The effects of enrofloxacin and tiamulin on serum haptoglobin and α-1-acid glycoprotein concentrations in modified medicated-early-weaned pigs

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Summary

Objective—*To ascertain the serum concentrations of two acute-phase proteins, haptoglobin (HPT) and α-1-acid glycoprotein (AGP) to medication in healthy weaned pigs. Acute-phase protein concentrations in healthy pigs were intended to serve as biomarkers, parameters used to indirectly detect the effect of medication on growth, independent of disease.*

Design and procedure—*In this experiment, piglets were medicated following a modified medicated early weaning (MMEW) regimen. Treatment included enrofloxacin (11.25 mg) intramuscularly preweaning for 3 consecutive days and tiamulin (180 mg per L) orally postweaning for 7 days. Control pigs were given only sterile water injections preweaning. Weaning age was at 3 weeks. Serum samples collected from each group were assayed for HPT and AGP before and after treatment.*

Results—*Serum HPT concentration was influenced by medication and postweaning time in young healthy pigs. Medication increased serum HPT, and had no effect on AGP concentrations in these animals.*

Implications—*Serum haptoglobin (HPT) was greater in medicated versus nonmedicated healthy pigs, but medication did not provide any benefit in performance during the nursery phase of growth.*

Keywords: *swine, acute-phase proteins, modified medicated early weaning (MMEW), growth performance*

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Haptoglobin (HPT) and α-1-acid glycoprotein (AGP) are two acute-phase proteins found in normal porcine serum.[1–5] They are known as acute-phase proteins because basal serum concentrations change during the acute phase of inflammation. The function of porcine AGP or HPT is not completely known, but like other acute-phase proteins, a homeostatic function is hypothesized. In human medicine, very early increases in basal HPT and AGP may be detected after inflammatory insults from infection, trauma, or necrosis.[6,7] Elevations of HPT and AGP in growing and adult swine have been reported in response to experimental or natural infection, mycotoxin exposure, and production stressors in high- and low-health herds.[8–14]

Medicated early weaning (MEW) and modified medicated early weaning (MMEW) are common strategies used to control disease in swine production.[15–17] Various modifications of these strategies exist, but all depend on an extensive use of broad-spectrum antibiotics in pre- and postweaned piglets. Medication is used as an adjunct to early weaning. Medication may minimize or eliminate the influence of suspected indigenous pathogenic microorganisms (*Mycoplasma* spp, *Actinobacillus* spp, *Streptococcus* spp, *Pasteurella* spp, *Bordetella* spp) on biological performance. By using a MEW or MMEW strategy, higher-health swine with enhanced potential for growth may be produced from a lower-health herd. However, the use of antibiotics in MEW schemes is controversial because some drugs used in protocols lack United States Food and Drug Administration (FDA) approval and/or may be potentially toxic to neonatal pigs, or their benefits may not outweigh costs. Medication often occurs at a very early age (< 3 weeks), when metabolic enzyme systems are not fully developed. Prophylactic use of antibiotics may have little effect on health or performance under certain conditions (e.g., swine that are healthy within a high-health herd).

The objective of this study was to assess the influence of medication on two acute-phase proteins, haptoglobin (HPT) and α-1-acid glycoprotein (AGP) in 80 healthy medicated and nonmedicated weaned pigs. Since these acute-phase proteins are sensitive to inflammatory change in swine, they may serve as biomarkers in weaned pigs of potential adverse effects caused by antibacterial therapy. The use of high-health pigs may provide baseline information regarding the use of HPT or AGP as potential biomarkers independent of disease. Weaning weights, final weights, and average daily gain (ADG) were compared in each group as indicators for health and biological performance.

Materials and methods

Experimental population

A high-health confinement swine farm served as our experimental study facility. This herd was a closed (replacement pigs produced by Cesarean and cross-fostering), veterinary research swine herd (150 sows) that had a history of minimal disease. Sows were Landrace × Large White rotational cross females. Sporadic cases of regional ileitis, *Mycoplasma* spp, nonprogressive atrophic rhinitis, and *Streptococcus* spp had been diagnosed in nursery, growing, and finisher pigs. No medications or biologics were used on pigs within this herd. Twelve litters were farrowed monthly. After weaning, all litters remained intact. Each weaning was managed all-in–all-out (AIAO) until reaching market weight. Weaned pigs were fed a single, pelletized, nonmedicated corn-soy diet ad libitum. All facilities were washed and disinfected between groups of pigs. Performance records were maintained using PigCHAMP® computerized software (PigCHAMP®, University of Minnesota, College of Veterinary Medicine, St. Paul, Minnesota).

Experimental design

The experiment followed a single-factor (treatment or control), repeated-measures design. There were two replicates from consecutive farrowings evaluated. Two farrowing rooms (six litters per room) were randomly assigned to either treatment (11.25 mg enrofloxacin [Bayer Animal Health, Inc., Shawnee, Kansas] or 0.5 mL per pig intramuscularly [IM]) or control (sham injection) groups. Enrofloxacin injections were given prior to weaning when pigs were 18 days old. Control pigs were nonmedicated, but received placebo injections of sterile water (0.5 mL IM). Both treatment and control pigs were weaned at 21 days of age. Treatment and control groups were isolated in separate nursery rooms after weaning. Beginning at weaning, the treatment group pigs were offered water medicated with tiamulin (180 mg per L [Fermenta Animal Health, Kansas City, Missouri]) for 5 days. No water medication was given to the control group pigs. During the second replicate, nursery rooms for treatment and control pigs were also isolated but rooms were reversed.

Twenty pigs were selected randomly from each of the treatment and control groups in both trials. Piglets were ear tagged for identification. Blood was collected from each pig by jugular venipuncture on days 0, 7, 14, 21, and 28. Day 0 blood was collected prior to medication and weaning. Sera were harvested from each sample and stored at –70°C until analyzed for HPT and AGP concentrations.

During both replicates, weaning weights and weight 28 days postweaning were recorded.

Acute-phase protein assays—HPT, AGP

To quantify serum HPT, the cyanmethemoglobin binding capacity (CHBC mg per dL) previously described was used.[14]

A commercially prepared porcine single radial immunodiffusion assay system (Development Technologies International, Inc., Frederick, Maryland) was used for serum AGP detection. Five mL of porcine sera were added to each of 10 wells in antiporcine AGP-impregnated agarose plates. Plates were incubated at 37°C for 48 hours. Precipitin rings were measured on an electronic plate reader (RID Plate Reader, Alta Diagnostic Machine, Ltd., England) after incubation. The quantity of AGP was calculated using a computerized spreadsheet derived from the reference curve corresponding to porcine AGP standards (250 and 1000 µg per mL) supplied with each kit. The diameter squared (d^2) of the precipitin rings obtained from the AGP standards were plotted against their prospective concentrations to generate the standard curve.[18,19] Internal control was maintained using the supplied standards during each assay run of 20 serum samples.

Statistical analysis

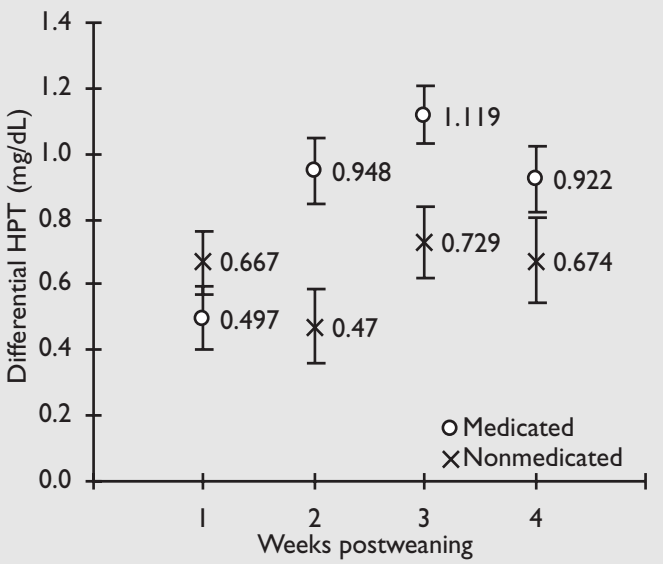
The effects of medication on serum acute-phase protein concentration (HPT, AGP) in weaned pigs were examined using multivariate repeated measures analysis of variance.[20] Analysis was conducted using SYSTAT® software (Systat®, Inc., Evanston, Illinois). The between-subject factors were: treatment (medicated or nonmedicated) and replicate. The within-subject factors were four levels of time (7, 14, 21, and 28 days post-treatment). The model-dependent variables were either serum HPT or AGP. To detect change in the acute-phase reaction, differential serum acute-phase protein concentrations (difference from baseline) were calculated and used as the dependent variables. Data was log transformed to correct for heterogeneity of variance. Differences in weaning weight, final weight, and ADG in each group and replicate were determined using two-factor ANOVA. In all statistical tests an α level of 0.05 was used.

Results

Haptoglobin (HPT)

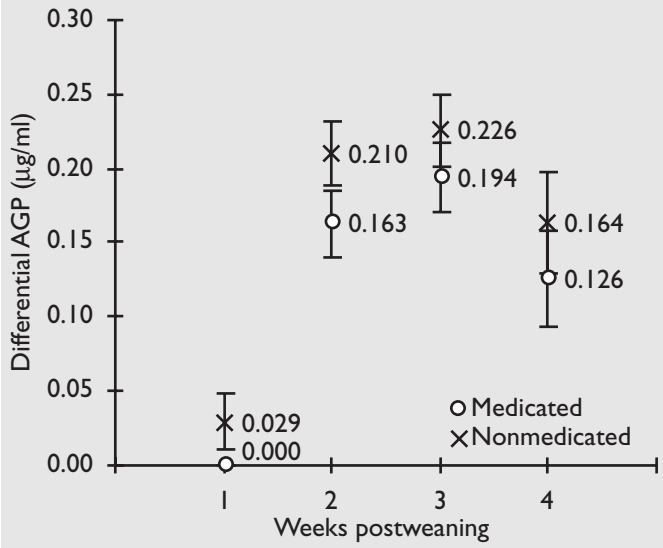
Overall, a significant time effect was detected ($P<.005$). A plot of the differential serum HPT concentration indicated a positive change from

Figure 1



Differential (preweaning from postweaning) serum haptoglobin (HPT) concentration in 80 3-week-old healthy medicated and nonmedicated pigs. Differential HPT concentrations are log transformed.

Figure 2



Differential (preweaning from postweaning) serum α-1-acid glycoprotein (AGP) concentration (mg/mL) in 80 3-week-old healthy medicated and nonmedicated pigs. Differential AGP concentrations are log transformed.

baseline concentration (Figure 1). An increasing trend in this acute-phase protein over postweaning time was also noted. A significant treatment × time interaction was detected ($P<.005$). Medication increased serum HPT concentrations in pigs at a greater rate and magnitude than those left unmedicated. A significant main effect was found ($P<.05$). Higher serum HPT was observed in the medicated versus nonmedicated pigs. A significant replicate effect ($P<.005$) was also detected, indicating serum HPT was greater in replicate one than in replicate two.

α-1-acid glycoprotein (AGP)

A significant temporal effect in serum AGP was found in pigs postweaning ($P<.0001$). Serum AGP increased from baseline and tended to increase postweaning (Figure 2). No treatment × time interaction or treatment main effect were detected.

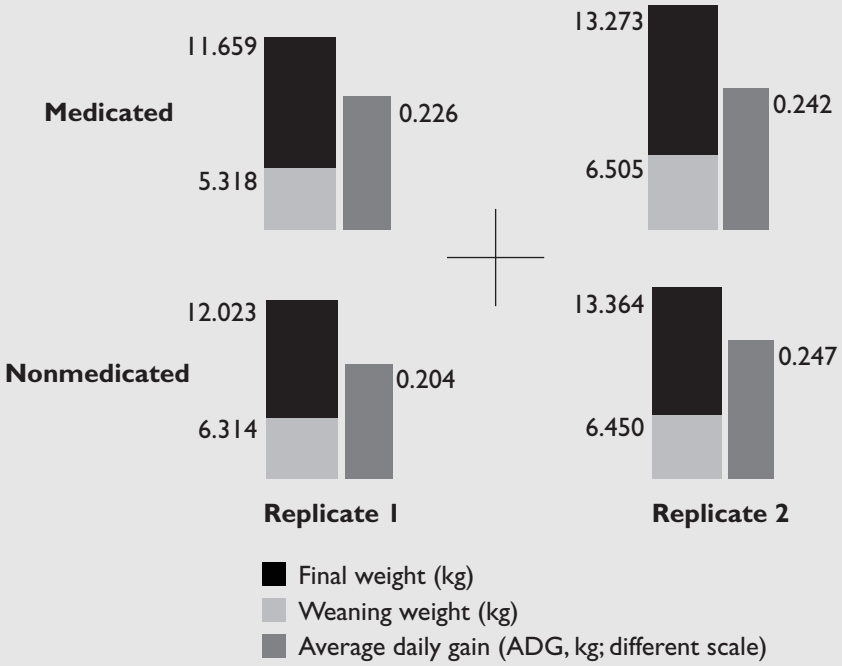
Performance

No significant differences were found within weaning weights and final weights between treatments, although treatment pigs were lighter than controls during replicate one ($P<.10$) (Figure 3). Significant differences in weaning and final weights were found between replicates ($P<.05$), indicating pigs were heavier at weaning and at the end of the experiment during replicate two. No significant difference in average daily gains were detected between treatment or replicate.

Discussion

Enrofloxacin and tiamulin were chosen as the medications used in this MMEW experiment because of their potency, availability, and efficacy against swine diseases.[21–25] Tiamulin is an FDA-approved antibiotic. Tiamulin is a semisynthetic derivative of the antibiotic pleuromutilin, which is a diterpene antibiotic with bacteriostatic activity.[21,22] When added to the drinking water, tiamulin may be used to treat *Serpulina hyodysenteriae* or *Actinobacillus pleuropneumoniae*.[22,23] Experimental investigations have also shown that tiamulin may be effective in controlling *Mycoplasma hyopneumoniae*.[23] These swine diseases

Figure 3



Performance statistics in 80 3-week-old healthy medicated and nonmedicated weaned pigs. Treatment pigs were lighter than controls in replicate one only ($P<.10$); pigs were heavier in replicate two at weaning and at the end of the experiment compared to replicate one ($P<.05$).

have been targeted for MEW or MMEW protocols.[15–17]

Enrofloxacin, a fluoroquinolone antibiotic, does not have current FDA approval for use in swine.[24–27] Enrofloxacin has a wide spectrum of activity against Gram-negative bacilli, including enterotoxigenic *Escherichia coli* and *Salmonella* spp.[24–27] However, the dosage, administration, and contraindications of this drug may restrict its use in veterinary medicine. In small and medium dog breeds during rapid phases of growth, enrofloxacin is contraindicated.[28,29] Fluoroquinolones may cause articular cartilage defects in these animals. Joint swelling and tendonitis have also been reported in humans exposed to certain fluoroquinolones.[30] Weaned pigs grow fast, and have been reported to have a high incidence of osteochondrosis dessicans (OCD) at the time of slaughter.[31] The dosage of injectable enrofloxacin solution is limited to a single dose. It is not known by the authors whether repeated enrofloxacin IM injections in dogs or cats result in tissue inflammation that preclude multiple administration by this route.

It is unknown whether tiamulin, offered orally, may have any potential adverse effects on articular cartilage or organ systems. In rare cases, tissue hypersensitivity, characterized by hyperemia, has been detected in growing swine.[32] Transitory hypersalivation and vomition have been reported to result from an overdose of tiamulin.[32] A calming effect in swine has also been noted in overdoses.[32]

Although in the original investigation of MEW, Alexander, et al., reported successful control of enzootic pneumonia in MEW pigs, nonmedicated pigs performed equally well.[16] Another striking finding in the Alexander study was mortality rate. They observed that medicated pigs had much greater mortality postweaning (18%) than nonmedicated controls (4.4%). Anorexia, diarrhea, and dehydration were common findings in their medicated groups, especially in lighter-weight pigs. They were unable to determine whether medication provided any additional effects for controlling disease or whether medication was necessary.

In our experiment, serum HPT concentrations were greater in pigs receiving antibiotics than placebos. No difference in serum AGP was found between treatment and controls. Although not significant, AGP concentrations were lower in the treatment group. The physiological mechanism favoring the detectable differences in serum HPT but not AGP after antibiotic administration is not known. One possible explanation may be that HPT is a more sensitive indicator of inflammatory change in swine than is AGP. It has been reported that the pattern of plasma proteins changes after tissue is injured or becomes inflamed. The magnitude, duration, and type of acute-phase protein released depends upon the species and the cause of inflammation. For example, serum HPT is not found to any extent in sera from healthy cattle but is readily detected in animals clinically affected with a variety of medical disorders.[33] Viral infections normally do not evoke acute-phase responses, unless severe inflammatory changes occur.[7] The specific reason why we observed higher serum HPT concentrations in medicated pigs cannot be determined from this study. No reactions due to antibiotic injection or from offering water medication were noted in these pigs during this experiment. Nor were any histopathological studies conducted on tissues from these pigs. However, articular cartilage defects or tissue hypersensitivity are possibilities for the response in serum HPT that we found.

The overall temporal change in both AGP and HPT concentrations in each group of weaned pigs is consistent with reports from other investigators.[8–14] Increases from baseline serum HPT and AGP in both medicated and nonmedicated pigs were found. These proteins are dynamic, increasing or decreasing in response to a variety of stimuli.[8–14] Mixing pigs from different litters disrupts dominance hierarchy, causing pigs to fight until a dominance hierarchy has been reestablished. Any fight wound could be a sufficient stimulus for an acute-phase response. Stress due to weaning or handling during injection may produce enough basal glucocorticoid to trigger an acute-phase protein release.[1,2,14] The observed peak in serum HPT and AGP that occurred 3 weeks postweaning requires further investigation. This was a consistent finding in both medicated and nonmedicated groups. This may indicate the decline of circulating maternal antibodies, or exposure to some endemic microorganism on this farm.

The replicate effect in serum HPT may be due to the weaning weights of pigs. There were significant differences in weaning weights, although there was no difference in the parity distribution of the sows (3.2 versus 3.0). Larger weaning weights produced heavier pigs at the end of the nursery phase of growth (3–7 weeks old). Pigs that are heavier may have lower serum HPT due to increased blood and serum volume, thereby diluting the basal concentration. Serum HPT concentration has been shown to correlate with weight gain in pigs.[10] Faster-growing pigs monitored from herds where health status was high had lower serum HPT concentration than slower-growing, conventional pigs.

Because differences in serum HPT were found in the treated pigs, serum HPT may be more reliable to monitor the effects of medication than AGP or weight gain. Average daily gains were not significantly greater in medicated and nonmedicated pigs in this experiment. Since the nonmedicated pigs had lower serum HPT concentrations and grew as fast as their medicated cohorts, we concluded that serum HPT represented a negative biomarker for antibiotic administration, at least in healthy pigs. Serum AGP concentration did not follow this same pattern. However, serum HPT or other acute-phase proteins may have limitations as biomarkers. In general, these proteins are nonspecific; other factors may trigger their increase (i.e., stress, mixing, disease, age, weight). More research is needed to evaluate the sensitivity and specificity of acute-phase proteins in swine related to antibiotic administration. A sensitive and specific biomarker may provide the veterinary clinician more objective evidence for antibiotic administration, effectiveness, and withdrawal. In many large herds, swine producers continue to medicate pigs even after disease outbreaks have resolved, in fear of disease recrudescence. Strategic serum acute-phase protein assessment may provide more complete information regarding herd health.

Implications

- Serum haptoglobin (HPT) was greater in medicated versus nonmedicated healthy pigs.
- Serum α-1-acid glycoprotein (AGP) was not different in medicated versus nonmedicated healthy pigs.
- In healthy pigs, medication did not provide any benefit in performance during the nursery phase of growth.
- Further investigations are needed to determine whether serum HPT or other acute-phase proteins can be used as biomarkers for strategic antibiotic therapy in MEW or MMEW protocols.

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