STUDY SUMMARY: STUDY 1U01FD006804-01

Duration of Use Evaluation Study Conducted through Funding Opportunity Announcement # RFA-FD-19-024; Award Project # 1U01FD006804-01

(tylosin phosphate Type A medicated article)

Type A medicated article to be used in the manufacture of Type B and Type C medicated feeds

Beef cattle

For reduction of the incidence of liver abscesses associated with *Fusobacterium necrophorum* and *Arcanobacterium pyogenes*.

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I. EXECUTIVE SUMMARY

Tylosin phosphate Type A medicated article is approved for use in beef cattle for reduction of the incidence of liver abscesses associated with *Fusobacterium necrophorum* and *Arcanobacterium pyogenes*. The approved dosage regimen is 60 to 90 mg tylosin/head/day fed continuously as the sole ration (see 21 CFR 558.625). The approved conditions of use do not define a duration of use for tylosin phosphate for this indication.

In 2019, FDA's Center for Veterinary Medicine (CVM) awarded Kansas State University (KSU) a grant through Funding Opportunity Announcement (FOA) number RFA-FD-19-024[1] titled, "Conduct Studies to Establish More Targeted Durations of Use for Certain Approved Antimicrobial New Animal Drugs in Food Animals." The award project number was 1U01FD006804-01[2]. This study was intended to generate publicly available data that may be used to help define the duration of use for tylosin phosphate to reduce the incidence of liver abscesses in beef cattle, in support of revisions to the conditions of use for tylosin-containing medicated feeds. This document summarizes the study conducted by KSU (Study 1U01FD006804-01) and CVM's evaluation of the study results.

Study 1U01FD006804-01 evaluated the effectiveness of tylosin for reducing the incidence of liver abcesses in beef steers when fed at a targeted dosage of 60 mg tylosin/head/day *ad libitum* for the first 30 or 60 days on feedlot rations (transition and finishing rations). The incidence of liver abscesses was not statistically different among treatment groups. While not conclusive, the results of this study suggest that 60 mg tylosin/head/day administered only for the first 30 or 60 days on feedlot rations may not be effective in reducing the incidence of liver abscesses in Angus-type steers. The information and observations from this study, along with other available information, may be useful in helping veterinarians decide when and how long to administer tylosin for this indication, or in developing future studies to further evaluate and optimize the timing and duration of use.

II. EFFECTIVENESS STUDY SUMMARY

1. **Title: "Effect of Targeted Durations of Use for Tylosin Phosphate in Feedlot Steers to Control Liver Abscesses" (Study No. 1U01FD006804-01)**

2. **Study Dates: approximately January 22, 2021, to December 30, 2022**

3. **Study Locations: Manhattan, Kansas, USA (feedlot); (b) (4) (processing facility)**

4. **Study Design:**

   Objective: To determine if administering tylosin phosphate to feedlot steers for the first 30 or 60 days on feed is effective in reducing incidence of liver abscess caused by *Fusobacterium necrophorum* and *Arcanobacterium (Actinomyces) pyogenes.*

[1] https://grants.nih.gov/grants/guide/rfa-files/RFA-FD-19-024.html
[2] https://reporter.nih.gov/project-details/9942089

Study Animals: A total of 462 healthy beef (predominantly Angus-type) steers weighing an average of 832 lb (377 kg) were enrolled in the study and housed in outdoor concrete-surfaced pens. Steers were fed twice daily (targeting *ad libitum* feed intake) and had *ad libitum* access to water.

Experimental Design: This was a single site, negative controlled, randomized clinical field study. The study was generally conducted in accordance with Good Clinical Practice (GCP).

Steers meeting enrollment criteria were divided into two cohorts of equal number based on sequential order of processing on study day (SD) -1. Steers were blocked by SD -1 body weight (BW) and randomly assigned to pen and treatment group. There were 22 weight blocks (11 blocks per cohort), with 3 pens per weight block and 7 steers per pen. Each treatment group had 22 pens. Treatment was initiated on SD 0 (cohort 1) or SD 2 (cohort 2). Cattle were shipped for processing and evaluation of liver abscess incidence after 161 days on feed (SD 161 and SD 163 for cohort 1 and cohort 2, respectively).

Drug Administration: Tylan™ 100 (tylosin phosphate Type A medicated article, Elanco US Inc., approved under NADA 012-491) was used to manufacture Type C medicated feed formulated to target a dose of 60 mg tylosin/head/day for the first 30 days on feed (Treatment Group [TG] Z) or the first 60 days on feed (TG Y). An identical diet that did not contain tylosin was used as the control article (TG X). The control article was also fed following the first 30 or 60 days on feed until processing for TG Z and TG Y, respectively. Treatment groups are described in Table II.1.

Table. II.1. Treatment groups

Treatment Group (TG)	Treatment Regimen	Number of Animals
TG X	0 mg tylosin/head/day for the entire feeding period	154 steers
TG Z	Tylosin phosphate fed targeting a dose of 60 mg/head/day* for the first 30 days on feed, followed by 0 mg tylosin until processing	154 steers
TG Y	Tylosin phosphate fed targeting a dose of 60 mg/head/day* for the first 60 days on feed, followed by 0 mg tylosin until processing	154 steers

*Due to the body weights and expected feed consumption of the study animals, the inclusion rate in medicated feed used to achieve these doses was below the lowest approved inclusion rate of 8 g tylosin/ton, which was consistent with CVM's position on complying with the approved conditions of use.[3]

[3] FDA/CVM. (2018, December 19). FDA Clarifies Use of Tylosin Phosphate in Beef Cattle – Veterinarians. Available at https://www.fda.gov/animal-veterinary/product-safety-information/fda-clarifies-use-tylosin-phosphate-beef-cattle-veterinarians

Planned concomitant therapies were consistent with standard industry practice and administered to all steers in each treatment group. Steers were implanted with Revalor®-XS (trenbolone acetate and estradiol extended-release implants) on SD 0 (cohort 1) or SD 2 (cohort 2). Rumensin™ 90 (monensin Type A medicated article) was included in the diet for the duration of the study; and Optaflexx™ 45 (ractopamine hydrochloride Type A medicated article) was included in diet for the last 42 days on feed.

Feeding and Rations: Steers received a high roughage receiving diet during acclimation (approximately 4.5 to 6 weeks). Beginning on SD 0 (cohort 1) or SD 2 (cohort 2), steers were adapted to increasing levels of energy in the diet by feeding a series of three step-up diets. The finishing ration was approximately 79% steam flaked corn, 12% corn steep liquor, 6% alfalfa hay, and 3% vitamin/mineral supplement (as-fed basis). With one minor exception, the finishing ration was fed from SD 21 (cohort 1) or SD 23 (cohort 2) through the remainder of the feeding period.

Measurements and Observations: Study procedures for steers in cohort 1 and cohort 2 were staggered relative to treatment initiation.

The amount and type of feed delivered to each pen was recorded twice daily. Feed refusal at the pen level was generally measured at the time of diet change (e.g., weekly for the first month) then monthly or as needed (e.g., wet, or soiled feeds). Individual body weights and doppler ultrasound liver abscess measurements were recorded monthly beginning on the day of treatment initiation (i.e., SD 0 or SD 2, SD 30 or SD 32, SD 60, or SD 62, etc.). Body weights were recorded per pen at monthly intervals beginning on SD 30 and SD 32 (for cohort 1 and cohort 2, respectively) and on the date of shipment to the processing facility on SD 161 and SD 163 (for cohort 1 and cohort 2, respectively). Steers were observed for general health once daily during the entire study.

Mixer validation samples for Type B and Type C medicated feeds were collected. Composite samples obtained from each study diet (i.e., step up diets and final ration) and a composite sample representing the first month of study diets fed to the 30- and 60-day treatment groups (TG Z and TG Y) were assayed for tylosin levels using (b) (4) method (b) (4).

Presence or absence of liver abscesses was determined by a U.S. Department of Agriculture Food Safety Inspection Service (USDA/FSIS) inspector at the processing facility.

Data for liver abscess severity score, hot carcass weight, longissimus muscle area, 12th rib fat thickness, marbling score, USDA yield and quality grades were also collected at processing. Additionally, feedlot performance data were calculated (average daily gain, dry matter intake, feed efficiency).

5. Statistical Methods:

The pen was the experimental unit. There were 22 replicates per treatment. The primary variable was the incidence of liver abscesses.

A generalized linear mixed model analysis was performed on the primary variable using the PROC GLIMMIX procedure of SAS®, where treatment was included as a fixed effect, and weight block and pen were included as random effects. The analysis was performed using the binomial distribution with the logit link. Liver abscess incidence rates estimated from the statistical analysis model are summarized in the Section 6 Results.

6. Results:

The incidence of liver abscesses was not statistically different among treatment groups ($p = 0.91$). The 30-day treatment group (TG Z) estimated incidence of liver abscess was 0.41. The 60-day treatment group (TG Y) estimated incidence of liver abscess was 0.43. The control group (TG X) estimated incidence of liver abscess was 0.44. The values reported here differ slightly from the values found in the final report because CVM removed one animal from TG Z that did not meet study enrollment criteria.

Assayed levels of tylosin in medicated study feeds fed to TG Y and TG Z ranged from less than 60.6% to more than 168% of targeted concentration. The approved permitted assay limits for tylosin in Type C medicated feeds were 75-125% of target[4]. The composite sample representing the first month of all study feeds assayed at 124% of target for study group TG Y and at 127.3% of target for study group TG Z. Though mixer validation samples demonstrated uniform mixing of the Type B medicated feeds that were used to prepare the Type C medicated feeds, mixer validation information for the Type C medicated feeds did not demonstrate uniformity.

Additional data collected (body weights, liver ultrasound, carcass data, feedlot performance data) were not used in the duration of use evaluation.

7. Adverse Reactions: No adverse reactions attributable to the test article were reported.

8. Discussion:

The results of Study 1U01FD006804-01 were not conclusive, but suggest that 60 mg tylosin/head/day administered only for the first 30 or 60 days on transition and finishing feedlot rations may not be effective in reducing the incidence of liver abscesses in Angus-type steers.

Limitations of this study prohibit drawing any definitive conclusions. Tylosin assay results for the Type C medicated feeds fed during this study could not be used to confirm dosing, in part because the (b) (4) method (b) (4) is not validated at the lower inclusion rates necessary for use in this study and because the Type C medicated feeds used in the study may not have been uniformly mixed; therefore, the reported assay results may not be reliable. Additionally, this single-site study using only Angus-type steers is insufficient to evaluate repeatability of the study results, particularly across different cattle breeds and different geographic locations.

[4] 21 CFR 558.4(d)

The information and observations from this study, along with other available information, may be useful in helping veterinarians decide when and how long to administer tylosin for this indication, or in developing future studies to further evaluate and optimize the timing and duration of use.