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# The effect of diet and oral antibiotic therapy on immune function and productivity in young pigs

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### Introduction

Medicated early weaning programs have been shown to be an excellent method to control disease incidence in the young pig. Additional research by Dritz, et al[1], showed that early weaning at 7-10 days without medication resulted in significant weight gains over conventional weaning at 14-17 days. 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 in a commercial operation. Previously, we had tested this treatment at a research facility and had shown increased production and a decreased polyclonal immunological response in the treated animals[23]. The use of such a program would be a benefit to producers who do not have the production facilities that allowing for early weaning (7-10 days) and/or multisite production.

A study was conducted to determine the effect of Aureomycin™ and Aureo-Sulmet™ on production. The study was a 2X2 factorial experiment to determine the effect of weaning treatment and nutrition level on immune response.

### Experimental Procedure

*Animals and Management:* A commercial 150-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 remodeled barn containing 27 crates. The crates and flooring had been replaced in the last year. Pigs were processed at 1 day of age (tails removed, iron dextran injections, needle teeth clipped).

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At 7 days of age, the pigs were vaccinated for *Mycoplasma hyopneumoniae* (Respisure, Pfizer) and the boars were castrated. Prior to vaccination, a 3 ml blood sample was collected. At day 17-24 (weaning day), the pigs were re vaccinated with M. hyopneumoniae and a 10 ml blood sample was collected for cellular immunological assays. Control pig diets were managed using the farm's husbandry procedures and all control creep and weaning diets contained 40 grams/ton of Apralan™.

The nursery facility was connected to the farrowing facility via a hallway and consisted of 10 raised deck pens. Eight pens were divided in 1/2 and each pen contained 10 pigs.

### Antibiotic Treatment and Nutrition

The treatment regimen consisted of feeding 400g/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 27-30 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 feed. The control group was managed using the farm's normal husbandry and feeding procedures and all control creep and weaning diets contained 40 grams/ton of Apralan™.

Sixteen litters (160 pigs) were randomly assigned to one of 4 treatment groups: 1) Control Standard nutrition; 2) Control-High nutrition; 3) Treatment-Standard nutrition and 4) Treatment High nutrition. Commercial diets were utilized. Traditional phase I, II & III nursery diets 1 were used for the standard feeding

regime while the high feeding regime consisted of pre-phase I (day 7 diet), phase I & phase II 1 Diet changes were made at 7 and 21 days post weaning. There were 4 replicates/ treatment. The pigs were weighed weekly through day 56 post farrowing. All feed was weighed to obtain feed efficiency data.

Serology and Immunology

Pigs were bled at 7 days of age, 28-31 days of age and 56-58 days of age for serology and immunological assays. Lymph node cells were harvested from the pigs sacrificed at day 56. Serology was performed at Oxford Diagnostic Laboratories, Worthington, MN using a commercial *M. hyopneumoniae* ELISA.

Mitogen proliferation assays were conducted in the Clinical Immunology Laboratory SDSU. The plant lectins, concanavalin A (ConA) at 1 µg/ml, phytohemagglutinin A (PHA) at 1 µg/ml and pokeweed mitogen (PMA) 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[4]. Briefly, 1 ml of mononuclear cells at 3 X 106/ml were incubated in 12X75 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).

At day 56, 10 pigs from each group were transported to the Diagnostic Laboratory at South Dakota State University (SDSU) euthanized and necropsied. The spleen, thymus, abdominal and lung lymph nodes were removed and weighed.

Results

*Production:* These results indicated a 4.5% increase in daily gain of the treated vs the control pigs on the standard nutrition program and 13.6% increase in daily gain of the treated vs controls on the high nutrition program (Table 1). Feed/gain was 9.6% lower for the treated vs the control pigs on the standard nutrition program. There was no significant advantage in gain between the standard and high nutrition program to offset the increased cost of the high nutrition ration. There was no significant difference ($P<.05$) in any of the parameters measured (Table 1).

Immunology

Thymus, spleen, and pulmonary and abdominal lymph nodes were removed from each group. Identification of thymic tissue and the variability in of the number of lymph nodes made accurate analysis of these immune organs difficult. There was no significant difference in the lymph nodes and the spleen (Table 2). Spleens were consistently smaller in the treated vs the control pigs (Table 2). There were no gross lesions present in any of the pigs.

Serology indicated a single statistical significant difference between the control-standard nutrition group and the treatment-standard nutrition group at day 56 (Table 3). The entire group had pre-immunization antibody to M. hyopneumoniae (Table 3). All groups of pigs developed higher antibody titers following immunization and a majority (>60%) of each group responded. The antibody decay was much faster in the treated pigs (Table 3).

The ConA and PHA mitogenic assays were used to measure the immunological capacity of peripheral blood T cells (PBL)(Figure 1 A & B). There was no statistical significant difference between any of the groups at either day 28 or day 56. The trend of this experiment was that the pigs fed the standard diet had a higher immune activation throughout the trial (Figure 1 A & B).

T and B cell mitogenic assays were also used to assess lymph node cells (Figure 2). These studies differed from the PBL studies in that the treated pigs and in particular the treatment-standard group consistently had higher activation to both T and B cell mitogens

(Figure 2). There was no statistical significant difference among any of the groups. The proliferative B cell capacity was measured following stimulation with PWM (Figure 3). The treatment pigs under both sets of nutritional conditions had higher level of proliferative response. There was no statistically significant difference between any of the groups at either day 28 or day 56. The ability of the cells to produce antibody was measured in a PWM forced antibody test (Figure 4). At day 28, the treated animals had higher antibody production. However by 56 days, there was no difference between groups. There was no statistical significant difference among any of the groups at day 28.

## Discussion

This trial showed that low levels of conventional water and feed grade antibiotic treatment increased daily gain and lowered feed/gain of young pigs in a commercial environment. Although these changes were not statistically significant, the study did show a 5-15% advantage in using oral antibiotics in weaning pigs. The trend seen in this study was consistent with the results obtained in our earlier studies (Libal et al, 1994). On the basis of improved growth and feed efficiency alone, the minimal cost of this supplementation was clearly justified (Libal et al, 1994).

The immunological results of this study were very interesting. In our initial study (Libal et al., 1994), we found that animals receiving the oral medication program had less peripheral T cell response capacity (demonstrated by lectin responses) and greater B cell response capacity (demonstrated by response to Pokeweed Mitogen). In this study, similar results were obtained, but the variability of the control and treated pigs was great enough so that we did not find these differences to be statistically significant. In addition to the basic peripheral blood mitogen assays performed in the first study, we also tested several other immunological parameters. We tested pokeweed mitogen "forced production" of antibody by multiple clones of B cells, responses of lymph node mononuclear cells to the T and B cell mitogens and specific humoral responses to M. hyopneumoniae vaccine. As would be predicated from our B cell proliferation data, the animals were able to produce a large quantity of antibody from peripheral B cells at 28 days of age when treated under the Cyanamid oral protocol. All the animals in the study produced similar amounts of antibody at 56 days. This level was equivalent to that produced by the treated pigs at 28 days. This suggests that the treated animals could mount a competent antibody response by the day 28, but it took longer for the control animals to achieve a similar capacity.

We found that treated animals had a generally higher capacity to respond to both T and B cell stimulation in lymph node cells. This may reflect a more complete development of secondary lymphoid tissue in the treated pigs. The major role of secondary lymphoid tissue is the organized expansion of activated clones. A well organized lymph node should contain a near optimal ratio of antigen presenting cells, helper cells and T and B effector cells. This allows for the most efficient antigen specific responses in an animal. The polyclonal stimulators used in this study measure the overall capacity to mount a proliferative response by the cells in the culture. The increased responsiveness here suggests that the protective immunological "environment" may be more mature in the treated pigs. A more efficient production of necessary cytokines or a more optimal ratio of antigen presenting cells, helper cells and effector cells (that go on to proliferate) would yield higher rates of proliferation. We measured the weights of thymus (a primary lymphoid organ) and spleen, pulmonary and mesenteric lymph nodes (secondary lymphoid organs). We found that the mesenteric lymph nodes were apparently more developed in the treated animals (Table 2). The nodes were larger in treated animals and these nodes were used in the mitogen studies. The pulmonary lymph nodes and spleens of the animals were not different among the groups. This is consistent with the proposed enteric effect of the oral treatment and its ability to promote quicker and more complete response to enteric antigens. No clear effect on primary lymphoid organs was expected. The control animals with high nutrition had large thymus compared to the other groups. The meaning of this finding is not clear to us at this time.

The specific immune response to the *M. hyopneumoniae* occurred in all animals. The duration of antibody titers was significantly shorter in the treatment group than the controls (Table 3). This may reflect an "environment"

less conducive to collateral stimulation in the treated pigs. The treated pigs showed a greater overall capacity to make antibody than the controls, but in this case, where no exposure to antigen after vaccination was indicated, they did not persist in producing high levels. We interpret these results as a redirection of resources into the growth of the pigs away from a sustained inflammatory response, which promotes an extended production of antibody no longer sustained by the presence of antigen stimulation. The differences in mucosal lymph node maturation may also play a role here. If optimal levels of antigen clearance and antibody production were reached earlier in treated animals, the need for further response would be removed and the control animals may just be slower in their overall responses.

These studies offered further evidence that management of the enteric and respiratory "environment" with the use of oral antibiotics as weaning supplement enhances feed utilization, growth and appears to improve the development of the secondary immune tissues. The treated animals had a small, but reasonable improvement in their ability to mount a humoral immune response. Evidence that the effect was most pronounced in the mucosal compartment included the differences seen between treated and control animals in lymph node development, ability to produce near optimal levels of antibody at a younger age, apparent enhancement of the activation environment with cells from the mesenteric lymph nodes tested, and a rapid peak and reduction in the antibody response to H. hyopneumoniae in the treated animals compared to the controls which had a much more prolonged rise in titer and a longer post vaccination reactivation period. The very low cost of this supplementation in the water and of the treatment of the sow are easily justified by the approximately 10% enhancement in growth. The herd in which this study was conducted was of reasonably high health status and there was little room for improvement in general health related growth. We feel that a lower health status herd would have had a greater level of difference in both growth and measured immunological activation. Chronically challenged animals are at a higher state of "inflammatory ready" than better managed herds. This allows for greater effect of the antibiotic supplementation in controlling the level and duration of enteric challenge and post challenge colonization.

We would recommend further studies of the comparative development of the secondary immune organs. A special emphasis should be place on the development of mucosal immune capacity and diversity. Some measures of general inflammatory state on mucosal surfaces and in systemic responses may also be valuable. We believe that the immune system develops better if the diversity of exposure is great, but the challenge load is limited. MEW programs have been successful because the systemic medications controlled the level of challenge in the respiratory tract. However, they are flawed in that they eliminated a broad diversity of exposure. This Cyanamid oral supplementation technique is better because it manages the total challenge exposure without reducing the diversity of exposure needed to successful development of a mature, functional immune system.

**References**


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TABLE 1. TRIAL 1-WEANING PERFORMANCE (DAYS 17-56)

| | Control Std[1] Nutrition | Treatment Std[1] Nutrition | Control High Nutrition | Treatment High Nutrition |
|---|---|---|---|---|
| Daily Gain, lbs[a] | 0.768 | 0.803 | 0.748 | 0.850 |
| Daily Feed, lbs[b] | 1.488 | 1.380 | 1.163 | 1.388 |
| Feed/Gain[c] | 1.913 | 1.728 | 1.568 | 1.620 |
| Survival, %[d] | 91.25 | 98.00 | 100.00 | 100.00 |

[1]Standard
[a]P=.6106 [c]P=.0774
[b]P=.3095 [d]P=.1430

TABLE 2. IMMUNOLOGICAL TISSUE WEIGHT (GRAMS)

| | Control Std[1] Nutrition | Treatment Std[1] Nutrition | Control High Nutrition | Treatment High Nutrition |
|---|---|---|---|---|
| Thymus, g[a] | 1.81 | 2.49 | 6.22 | 2.71 |
| Spleen, g[b] | 29.16 | 27.65 | 32.79 | 27.93 |
| Pulmonary LN2, g[c] | 0.79 | 0.76 | 1.17 | 0.81 |
| Mesenteric LN2, g[d] | 2.81 | 3.41 | 2.70 | 4.19 |

[1]Standard 2 Lymph node
[a]P=0.0008 but Diet Weaning Regime significant at P=0.0084.
[b]P=0.400
[c]P=0.185
[d]P=0.190

TABLE 3. SEROLOGICAL TITER (LOG10) TO *MYCOPLASMA HYOPNEUMONIAE*
(number of pigs seropositive/total number of pigs tested)

| Days of age | Control Std[1] Nutrition | Treatment Std[1] Nutrition | Control High Nutrition | Treatment High Nutrition |
|---|---|---|---|---|
| Day 7 | 1.18±1.25 (5/10) | 0.56±1.18 (2/10) | 0.89±1.16 (4/10) | 1.08±1.40 (4/10) |
| Day 28 | 2.33±1.27 (8/10) | 2.14±1.51 (7/10) | 1.82±1.59 (6/10) | 2.02±1.11 (8/10) |
| Day 56[a] | 2.18±1.27 (7/9) | 0.22±0.67 (1/10) | 1.44±1.41 (5/9) | 0.83±1.34 (3/10) |

[1]Standard
[a]Treatment Std Nutrition is different from Control Std Nutrition (P<.001)