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The effects of oral antibiotic therapy on productivity and immune function following challenge with E. *coli* and rotavirus

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Introduction

Early weaning programs have been aimed at the control and elimination of respiratory infections in the young pig. Segregation from their dams at less than 21 days, batch rearing with all-in all-out by room, building, or site, and proper biosecurity (cleaning, disinfecting and quarantine) are mandatory to implement early weaning programs[1]. Early weaning with all its components gives a tremendous economic advantage to those who use this technology. This technology however does not come without a cost. This requires necessary building sites, scheduling and a high level of management. Enteric infections such as neonatal coccidiosis and post weaning diarrhea problems have not been prevented by early wean programs. Although the Pork Quality Assurance Program has been developed to achieve the highly desirable goal of reduced antibiotic use, there are feed and water medication is needed for prevention and control of enteric infections.

We were interested in the effects of low levels of conventional water and feed grade antibiotic treatments on performance and immunological parameters of the young pig infected with the common enteric pathogens, E. coli and rotavirus. Previously, we had tested this treatment at both a research facility and a commercial operation and had shown increased production and decreased immunological response in the treated animals[2,3]. We have established that the use of such a program would be a benefit to those producers who do not have the production facilities that would allow early weaning (7-10 days) and/or multi-site production. However the effect of these oral treatments on minimizing production losses and activation of the immune system following infection with enteric infections has not been established.

The purpose of this study was to measure production and immunological parameters in orally medicated and control animals following a post weaning E. coli and rotavirus challenge.

Experimental Procedure

Animals and Management

Farrowing and neonatal pig management: A commercial 130-head sow herd of known health status was chosen for this trial. This is a herd whose health status is monitored by Dr. Chase. The farrowing facility is a two-year-old building with 2-10 crate rooms. Pigs were processed at 1 day of age (tails removed, iron dextran injections, needle teeth clipped). The pigs were vaccinated for Mycoplasma hyopneumoniae at 7-11 days of age and the boars were castrated. Prior to vaccination on day 7-11, a 3 ml blood sample was collected. At day 17-21 (weaning day) the pigs were re vaccinated with M. hyopneumoniae and a 10 ml heparinized blood sample were collected from 10 pigs in each group.

Ten sows (ten litters-92 pigs: 5 litters/group) were randomly assigned to one of two groups: 1) control and 2) treatment. The treatment regimen consisted of feeding 400 g/ton of Aureomycin™ in the gestation-lactation ration for 1 week before and 1 week after farrowing. The baby pigs' water was treated with water soluble Aureomycin™ to deliver 10 mg/# body weight beginning day 2 post farrowing through day 9 post farrowing. Auero-Sulmet™ soluble was added to the pigs' drinking water at rate of 250 mg chlortetracycline/250 mg sulfamethazine per gallon from day 10 post farrowing through day 27-34 post farrowing (10 days postweaning). ASP250™ at a rate of 100 gm Chlortetracycline/100 gm sulfamethazine/50 gm penicillin per ton was used in the creep feed.

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[3] SDSU and Rural Technologies Inc., Brookings, SD
[4] American Cyanamid Company, Princeton, NJ

Control pigs diets were managed using the farm's husbandry procedures and all control creep contained 40 grams/ton of Apralan™.

Nursery management: The pigs were weaned at 17-21 days of age and transported 100 miles from Brookings, SD to Beresford, SD to an newly remodeled nursery-finisher isolation facility. The nursery facility contained 2 rooms each room contained 10-6' ft X 16' pens. The 46 treatment and 46 control pigs were each divided into 8 replicates. Eight pens in one room were divided with plywood and plastic coated wire was placed over the slats to prevent injury to the young pigs. Treated pigs were placed in one half and control pigs were placed in the other half of each pen. The treated pigs received Auero-Sulmet™ soluble in the drinking water at rate of 250 mg chlortetracycline/250 mg sulfamethazine per gallon for 10 days post weaning (day 27-34 post farrowing). All the pigs received traditional phase I, II, & III commercial nursery diets[4]. Diet changes were made at 7 and 21 days post weaning. The treated pigs nursery diet contained ASP250™ at a rate of 100 gm chlortetracycline/ 100 gm sulfamethazine/ 50 gm penicillin per ton. Control pigs weaning diets contained 40 grams/ton of Apralan™. All pigs were switched to 20 grams/ton of Tylan 10 for the grower/finisher phase and these diets were standard grow finish diets. The pigs were weighed weekly through day 160. All feed was weighed to obtain feed efficiency data.

Microbiological challenge: Rotavirus (OSU strain) was obtained from Dr. David Benfield, Dept. Vet. Sci., South Dakota State University (SDSU). The stock virus had been passed through pigs. The virus was grown on MA104 cells, harvested and titrated. The tier of the challenge virus was 6.25 X 109 TCID50. The virus was diluted with minimum essential media (MEM) to give each pig a 2 ml challenge dose of 107 of TCID50 rotavirus p.o. The O111 strain of E.*coli* was grown in broth cultures and diluted to 250 ml with PBS and each pig was given a 2 ml dose p.o. that containing 108 E.*coli* O111. The pigs were challenged 2 days after arriving at the nursery facility (19-23 days of age).

Clinical indices: Clinical signs (fever and diarrhea) were recorded. The temperature of the same one or two pigs/pen was taken for 15 days. Diarrhea was assessed daily in each pen on a scale of 0-4 (0-firm, formed feces; 1-pasty feces; 2-pudding feces; 3-slightly runny feces; and 4-watery feces) for 29 days.

Microbiological tests: A fecal sample was collected using a sterile swab from one pig in each pen on the day of challenge and 3, 10, 16, 24, 28, and 35 days PC.

Bacteriology: The swabs were streaked by the SDSU Diagnostic Bacteriology Laboratory on differential plates. The number of hemolytic *E. coli* was estimated. E.*coli* isolates were then typed for O111.

Virology: The fecal swabs were then pooled according to treatment groups and the samples examined with an electron microscope for the presence of rotavirus by the SDSU Electron Microscopy Laboratory.

Serology: Blood was collected and serum samples were harvested at 7-11 and 53-57 days of age (35 days PC) from 10 pigs in each group. Serum samples were tested for *M. hyopneumoniae* antibodies using as commercial *M. hyopneumoniae* ELISA performed by Oxford Diagnostic Laboratories, Worthington, MN.

*Clinica Immunology:*10 ml-heparinized blood was collected and lymphocytes harvested at 17-21 (weaning day), 23-27 (4 days PC), 30-34 (11 days PC) and 53-59 days of age (34 days PC) from 10 pigs in each group. Mitogen proliferation assays were conducted in the SDSU Clinical Immunology Laboratory. The plant lectins, concanavalin A (ConA) at 1 μg/ml, phytohemagglutinin A (PHA) at 1 μg/ml and pokeweed mitogen (PWM) at 5 μg/ml were used to stimulate isolated peripheral blood lymphocytes. The lymphocytes were cultured for 44 hours and pulsed for 4 hours with tritiated (3H) thymidine and harvested at 48 hrs in a cell collector. The disks were counted in a liquid scintillation counter. All cultures were done in triplicate and the values represent the mean specific incorporation (sample mean-unstimulated cell mean) of the triplicate samples. Forced antibody production was performed after the protocol of Hammerburg et. al[5]. Briefly, 1 ml of mononuclear cells at 3 X 106/ml were incubated in 12 X 75 mm sterile plastic tubes containing 1 ml of RPMI with 10 μg/ml of PWM for 72 hours. The tubes were centrifuged and the supernatant was collected. The supernatant was diluted in a ten fold series

and a polyclonal-polyclonal sandwich capture ELISA was used to measure the total amount of immunoglobulin present. The assay was standardized with a preparation of porcine IgG (Sigma, St. Louis, MO).

Results

Production Results from the growth trial are presented in Table 1[a,e]. In the starter phase, the treatment protocol improved daily gain (P<.124) and feed efficiency (P<0.079) (Table 1[a]). In the grower, finisher, and overall growth phases, performance was not affected by treatment (P>.10) (Tables 1[b,d]). Ultrasonic measurements of 10th rib backfat thickness and loin eye area at 240 lbs. were unaffected by treatment (P>.10) (Table 1[e]).

Clinical Signs: The temperatures of the treated pigs were not significantly different from the control group from day 1 to day 15 PC) (Figure 1). The temperatures of the treated pigs were in the normal range throughout the trial (Figure 1). From Day 6 to Day 15, the temperatures of the treated pigs were lower than the control. The diarrhea scores were similar between control and treatment groups. The diarrhea was biphasic in the control group at 4-5 days PC and at 18-24 days PC. The highest amount of diarrhea occurred at Days 18-22 PC (Figure 2).

Microbiology: No rotavirus was identified prior to challenge (Table 2). Rotavirus was identified at Day 3 PC in both control and treated pigs. Rotavirus was also identified at Day 10 and Day 16 PC (Table 2) in the control pigs. Hemolytic *E.coli* was isolated from 1 treatment pen prior to beginning the study (Table 3). Tests are under way to determine the serotype. Hemolytic E.*coli* at moderate levels were isolated at Days 16 and 24 PC. No hemolytic E.*coli* was isolated from the control pigs prior to the study. Hemolytic E.*coli* was present in through out the trial in the control group (Table 3). Serotyping of the E.*coli* established that the challenge was O111 but subsequent isolations throughout the trial were negative for O111.

Serology: There was no serological response to the *M. hyopneumoniae* vaccination in the treated pigs and a very small response (1/10) of the control pigs (Table 4). Three of the control pigs had passive *M. hyopneumoniae* titers and none of the treated pigs and passive titers (Table 4).

Clinical immunology: T cell mitogen activity, B cell mitogen activity and induced immunoglobulin (Ig) were measured in the treatment and control groups (Figures 3-5). The immunological response with the T cell mitogens with both phytohemagglutinin (PHA) and concanavalin A (ConA) was lower in the treatment group (Figure 3). The ConA response was significantly lower (P<0.05) at 17, 30 and 56 days of age (Figure 3). The B and T cell mitogen pokeweed mitogen (PWM) (Figure 4) and the forced antibody production assays (Figure 5) had a different pattern from the T cell assays. Prior to challenge on day 17, B cell proliferation (Figure 4) and induced antibody production (Figure 5) were similar in both groups. At 23 days of age (4 days PC) both parameters were depressed. At 30 days of age, the B cell mitogen activity (Figure 4) and the induced antibody production (Figure 5) were higher in the treatment group. At 56 days of age, the B cell mitogen activity was similar in both groups and the forced antibody production was higher in the control group.

Discussion

The production results indicate again an early advantage in rate of gain and feed efficiency in the treatment group (Table 1[a]). However by the end of the trial the two groups were similar in these parameters (Table 1[cd]). Data from this trial indicate that this MMEW protocol improves nursery performance but compensatory performance masks those benefits in the grower and finisher phases. These results may represent what would happen under field conditions following removal from water antibiotic therapy. One of the main design issues is that the control and treatment animals were housed in adjacent pens throughout the trial. The treated pigs could have become infected after the end of the treatment resulting in similar production results at the end of the study. In the future, strict segregation should be used to determine the long-term effect of this antibiotic therapy.

Clinical scores were similar for both groups. The initial design called for monitoring temperatures for 14 days. The data indicates that temperatures were on the rise and with the occurrence of diarrhea at 18 days temperatures

should be monitored concurrently with diarrhea. The diarrhea scores indicate that the treated pigs were partially protected from the initial diarrheal phase at 4-5 days PC. Duration and severity of the second phase was similar between the two groups. Again the contamination and infection of the treated pigs by the adjacent control pigs following end of antibiotic therapy could have played a factor in the resulting second diarrheal phase. The immune response to diarrheal disease in the control animals was not effective in preventing the second diarrheal phase.

The microbiological data was interesting in this trial. Rotavirus recovery was much lower in the treated pigs. The sensitivity of the electron microscopy detection is 105 particles/ml. The decreased rotavirus detection indicates lower replication of rotavirus in the treated pigs. *E.coli* detection was also lower with no organisms detected 3 days PC indicating that the treatment reduced the load of *E.coli*. Subsequently the levels of *E.coli* recovery during the second diarrheal phase were similar between the two groups. The challenge O111 serotype could not be recovered after the initial infection. We have no explanation for these phenomena.

The serological data in this experiment was not useful. There was a single control pig that responded to the *M. hyopneumoniae* vaccine. The vaccine used in this trial (*M. hyopneumonia* vaccine, Oxford Laboratories) was a different product from that used in the previous trial[3] (Respisure, Pfizer). The manufacturer of the vaccine was the same company that performed the *M. hyopneumoniae* ELISA test. Both the efficacy of the vaccine and the sensitivity of the ELISA testing method are questionable and neither the vaccine nor the tests are available.

The immunological data was similar to that seen in our two previous studies [2,3,6]. There was a decreased T cell response indicating a lower inflammatory response. This decreased inflammatory response has a sparing effect that allows increased growth in the treated pigs. The antibody capacity and production of the treated animals as measured by B cell mitogen activity and forced immunoglobulin should allow them to respond to a challenge.

Conclusions

These results indicate an increase in daily gain and feed efficiency with pigs treated with an oral antibiotic regiment. The *E.coli*-rotavirus challenge model indicated an advantage to the treated pigs with a lower fever response increased clearance of *E.coli* and rotavirus and decreased T cell activation. These decreased physiological and immunological responses and higher pathogen clearance are all factors that could result in the increased production parameters in the treatment group.

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TABLE 1[a]. WEANING PERFORMANCE (STARTER PHASE-DAYS 17-61, 10-46 LBS.)

	Control	Treatment	P value
Daily gain, lbs.	0.756	0.827	0.124
Daily feed. lbs.	1.418	1.440	0.542
Feed/gain	1.900	1.742	0.079

TABLE 1[b]. PERFORMANCE (GROWER PHASE-DAYS 62-105, 46-118 LBS.)

	Control	Treatment	P value
Daily gain, lbs.	1.647	1.689	0.465
Daily feed lbs.	4.083	4.248	0.258
Feed/gain	2.489	2.511	0.717

TABLE 1[c]. PERFORMANCE (FINISHING PHASE-DAYS 106-160, 118-234 LBS.)

	Control	Treatment	P value
Daily gain, lbs.	2.052	2.026	0.762
Daily feed. lbs.	6.356	6.377	0.911
Feed/gain	3.116	3.153	0.595

TABLE 1[d]. OVERALL PERFORMANCE (DAYS 17-160, 10-234 LBS.)

	Control	Treatment	P value
Daily gain, lbs.	1.529	1.554	0.570
Daily feed. lbs.	4.154	4.222	0.435
Feed/gain	2.730	2.717	0.793

TABLE 1[e]. CARCASS PERFORMANCE (234 LBS.)

	Control	Treatment	P value
10th Rib Fat Thickness, in.	0.849	0.849	0.999
Loin Eye Area, sq. in.	5.175	5.076	0.405

TABLE 2. ROTAVIRUS DETECTION IN PIGS

	Days Post Challenge						
	Day 0	Day 3	Day 10	Day 16	Day 24	Day 28	Day 35
Control	neg.	pos.	pos.	pos.	neg.	neg.	neg.
Treated	neg.	pos.	neg.	neg.	neg.	neg.	neg.

pos-virus detected in fecal samples
neg-no virus was detected in fecal samples

TABLE 3. E.*coli* DETECTION IN PIGS

	Days Post Challenge						
	Day 0	Day 3	Day 10	Day 16	Day 24	Day 28	Day 35
Control	0	1.1	1.3	1.1	2.3	1.3	0.1
Treated	0.1	0	0.9	1.6	2.0	0	0.4

0-no hemolytic E.*coli*
1-few hemolytic E.*coli*
2-moderate hemolytic E.*coli*
3-many hemolytic E.*coli*

TABLE 4. SEROLOGICAL RESPONSE (LOG10) TO *Mycoplasma hyopneumoniae* (NUMBER OF PIGS SEROPOSITIVE/TOTAL NUMBER OF PIGS TESTED)

Days of age	Treatment	Control
Day 10	0 (0/10)	0.77±1.25 (3/10)
Day 56	0 (0/10)	0.32±1.01 (1/10)