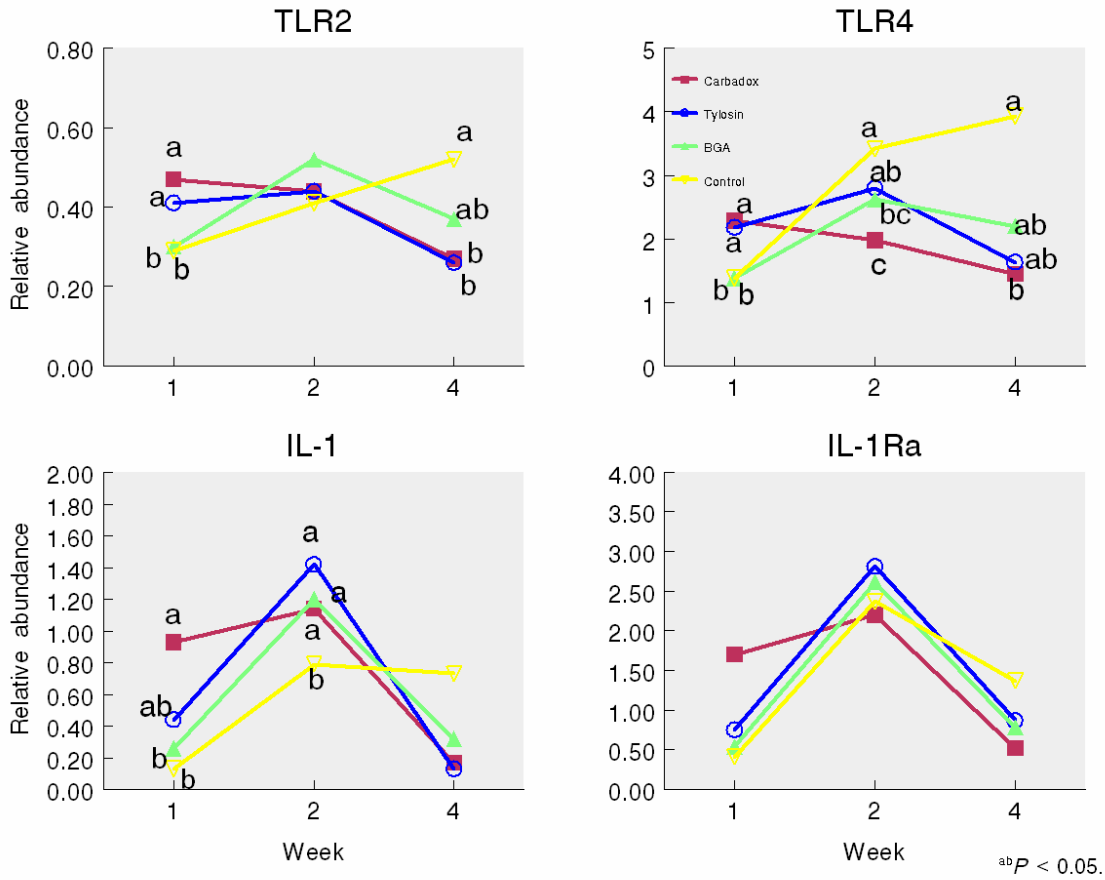


Figure 4. Relative abundance of expression of the anti-microbial peptide, PR-39 in lung and mesenteric lymph node tissues during a post-weaning 4-wk period.

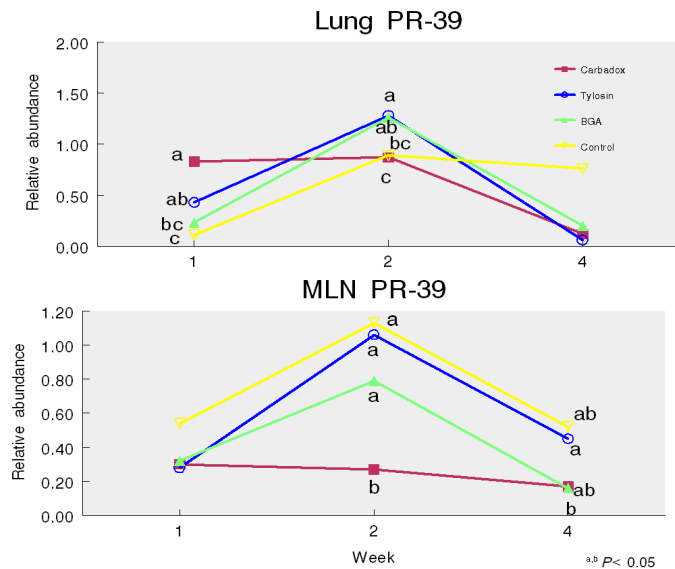


Figure 5. Relative abundance of expression of toll-like receptors 2 and 4 and acute phase cytokine IL-1 and its receptor antagonist in mesenteric lymph node tissues during a post-weaning 4-wk period.

