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The efficacy of extended-release eprinomectin for the reduction of horn
flies, face flies, and fecal egg counts of parasitic nematodes in
replacement beef heifers

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Presented to the Faculty of
Dale Bumpers Agricultural, Food, and Life Science Honors College

In Partial Fulfillment of Requirements
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Table of Contents

Introduction.....	1
Literature Review.....	3
Methods.....	10
Participants and Treatment	10
Data Collection	10
Results and Discussion	11
Conclusions.....	16
References.....	18

List of Figures

Figure 1. Mean nematode egg count by treatment and date.	12
Figure 2. Mean face fly count by treatment and date.	13
Figure 3. Mean horn fly count by treatment and date.....	14
Figure 4. Heifer conception rate	16

Introduction

Parasites are one of the most devastating elements affecting cattle production today. Both internal and external parasitism affects cattle in every part of the world causing a global concern of how to decrease the prevalence of flies and helminths in order to increase the health and welfare of livestock. Parasitic worms are extremely accomplished in infecting an entire herd of cattle in a field and staying prevalent in their hosts and environment through their unique life cycle. The list of helminths plaguing cattle includes nematodes, cestodes, and trematodes (O'Donoghue, 2010). Horn flies (*Haematobia irritans* (L.)) and face flies (*Musca autumnalis* (De Geer)) also have interrelated life cycles causing them to be a particular nuisance in the spring, summer, and fall seasons. Cattle heavily infected with worms develop compromised immune systems, lose productivity, lose body weight, and even lose reproductive efficiency (Andresen, 2018). External parasites such as horn flies and face flies are detrimental to cattle health through increasing the stress on animals resulting in a decrease in feed efficiency and overall production (DeRouen, 2003).

The pharmaceutical company Merial has produced an extended-release, injectable cattle parasiticide, Long RangeTM (eprinomectin), in the last couple of years (Seibert, 2016). This new formulation of eprinomectin claims to have the ability to protect cattle for 100 to 150 days post-treatment (Boehringer, 2012). Eprinomectin is an effective drug against both immature and adult endoparasites as well as ectoparasites (Shoop, 1996). The slow release of extended-release eprinomectin in treated cattle causes the parasite life cycle to be broken thereby preventing future infections (Boehringer, 2012). The extended-release activity also allows a greater level of convenience for both veterinarians and producers who only have to administer one injection per 150 days therefore limiting handling stress on cattle (Forbes, 2013). Excessive handling

instigates higher stress on cattle which has the ability to lower immunity, reduce productivity, and animal wellbeing (Mader, 2006). Due to the convenience in administration, endectocide quality, and slow release mechanism, extended-release eprinomectin is a promising drug in today's market.

There has been recent industry dogma that extended-release eprinomectin is efficacious in reduction of horn fly and face fly populations. Some producers utilize extended-release eprinomectin not only to combat parasitic nematodes in their cows, but also rely on the drug to decrease fly populations in treated animals. Although the drug is labeled as an endectocide for worms, grubs, and mites, it is not labeled for fly control. In order to evaluate the industry dogma regarding extended-release eprinomectin, this study tested the drug's efficacy against labeled parasites such as parasitic nematodes and non-labeled parasites such as horn and face flies.

The purpose of this study was to evaluate extended-release eprinomectin for the reduction of horn flies, face flies, and parasitic nematodes in adult, Angus-crossbreed replacement heifers.

Literature Review

Parasitic helminths are ubiquitous organisms that affect every species of vertebrates on this planet (O'Donoghue, 2010). Nematodes (roundworms), trematodes (flukes), and cestodes (tapeworms) all infect cattle populations. The most common worms that infect cattle include gastrointestinal nematodes such as *Cooperia oncophora*, *Ostertagia ostertagi*, and *Haemonchus placei* (Fox, 2018). One of the main reasons helminths are universal in livestock herds is due to their effective life cycle (O'Donoghue, 2010). Nematodes, trematodes, and cestodes can be spread from host organism to host organism through arthropods such as snails, per os during grazing, or by a transcutaneous route (Moreau, 2010). Helminths have three main stages in their life cycles which include eggs, larvae, and adults (O'Donoghue, 2010). Eggs are deposited in fields through an infected animal's feces. From there, L1, L2, and infective L3 larval stages are developed in the grass. A ruminant then ingests the larvae during grazing allowing the infective L3 larvae to become parasitic L4 in the gastrointestinal mucosa. The larvae utilizes the host's nutrients to develop into an adult worm that has the ability to reproduce and lay eggs that are then passed on through the feces to repeat the life cycle. A higher pervasiveness of parasitic worms can be seen in climates with warm, sub-tropical ecosystems such as Australia, South America, and North America (Forbes, 2013). Pastures with a high density of ruminants can also contribute to overwhelming parasite burden (Siebert, 2016).

Most cattle infected with nematode burdens are asymptomatic; however, there is a significant loss of production due to subclinical infection (O'Donoghue, 2010). Factors such as weight gain, milk production, calving intervals, and feed conversion are all adversely affected by parasitism and act as markers to signify the severity of the infection (Corwin, 1997). Softening of the feces and diarrhea are clinical signs of an infected animal (Forbes, 2000). Nematodes not

only affect body condition, but also somewhat disable the host's immune system, making infected animals more susceptible to extraneous infections. Diseases such as parasitic gastroenteritis (PGE) and parasitic bronchitis are especially prominent in young cattle battling heavy parasite infections (Merial, 2015). Adult cattle are more resistant against infections such as PGE but still contain large numbers of parasitic worms which in turn contribute to the egg and larvae population on a pasture (Forbes, 2013). This loss of productivity through disease and decrease of body condition causes a substantial economic deficit for producers. Over \$330 million dollars are lost annually due to parasitism in American cattle (Seó, 2015). Due to the fact that cattle are a major source of income, large amounts of resources have been utilized to try and develop effective drugs to combat the populations of parasites distressing cattle.

Horn flies (*Haematobia irritans* (L.)) are arguably the most economically important ectoparasites affecting cattle populations in the USA (Trehal, 2017). Their need for constant blood meals causes them to spend the majority of their life cycle on their host inflicting pain and stress to the infested animal. This increase in stress attributes to lower feed efficiency, reduction in body mass, and decreased production in cattle (Derouen, 2003). During spring, summer, and early fall, female horn flies will lay 15 to 20 eggs in fresh manure for development (Smythe, 2018). The fly larvae will hatch and develop through three larval instars in the manure pat and then migrate to drier terrain to begin pupation. Once pupation and/or diapause are complete, the adult fly will emerge and begin taking blood meals from a host (Smythe, 2018). Once the economic threshold for horn flies (N=200 flies per animal) is reached, treatment to reduce horn fly numbers is encouraged. If left untreated, horn fly populations will rapidly increase due to new eggs being laid everyday causing cattle herds to quickly be overwhelmed creating economic loss for producers (Trehal, 2017).

The face fly (*Musca autumnalis* (De Geer)) is another prominent ectoparasite causing increased stress and disease in cattle from April to late October (Teskey, 1969). The only times face flies are absent from pasture are during the winter months when they hibernate in houses and barns (Boxler, 2015). Female flies oviposit approximately 30 eggs in fresh manure pats every couple of days (Wang, 1964). The larvae hatch after 24 hours and develop through three larval instars before pupation. Nutrient requirements for adults vary depending on the sex of the fly. Male face flies utilize nectar and dung liquids while female flies gather around wounds or eyes of the animal and feed on blood and various other exudates (Boxler, 2015). The mechanical damage done by the prestomal teeth of the fly during feeding are the primary cause of increased stress on affected cattle, the spread of pink eye bacteria (*Moraxella bovis*), and wound development around the face (Brown, 1972). Face flies cause \$150 million worth of loss each year due to the spread of keratoconjunctivitis, loss of production, and treatment costs to try and decrease face fly numbers in cattle herds (Boxler, 2015).

Livestock are easy hosts for helminths to contaminate due to the direct access of animals to contaminated pastures through grazing (Mckellar, 2004). Horn flies and face flies have an even more direct access to hosts by simply flying and landing on the exterior of cattle. Although it is nearly impossible to completely eradicate horn fly, face fly, and nematode parasitisms in a herd of cattle grazing in a field, it is important to keep parasite levels at a sub-economic level so that the health of cattle are not dramatically compromised (Vercruyssen, 2001). Cows infected with a heavy parasite burden will begin to lose weight, thus negatively affecting production performance. This decrease in production will cause profit loss for the producer and an eventual increase in drug treatment cost for the infected cattle if and when treatment is attempted (Moreau, 2010).

Cattle are normally treated in early spring with anthelmintics and insecticides for nematode and fly parasitisms. Chemicals such as benzimidazoles, imidazothiazoles, and macrocyclic lactones have all been proven to be effective in lowering internal parasite numbers in cattle and have therefore improved the productivity of livestock (Seibert, 2016). These three drug groups were proven to have high efficacies when they originated between the 1960's and 1980's. However, currently nematodes and flies have developed resistance to these major drug groups (Mckellar, 2004).

This resistance can partially be attributed to misuse. It is common for producers to routinely treat their entire herd at indiscriminate time intervals unrelated to strategy for effectiveness (Corwin, 1997). This improper use of dewormers and insecticides has aided both internal and external parasites to develop resistance to drugs. Parasites have also been aided in developing resistance to chemicals due to both undertreatment and overtreatment (Vercruysse, 2001). Resistance is declared when the reduction of the parasite population is <95% (Claerebout, 1998). A drug that is unable to eliminate 95% of a parasite population is said to have reduced efficacy (decreased effectiveness). Resistance is generally due to selection of resistant alleles (Claerebout, 1998). Eventually, nearly the entire parasite population will contain resistant genes rendering the drug useless. There has been no evidence of reversion to susceptibility once a population of worms has attained resistance to a certain type of drug (Claerebout, 1998). Solutions designed to keep parasite populations at bay involve new medications and sustainable use.

Resistance can be avoided if the correct program for parasite control is implemented to stop overtreatment, undertreatment, and misuse (Vercruysse, 2001). It is imperative not to overexpose a population of endoparasites or ectoparasites to the same drug. If parasites still

susceptible to the drug are left in a parasite population, refugia will be maintained (Seibert, 2016). This means the genes of those still susceptible to the drug will be passed on to the next generation allowing drug efficacy to still be preserved in a population of worms or flies (Van Wyk, 2001).

In order to break the parasitic life cycle and reduce the amount of eggs on pasture, 100 days of parasite management is required (Boehringer, 2012). The lack of long acting therapeutic levels in most dewormers fails to reduce the number of GIN L3 nematodes which are the life stages of worms that contribute to diseases (Seibert, 2016). To reach this goal of reducing pasture contamination, it would require drug administration at three week inter-dosing intervals (Mckellar, 2004). Not only does this continuous administration of chemicals cause excess work for the producer, but it also causes more stress for the animal. An increase amount of stress could add to an already lowered immune system due to parasite burden and lead to a higher chance of contracting diseases (Mader, 2006).

In order to increase protection against nematodes and ectoparasites while lowering the amount of injections needed, the animal health company Merial, created an extended-release injectable cattle dewormer Long RangeTM (eprinomectin) (Boehringer, 2012). One dose provides 100 to 150 days of parasite control which is enough time to break the parasite life cycle and reduce egg populations in pastures (Rehbein, 2013). Extended-release eprinomectin is a subcutaneous injection to the neck which injects a 5% solution of eprinomectin that is dispensed at a rate of 1 mg/kg body weight (Forbes, 2013). Each mL of drug contains 50mg of eprinomectin in a solution paired with a 50mg polymer (poly-lactide-co-glycolic-acid 75:25), which slowly releases the medication providing a longer duration of effectiveness in an organism (Merial, 2015). According to the director of Merial Field Veterinary Services, Dr. Joe

Dedrickson, the Theraphase Technology produces therapeutic peaks following the initial injection and 70 days after treatment to provide season-long control (Boehringer, 2012). The chemical formula contains macrocyclic lactones that attach to glutamate-gated chloride ion channels in parasites causing the nerve and muscle cells to become more permeable to chloride ions (Merial, 2018). This hyperpolarization of the cells causes the paralysis and eventual death of affected parasites (Merial, 2018).

Past studies have proven that eprinomectin extended-release injectable (ERI) reduced the egg count by >94% and reduced the nematode counts by >92% in treated cattle (Rehbein, 2013). Eprinomectin successfully eradicated 99-98% of adult helminths (Shoop, 1996). At this dosage level, helminths such as *Cooperia oncophora*, *Haemonchus placei*, and *Ostertagia ostertagi* populations can be expected to be cleared from a treated cow's system (Shoop, 1996). Eprinomectin has been proven to have a three times greater efficacy against helminth control compared to ivermectin as shown in dose titration studies (Rehbein, 2013; Shoop et al., 1996; Shoop and Soll, 2002). Extended-release eprinomectin is categorized as a broad spectrum endectocide due to the fact that it has been proven to be effective against the eradication of endoparasite and ectoparasite populations (Shoop, 1996.) This specific drug has the ability to reduce the number of arthropods on cattle such mites and grubs (Shoop, 1996). The high efficacy, extended half-life, and endectocide quality of extended-release eprinomectin causes it to be a powerful tool in any producer's or veterinarian's arsenal for parasite control (Forbes, 2013).

Not only has extended-release eprinomectin proven to be effective in reducing the number of helminths and ectoparasites, but it also has a very high margin of safety for cattle. Mammals lack glutamate-gated chloride ion channels that are utilized by macrocyclic lactones to

cause paralysis in helminths (Merial, 2018). Macrocyclic lactones also lack the ability to cross the blood-brain barrier in treated cattle (Merial, 2018). In a test treating 118 cattle with eprinomectin, the main ingredient of the drug, there were no adverse side effects or reactions observed (Shoop, 1996). Eprinomectin has even been sanctioned as safe in the treatment of lactating cattle without a milk withholding period (Mckellar, 2004). Multiple studies have also shown with statistical significance, that extended-release eprinomectin contributes to more weight gain in treated cattle compared to cattle in control groups (Boehringer, 2012). Finally, this particular drug offers convenience to producers and veterinarians due to the chemical's long acting formula (Forbes, 2013). Due to the single administration quality of the drug, producers are able to lower labor and equipment costs from repeated gathering of cattle for treatment (Rehbein, 2013).

Due to the fact that extended-release eprinomectin is an endectocide, some producers believe that the drug can be utilized to lower horn fly and face fly populations. However, this particular drug is only labeled for protection against parasitic nematodes, grubs, and mites. By relying on a drug that claims to have no fly control abilities, producers are leaving their cattle open to fly parasitism which will in turn increase the stress of their animals and decrease overall health and welfare. By allowing parasitism to reach economic injury level, producers must then spend money combating not only the overwhelming number of parasites, but also diseases introduced to their herds through flies such as pink eye. Therefore, it is imperative that producers understand which drugs are effective against which types of parasites to avoid economic injury of their animals. This study examined the capability of extended-release eprinomectin in reducing horn fly, face fly, and fecal egg counts of parasitic nematodes in mixed-breed beef heifers.

Methods

Participants and Treatment

The study began May 4th, 2018 and ended August 23rd, 2018. Angus-crossbred replacement heifers (N=54; BW=392.35[±] kg) were located at the University of Arkansas Parasitology research unit on Jack Perkins Lane, Fayetteville, AR. All animals were identified by numbered ear tags. Heifers were randomly divided into three groups (N=18). Each of the groups was then placed on three different pastures for separate grazing throughout the study. Individual breeding bulls were placed in each pasture (1-May), three days before the study began. Bulls were taken out at the end of the study. Group 1 received a labeled dose of extended-release eprinomectin via a subcutaneous injection dosed at 1ml/110lbs on day 0 (4-May). Group 2 served as an untreated control. Group 3 received a labeled dose of extended-release eprinomectin once one fourth of the group reached threshold level for horn fly infestation (N=200 flies/animal; 14-Jun). Due to the high number of face flies, all heifers in every group were treated with a combination of Permethrin® and Co-Ral® dusts twice during the study (27-Jun and 12-Jul).

Data Collection

Horn and face flies were visually monitored between 7-10 AM and recorded eleven times during the study. Whole body fly counts were performed weekly for two weeks following treatment; then biweekly until the end of the study. Horn fly counts were performed by examining each animal. When fly counts exceeded twenty five, the fly populations were estimated. Face fly counts were performed by counting all flies flying and landing about the face. Fecal samples were taken from all heifers on day 0 (4-May), approximately every two weeks for the first four collections and then monthly for the final two collections. The fecal samples were examined using a direct centrifugation flotation technique. One gram of fecal material was

homogenized in a MgSO_4 solution, filtered using a tea strainer, then centrifuged in a 15-mL tube with a cover slip on top at $200 \times g$ for 3 minutes (Yazwinski, 1994). Egg counts were performed by placing the cover slip on a microscope slide and examining at 100X. On day 144 (25-Sep) post treatment, each animal was restrained in a WW squeeze chute for pregnancy evaluations. Pregnancy evaluations were performed using an Ibex veterinary ultrasound imaging system. Horn fly, face fly, and nematode fecal egg count data were analyzed using the MIXED procedure of SAS, while pregnancy data were analyzed using the GLIMMIX procedure. The fixed effect of the study was treatment. The model included main effect of treatment for nematode egg count control, horn fly control, and face fly control. Significance for all data was declared at $P \leq 0.05$.

Results and Discussion

Although nematode egg counts were low throughout the study, significant differences ($P < 0.01$) were noted between all three treatment groups. Group 1 consistently showed lower nematode egg counts compared to groups 2 and 3 throughout the study (Figure 1). Group 3 heifers that received treatment on 14-Jun recorded 7.1 eggs per gram. During the next fecal collection date (day 69) fecal egg counts for group 3 were significantly reduced ($P < 0.01$) due to treatment to 2 eggs per gram. However, on 23-Aug the nematode population returned to 9.1 eggs per gram. At study conclusion (23-Aug), group 1 heifers experienced 2.3 eggs per gram, group 2 had 7.4 eggs per gram, and group 3 had 9.1 eggs per gram. As demonstrated in figure 1, the prolonged period of low nematode egg counts experienced by group 1 and the reduction of nematode egg counts seen in group 3 after treatment demonstrated that extended-release eprinomectin was efficacious against low levels of parasitic nematodes in heifers. The heifers in this study may have experienced low internal parasite counts due to the fact that the pastures they were located on were not heavily grazed before this experiment likely resulting in a low amount

of pasture contamination at the beginning of the study. Another contributing factor may have been that there were only 18 heifers per pasture allowing them ample room to graze in areas not heavily contaminated with parasite eggs.

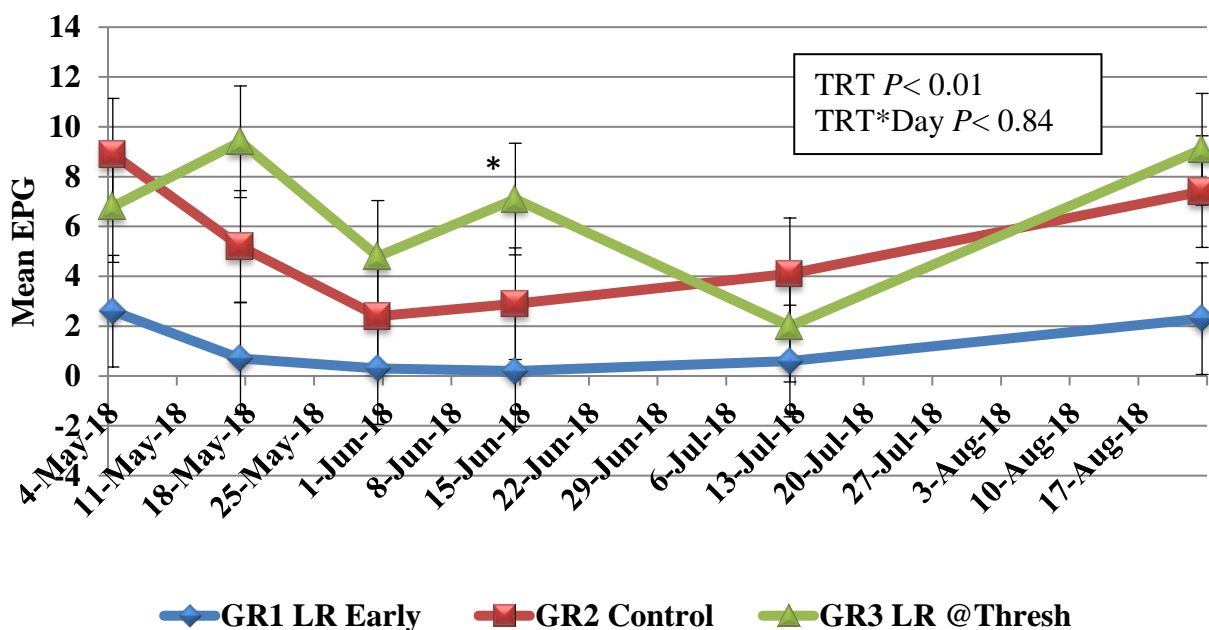


Figure 1. Mean nematode egg count by treatment and date.

*Indicates time of extended-release eprinomectin treatment for group 3 heifers (14-Jun).

Extended-release eprinomectin had little impact on face fly numbers (Figure 2) ($P < 0.42$). Heifers in group 1 experienced almost the same, if not higher levels of face flies when compared to heifers in group 2 and 3. Face fly counts for all three groups continued to rise until insecticide dusts were administered to reduce face fly populations and decrease the risk of pink eye. After insecticide dusts were administered, face fly levels stayed below threshold level for all heifer groups on 12-Jul till the end of the study. One hypothesis as to why extended-release eprinomectin had such poor results on decreasing face fly numbers was that face flies do not take blood meals unless an open wound is bleeding. Instead they rely upon lachrimation and nasal secretions which did not contain therapeutic levels of the drug.

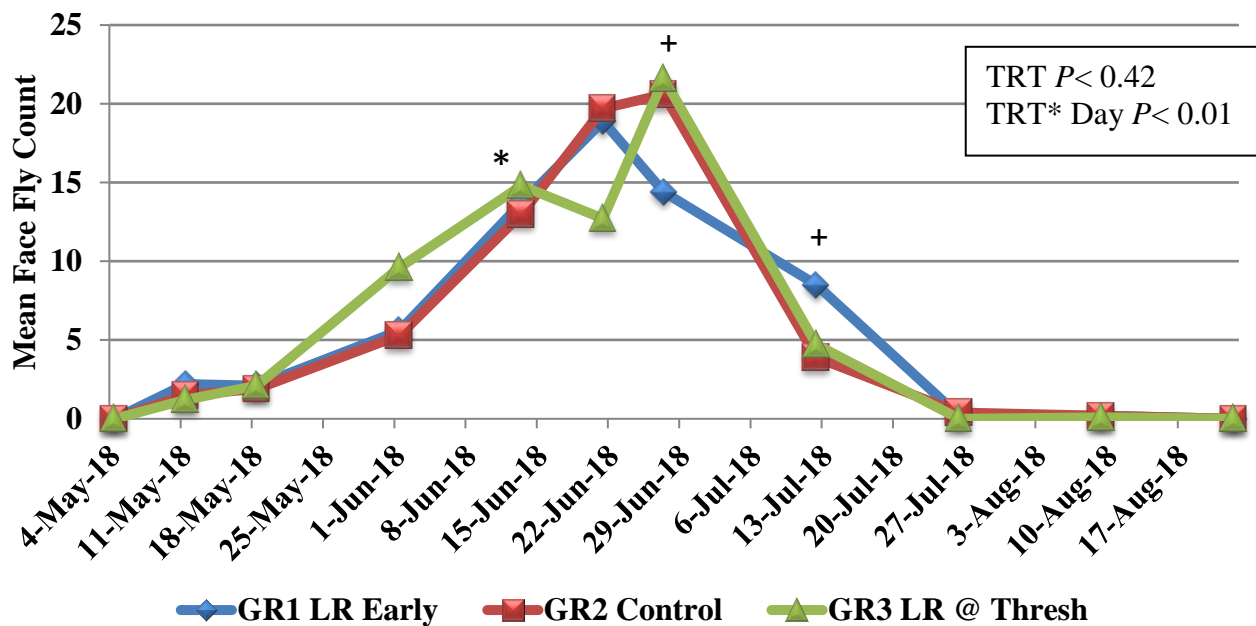


Figure 2. Mean face fly count by treatment and date.

*Indicates time of extended-release eprinomectin treatment for group 3 heifers (14-Jun).

+Indicates treatment day for all groups of heifers with Permethrin® and Co-Ral® dusts for face fly control (21 and 27 of June).

Extended-release eprinomectin when applied before threshold level (group 1) showed activity against horn flies for about six weeks (Figure 3). Mean horn fly counts were lower than the control group (group 2) and for the yet to be treated group 3. However, the population reductions were small, less than 80%. Horn fly counts dropped from 150-30 in animals who experienced threshold level for horn flies (group 3). As previously stated, all animals were dusted due to the lack of ability of extended-release eprinomectin to control face flies. After two weeks post dusting, horn fly populations grew steadily for control and threshold treatment animals (Figure 3). Horn fly populations grew at a slower pace for heifers who received the extended-release eprinomectin treatment at the beginning of the study. On the final day (22-Aug), all heifers were above economic threshold level and suffered economic injury (group 1 405.6; group 2 680.6; group 3 680.6). When economic injury level is reached, producers must expect to lose money

treating their animals due to the decrease in animal production caused by parasitism.

Nevertheless, it should be noted in Figure 3 that there was an extended period of protection for heifers in group 1 who had statistically ($P < 0.01$) lower fly counts (405.6) compared to the untreated group 2 (680.6) and later treated group 3 (680.6). This data suggests that extended-release eprinomectin disrupted the horn fly life cycle and was able to reduce the amount of horn fly eggs laid in the fecal pats in the pasture of group 1. Similar results were described in a study conducted by Kansas State University researching horn fly control using LongRange™ in crossbred stocker heifers. Researchers found that extended-release eprinomectin offered up to ten weeks of horn fly control in treated heifers (Trehal, 2017). In comparing the last six weeks with the last five weeks all groups followed the same trend, with a faster population growth in the last period. This delayed increase in group 1 was most likely the result of the suppressed horn fly population in the manure pats. In groups 2 and 3 however, a higher level of fly eggs survived in the fecal pats, resulting in a faster population growth rate.

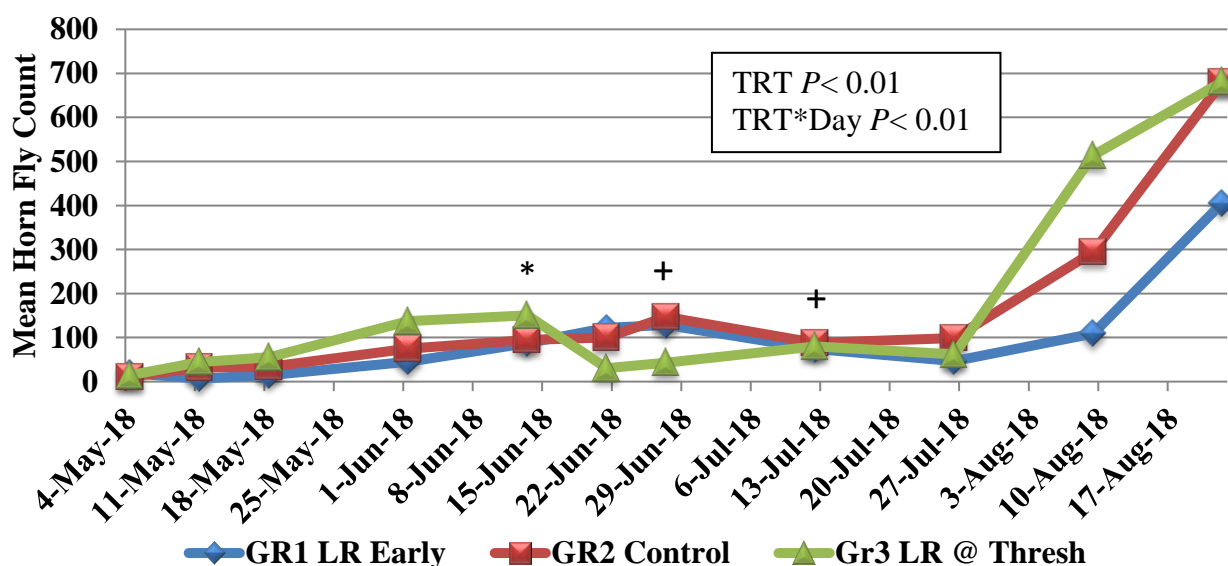


Figure 3. Mean horn fly count by treatment and date.

*Indicates time of extended-release eprinomectin treatment for group 3 heifers (14-Jun).

+Indicates treatment day for all groups of heifers with Permethrin® and Co-Ral® dusts for face fly control (21 and 27 of June).

In a previous study performed by the American Registry of Professional Animal Scientists (ARPAS), extended-release eprinomectin was evaluated based on cow-calf performance and reproduction success in a beef fall-calving herd (Andresen, 2018). Researchers concluded that cows treated with extended-release eprinomectin not only demonstrated a larger body weight difference compared to cows who received conventional anthelmintic products, but also higher pregnancy success rates. Our study supports these findings. At the conclusion of the study, pregnancy evaluations were conducted to assess the correlations between parasite burden in heifers and their ability to breed. Group 1 had significantly ($P<0.05$) higher conception rates (89%) compared to the control heifers (56%) (Figure 4). Group 3 who received the later treatment of extended-release eprinomectin exhibited an intermediate rate (72%). This phenomenon is logical since group 1 experienced lower horn fly counts while nematode eggs per gram were too low to be considered. This data suggested that decreased parasite burdens allowed heifers to have lower amounts of stress since they were not battling flies or experiencing internal damage through parasitic worms. Heifers could then utilize their energy to sustain a healthy immune system and have the energy to support a pregnancy. Group 2 heifers that were not treated with the drug experienced higher parasite burdens and therefore spent energy trying to throw off flies. It should be noted that the breeding bull in group 2 experienced lameness in mid-July which could have contributed to the decreased pregnancy success. The bull was immediately treated and left in pasture to continue breeding. However, past literature examining horn fly control on growth and reproduction of beef heifers found no difference in pregnancy success in heifers treated with insecticide vs. non-treated heifers (Derouen, 2003). Due to these conflicting results, more research should be conducted assessing the correlation between parasitism and pregnancy success.

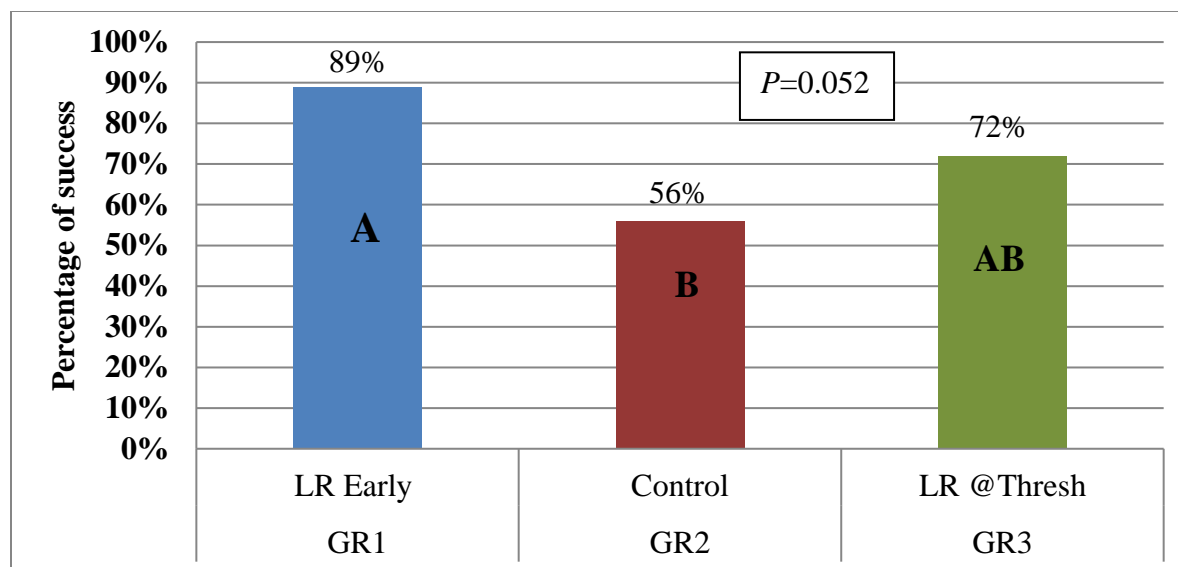


Figure 4. Heifer conception rate.

Conclusions

Extended-release eprinomectin was efficacious against low amounts of nematode parasitism as seen in the initially treated group 1 heifers and the heifers in group 3 treated later during the study. The drug also had some prophylactic effect with treating heifers for horn flies and was able to suppress and reduce established horn fly populations for around 60 days. However, due to dust treatment we are unable to say how long the drug was efficacious. The endectocide did have difficulty battling high amounts of horn fly populations. Extended-release eprinomectin did not decrease face fly numbers below threshold level. Producers should not rely solely on extended-release eprinomectin to control face fly populations. Therefore, it is recommended that when producers use extended-release eprinomectin, additional insecticides for fly control may be necessary. Heifers treated with the endectocide had decreased parasitism resulting in higher pregnancy rates compared to nontreated heifers. However, additional studies are needed to confirm these findings. If a producer wished to increase reproductive success in heifers, it is imperative to control for both internal and external parasites. Results indicated that although

treatment with extended-release eprinomectin did not decrease the face fly burdens, it did have an effect on fecal egg counts, horn fly burdens, and subsequent pregnancy status at the conclusion of the study.

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