

Title: Alternative Strategies for Utilizing Antimicrobials as Growth Promotants

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Abstract: Recently there has been concern that antimicrobial resistant bacteria developed in livestock fed subtherapeutic levels of antimicrobials could ultimately pose a threat to human health. Consequently, there has been increasing pressure to eliminate the use of antimicrobials as growth promotants in meat-producing animals. The Center for Disease Control and Prevention has stated that the use of antimicrobials as growth promotants, at low levels and for extended periods of time, is not prudent and judicious. As such, the future use of antimicrobials as growth promotants is uncertain. Consequently, there has been a push to identify viable alternatives to antimicrobial supplementation that would maintain the benefits of improved growth and health of the animal without facilitating the development of resistant bacteria. The goal of this research project was to determine whether feeding therapeutic levels of an antimicrobial for a short period of time stimulates the growth of swine to the same or greater extent, than feeding subtherapeutic levels of an antimicrobial for an extended period of time. In order to accomplish this goal we assessed the growth and health status of 1) pigs fed a control diet, 2) pigs fed a control diet supplemented with subtherapeutic levels of antimicrobials and 3) pigs fed a control diet and injections of therapeutic levels of antimicrobials at specific times.

The study was conducted; growth performance data and blood samples were collected. However, the pigs receiving antimicrobials did not grow faster and more efficiently than did the pigs fed the control diet. There was no difference in blood borne growth factors or acute phase proteins across treatments. Consequently the results from this study suggest that therapeutic doses of antimicrobials do not enhance growth of swine. However, since feeding the subtherapeutic levels of antimicrobials did not enhance growth either, the results are not unequivocal. It has been estimated that subtherapeutic levels of antimicrobials do not enhance the growth of swine in approximately 30% of the studies conducted. This study falls into that category. Therefore, within the context of this study it is not possible to draw definitive conclusions as to whether strategically timed administration of therapeutic levels of antimicrobials could be a viable alternative to subtherapeutic antimicrobials.

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Introduction: Antimicrobials have been used at subtherapeutic levels as feed supplements for almost 50 years to increase rate of gain and improve feed efficiency in young pigs. Recently there has been concern that antimicrobial resistant bacteria developed in livestock fed subtherapeutic levels of antimicrobials could ultimately pose a threat to human health. Consequently, there has been increasing pressure to eliminate the use of antimicrobials as growth promotants in meat-producing animals. The Center for Disease Control and Prevention has stated that the use of antimicrobials as growth promotants, at low levels and for extended periods of time, is not prudent and judicious. As such, the future use of antimicrobials as growth promotants is uncertain. Consequently, there has been a push to identify viable alternatives to antimicrobial supplementation that would maintain the benefits of improved growth and health of the animal without facilitating the development of resistant bacteria.

There have been two schools of thought as to how to best approach the question of finding a viable alternative to subtherapeutic antimicrobial supplementation. One school has proposed testing a wide variety of feed additives/gastro-intestinal inoculums as possible substitutes. Many of those choices have yet to be satisfactorily evaluated, but the preliminary data available would suggest that no single alternative can act as a substitute. The second school of thought has concentrated on understanding the underlying fundamental principle by which antimicrobials are able to enhance growth i.e., elucidation of the mechanism of action.

Historically there has been data to support three major hypotheses (Hays, 1991) which have been proposed to explain the growth promotion effect of feeding subtherapeutic levels of antimicrobials to young, rapidly growing animals: (1) the disease control effect (2) the nutrient-sparing effect and (3) the metabolic effect. Although all the hypotheses are still under consideration, it is generally agreed that antimicrobials enhance rate and efficiency of growth by altering the gastro-intestinal microflora. It has been determined that 400-500 different species of microorganisms reside in the gastro-intestinal tract. Identifying a change in a specific population(s) of microorganisms responsible for growth promotion would be extremely informative, but with existing technologies would be a daunting task.

We believe that the microorganisms that are altered in response to antimicrobial supplementation are pathogenic bacteria that are ubiquitously present in the gastro-intestinal tract, albeit in rather low abundance, since overt disease symptoms are not apparent. We believe that feeding antimicrobials to pigs promotes growth by reducing the overall pathogenic bacteria load, thereby reducing the immunological stress on the animal. This theory is supported by the following data: (1) very diverse antimicrobials have been shown to be effective growth promotants; (2) antimicrobials with the broadest spectrum of activity have been shown to be the most effective growth promotants; (3) the use of subtherapeutic levels of antimicrobials have been shown to reduce morbidity and mortality rates; (4) the effectiveness of antimicrobials as growth promotants is greatest in environments with the greatest pathogen contamination; (5) antimicrobials reduce the lymphatic tissue in the gut; and (6) the magnitude of benefit varies from animal to animal, commensurate with the unique profile of microorganisms across animals.

The presence of pathogenic bacteria in the gastro-intestinal tract is a threat to the health status of the animal. When an animal is immunologically challenged many metabolic changes occur, including an increased concentration of the pro-inflammatory cytokines interleukin-1 (IL-1) and interleukin-6 (IL-6) and an increased concentration of the acute phase proteins. In turn there is a decrease in serum insulin-like growth factor-1 (IGF-I) levels, and a decrease in both voluntary feed intake and the efficiency of nutrient utilization (Lang et al, 1998). As a consequence, growth rates decline. Each of these conditions is reversed when antimicrobials are incorporated into the diet of young, rapidly growing pigs (Hathaway et al., 1996). We proposed to accomplish the first 3 objectives with the original funding from the National Pork Board and the last 2 objectives with the supplemental funding from MPPA.

Objectives:

- (1) To determine whether feeding therapeutic levels of an antimicrobial for a short period of time stimulates the growth of swine to the same or greater extent than feeding subtherapeutic levels of an antimicrobial for an extended period of time.
- (2) To determine whether serum IGF-I levels are enhanced by feeding therapeutic levels of an antimicrobial for a short period of time as compared to feeding subtherapeutic levels long term.
- (3) To evaluate the types and levels of acute phase proteins present in the serum of animals fed antimicrobials for a short period of time or subtherapeutic levels of an antimicrobial for an extended period of time.
- (4) To measure IL-6 concentrations in the serum of animals fed antimicrobials for a short period of time or subtherapeutic levels of an antimicrobial for an extended period of time.
- (5) To collect intestinal tissue secretions and samples from sacrificed pigs in order to study the immunological response occurring very specifically at different sites along the gastro-intestinal tract.

Procedures:

Procedure to accomplish objective 1: A study was conducted in which a group of pigs was monitored from weaning through finishing to determine whether short treatments with therapeutic levels of antimicrobials at strategic points in time would be as efficacious as a treatment with subtherapeutic levels of antimicrobials for an extended period of time. The antimicrobial chlortetracycline, which is approved at subtherapeutic levels as a growth promotant and at therapeutic levels to treat disease conditions, was used. At weaning 210 pigs were allotted to 3 treatment groups (70 pigs/treatment), based on gender, litter, weight and average daily gain. The treatment groups consisted of pigs fed (1) a control diet without antimicrobials from weaning to finish, (2) an antimicrobial diet supplemented at subtherapeutic levels, i.e., 50 g/ton from weaning to finish and (3) an antimicrobial diet supplemented at therapeutic levels, i.e., 400 g/ton for 1 week of each major growth phase, i.e., during the first week in the nursery, the first week in the grower facility and the first week in the finisher facility. After the initial period of therapeutic treatment within each major growth phase, the pigs on treatment 3 were fed the control diet without the antimicrobial. Blood samples were drawn via vena puncture of the jugular vein, at bi-weekly intervals. Pig weights and feed intakes were recorded weekly. The growth performance of the pigs (i.e., average daily gain and feed efficiency) on the 3 different treatments was compared. In the past, it has been reported that the withdrawal of subtherapeutic levels of antimicrobials from the diets of pigs could result in loss of all growth-promoting benefits otherwise gained. This study differs significantly from those in that the pigs are being supplemented at therapeutic levels for short periods of time at strategic, immunologically-stressful points during the wean to finish period.

Procedure to accomplish objective 2: In order to determine whether feeding therapeutic levels of an antimicrobial for a short period of time increases the serum IGF-I level to a greater extent than does feeding either a control diet or subtherapeutic levels of an antimicrobial for longer period of time, the blood samples drawn in objective 1 were analyzed for serum IGF-I using a radioimmunoassay. This assay is in routine use in our laboratory. Comparisons of IGF-I levels will be made between pigs fed the control diet and the diets containing both levels of antimicrobial in objective 1. Increases in sera IGF-I levels are highly correlated with increases in growth. Each blood sample obtained from each pig was analyzed.

Procedure to accomplish objective 3: There have been reports in the literature that serum levels of acute phase proteins can be used as indicators of health status in pigs. Moreover, by calculating the ratios of certain acute phase proteins it is possible to determine whether the immunological challenge is chronic or acute in nature. In order to determine whether feeding therapeutic levels of antimicrobial is capable of altering the immunological status of the pig, acute phase protein levels will be

measured. There are commercially available assays for porcine acute phase proteins: C-reactive protein (ELISA), haptoglobin (immunodiffusion), serum amyloid-A (ELISA) and acid-alpha glycoprotein (immunodiffusion). Based on the literature the levels of haptoglobin, C-reactive protein, alpha acid-glycoprotein and serum amyloid-A should be indicative of the health status of the pig. The haptoglobin/serum amyloid-A ratio will allow us to determine whether the immune challenge is chronic or acute leading to better overall understanding of the immunological challenges faced by the animal.

Procedure to accomplish objective 4: In order to determine whether injecting therapeutic doses of an antimicrobial at strategic points in time decreases the serum IL-6 level to a greater extent than does feeding either a control diet or subtherapeutic levels of an antimicrobial for longer period of time, the blood samples drawn in objective 1 would be analyzed for serum IL-6 using an ELISA which is commercially available. Comparisons of IL-6 levels will be made between pigs from all treatment groups in objective 1.

Procedure to accomplish objective 5. In order to study the immunological response occurring very specifically at different sites along the gastro-intestinal tract and better characterize the localized immunological response we would collect intestinal tissue secretions and samples from sacrificed pigs. Small intestine will be removed, flushed and wicked according to the method of Haneberg et al. 1994. Total IgA will be measured in collected secretions by sandwich ELISA using a peroxidase-labelled anti-IgA and TMB detection. We would be looking for differences in IgA concentrations in intestinal tract secretions. Total RNA will be isolated using the single step guanidinium thiocyanate procedure. Quantitative real-time RT-PCR will be used to determine the level of specific mRNA for various cytokines in the intestinal tract tissues. These procedures are in routine use in our laboratory.

Results:

Initially there was a numerical increase in body weight for the pigs fed a control diet and receiving the therapeutic injections of the antimicrobial compared to the pigs fed the control diet during the first week on study. However, no differences in average daily gain were detected in the subsequent weeks (Table 1). There was no difference in Average daily feed intake or feed per pound of gain by treatment (Tables 2 and 3). There was no difference in serum IGF-I concentrations across treatments. Blood samples from each pig at each time point were analyzed in the RIA. Values shown are the result of averaging all pigs within a treatment for the indicated phase of growth. Because there was no apparent difference in serum IGF-I concentrations across treatments during the nursery and growing period, blood samples were not drawn during the finishing period of the study (Table 4). There were no apparent differences in C-reactive protein concentration across treatments at any of the time points (Table 5). All values fell below detection limits in the serum amyloid A assay. Because the pigs fed the subtherapeutic levels of antimicrobials and the pig receiving the injections of therapeutic levels of antimicrobials did not grow faster or more efficiently than the control pigs, and because the serum IGF-I levels and the acute phase proteins did not differ across treatments, the IL-6 assays and the collection of intestinal tissue data was not conducted.

Table 1. Average Daily Gain (lbs/day)

Day on Study	7	14	21	28	35	42	56	70	84	98	112	126	140
Control ^a	0.17	0.59	0.96	1.34	1.43	1.26	1.71	2.12	2.19	2.19	2.11	2.03	1.77
CTC ^b	0.19	0.58	0.88	1.28	1.45	1.37	1.68	2.18	2.24	2.26	2.24	2.09	1.75
Excenel ^c	0.26	0.59	0.94	1.31	1.40	1.35	1.67	2.17	2.22	2.23	2.18	2.06	1.77

^a Pigs fed a control diet without antimicrobials.

^b Pigs fed a diet supplemented with subtherapeutic levels of chlortetracycline.

^c Pigs fed a control diet but injected IM with Excenel.

Table 2. Average Daily Feed Intake (lbs/day)

Day on Study	7	14	21	28	35	42	56	70	84	98	112	126	140
Control ^a	.24	.72	1.26	1.97	2.44	2.28	3.14	4.54	5.47	5.74	6.13	6.42	6.39
CTC ^b	.25	.72	1.26	2.01	2.41	2.44	3.02	4.54	5.53	5.98	6.59	6.66	6.57
Excenel ^c	.31	.70	1.2	2.00	2.38	2.40	3.09	4.61	5.49	5.83	6.28	6.57	6.57

^a Pigs fed a control diet without antimicrobials.

^b Pigs fed a diet supplemented with subtherapeutic levels of chlortetracycline.

^c Pigs fed a control diet but injected IM with Excenel.

Table 3. Feed per Pound of Gain (lbs/day)

Day on Study	7	14	21	28	35	42	56	70	84	98	112	126	140
Control ^a	1.44	1.22	1.31	1.47	1.72	1.81	1.84	2.14	2.5	2.63	2.92	3.18	3.63
CTC ^b	1.44	1.24	1.41	1.54	1.70	1.78	1.80	2.07	2.45	2.62	2.91	3.22	3.67
Excenel ^c	1.20	1.20	1.30	1.53	1.70	1.78	1.84	2.13	2.48	2.61	2.87	3.20	3.71

^a Pigs fed a control diet without antimicrobials.

^b Pigs fed a diet supplemented with subtherapeutic levels of chlortetracycline.

^c Pigs fed a control diet but injected IM with Excenel.

Table 4. Serum IGF-1 concentration in ng/ml.

Time point	Excenel ^c	CTC ^b	Control ^a
Weaning	173	160	171
Nursery phase	70	75	73
Growing phase	152	163	168

^a Pigs fed a control diet without antimicrobials.

^b Pigs fed a diet supplemented with subtherapeutic levels of chlortetracycline.

^c Pigs fed a control diet but injected IM with Excenel.

Table 5. Serum C-Reactive concentrations in micrograms/ml.

Time point	Excenel ^c	CTC ^b	Control ^a
Weaning	0	-3	-2
Nursery phase	50	51	54
Growing phase	65	61	66

^a Pigs fed a control diet without antimicrobials.

^b Pigs fed a diet supplemented with subtherapeutic levels of chlortetracycline.

^c Pigs fed a control diet but injected IM with Excenel.