June 2006

Executive Summary

A comparative study was conducted in 2005 to assess the clinical and economic effects of lincomycin feed medication (Lincomix®, Pfizer Animal Health) and/or vaccination with an ileitis vaccine (Enterisol® Ileitis, Boehringer Ingelheim) administered to pigs artificially challenged with *Lawsonia intracellularis*.1 Pigs were evaluated for clinical effects of disease, growth performance, and intestinal lesions attributed to *L. intracellularis*. Following challenge, pigs in the Lincomix + Enterisol and the Lincomix alone groups had significantly (P ≤0.05) fewer diarrhea days than pigs receiving Enterisol alone and produced significantly (P ≤0.05) more marketable pounds of pork than pigs in the control or Enterisol alone groups. The average per pig value of the Lincomix, Enterisol, and Lincomix + Enterisol groups had numerical advantages of $9.44, $7.99, and $12.13, respectively, compared to pigs in the untreated control group.

More than 70 years after the lesions of porcine proliferative enteropathy (PPE), or ileitis, were first described in pigs in Ames, Iowa, the disease remains one of the most serious threats to the health and profitability of the U.S. swine herd. Results of a National Animal Health Monitoring Service (NAHMS) report indicated that 96% of U.S. swine herds and 28% of pigs are seropositive for the obligate intracellular bacterium *Lawsonia intracellularis*, the causative agent of PPE.2

The majority of ileitis cases are subclinical infections characterized by decreased daily gains, poor feed efficiency, and increased days to market. Primarily affected are 6- to 20-week-old grower pigs exhibiting reduced appetite, slow and uneven growth, and moderate diarrhea.3 Secondary enteric agents, including *Escherichia coli*, *Campylobacter* spp., *Salmonella* spp., and *Brachyspira pilosicoli*, can often be found in concert with *L. intracellularis*.

In its acute form, ileitis most frequently occurs in older finishing pigs between 4 and 12 months of age and is associated with bloody diarrhea, weakness and depression, and death loss of 5 to 6%, although mortali-
ty in some young adult swine has been reported to be as high as 50%. Sows and gilts that are pregnant when infected may abort, usually within six days of the first appearance of clinical signs. The disease is transmitted through fecal shedding and, left unchecked, can quickly spread throughout a herd. Herd histories reveal that clinical outbreaks often are preceded by the occurrence of major stressors (e.g., movement, mixing different populations, and sudden changes in weather). An intestinal lesion—thickening of mucosa with an irregular nodular or folded appearance—of the ileum and jejunum is the most characteristic clinical sign.

Taken together, the economic effect of clinical and subclinical ileitis is estimated to cost between $2 and $22 per affected pig, depending upon the severity and duration of disease. The best estimate of the annual cost to the U.S. pork industry as a whole is approximately $98 million.

No therapy has been discovered that can completely eliminate ileitis from a herd; however, antimicrobials can effectively control outbreaks. Standard strategies with approved antimicrobials (e.g., tiamulin, tylosin, chlortetracycline, lincomycin) include:

- **Feed medication.** In acute outbreaks, veterinarians sometimes use a combination of feed plus water and/or injectable antimicrobial therapy, although no water or injectable medications are approved for treatment or control.
- **Biosecurity and sanitation measures** to minimize bacterial spread
- **"Pulse" anti-infective therapy** that delivers treatment after animals are infected but before significant lesions develop. Success depends upon maintaining the pulse dose for a period long enough to suppress disease.

An alternative approach to controlling ileitis is vaccination with an oral (drinking water or drench applications) vaccine (Enterisol® Illeitis), which contains avirulent live *L. intracellularis* bacteria. The vaccine is designed to stimulate an active immune response in weaned pigs from 3 weeks of age and older to help reduce intestinal lesions caused by *L. intracellularis* infection. Because the vaccine is a live bacterium, simultaneous use of antimicrobials effective against *L. intracellularis* should be avoided for a minimum of three days before and after vaccination. Additionally, all materials used in administering the vaccine must be free of antimicrobials, detergents, or disinfectant residues to prevent inactivation of the live bacterium. The vaccine is not approved for use in pregnant sows and gilts or breeding boars.

The study presented here was conducted to determine the clinical and economic effects of lincomycin feed medication (Lincomix) and/or vaccination with Enterisol in pigs from a herd with no clinical history of ileitis and artificially challenged with a disease-producing strain of *L. intracellularis*.

### Study Overview: Infection Model, Assessments, and Analysis

#### Study Design

A total of 192 clinically healthy 3- to 4-week-old weaned barrows (n = 96) and gilts (n = 96) were enrolled in the study. All pigs originated from a herd that had been screened negative for ileitis by fecal polymerase chain reaction (PCR). Upon arrival at the study site, pigs were ear tagged, sexed, weighed, and allocated to treatments and pens according to a randomized complete block design (Table 1). Altogether, there were 32 pens and 4 treatment groups, with one complete block comprising 4 pens. Prior to initiation of the study, pigs were acclimated for a period of six days in the study facility and were examined daily by the attending veterinarian to confirm suitability for the trial.

#### Challenge Materials

The mucosal challenge model used in the study was that of Dr. Nathan Winkelman (Swine Services Unlimited, Inc., Morris, Minnesota). Prior to challenge, a sample of the mucosal challenge material was submitted to Dr. Connie Gebhart of the University of Minnesota to test for the

<table>
<thead>
<tr>
<th><strong>Group</strong></th>
<th><strong>No. Pigs</strong></th>
<th><strong>Dosage</strong></th>
<th><strong>Regimen</strong></th>
<th><strong>Route of Administration</strong></th>
<th><strong>Challenge Day</strong></th>
<th><strong>Evaluations</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated control</td>
<td>48</td>
<td>Not applicable</td>
<td>Not applicable</td>
<td>Not applicable</td>
<td>28</td>
<td>Clinical signs, intestinal lesions, growth performance, market value</td>
</tr>
<tr>
<td>Lincomix</td>
<td>48</td>
<td>100 g/ton + 40 g/ton</td>
<td>21 consecutive days from challenge + Pulse regimen*</td>
<td>In feed</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Enterisol</td>
<td>48</td>
<td>1 dose (2 mL/pig)</td>
<td>Once (28 days prior to challenge)</td>
<td>In water</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Lincomix + Enterisol</td>
<td>48</td>
<td>100 g/ton + 40 g/ton</td>
<td>21 consecutive days from challenge + Pulse regimen*</td>
<td>In feed</td>
<td>28</td>
<td></td>
</tr>
</tbody>
</table>

*Pulse regimen: 7 consecutive days out of every 28 days until slaughter. First pulse given 3 weeks after end of initial dose of 100 g/ton.
presence and enumeration of *L. intracellularis* and to confirm the absence of secondary enteric bacteria.

**Assessments**

Prior to medicating the pigs, batches of medicated feed and non-medicated control feed were tested to confirm the correct level of lincomycin and absence of lincomycin, respectively.

During the course of the study, the health status of the pigs was monitored daily. Pen diarrhea scores were recorded from challenge on Study Day 28 through the end of the study. Each pen was scored daily according to a scale where:

- 0 = normal feces
- 1 = presence of mild diarrhea, no blood
- 2 = presence of watery diarrhea, no blood
- 3 = presence of mild or watery diarrhea with blood
- 4 = presence of mild or watery diarrhea with very dark, tarry feces.

Blood and fecal samples were taken from two randomly selected pigs per pen at enrollment on Study Day 0, prior to challenge on Study Day 27, on Study Day 47, and approximately monthly for the remainder of the study for serological and PCR assay, respectively. Also on Study Day 47, 2 additional pigs from each pen were randomly selected and euthanized. The small intestines of these pigs were evaluated at the University of Minnesota by Dr. Gebhart for proliferative lesions associated with *L. intracellularis* infection. The lesions were scored for severity with 1 indicating no gross lesions and 4 indicating severe nodular lesions.

All pigs were weighed on arrival (Study Day -6), the day before vaccination (Study Day -1), the day before challenge/lincomycin treatment (Study Day 27), the 2-pig/pen necropsy day (Study Day 47), on Study Day 119 and 132, and on the day before the last day each pen was on study (when the average weight of the block was 265 pounds).

Additionally, all pigs removed from the study for any reason were also weighed.

Feed consumption (pounds/day) was measured by pen from Study Day 0 through the end of the study. Adjustments for mortality were made by calculating consumption by pig-day.

**Market Evaluation**

Market values in this study were determined at the time of slaughter according to the grid in Table 2. Animals that died or were removed due to ileitis were assigned a value of $0.00.

**Study Analysis**

Analysis of all variables was performed with SAS Version 8.2 (SAS Institute, Cary, North Carolina), and a significant overall treatment effect was required before pairwise treatment comparisons were conducted. Within each analysis, linear combinations of treatment effects were used to estimate and test the main effects of Lincomix and Enterisol as a 2x2 factorial treatment structure (see sidebar below). The 5% level of significance (P≤0.05) was used to assess all statistical differences.

**Results**

**Pre-challenge results**

The challenge dose of *L. intracellularis* was quantified at 1.4 x 10⁸ organisms per pig, a level of challenge previously shown to produce clinical signs and pathology typical of field cases of PPE.¹⁰ *B. hyodysenteriae, B. pilosicoli, Salmonella choleraesuis, and ß-hemolytic Escherichia coli* were not identified in the mucosal challenge material.

Confirmatory testing of test diet lots for the presence of lincomycin, both medicated and control, showed all lots within acceptable ranges or negative as appropriate.

Fecal samples collected from pigs at the source farm, prior to shipping to the study site, were negative for *L. intracellularis* by PCR. All study animals were negative by fecal PCR for *L. intracellularis* and for serum IgG antibodies to *L. intracellularis* on Study Day -1.

Additionally, there was no significant difference in average daily gain (ADG), body weights, feed consumption, feed conversion (pounds of feed/pounds of gain), or diarrhea days between treatment groups prior to challenge.

**PCR and Serology**

No statistical comparisons were made for the fecal PCR or serology variables. Results of PCR testing for the presence of *L. intracellularis* in fecal samples showed that 12.5% of control, 6.7% of Lincomix, 12.5% of Enterisol, and 28.6% of Lincomix + Enterisol group pigs were negative for *L. intracellularis* DNA at 21 days after challenge. At 105 days after challenge, 33.3% of the controls, 81.8% of Lincomix, 41.7% of Enterisol, and 66.7% of Lincomix + Enterisol group pigs were negative.

At 21 days after challenge, 31.3% of control, 6.3% of Lincomix, 43.8% of

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**Table 2—Current industry grid for market weight value**

<table>
<thead>
<tr>
<th>Live Weight Range (lb)</th>
<th>Estimated Market Value (US$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;200</td>
<td>$42.95/cwt</td>
</tr>
<tr>
<td>201-210</td>
<td>$46.31/cwt</td>
</tr>
<tr>
<td>211-220</td>
<td>$48.49/cwt</td>
</tr>
<tr>
<td>221-230</td>
<td>$52.00/cwt</td>
</tr>
<tr>
<td>231-290</td>
<td>$52.93/cwt</td>
</tr>
<tr>
<td>291-300</td>
<td>$50.71/cwt</td>
</tr>
<tr>
<td>301-310</td>
<td>$49.04/cwt</td>
</tr>
<tr>
<td>311-320</td>
<td>$45.54/cwt</td>
</tr>
<tr>
<td>&gt;321</td>
<td>$42.02/cwt</td>
</tr>
</tbody>
</table>

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**Why Factorial Design?**

Studies are often designed to look at more than one treatment factor, and each treatment factor can be included at multiple levels. A factorial treatment structure is an experiment in which all factor-level combinations are studied and is useful for examining the factors as well as the interaction of the factors. A study with 3 treatment factors each included at 2 levels each would be called a 2x2x2 factorial.
Enterisol, and 18.8% of Lincomix + Enterisol group pigs were negative for IgG antibodies to *L. intracellularis* in serum samples. At 49 days after challenge, the percent negative was 0.0% for controls, 6.3% for both Lincomix and Enterisol, and 6.7% for Lincomix + Enterisol. At 105 days after challenge, 12.5% of the control, Lincomix, and Enterisol groups were negative, whereas 25% of the Lincomix + Enterisol group was negative.

**Mortality**

Mortality rate was not analyzed in the study due to the low number of deaths attributable to ileitis: 6.3% (3/48) for controls, 2.1% (1/48) for Lincomix, 0% (0/48) for Enterisol, and 2.1% (1/48) for Lincomix + Enterisol.

**Intestinal Lesions**

Nineteen days after challenge, 2 pigs per pen (16 per treatment) were randomly selected for euthanasia and evaluation of the jejunum/ileum lesions and determination of lesion length. There were no significant differences between treatment means in lesion length. Lesion lengths in inches were 19.73 for controls, 16.48 for Lincomix, 12.69 for Enterisol, and 16.47 for Lincomix + Enterisol. In the factorial analysis, lesion lengths in inches were 16.47 for lincomycin, 16.21 for non-lincomycin, 14.58 for vaccine, and 18.11 for non-vaccine effects. There was no significant treatment effect. Jejunum and ileum lesion scores are presented in Figures 1 and 2.

**Body Weights and Average Daily Gain**

Figure 3 presents the mean body weights recorded for each treatment group during the study. At 21 days after challenge (Study Day 47), weights for the Lincomix (77.35 lb) and Lincomix + Enterisol (78.26 lb) groups were significantly higher than the control group (72.04 lb), and for the same period, the Lincomix and Lincomix + Enterisol groups were significantly higher than the group receiving Enterisol alone (73.08 lb). At 91 and 105 days after challenge (Study Days 119 and 132), the Lincomix + Enterisol group was significantly higher than the group receiving Enterisol alone. In the factorial analysis, a significant lincomycin effect on body weight was observed at 21, 91, and 105 days after challenge, compared with no lincomycin. Significant vaccine effects for body weight, compared with no vaccine, were not observed during the study.

Table 3 (see page 5) presents the mean ADG for each treatment group by study period and analysis method. For the first 21 days after challenge, ADG for the Lincomix (1.57 lb) and Lincomix + Enterisol (1.58 lb) groups was significantly higher than the control group (1.23 lb). For the same period, ADG for the Lincomix and Lincomix + Enterisol groups was significantly higher than the group receiving Enterisol alone (1.31 lb).

From 21 to 91 days after challenge, the Lincomix + Enterisol (2.04 lb) group was significantly higher than the group receiving Enterisol alone (1.93 lb). From challenge to 105 days after challenge, ADG was 1.89 lb for controls, 1.93 lb for Lincomix, 1.83 lb for Enterisol, and 1.99 lb for Lincomix + Enterisol. For this period, the ADG of the Lincomix + Enterisol group was significantly higher than the group receiving Enterisol alone.
From initiation of the study through 105 days after challenge, ADG was 1.72 lb for controls, 1.74 lb for Lincomix, 1.67 lb for Enterisol, and 1.80 lb for Lincomix + Enterisol. Thus, for the duration of the test period, ADG for the Lincomix + Enterisol group was significantly higher than the group receiving Enterisol alone. By factorial analysis, a significant lincomycin effect occurred during the 21-day post-challenge period, for 105 days after challenge, and for the entire study period. By the same analysis, there were no significant vaccine effects on ADG for any of the study periods, compared with no vaccine.

**Feed Intake and Feed Conversion**

Mean daily feed intake (lb/day) results for each study period, treatment group, and analysis method are presented in Table 4. For the first 21 days after challenge, feed intake was significantly higher for the Lincomix (2.86 lb) and Lincomix + Enterisol (2.91 lb) groups compared to the control (2.47 lb) and Enterisol alone (2.56 lb) groups. There was no difference between the control and Enterisol groups. While there was no significant treatment effect on feed intake from 21 to 91 or 92 to 105 days after challenge, there was a significant effect from challenge to 105 days after challenge as both the Lincomix (4.65 lb) and Lincomix + Enterisol (4.68 lb) groups had significantly higher feed intake than controls (4.34 lb). During the same period, feed intake of the Lincomix + Enterisol group was significantly higher than the Enterisol (4.41 lb) group, and the Enterisol group did not differ from the control group. From the beginning of the study through 105 days after challenge, there was no significant treatment effect for feed intake on the following values: controls, 3.59 lb, Lincomix, 3.81 lb, Enterisol, 3.62 lb, and Lincomix + Enterisol, 3.80 lb. Additionally, from initiation of the study through marketing, there was no significant treatment effect for feed intake.

In the factorial analysis, there was a significant lincomycin effect on feed intake during the first 21 days after challenge, for 105 days after challenge, from study initiation to 105 days after challenge, and for the entire study period (Study Day -1 to market), compared with no lincomycin. Compared with no vaccine, there was no significant vaccine effect on feed intake for any of the study periods. There was no significant treatment effect for feed conversion during any of the study periods for any treatment groups or analysis methods (data not shown).
**Percent Diarrhea Days**

Percent diarrhea days was determined from the day of challenge through 105 days after challenge, and treatment effects were only tested for the entire period. Figure 4 shows that there was a significant treatment effect for the duration of the study. The percent diarrhea days was 17.5% for controls, 10.4% for Lincomix, 21.2% for Enterisol, and 11.2% for Lincomix + Enterisol. Lincomix, Enterisol alone, and Lincomix + Enterisol were not significantly different from controls; however, Lincomix and Lincomix + Enterisol had significantly fewer diarrhea days than Enterisol alone.

In the factorial analysis, the percent diarrhea days was 10.8% for lincomycin, 19.3% for non-lincomycin, 16.2% for vaccine, and 13.9% for non-vaccine effects. The lincomycin effect on percent reduction of diarrhea days was significant, but the vaccine effect was not.

**Market Evaluation**

The average market value (dollars) for all pigs in each treatment group is presented in Table 5. No significant treatment effect for average market value per pig was observed (P = 0.40): $127.04 for controls, $136.48 for Lincomix, $135.03 for Enterisol, and $139.17 for Lincomix + Enterisol. In the factorial analysis, average market value per pig was $137.82 for lincomycin, $131.04 for non-lincomycin, $137.10 for vaccine, and $131.76 for non-vaccine effects. The overall treatment effect for lincomycin (P = 0.20) and vaccine (P = 0.31) for average market value per pig was not significantly different.

Figure 5 (see page 7) shows where pigs landed on the slaughter weight distribution matrix. More pigs treated for ileitis with Lincomix or Lincomix + Enterisol landed in the highest value matrix—231 to 290 pounds—and above the highest value matrix than controls or pigs vaccinated with Enterisol alone. Improved sorting technique can assist in reducing the number of pigs that fall above the high value matrix and ensure greater returns.

Additionally, Lincomix and Lincomix + Enterisol generated fewer pounds of pig in the light-weight range compared to Enterisol alone or controls. The vaccinated and control groups produced similar levels of pig pounds below the high value matrix. Lincomix alone produced approximately 50% fewer lightweight pig pounds than the control and Enterisol groups, whereas Lincomix + Enterisol produced no lightweight pig pounds.

Lincomix and Lincomix + Enterisol generated more marketable pounds of pig in and above the high value matrix than controls or Enterisol alone and fewer pig pounds in the lightweight range than controls or Enterisol alone.

### Discussion

Two options—antimicrobial therapy and vaccination—are currently available to swine producers for addressing the effects of porcine proliferative enteropathy, or ileitis. Results of U.S. trials conducted with one of the antimicrobials labeled for ileitis, Lincomix Feed Medication, established that treatment:9, 11

- Significantly reduced the incidence of diarrhea and abnormal clinical scores (at 40 g/ton and 100 g/ton of feed)
- Improved average daily gain and feed conversion efficiency (40 g/ton and 100 g/ton)
- Significantly reduced shedding of the causative agent, *L. intracellularis* (40 g/ton and 100 g/ton)
- Lowered mortality (100 g/ton)
- Significantly improved the amount of feed pigs consumed (40 g/ton and 100 g/ton)
- Delivered a higher marginal value for treated pigs (40 g/ton and 100 g/ton).

The second option is the avirulent live vaccine Enterisol, which is approved in the U.S. as an aid in the prevention and control of ileitis caused by *L. intracellularis*. In clinical trials, the vaccine was shown to:

- Significantly reduce gross and microscopic intestinal lesions of ileitis
- Significantly reduce colonization by *L. intracellularis* following challenge with disease-producing microorganisms
- Stimulate immunity following vaccination persisting for at least 7 weeks.

In the European Union, Enterisol is also licensed to reduce growth variability and loss of weight gain associated with ileitis. This study was conducted to evaluate the clinical and economic effects of Lincomix Feed Medication and/or

### Table 5—Average per pig market value (dollars) at slaughter

<table>
<thead>
<tr>
<th>Analysis Method</th>
<th>Treatment</th>
<th>Mean Dollars</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individual</td>
<td>Control</td>
<td>127.04*</td>
</tr>
<tr>
<td>Treatments</td>
<td>Lincomix</td>
<td>136.48*</td>
</tr>
<tr>
<td></td>
<td>Enterisol</td>
<td>135.03*</td>
</tr>
<tr>
<td></td>
<td>Lincomix + Enterisol</td>
<td>139.17*</td>
</tr>
<tr>
<td>Factorial</td>
<td>Lincomycin effect</td>
<td>137.83*</td>
</tr>
<tr>
<td></td>
<td>Non lincomycin effect</td>
<td>131.04*</td>
</tr>
<tr>
<td></td>
<td>Vaccine effect</td>
<td>137.10*</td>
</tr>
<tr>
<td></td>
<td>Non vaccine effect</td>
<td>131.76*</td>
</tr>
</tbody>
</table>

Within each analysis (separated by bold black lines), values within a column with different superscripts are significantly (P≤0.05) different.
Enterisol Ileitis vaccine administered to pigs artificially challenged with *L. intracellularis*. The primary variables analyzed were those often considered most important to swine producers—mortality, percent diarrhea days, average daily feed intake, feed conversion efficiency, average daily gain, and market value at slaughter. Also evaluated were intestinal lesion scores and lengths associated with *L. intracellularis* infection.

Infection of pigs with *L. intracellularis* resulted in disease typical of a chronic field infection. Minimal death loss was evident in all treatment groups, and there were no significant differences between treatment means in lesion lengths. However, pigs receiving Lincomix alone and Lincomix + Enterisol had a significantly lower percentage of diarrhea days than the pigs receiving Enterisol alone. Subsequent factorial analysis established that lincomycin had a significant effect upon reducing the number of diarrhea days, but the vaccine did not.

There was a similar overall effect for body weights as pigs in the Lincomix and Lincomix + Enterisol groups were significantly heavier at each assessment point than pigs in the group receiving Enterisol alone. Factorial analysis confirmed a significant lincomycin effect upon increased body weight, but no vaccine effect.

Analysis of other production variables showed no significant overall effect of treatment; however, significant feed intake and ADG differences were detected for individual assessment periods. By factorial analysis, there was a significant lincomycin effect on increased feed intake at 21 days after *L. intracellularis* challenge, 105 days after challenge, and for the entire study period. By contrast, there were no significant vaccine effects for any of the study periods. The factorial analysis also showed a significant lincomycin effect on ADG at the 21-day and 91-day post-challenge assessment intervals, as well as for the entire study period, but no significant vaccine effects for any of the study periods.

Assessments of pig and market values showed no significant differences among treatments, but animals receiving Lincomix had the highest overall market value by factorial analysis.

**Conclusions**

- In this comparative study, both Lincomix alone and Lincomix + Enterisol were effective in controlling diarrhea and production losses due to artificial challenge with *L. intracellularis*.
- By factorial analysis, Lincomix alone had a significant effect on body weight, ADG, feed intake, and percent diarrhea days, whereas the vaccine did not.
- Lincomix and Lincomix + Enterisol treated pigs generated more pounds of pork in the higher value and heavy-weight profitable ranges of the current industry grid for market weight value than controls or Enterisol alone.
- Lincomix and Lincomix + Enterisol generated fewer pounds of pork in the lightweight range of the current industry grid for market weight value than controls or Enterisol alone.
- These data confirm the results of previous reports assessing the efficacy of Lincomix Feed Medication in controlling the clinical signs and production impact of ileitis.
- The combination of Lincomix and Enterisol provided the maximum production improvement and financial return.

**References**

1. Data on file, Study Number 1921R-60-04-231, Pfizer Inc.


This study was conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee of Veterinary Resources, Inc.