Date of Approval: November 26, 2001

FREEDOM OF INFORMATION SUMMARY

SUPPLEMENTAL NEW ANIMAL DRUG APPLICATION

NADA 139-472

For the use of
DENAGARD® (Tiamulin) Type A Medicated Article

"…for control of porcine proliferative enteropathies (PPE)
associated with *Lawsonia intracellularis* "

Sponsored by:

Boehringer Ingelheim Vetmedica, Inc.

Date of Approval: November 26, 2001

I. GENERAL INFORMATION

NADA Number:	139-472
Sponsor:	Boehringer Ingelheim Vetmedica, Inc. 2621 North Belt Highway St. Joseph, Missouri 64506-2002
Established Name:	tiamulin hydrogen fumarate
Proprietary Name:	Denagard® (Tiamulin) Medicated Premix
Marketing Status:	Over-the-counter
Effect of Supplement:	This supplemental application amends the approved NADA to provide for the use of DENAGARD® (Tiamulin) Type A Medicated Article "for control of porcine proliferative enteropathies (ileitis) associated with *Lawsonia intracellularis*."

II. INDICATIONS FOR USE

For increased rate of weight gain and improved feed efficiency.

For treatment of swine dysentery associated with *Brachyspira* (formerly *Serpulina* or *Treponema*) *hyodysenteriae* susceptible to Tiamulin.

For control of swine dysentery associated with *Brachyspira* (formerly *Serpulina* or *Treponema*) *hyodysenteriae* susceptible to Tiamulin.

For the control of porcine proliferative enteropathies (ileitis) associated with *Lawsonia intracellularis*.

III. DOSAGE

Dosage Form:	Type A Medicated Article
Route of Administration:	Oral, in feed
Recommended Dosage:	Increased rate of weight gain - feed 10 g/ton continuously Treatment of swine dysentery - feed 200 g/ton for 14 days Control of swine dysentery - feed 35 g/ton continuously Control of PPE 35 g/ton - feed 35 g/ton for not less than 10 days

IV. EFFECTIVENESS

DOSE JUSTIFICATION

A. Dose Justification Study I

1. Type of Study: A clinical field study was initiated to evaluate the effectiveness of tiamulin hydrogen fumarate in the control of *Lawsonia intracellularis* (porcine proliferative enteropathies/ileitis) infection induced in growing pigs in a disease model system.

2. Investigator: Lynn Joens, Ph.D., University of Arizona, Department of Veterinary Science, Building 90, Room 201, Tucson, Arizona 85721

3. General Design:

   a. Purpose of Study: The purpose of this study was to make an assessment of the effectiveness of tiamulin hydrogen fumarate administered in the water followed by tiamulin hydrogen fumarate in the feed for the prevention of an induced porcine proliferative enteropathy (PPE) infection in swine.

   b. Test Animals: Twenty-four healthy 4-week old pigs from a swine herd with no clinical or histologic evidence of PPE, swine dysentery, or salmonellosis were utilized for the study.

   c. Design and Test Article Administration: Prior to induction of infection, the pigs were allocated to six groups of four pigs. This resulted in three replications (pens) of two treatment groups. Within each replicate, treatments were assigned to pens randomly. The two treatment groups were: (1) infected, nonmedicated control and (2) infected, medicated treatment. The pigs were challenged with a pure culture *Lawsonia intracellularis* inoculum by gastric intubation. The therapy regime was 180 ppm tiamulin hydrogen fumarate (THF) in drinking water for 5 days followed by 35 g/ton THF in feed for 20 days. The study included a 25-day post-challenge observation period.

   d. Measurements and Observations: Each pig was weighed on study Days 0, 4, 11, 18, and 25. Each pig was observed and scored daily for diarrhea severity (scale 0-3/normal, mild, moderate or severe; and presence or absence of blood) and general appearance (scale 0-2/normal, depressed or moribund).

   e. Post-Treatment Microbiology: At necropsy, samples of the small intestine and large intestine from all pig were cultured for *Brachyspira hyodysenteriae* and *Salmonella* spp.

4. Results:

Pigs in the nonmedicated group started to have a depressed appearance on Day 3 and developed a mild diarrheal condition on Day 5. This condition persisted and changed to moderate/severe diarrhea in the majority of the nonmedicated pigs on Day 9. The severe diarrhea persisted in the nonmedicated group through the end of the study and mucus was observed intermittently. The treated group appeared normal through Day 17 with two pigs exhibiting a mild diarrhea after Day 17.

Gross examination of intestinal tissue at necropsy revealed lesions of edema (flaccid appearance), fibrin, mucus, reticulation, hyperemia, and occasional hemorrhage in the majority (9 of 12) of the nonmedicated pigs. Four of the 12 pigs receiving medication exhibited intestinal lesions typical of ileitis (fibrin, edema, and hyperemia), however evidence of hemorrhage was not observed in the treated group. Two of the 12 pigs died in the medicated group but had no gross lesions of ileitis (their cause of death is not stated). Four of the 12 nonmedicated pigs died during the study with typical lesions of ileitis.

Microscopic lesions consisted of epithelial cell hyperplasia with branching of crypts along with the presence of edema, inflammation, and fibrin. Epithelial cell erosion was also observed. There appeared to be a relationship between gross and microscopic lesions of the small intestinal tissue when comparisons were made between the pigs from both groups.

The results of the clinical parameters as well as the gross and microscopic lesions are summarized in Table 4.1.

Table 4.1: Effect of THF
for control of a severe PPE challenge

	nonmedicated	THF
Average daily appearance score, 0-2	0.52	0.19
Average daily diarrhea score, 0-3	1.43	0.34
Pig days with diarrhea, %	63.3	24.8
Gross lesions: small intestine	9/12	4/12
Gross lesions: large intestine	7/12	3/12
Microscopic lesions: small intestine	6/12	2/12
Microscopic lesions: large intestine	10/12	10/12
Average daily weight gain, g	34	96
Mortality	4/12	2*/12

*Not PPE related.

Pre-treatment and post-treatment microbiological cultures were found to be negative for *Brachyspira hyodysenteriae* and *Salmonella* spp. The presence of

Lawsonia intracellularis was confirmed by intestinal scrapings from nonmedicated control pigs.

5. Conclusions:

   The dose level of THF in feed used in this study is the dose used in the proposed claim. The results of this study corroborate the reported *in vitro* activity and *in vivo* effectiveness of tiamulin hydrogen fumarate against *Lawsonia intracellularis*.

B. Dose Justification Study II: Clinical Field Study

1. Type of Study: A clinical field study was initiated to evaluate the effectiveness of tiamulin hydrogen fumarate in the control of *Lawsonia intracellularis* (porcine proliferative enteropathy/ileitis) infection induced in growing pigs in a disease model system.

2. Investigator: Kent Schwartz, D.V.M., M.S., TEAM Associates, Route 2, Box 92, Story City, Iowa 50248.

3. General Design:

   a. Purpose of Study: The purpose of this study was to make an assessment of the effectiveness of two dietary inclusion rates of tiamulin hydrogen fumarate for the prevention/control of porcine proliferative enteropathies (PPE).

   b. Test Animals: Thirty-two healthy 4-week old pigs of mixed breed and sex from a closed swine herd with no clinical, microbiological, or pathological history of PPE, swine dysentery, or salmonellosis were utilized for the study.

   c. Design and Test Article Administration: Prior to induction of infection, the heaviest 16 pigs were allocated on the basis of weight and gender to Pens 1-4 and the lightest 16 pigs were allocated to Pens 5-8. This resulted in two replications (pens) of four treatment groups. Within each replicate, treatments were assigned to pens randomly. The four treatment groups were: (1) noninfected, nonmedicated (NI-NM) control, (2) infected, nonmedicated (I-NM) control, (3) infected tiamulin hydrogen fumarate at 35 g/ton (I-35) and (4) infected, tiamulin hydrogen fumarate at 50 g/ton (I-50). Pigs were started on medicated feed on the Day 0 and were fed 35 g/ton THF in feed for 35 consecutive days. The pigs were challenged with a pure culture *Lawsonia intracellularis* inoculum by gastric intubation on study Day 7. The study included a 28-day post-challenge observation period.

d. Measurements and Observations: Each pig was weighed on Days 0, 7, 14, 21, 28, and 35. Feed consumption was measured at these same weekly intervals. Rectal swabs were taken on Days 7 (before challenge), 14, 21, 28, and 35 and used for polymerase chain reaction (PCR) detection of *Lawsonia intracellularis* (+or -). Each pig was observed and scored daily for diarrhea severity (scale 0-3/normal, mild, moderate, or severe; and presence or absence of blood) and general appearance (scale 0-2 / normal, depressed, or moribund). Mortality data was also recorded.

   On study Day 35 all remaining live pigs (31/32) were euthanized by electrocution, necropsied, and samples of ileum, jejunum, and colon were collected for histopathological examination by hematoxylin and eosin (H&E) and silver staining. H&E slides were used for identifying and scoring the presence and degree of crypt cell hyperplasia (scale 0-4; a pathognomonic PPE lesion when intracellular curved bacteria are present) and increased mononuclear cell infiltration of the lamina propria and submucosa (scale 0-4; indicator of inflammation, nonspecific for *Lawsonia intracellularis*). Silver staining was used to detect the presence and relative amount of intracellular curved bacteria indicative of *Lawsonia intracellularis* infection (scale 0-4).

   PCR (+ or -) and indirect fluorescent antibody (IFA; scale - to ++++) tests on ileal and colonic mucosal scrapings were done for detection and specific confirmation of the presence of *Lawsonia intracellularis* at necropsy. Blood samples were also collected from all pigs on post-challenge Days 0, 14, 21, and 28 for serological detection of IgG specific for *Lawsonia intracellularis* using an indirect fluorescent antibody (IFA) test.

e. Post-Treatment Microbiology: At necropsy all pigs were cultured (tissues: mesenteric lymph node, ileum and colon for *Salmonella* spp., ileum for *E. coli*, and colon for *Brachyspira* spp.)

4. Results:

   The prevalence or severity of ileal PPE-specific gross or microscopic lesions were reduced in the treated groups compared to infected unmedicated controls. A detailed summary of these results is contained in Table 4.2.

Table 4.2 Effect of Tiamulin on the Prevalence of Specific Gross and Microscopic PPE Lesions 28 Days Post-Challenge

	NI-NM	I-NM	I-35	I-50
Gross lesions - small intestine/ large intestine	0/8 0/8	5/8 2/8	0/8 0/8	0/8 0/8
Microscopic lesions - small intestine/large intestine	0/8 0/8	7/8 6/8	2/8 0/8	2/8 0/8
Microscopic lesions, ileal crypt cell hyperplasia (pathognomonic lesion)	0/8	7/8	1/8	2/8
Microscopic lesions, jejunal crypt cell hyperplasia	0/8	6/8	0/8	0/8
Microscopic lesions, colonic crypt cell hyperplasia	0/8	6/8	0/8	0/8
Microscopic lesions, intracellular curved bacteria, ileum	0/8	7/8	1/8	2/8
Microscopic lesions, intracellular curved bacteria, jejunum	0/8	5/8	0/8	0/8
Microscopic lesions, intracellular curved bacteria, colon	0/8	6/8	0/8	0/8

Clinical scores including the daily incidence and severity of diarrhea (scale 0-3) and daily general appearance scores (scale 0-2) of the pigs were evaluated on a pen basis.

Diarrhea was sporadic and blood was present only occasionally. Diarrhea and general appearance scores were significantly improved by tiamulin medication compared to the I-NM group.

A composite summary of the clinical scoring results for the 28-day post-challenge period is in Table 4.3.

Table 4.3 Effect of Tiamulin on Composite Diarrhea and Appearance Scores for the Combined 28-day Post-Challenge Period

	NI-NM	I-NM	I-35	I-50
Average daily appearance score, 0-2	0.018	0.141	0.0	0.0
Pig days with appearance scores >0, %	1.785	12.055	0.0	0.0
Average daily diarrhea score, 0-2	0.031	0.329	0.0	0.04
Pig days with diarrhea scores >0, %	3.125	28.930	0.0	0.0445

Productivity data was also collected and evaluated. Consistent trends in the data suggest decreased productivity as a result of infection and a protective effect of medication.

Post-treatment microbiological cultures were found to be negative for *E. coli, Salmonella* spp., and *Brachyspira* spp. This verified that this study was an evaluation of an uncomplicated PPE/LI infection.

5. Conclusions:

   Tiamulin hydrogen fumarate at rates of 35 and 50 g/ton proved to be effective in controlling the specific effects of LI infection (PPE/ileitis) in this clinical study for the prevention/control of PPE.

DOSE CONFIRMATION - Clinical Field Trial

A. Type of Study: A clinical field study was conducted to evaluate the effectiveness of tiamulin hydrogen fumarate in the control of *Lawsonia intracellularis* (porcine proliferative enteropathies/ileitis) infection induced in growing pigs in a disease model system.

B. Investigator: Kent Schwartz, D.V.M., M.S., TEAM Associates, Route 2, Box 92, Story City, Iowa 50248.

C. General Design:

1. Purpose: The purpose of this study was to make an assessment of the effectiveness of tiamulin hydrogen fumarate at 35 grams per ton for the control of porcine proliferative enteropathies (PPE/ileitis).

2. Test Animals: Forty-eight healthy 3 to 5 week old pigs of mixed breed and sex were purchased from a closed swine herd that had no clinical or diagnostic history of porcine proliferative enteropathy (PPE due to LI), *Salmonella*, or *Brachyspira* infections.

3. Design and Test Article Administration: Prior to the induction of infection, the heaviest six pigs were allocated on the basis of weight to Pens 1 and 2 (3 pigs/pen) which comprised Block 1. This process was repeated until all pigs had been allocated to one of eight blocks. This resulted in eight replications (pens) of two treatments balanced as closely as possible for weight. Gender was also balanced across treatment groups but it was not always possible to balance for gender within replicates. Within each replicate, treatments were assigned to pens randomly.

   The two treatment groups were: (1) infected, unmedicated control and (2) infected, tiamulin hydrogen fumarate at 35 grams per ton of feed. The pigs were challenged with a pure culture *Lawsonia intracellularis* inoculum by gastric intubation on study Day 0. Animals were started on medicated feed on Day 9, when 15% of the herd demonstrated clinical signs of disease. THF was administered for 28 consecutive days at 35 g/ton in complete feed. The study included a 37-day post-challenge observation period.

   Each pig was weighed on study Days 0 (infection), 9 (treatment initiation), 16, 23, 30, and 37 (weekly intervals for 4 weeks post-treatment initiation).

Feed consumption was measured for these same weekly intervals. Rectal swabs were taken on the same days body weights and feed measurements were taken and used for PCR detection of LI (+ or -). Each pig was observed and the individual pigs were scored daily for diarrhea severity (scale 0-3/normal, mild, moderate, or severe; and presence or absence of blood) and general appearance (scale 0-2/normal, depressed, or moribund). Mortality data was also recorded daily.

On study Day 37 all remaining live pigs were euthanized by electrocution, necropsied, and samples collected. Samples of ileum, jejunum, and colon were collected for histopathological examination by hematoxylin and eosin (H&E) and silver staining. H&E slides were used for identifying and scoring the presence and degree of crypt cell hyperplasia (scale 0-4). Silver staining was used to detect the presence and relative amount of intracellular curved bacteria indicative of LI infection (scale 0-4). Indirect fluorescent antibody (IFA, scale - to ++++) tests on ileal and colonic mucosal scrapings were done for detection and specific confirmation of the presence of LI at necropsy.

At necropsy all pigs were cultured (tissues: mesenteric lymph node, ileum and colon for *Salmonella* spp., ileum for *E. coli*, and colon for *Brachyspira* spp.)

4. Statistical Analysis: All statistical tests with pig-specific data were stratified by block, thus, controlling for baseline weights. The growth performance analyses were based upon pen-specific analyses instead of pig-specific analyses.

5. Decision Criteria: Gut lesions scores and daily clinical observation scores were the primary decision criteria for determining effectiveness. Gut lesions relevance to overall clinical outcome was validated by demonstration of numerical superiority of the treated animals as compared to the control animals in average daily gain (ADG). Growth performance measures were considered as corroborative evidence.

D. Results:

1. Lesion Scoring: The prevalence and the severity of ileal PPE-specific gross or microscopic lesions in the medicated group was significantly reduced, ($p = 0.0298$) and ($p = 0.0238$), respectively, compared to unmedicated controls. In addition, there was a significant reduction in the prevalence ($p = 0.0375$) and severity ($p = 0.0169$) of large intestine gross or microscopic lesions.

Table 4.4: Comparison of Prevalence and Severity of PPE-Specific Lesions at Necropsy

	Overall Prevalence Rates		Comparisons of Prevalence Rates		Comparisons of Severity
Site and Type	**Tiamulin hf Treated Pigs**	**Unmedicated Control Pigs**	**Mantel-Haenszel Odds-Ratio**	**Exact p-value* (Stratified)**	**Exact p-values# (Stratified)**
Small Intestines (Ileum)					
Any Lesions	**8.7% (2/23)**	**37.5% (9/24)**	**4.96**	**0.0298***	**0.0238***
Gross Lesions	**4.3% (1/23)**	**29.2% (7/24)**	**6.60**	**0.0422***	**0.0281***
Microscopic Lesions	**8.7% (2/23)**	**33.3% (8/24)**	**4.36**	**0.0557**	**0.0490***
Crypt Cell Hyperplasia	**8.7% (2/23)**	**33.3% (8/24)**	**4.36**	**0.0557**	**0.0623**
Intracellular Curved Bacteria	**8.7% (2/23)**	**33.3% (8/24)**	**4.36**	**0.0557**	**0.0569**
Large Intestines					
Any Lesions	**4.3% (1/23)**	**29.2% (7/24)**	**9.40**	**0.0375***	**0.0169***
Gross Lesions	**0.0% (0/23)**	**16.7% (4/24)**	**+Infinity**	**0.0750**	**0.0750**
Microscopic Lesions	**4.3% (1/23)**	**29.2% (7/24)**	**9.40**	**0.0375***	**0.0169***
Either Large or Small Intestines					
Any Lesions	**8.7% (2/23)**	**37.5% (9/24)**	**4.96**	**0.0298***	**0.0091***
Gross Lesions	**4.3% (1/23)**	**33.3% (8/24)**	**7.60**	**0.0206***	**0.0131***
Microscopic Lesions	**8.7% (2/23)**	**33.3% (8/24)**	**4.36**	**0.0557**	**0.0204***

*Significantly greater for unmedicated control pigs than for tiamulin hydrogen fumarate. ($p<0.05$)

2. Clinical Signs: - The comparisons of the individual daily clinical scores during the treatment period are summarized in Table 4.5.

Table 4.5: Comparison of Clinical Scores

Day	Tiamulin hf medicated Non-zero Scores (n = 23)	Unmedicated Non-zero Scores (n = 24)	One-sided p-values
1	1,1,1,1,2,2,4,4	1,1,1,1,1,2,2,2	1.0000
2	2,2,3	1,1,1,2,2,2	0.2869
3	1,1,1,1,3,3	1,1,1,1,1,1,1,1,1,2	0.2189
4	1,1,2,2	1,1,1,1,1,1,1,2	0.2854
5	1,2,2	1,1,1,1	0.5325
6	1,1,1,2	1,1,1,1,1,1,2,2,3	0.0752
7	1,2	1,1,1,1,1,1,1,1,1,1,2	0.0031*
8	1,2	1,1,1,1,2,3	0.1132
9	1,1,1	1,1,1,1,1,2,2	0.0744
10	-	1,1,1,1,2,2,3,3	0.0025*
11	2	1,1,1,1,2,2,2	0.0275*
12	1,1,1	1,1,1,1,1,1,1,1,2,3,4	0.0050*
13	1,1,1	1,1,1,1,1,1,3,3,4	0.0355*
14	-	1,1,1,1,1,2,3,3,5	0.0009*
15	1	1,1,1,1,1,1,1,2,2,3,3,4,5	0.0001*
16	-	1,1,1,1,1,1,1,1,2,2,3,3,4	0.0000*
17	1,1	1,1,1,1,1,1,2,2,2,3,4	0.0012*
18	1	1,1,1,1,2,2,3,3,4,5	0.0009*
19	1	1,1,2,3,4,4,4,5	0.0055*
20	-	1,1,1,1,1,1,1,2,3,3,5,5,5	0.0000*
21	-	1,1,1,1,1,3,3,4,4,5	0.0004*
22	1	1,1,1,1,4,5,5,5	0.0070*
23	-	1,1,1,1,1,3,5,5,6,6	0.0002*
24	-	1,1,1,1,1,5,5,5,6	0.0010*
25	-	1,5,5,5,5	0.0200*
26	-	1,1,1,5,6,Dead,Dead	0.0062*
27	-	5,5,Dead,Dead	0.0500*
28	-	5,5,Dead,Dead	0.0500*

*Significantly greater for unmedicated control pigs than for tiamulin hydrogen fumarate, $p < 0.05$

Clinical signs included diarrhea, general appearance, and blood in feces. These signs were used to create individual animal clinical scores. The individual clinical scores were added together into an overall 'clinical sign score' for comparison between treated animals and control animals (*see* Table 4.5). Daily clinical scores were used to establish minimum required treatment duration. The minimum duration was set at 10 days because it was the day in which the improvement of the treated group demonstrated statistically significant improvement over the control group for a sustained period of days. In this trial the scores for the treated group remained statistically improved over the control group for the entire remainder of the trial.

3. Productivity (Supportive) - For the overall 28-day treatment period, the average daily feed consumption, average daily weight gain, and feed efficiency were greater for the tiamulin hydrogen fumarate medicated pigs.

E. Conclusions: Tiamulin hydrogen fumarate fed at 35 grams per ton for not less than 10 days proved to be effective in the control of porcine proliferative enteropathies (ileitis) associated with *Lawsonia intracellularis* in this well controlled clinical study.

V. ANIMAL SAFETY

Target animal safety data were presented in the original NADA 139-472 FOI Summary dated July 17, 1987, and supplemental FOI summary dated July 7, 1994. No additional data were required for approval of this supplement.

VI. HUMAN FOOD SAFETY

All human safety information appears in the original NADA 139-472 FOI Summary. It was determined by the Agency that this submission requesting additional label claim for DENAGARD® (tiamulin) Type A medicated article has at this time satisfied the requirements for microbial safety with respect to resistance and pathogen load issues. No additional information was required for this supplemental approval.

VII. AGENCY CONCLUSIONS

The data submitted in support of this supplemental NADA satisfy the requirements of Section 512 of the Federal Food, Drug, and Cosmetic Act and 21 CFR Part 514 of the implementing regulations. The data demonstrate that DENAGARD® Type A Medicated Article is effective for the control of porcine proliferative enteropathies (ileitis) associated with *Lawsonia intracellularis* when administered at 35 g/ton in swine feed for not less than 10 days.

The Agency has concluded that this product shall retain over-the-counter marketing status because adequate directions for use have been written for the layman and the conditions for use prescribed on the label are likely to be followed in practice.

Under section 512(c)(2)(F)(iii) of the FFDCA, this approval for food-producing animals qualifies for THREE years of marketing exclusivity beginning on the date of approval because the supplemental application contains substantial evidence of the effectiveness of the drug involved, any studies of animal safety, or, in the case of food-producing animals, human food safety studies (other than bioequivalence or residue studies) required for the approval of the application and conducted or sponsored by the applicant. The three years of marketing exclusivity applies only to the new claim for which the supplemental application is approved.

In accordance with 21 CFR 514.106(b)(2)(v), this is a Category II change which did not require a reevaluation of the safety or effectiveness data in the parent application.

There are currently no U.S. patents DENAGARD® Type A Medicated Article.

VIII. APPROVED PRODUCT LABELING

Copies of facsimile Type A medicated article labeling and specimen (Blue Bird) Type B and Type C medicated feed labels are attached to this document.

A. DENAGARD® Type A Medicated Article

B. Blue Bird DENAGARD® Type B Swine Feed

C. Blue Bird DENAGARD® Type C Swine Feed

Copies of applicable labels may be obtained by writing to the following:

Freedom of Information Staff (HFI-35)
Food and Drug Administration, Room 12A16
5600 Fishers Lane
Rockville, Maryland 20857