COMPARATIVE EFFICACY OF TWO IVERMECTIN POUR-ON ANTHELMINTICS
IN BEEF STEERS IN A COMMERCIAL FEEDYARD

by

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Abstract

A total of 400 head of naturally parasitized commercial feedyard cattle (subset of 40 hd/pen; 10 pens) were utilized to compare trade name ivermectin pour-on and a generic ivermectin pour-on. The efficacy of each product was measured by obtaining rectal fecal egg counts on day 0 and day 14 using a Modified Wisconsin sugar float with centrifugation from rectal fecal samples and calculating fecal egg reduction post treatment. There were no differences in net egg count reduction between treatments (P= 0.15) at 14 days post-treatment application. Regardless of treatment, only 26% of animals had a fecal egg count reduction of >90% and only 35% achieved a FECR of >80% which is low considering 90% reduction is the accepted efficacy level. Cattle treated with the generic pour-on had improved average daily gains (ADG) compared to cattle treated with the trade name pour-on P = 0.02. This study demonstrated decreased efficacy of both products with a FECR of less than 90% within a commercial feedyard environment.
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Preface

Chapter 2 entitled “Comparative Efficacy of Two Ivermectin Pour-on Anthelmintics in Beef Steers in a Commercial Feedyard” was written to be submitted to the Open Journal of Veterinary Medicine.
Chapter 1 - Literature Review

Gastrointestinal Parasitism

The vast majority of animals have a subclinical level of parasitism undetectable to the eye but quantified more accurately by improved performance due to anthelmintic treatment (eg, feed efficiency, nitrogen balance, weight gain, milk production) (Yazwinski et al. 2006a.). To correctly present a control strategy for nematode parasites, the knowledge of the relationship between the parasites and their hosts is necessary (Stromberg et al. 2006).

There are 10 species of cattle nematodes that are considered the most significant because of their prevalence and veterinary importance (Yazwinski et al. 2006a.). Cattle become infected with gastrointestinal parasites by grazing on contaminated pastures. The adult nematodes live within the gastrointestinal system of cattle, where the female nematodes shed eggs that are excreted from the animal through the feces. Once in the environment, larvae develop from the eggs and develop into the infective larvae. The infective larvae then migrate away from the feces onto blades of grass where they are consumed by grazing cattle. Pasture contamination is directly correlated to the stocking rate of cattle within the pasture (Stromberg et al. 2006). Survival of the infective stage of the larvae on the pasture is dependent on temperature and moisture conditions within the environment (Figure 1.1).

The following is an overview of several important nematode species that infect cattle and are most common in the literature. These are also the most common parasites with the ability to cause a clinical syndrome.

*Haemonchus placei* and *Haemonchus contortus*, also known as barber pole and wire worms respectively, have a direct life cycle through cattle. A direct life cycle involves a parasite that does not need an intermediate host in order to become infective to the host species. Strongyle type eggs are shed into the environment where they mature from the L1 larvae into the infective L3 larvae on the grass. Cattle, as they are grazing, ingest the infective L3 larvae to continue the cycle, where the L3 larvae then molt into the L4s, and then adults. This is known as the classical trichostrongyle life cycle. The pre-patent period from egg to infective larvae is 2-3 weeks. *Haemonchus* resides in the abomasum of cattle. The adults produce large numbers of eggs, and are also hematophagic, which leads to most of it’s pathogenicity. *Haemonchus* also has
some degree of seasonal inhibition and periparturient rise in egg shedding. It is prevalent in the southern US, likely due to increased egg survival over the winter and the increased numbers of eggs shed.

*Ostertagia ostertagi* is also known as the brown stomach worm. This is the most important parasite of cattle in the United States (Kaplan 2010, Yazwinski et al. 2006a.). *Ostertagia* follows the same general trichostrongyle type life cycle as most bovine gastrointestinal nematodes. The pre-patent period is about 3 weeks. The larvae molt within the gastric glands of the abomasum, causing cellular hyperplasia and loss of glands. This loss leads to less acid secretion and a higher pH within the abomasum which inhibits protein digestion. *Ostertagia* infections are marked by profuse watery diarrhea. There are two types of infection with *Ostertagia*. Type I occurs immediately upon uptake. Type II infections occur when L4 larvae remain arrested within the abomasum and evade immune response during harsh times of the year and then re-immmerge, damaging the gastric glands. Northern hypobiosis occurs during the winter, and southern inhibition occurs during the summer months. Protective immunity to clinical disease due to this parasite occurs generally after a full grazing season. Temperature and humidity, stocking rates and pasture rotation, seasonal hypobiosis, and immune response are all factors that contribute to the epidemiology of *Ostertagia*.

*Trichostrongylus axei* is also known as the small stomach worm. It follows the general trichostrongyle type life cycle. The L3 larvae on grass become infective after a pre-patent period of approximately 3 weeks. This nematode does not undergo hypobiosis or arrestment of any developmental stage as other abomasal parasites do. *Trichostrongylus* is usually of low prevalence, accounting for less than 10 percent of individual animal parasitic infections on most farms (Yazwinski et al. 2006). It can, however, be found in much larger numbers within animals. This nematode has the ability to elude the immune responses of cattle, making the prevalence of this nematode independent of animal age or prior exposure (Yazwinski et al. 2006a.). High numbers of this parasite leads to a debilitating watery diarrhea. It is often overlooked at necropsy due to its very small size at only 7mm. It is uncommon for *T. axei* to be a primary abomasal pathogen, but it is often present with *O. ostertagi*.

*Cooperia* species also follow the typical trichostrongyle life cycle. The pre-patent period of *Cooperia* is approximately 3 weeks. This nematode does not reside within the abomasum as the nematodes previously discussed, but rather reside within the proximal small intestines. This
species is associated with villous atrophy in the small intestines and diarrhea when found in large numbers. Cattle generally develop an immunologic resistance to *Cooperia* by the animal’s second grazing season (Yazwinski et al. 2006a.). Due to the immune response by the body, the significant worm burdens are only seen in animals 3 years of age or younger. Recently, greater attention has been given to *Cooperia* due to documented resistance to macrocyclic lactones (Anziani et al. 2001, Kaplan 2010, Vermunt et al. 1995). However, *Cooperia* species are dose dependently susceptible to the macrocyclic lactones. Years of highly efficacious use of these avermectins may have changed pasture population dynamics leaving inherently resistant parasites to populate the pasture.

**Economic Impact of Gastrointestinal Parasites**

Gastrointestinal parasitism is among the most costly diseases of the cattle industry, and conservative estimates suggest that over $2 billion per year are lost in productivity and increased operating expenses (Stromberg et al. 2006). This estimate takes into account the cost of treatment and loss of production. Nematodes within the gastrointestinal tract interfere with nutrient intake, digestion, and the host immune system, all of which translate into loss of production.

Destruction of gastric glands is a trademark function of *Ostertagia*. The larvae develop within the parietal gastric glands and in the process destroy them. Once destroyed, the cells are replaced by non-secreting rapidly dividing cells. Loss of the acid producing glands will increase pH levels within the abomasum and decrease pepsinogen activity (Taylor M. 2000), decreasing the ability of the abomasum to chemically break down feedstuffs. A clinical syndrome can also be produced by heavy loads of gastrointestinal parasites, known as parasitic gastroenteritis. This syndrome may cause weight loss, diarrhea, submandibular edema, and anemia (Kaplan 2010).

Decreased production and gain is documented in parasitized cattle. A decreased average daily gain (ADG) in parasitized cattle compared to anthelmintic-treated cattle has been seen on pasture as well as within the feedlot. On grazing yearling cattle, a decreased gain of 14.5 lbs. over a 143 day breeding season was reported (Mertz et al. 2005). Similar findings have been observed in several other studies (Rew 1998). In the feedlot setting, an unrealized ADG of 0.18lbs was noted when animals were not treated for nematodes on arrival at the feedlot (MacGregor et al. 2001). Gross pathology of the abomasum is significantly decreased if the animal is treated with an anthelmintic either on pasture or the feedlot (Taylor M. 2000). The
industry has pushed to maximize efficiency of our food producing animals through genetics, nutrition, implant utilization, and health protocols. As the animals become more efficient the impact of parasites becomes greater (Bliss et al. 2008).

The economic impact of parasites is well known by the producers of the United States. In the Cow/Calf Health and Productivity Audit by the Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA), producers answered questions about diseases that may have significant economic impact within their operation. Producers responded that internal parasites (47.3%) and external parasites (53.1%) cause a significant economic impact due to cost of treatment, cost of prevention, and lost production. In the National Animal Health Monitoring System (NAHMS) audit of health management and biosecurity on US Feedlots, nearly all feedlots use at least one parasiticide on their operation (98.9% small feedlots and 100% large feedlots).

**Parasite Laboratory Tests**

Several quantitative techniques have been established to obtain eggs per gram of feces. Each of these techniques has different sensitivities to detect eggs shed within the feces of the bovine. When choosing a test to measure fecal egg counts one must take into account several factors such as familiarity of the test, cost, time, and sensitivity. Two of these tests have become the most commonly used in detecting shed eggs in feces. They are the Modified Wisconsin Test and the Modified McMasters Test.

The Modified Wisconsin Test is sensitive to 10 eggs per gram. This technique begins with 5 grams of feces that is mixed with 22 mL of tap water. The mixture is then screened through a coarse sieve into a 15mL tube and centrifuged. The supernatant is discarded, and replaced with 12mL of sugar solution to break up the pellet. The sugar solution is added until a convex meniscus forms and a cover slip is placed on top of the tube and the mixture is centrifuged again. The eggs will then adhere to the cover slip. The ova counts are then divided by 5 and reported as eggs per gram (Gasbarre et al. 2009, Cox et al. 1962).

The Modified McMasters Test is sensitive to 50 eggs per gram. This technique begins with 3 grams of feces and 42 mL of water. The homogenized liquid is then poured through a mesh, collected, and centrifuged. The supernatant is removed and a Sodium Chloride solution is added to the tube to the same volume. The tube is then inverted (mix) five to six times. A pipette
is used to remove a sample to fill the chamber of a McMaster slide and this is repeated for the second chamber. All eggs are counted under the 2 grids and multiplied by 50 to give eggs per gram (Coles et al. 1992). This test is most often used when extremely high egg counts are expected.

Both of these tests have a proper place when evaluating gastrointestinal parasitism in cattle, depending on the expected nematode burden. Given the sensitivities of each test, they each have value depending on what the researcher may be looking for.

**Anthelmintic Efficacy Tests**

**Fecal Egg Count Reduction Test**

The Fecal Egg Count Reduction Test (FECR) is the widest used and most accepted test to monitor anthelmintic efficacy in the field (Cabaret et al. 2004, Coles et al. 2006, Taylor et al. 2002, Yazwinski et al. 2009a.). The test measures fecal egg counts before and after treatment with an anthelmintic, with calculation of the post treatment reduction. This test may not accurately estimate efficacy because egg output by gastrointestinal nematodes does not correlate well with the actual worm burden within the animal (Taylor et al. 2002). This is due to only measuring the effects of mature worms at the time of sampling. However, it is still accepted because the alternative, more accurate way to detect efficacy is quantification of parasites at necropsy (Yazwinski et al. 2009a.). Several authors have compiled different techniques to help maximize the effectiveness of this test.

To ensure accuracy, a random sampling of 10% of the herd or group should be conducted to give adequate herd analysis (Ballweber 2006, Yazwinski et al. 2009a.). Fresh fecal samples should be obtained rectally when possible for analysis (Ballweber 2006). Sample care by refrigeration or packing with cold packs for shipment within 24-48 hours to the laboratory is important (Ballweber 2006). Precise and accurate dosing and administration of the anthelmintic should be followed. All sampled animals must be identified to assure the same animals are sampled again following the post-treatment sampling interval. This interval should be at the point of maximum reduction of fecal eggs (Yazwinski et al. 2009a.). Fourteen days has been used by many researchers for macrocyclic lactones and at least 10 days for benzimidazoles (Coles et al. 2006, Gasbarre et al. 2009, Taylor et al. 2002, Yazwinski et al. 2004, Yazwinski et
al. 2009a.). Coprocultures should also be conducted on each individual sample that has ten eggs per gram or more.

The results of the FECR are given as a percentage. This correlates to the percent of total fecal eggs that were reduced following treatment. The accepted reduction for anthelmintics is 90% or greater with anything less indicating anthelmintic resistance (Bliss et al. 2008).

There are many variations of this test along with multiple uses for this test both in the field and in the experimental setting. Despite its limitations and known low limited accuracy, it still remains the most readily used test for anthelmintic efficacy.

**Controlled Efficacy Test**

This test is considered the gold standard in measuring efficacy of anthelmintics (Yazwinski et al. 2009a.). This tests the efficacy of an anthelmintic by comparing parasite populations in groups of treated and untreated animals. The animals are necropsied after a treatment interval and the parasites are recovered to be identified and counted (Wood et al. 1995). This test; however, is not used as often because it is also the most costly in terms of labor, expense, and loss of animal life (Taylor et al. 2002, Yazwinski et al. 2009a.).

To carry out the Controlled test, parasitized animals are randomly allocated into treated and non-treated groups with the chosen anthelmintic. Following the suitable interval after treatment the animals are necropsied and the parasites are recovered. The geometric mean of parasite counts is used to more accurately depict the degree of efficacy of the product (Wood et al. 1995).

Guidelines for proper necropsy procedure and counting of nematodes within the gastrointestinal tract were outlined in the publication of the World Association for the Advancement of Parasitology second edition of guidelines for evaluating the efficacy of anthelmintics in ruminants in 1995. Wood recorded in this publication that the interval between treatment and necropsy should be 4-7 days (Wood et al. 1995). This may be extended if there is persistent activity of the product. The necropsies should all be completed in the same day. Each animal should be properly identified and a thorough necropsy performed after humane euthanasia. Double ligature or clamps should be placed on the pyloric and omasal ends of the abomasum as well as the ileocecal junction prior to removal (Wood et al. 1995). The parasites are either soaked and washed from the mucosa using saline or digested from the mucosa with 3%
HCL. The recovered contents are passed through a sieve and the residue is retained. 5% aliquots (representative sample) are retained to be counted. The nematodes are stained with 45% iodine and cleared with 5% sodium thiosulfate to be counted. Then 100 larvae and 100 male nematodes (identified microscopically) are transferred to a slide to be identified (Wood et al. 1995).

The controlled test is a very laborious and expensive test. However it is the most accurate test to use when measuring efficacy of an anthelmintic. This test is seldom used for efficacy trials due to the costs and labor.

**Available Anthelmintic Products for Cattle**

Three classes of anthelmintics are available for use in cattle. They are the Macrocyclic Lactones, Benzimidazoles, and the Imidazothiazoles and Tetrahydropyrimidines. Each of these classes has different modes of action, slightly different spectrum of coverage, and also come in different formulations.

The Macrocyclic Lactones are a group that consists of avermectins and milbemycins. These are regarded as the most effective and safest parasiticides yet developed (Lynn 2009). These compounds are the fermentation products of *Streptomyces avermilitis* and *Streptomyces cyanogriseus* microorganisms (Taylor M. 2000). The active compounds within this group consist of ivermectin, eprinomectin, doramectin, and moxidectin (Kaplan 2010). The avermectins (ivermectin, eprinomectin, doramectin) differ chemically from each other by side chain substitutions on the lactone ring, while milbemycins (Moxidectin) are absent a sugar on the lactone skeleton compared to avermectins (Taylor M. 2000). These compounds work by opening a glutamate-dependent chloride channel in neuromuscular membranes of parasites resulting in paralysis (Prichard et al. 1994). All of these macrocyclic lactones are formulated in an injectable and pour-on formulation except for eprinomectin which is only offered in a Pour-on. All of the available compounds include nematodes, lungworms, grubs, and ectoparasites in their spectrum. The gastrointestinal nematodes included in the spectrum of coverage include *Ostertagia, Trichostrogyulus, Cooperia, Haemonchus, Nematodirus, Chabertia, Brunostomum, Oesophagostomum*, and *Strongyloides* species. All of the macrocyclic lactones also include the lungworm, *Dictyocaulus viviparous* in their spectrum of coverage (Lynn 2009).

Benzimidazoles include the compounds fenbendazole, oxfendazole, and albendazole. These compounds were first discovered in the early 1960s and have undergone many structural
changes to improve safety and spectrum of the products (Taylor M. 2000). This group of compounds works by binding to tuberin within the parasite, disrupting tubulin polymerization, leading to cell starvation (Taylor M. 2000). In general, these compounds are available most commonly in oral pastes and suspensions due to poor solubility. In The slow transit through the rumen makes it ideal for use in ruminants. The spectrum of this group includes nematodes, lungworms, and tapeworms: *H. contortus, H. placei, O. ostertagi, T. axei, B phlebotomum, N. helveticus, C. punctate, C oncophora, T. colubriformis, O. radiatum, and D. viviparous* (McKellar et al. 1990). Albendazole is also effective against liver flukes (*Fasciola hepatica*). Albendazole and Oxfendazole have been found to be teratogenic and are not recommended for use in pregnant animals.

Imidazolthiazoles and Tetrahydropyrimidines are a class of compounds that include levamisole and morantel. These compounds are nicotinic agonists, acting as cholinergic agonists to cause depolarization of nematode muscle bag membranes with an outflow of sodium (Lynn 2009, Prichard 1994), leading to tonic paralysis. Levamisole is supplied in oral, injectable and pour-on formulations. The levamisole spectrum includes *H. Placei, O ostertagi, T. axei, T. longispicularis, C. oncophora, C. punctate, N. spathiger, B. phlebotmum, O. radiatum, and D. viviparous*. Morantel only comes in a feed premixhas and has a spectrum including the nematodes species of *Haemonchus, Ostertagia, Trichostrongylus, Cooperia, Nematodirus,* and *O. radiatum* (Lynn 2009). Levamisole does have toxic side effects that include salivation, restlessness, and muscle fasciculation. At the labeled dosage, the occasional animal may show increased licking of the lips, and muzzle foam. At twice labeled dosage, calves may show signs of toxicity.

**Formulation Differences**

Pour-on anthelmintics are quite different in pharmacokinetics compared to injectable formulations. Pour-on products are formulated in a vehicle that transports the drug transdermally or down the shafts of the hair. Pour-ons have a unique application that gives owners a convenient way to administer the medication without the risk of injury or discomfort for the worker or the animal. Pour-on anthelmintics are applied to the topline of cattle from the withers to the tailhead.
Injectable formulations generally have a vehicle that directly influences the absorption process. An oily base to the product can result in a slower absorption from the subcutaneous space resulting in persisting concentrations within the body (Lifschitz et al. 2004).

A publication by Laffont in 2003 outlined the actual plasma and fecal disposition of ivermectin in cattle. Laffont previously made the connection in 2001 between grooming and licking by cattle and lower bioavailability of ivermectin in the system. In this study, twin cattle were used to compare injectable and pour-on formulation while controlling licking. Observations of highly variable systemic exposure were dependent on the ability of the cattle to lick or not. The model used within this study concluded that 58-87% of the topically applied ivermectin was actually ingested and not absorbed dermally. The conclusions of this study are that the oral absorption and not the dermal absorption is the main route for systemic levels of the ivermectin pour-on products used leading to inconsistent drug delivery (Laffont et al. 2003).

In 2004, Lifschitz conducted an experiment that evaluated not only several ivermectin injectable formulation, but also doramectin and abamectin. The pharmacokinetic differences between the different formulations were marked. The results of the study concluded that doramectin gave significantly higher plasma levels and extended residual levels in the blood compared to the several ivermectin formulations and abamectin. This was not a surprise considering that the largely oil based vehicle used as a carrier of doramectin leads to the higher concentration profiles in plasma. However, this does not directly indicate that the higher levels are more efficacious. The main significant finding in this study was the difference in kinetic properties between the ivermectin formulations. Different formulations only labeled as “generic” had significant differences not only in concentration maximum, but also in mean residence time (Lifschtiz et al. 2004).

Depending on the route of administration and the product used, different concentrations of the drug will be acting upon parasites. With pour-on formulations, a decreased systemic bioavailability is expected. With injectable products, systemic bioavailibility it is very dependent on the vehicle the product uses.

**Field Trials with Available Anthelmintics**

In anthelmintic field trials, several variables are investigated in aggregate to determine efficacy. These variables include environment, management, parasites, host, production gain,
economic interpretation and analysis (Corwin 1997). Recent field trial work has shown differences in ADG, and efficacy as depicted by fecal egg count reduction of several products. The main focus of this review will be on anthelmintic field trials that utilized feedlot and stocker cattle.

**Feedlot Environment**

The feedlot is a unique environment for anthelmintic trials. The animals are unable to get re-infected with further parasitism after arrival because of no exposure to L3 larvae on grass. Therefore, the gastrointestinal nematodes present on arrival are the same nematodes that will be affected by treatment and continued reinfection will not affect the results. Control of these parasites within the feedlot setting should increase the ability of cattle to digest feedstuffs by inhibiting the detrimental effects on the abomasum by gastrointestinal parasites.

In 2000, Smith and others compared the effects of deworming with fenbendazole on arrival at the feedyard compared animals that were either strategically dewormed while on pasture or kept as an untreated control during grazing (Smith et al. 2000). The strategic deworming during grazing involved administering an oral suspension at initial processing and then given a free-choice mineral that contained fenbendazole at day 28 and 56. The results of this study concluded that deworming on arrival into the feedyard increased ADG by 18.4% and feed:gain by 10.3% in control cattle and increased ADG by 5.7% and feed:gain by 2.3% in strategically dewormed cattle. This trial revealed the benefit of deworming on arrival into the feedyard regardless of prior deworming strategies. This study agrees with what MacGregor and others published in 2001. In this trial, ADG was 1.65kg/day in treated cattle compared to 1.57kg/day in controls over the entire feeding period with a p-value of <0.001. Yearling steers were utilized in this study after being pastured in California (MacGregor et al. 2001).

It was reported in the early 2000s that the use of an Avermectin (doramectin) surpassed an organophosphate pour-on (fenthion) in hot carcass weight (HCW) and ADG in groups of crossbred steers within a feedlot (Edwards et al. 2001). Schunicht and others published similar findings in 2000 comparing ivermectin to fenthion treated yearlings (Schunicht et al. 2000). These studies agree with the results from Nebraska experiment in 2000 that also published results of improved final weight, weight gain, ADG and dry matter intake to gain ratio in animals treated with ivermectin compared to a group treated with fenbendazole combined with
permethrin and fenthion pour-ons (Guichon et al. 2000). These reports concluded the observed differences in performance were due to the Avermectins improved control of both internal and external parasites, and showed it was beneficial to treat for gastrointestinal nematodes in calves as well as yearlings entering the feedyard.

Due to the increased use of Avermectins in the industry because of proven benefits to control internal and external parasites, much of the research has been focused on comparing products within the same class. Even though no single product available today effectively eliminates all economically important nematodes (Ives et al. 2007), these anthelmintics seem to give a relatively good cost benefit within production systems.

The idea of combining classes of anthelmintics was borne to maximize the benefit of both benzimidazoles and macrocyclic lactones during the feeding period. Ives and others conducted a study in Texas utilizing 2,647 sale barn origin feeder steers. This study focused on the performance of several deworming products including doramectin (injectable), moxidectin (injectable), and doramectin (injectable) with oxfendazole (drench). Each treatment had 10 pens of cattle. In this trial no difference in dry matter intake, ADG or intake to gain ratio was observed (Ives et al. 2007). It appears from this trial that the effectiveness of these products within the feedlot setting is similar, and that combining a macrocyclic lactone with a benzimidazole did not increase efficacy against nematodes or performance. This study disagrees with the previously reported observations in 2006 by Reinhardt and others that compared an ivermectin pour-on with the addition of fenbendazole to ivermectin pour-on or doramectin injectable alone (Reinhard et al. 2006). In this study, 1,862 yearling heifers that were treated with the combined products had a greater ADG than ivermectin pour-on (p-value: 0.01) and doramectin injectable (p-value: 0.06). It is interesting that these studies had different outcomes with the use of similar products. The data in Ives study did trend toward better performance with the combined products although it was not significant. This may have been due to using less replicates than Reinhardt and others.

Many studies have looked specifically at the efficacy of anthelmintics in the feedyard setting. These studies most often utilized the Fecal Egg Count Reduction test (FECR) to measure efficacy post treatment. Efficacy of the product is important because we observe the benefit of production and ADG by reducing the gastrointestinal parasite load within the animal, and FECR gives us an estimate of this reduction.
In 2009, Yazwinski and others conducted an anthelmintic efficacy trial utilizing naturally infected calves and measured efficacy with the FECR test and a controlled efficacy test. This trial was not conducted in a traditional feedlot setting, but the animals were confined to clean concrete floored pens during the trial and fed free choice hay. This environment does however give the closed parasite setting, similar to what would be encountered in a feedyard. The efficacy of injectable moxidectin, injectable ivermectin, oral oxfendazole, and oral fenbendazole was examined. All the mentioned products attained a greater than 90% FECR at day 14. However, the ivermectin treated group had less than a 90% reduction at the time of necropsy which was on days 35-39 while the other treatments maintained above the 90% level. It was concluded that oxfendazole and moxidectin were the only two treatments that maintained the ability to decrease fecal egg counts by at least 94% during the trial. Moxidectin did show the greatest efficacy reported by the controlled trial demonstrating greater than 96% efficacy on all nematode populations (Yazwinski et al. 2009b.). Another study compared different anthelmintics in a feedyard environment utilizing incoming steers to the yard. Ives and others did not observe any efficacy differences in the study between moxidectin (injectable), moxidectin (injectable) plus oxfendazole (oral), and doramectin (injectable). All of these treatment groups recorded FECR of at least 98% (Ives et al. 2007). This study reported similar efficacy of moxidectin along with equivalent efficacy of doramectin.

Reinhardt and others reported that treating with ivermectin pour-on plus fenbendazole compared to ivermectin pour-on had fewer worms per sample 98 days after treatment (p=0.06) (Reinhardt et al. 2006). They also reported that the combination treatment, when compared to doramectin injectable, had fewer worm eggs per sample at 35 days post treatment (p=<0.01) (Reinhardt et al. 2006).

The studies reporting results using feedlot cattle, have supported a positive economic benefit for anthelmintic treatment by its positive effects on ADG. However, the differences in efficacy between treatment products has not been as well documented.

**Stocker Environment**

The stocker environment, unlike the feedlot environment, has a continuous exposure to parasites. While cattle graze, they will consume grass that has infective L3 larvae attached. Stocker cattle have been used in multiple field trials to measure efficacy and the effect on
performance of different anthelmintics. While not a perfect model, stockers do give some insight to the feedlot environment because it is an all-in-all-out production process where all animals are placed as a group on pasture for a specified amount of time and removed as a group at the same time. The cattle are exposed to the same amount of parasite load for the same amount of time. The anthelmintics used on stocker cattle are many of the same products used on entry into the feedyard. The trials run on stocker cattle bring insight to efficacy, persistent effect, and performance enhancement of anthelmintics.

In the late 1990’s, Williams performed several direct comparison trials of anthelmintics on grazing stocker calves. In the first trial, doramectin injectable, ivermectin injectable, and ivermectin pour-on were compared to a control by utilizing FECR % and ADG with egg counts taken throughout the grazing period. The control group egg counts were significantly higher throughout the trial. Doramectin treated animals had a significant decrease in fecal egg shedding from day 28 through day 56 compared to the other treatments, while both groups of ivermectin treated animals did not differ. By the end of the experiment there were no significant differences between the treated groups. The ADG within the treated groups were not significantly different from each other at the end of the trial, but were significantly greater than the controls (0.60kg ADG in the controls; 0.98kg, 0.91kg, 0.92kg ADG for doramectin injectable, ivermectin injectable, and ivermectin pour-on, respectively) (Williams et al. 1997a.).

Williams reported findings in 1999 that differed from the previous trial described above (Williams 1999). When comparing topical formulations of doramectin, ivermectin, eprinomectin and moxidectin in a similarly run trial he found that at no time after treatment were ivermectin FECR greater than 75%, and that doramectin, eprinomectin, and moxidectin egg count reductions were significantly greater during the trial. The ADGs of doramectin, eprinomectin and moxidectin (1.31kg, 1.33kg, 1.37kg respectively) were significantly greater than ivermectin (1.25kg) and the control (1.14kg), while not being significantly different from each other. Ivermectin did show improved gains over the control, but was lower than the other treatment groups.

Similar findings were reported by Yazwinski (Yazwinski et al. 2006b). This trial compared the effectiveness of moxidectin injectable and ivermectin injectable to a control treatment in stocker cattle. Moxidectin treated calves significantly outgained the other two treatment groups over the grazing period of 150 days, with ADGs of 0.54, 0.44, and 0.44 kg for
moxidectin, ivermectin, and control respectively. The FECR for moxidectin treated calves was 99.9% at 22 days while the ivermectin treated calves reduction was only 83.4%. The authors also reported that 2 of the 52 moxidectin treated animals developed injection site swelling.

These studies have a trend of ivermectin having lower efficacy over the grazing period compared to other macrocyclic lactones. Ivermectin does, however, continually perform better than control groups but only in the early stages of grazing.

Although ivermectin may not have performed as well in decreasing fecal egg counts or ADG compared to other macrocyclic lactones, it is still considered the hallmark for this class of anthelmintics due to being the first developed and longest used product in the class. Williams also conducted a study comparing ivermectin pour-on to several benzimidazoles such as albendazole, oxfendazole, and fenbendazole on their efficacy against inhibited fourth stage larvae of *Ostertagia ostertagi*. Ivermectin pour-on was superior to the benzimidazoles in removing all stages of *Ostertagia* (Williams et al. 2007b). This study lends insight to the use of macrocyclic lactones as the most effective way to control one of the most costly gastrointestinal nematodes. However, the use of benzimidazoles still has an economic benefit to cattle on grazing production systems as shown by the work done by Smith and others who reported strategically dewormed steers gained 48 more pounds than did control steers during a grazing phase when treated with fenbendazole (Smith et al. 2000).

Gasbarre, et al., has reported an instance of poor anthelmintic efficacy of several products and classes of anthelmintics (Gasbarre et al. 2009). This trial compared injectable ivermectin, eprinomectin pour-on, injectable doramectin, moxidectin pour-on, and albendazole orally. None of the fore-mentioned treatments had fecal egg count reduction of at least 90%. The mean FECR at 14 days regardless of treatment was less than 85%. This was the first reported presence in the US of cattle parasites resistant to avermectins, moxidectin, and benzimidazole. The nematode species recovered from the abomasums within this trial were predominately Haemonchus and Cooperia, which are known to already have a lower susceptibility compared to other species. Haemonchus is also a high shedder of eggs which could have accounted for the decreased FECR.

**Generics**

The majority of the products that are used in comparative trials are the trade name products within their class of anthelmintic. The patent rights on ivermectin have expired and now
a number of generic products are on the market. Due to the extensive use of generic products, trade name products may not possess the major representation of product usage in certain parts of the country (Yazwinski et al. 2009b). The efficacy of generic anthelmintic products has been questioned due to the lack of trials for approval.

Yazwinski was one of the first to identify the need to have solid literature on the effectiveness of generic ivermectin in cattle (Yazwinski et al. 2004). Yazwinski, et al., conducted a trial evaluating three generic pour-on ivermectin products compared to the original proprietary product in stocker calves. In this trial, fecal egg count reduction of the original proprietary product was greater than any other ivermectin product tested at 14 days and 56 days. Ivomec Pour-On had a FECR of 96% at 14 days, while the other products ranged from 81-93%. On day 56, on the original proprietary product still had an egg reduction of 56% while the generics ranged from 1-38% reduction. All of the treatment products used significantly decreased the fecal egg shedding from the control group. However, there was not a significant difference in ADG between treatment groups. This study highlighted that although there were differences in FECR, there were no significant differences in ADG. This was the first documentation of generic formulations having a lower efficacy (measured by FECR) compared to the trade name product.

Yazwinski, et al., followed the previous study by including generic ivermectin injectable along with the original ivermectin injectable formulation in an efficacy trial conducted in 2009 (Yazwinski et al 2009a.). The authors concluded that the generic and trade name ivermectin were not significantly different from each other. Neither of the products reached greater than 89% fecal egg count reduction, which is inadequate effectiveness of the product as outlined by Bliss and others in 2008. During the same trial, a separate test was conducted comparing Ivomec Plus injectable to a generic ivermectin equivalent. In this test, the generic did perform significantly different. On day 17, Ivomec Plus reported a FECR of 83% while the generic ivermectin plus only achieved 66% reduction of fecal eggs. Again this test reported less than ideal fecal egg count reductions. By testing several generic products from different companies this trial concluded that the differences in formulations lead to the range of efficacy. One of the formulations performed as well as the original product, while the other did not.

Genchi and others also compared generic ivermectin to the trade name product (Genchi, et al., 2008). This study was based more on the effectiveness against psoroptic mange than gastrointestinal nematodes. In this study, generic ivermectin was significantly less effective than
the original proprietary ivermectin in control of mange. On day 25 post treatment, 9/11 animals treated with the generic product still showed clinical signs of mange while the trade name product-treated animals were all clinically cured. The ADG in these two groups were also significantly different between the generic group and the trade name group (1.07kg and 1.24kg respectively). The generic treated group in this study revealed an incomplete clinical and parasitological response for mange as well as a lower ADG.

The final study that compared generic ivermectin to the original product was conducted by Anziani and others in Argentina (Anziani, et al., 2001). Eighty calves with histories of ill thrift and persistent fecal egg counts after treatment with ivermectin were utilized in this trial. This study only yielded a FECR of 49% for the generic product. The trade name product FECR was 65%. While both products never reached 90% reduction, the difference was statistically significant. This study agrees with the previously discussed results of decreased efficacy of generic ivermectin products.

The available literature has depicted generic ivermectin products as being less efficacious compared to the original trade name product. However, there have been reports of equivalent efficacy in certain trials. Due to the wide price discrepancy between generic and trade name products many producers may continue to use generics despite the possible decrease in efficacy.

Objectives

The use of anthelmintics in cattle is based on the expectation of control of gastrointestinal nematodes and improved performance post-application. In the current literature, there has been a focus on residual protection of anthelmintics during grazing (Gasbarre et al. 2009, USDA 2009, Yazwinski et al. 2004, Yazwinski et al. 2009a., Williams et al. 2007a., Williams et al. 2007b., Williams et al. 2009).

The available literature concerning feedlot applications suggests that deworming upon entry into feedyards has a cost benefit by increasing average daily gain and feed conversion (Edwards et al. 2000, Guichon et al. 2000, Ives et al. 2007, Reinhardt et al. 2006, Smith et al. 2000). However, little information has been published on the comparative anthemintic efficacy of products within the feedyard setting. Most of the information about comparative efficacy of products has been extrapolated from pasture or modified environments such as the study conducted by Yazwinski in 2009. The use of generic products has decreased costs in a
production system that operates on a tight margin. Research has indicated that some generic products do not have the same anticipated efficacy as the name brand products (Anziani et al. 2001, Genchi et al. 2008, Yazwinski et al. 2004, Yazwinski et al. 2009a.). Of these studies available, only one utilized feedlot cattle and it was conducted to measure the effect on psoroptic mange. Due to the dearth of literature specific to generic anthelmintic use in feedlot cattle, the trial outlined in Chapter 2 was conducted.
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Chapter 2 - Comparative Efficacy of Two Ivermectin Pour-on Anthelmintics in Beef Steers in a Commercial Feedyard

Introduction

Parasitism leads to decreased performance, subclinical, and clinical disease in cattle (Corwin 1997, Kaplan 2010). The most common gastrointestinal parasites of cattle are Cooperia, Ostertagia, and Haemonchus, and to a lesser extent Trichostrongylus, Oesophagostomum and Nematodirus (Williams et al. 1997a, Williams et al. 1997b, Williams et al. 1999, Yazwinski et al. 2004, Yazwinski et al. 2009a, Yazwinski et al. 2009b). Producers often treat or prevent parasitism with a number of approved trade name and generic anthelmintics. Generic products generally have a cost advantage for beef producers when compared to name-brand products; however, several studies have reported that generic macrocyclic lactones are less efficacious than the original name-brand products (Anziani et al. 2001, Genchi et al. 2008, Lifschitz et al. 2004, Yazwinski et al. 2004, Yazwinski et al. 2009a).

Research comparing generic to trade-name ivermectin products has been completed in many different areas. A pharmakokinetic study observed four generic formulations of ivermectin to have marked kinetic differences between each other (Lifschitz 2004). These differences in systemic availability and drug disposition kinetics among generic formulations may affect the efficacy and persistence of generic products’ antiparasitic activity. In studies conducted in Argentina and the U.S., generic injectable ivermectin was less efficacious compared to the name-brand product with a decrease in fecal egg count reduction coorelated with a decrease in efficacy by a decrease in FECR (Anziani et al. 2001, Yazwinski et al. 2009a). Trade name ivermectin” pour-on had improved fecal egg count reduction compared to generic ivermectin in a study utilizing female stocker calves in Arkansas, however there were no reported differences in average daily gain (ADG) over the 56 day experimental period (Yazwinski et al. 2004).

Since the 1990’s there have been a number of studies reporting a decrease in the efficacy of all ivermectin compounds in cattle (Vermunt et al. 1995, Waller 1997, West et al. 1994). Resistance to modern anthelmintics by ruminant nematode parasites is an increasing problem throughout the world (Gasbarre et al. 2009). Emerging reseistance to other common
anthelmintics such as avermectins, moxidectin and a benzimidazole have also been reported (Gasbarre et al. 2009, USDA 2009). The most reported resistant parasite species are strains of Cooperia and Haemonchus (Gasbarre et al. 2009, Vermunt et al. 1995, West et al. 1994, Yazwinski et al. 2009a.). This reported resistance may be due to a change in pasture nematode population shifts. These species had inherent resistant properties since these products were developed. However, no literature has been published on nematode new resistance to ivermectins in the feedlot environment.

Often times, the decision to use an anthelmintic is based on the dogma or expectation of improved performance post-application. Yet a majority of the literature focuses on the residual protection from the constant parasitism that occurs in the pasture setting (Gasbarre et al. 2009, USDA 2009, Yazwinski et al. 2004, Yazwinski et al. 2009a., Williams et al. 2007a, Williams et al. 2007b, Williams et al. 2009). There are fewer published studies examining the efficacy of anthelmintics and the corresponding performance effects on animals in the feedlot (Ives et al. 2007, Reinhardt et al. 2006, Smith et al. 2000). Therefore, the objective of this experiment was to determine the relative efficacy of a generic pour-on and the trade name pour-on to assess the effects of these products on ADG of feedlot cattle.

**Materials and Methods**

Ten pens of 200 steers with an average body weight of 676.2lbs (307.4kg) and a standard deviation of 173.2lbs (78.7kg), of newly arrived feedlot cattle were selected for the experiment based on animals of similar origins, breed, age, body weight, and health status within pen. No information was available on previous history. A subset of cattle (40 hd/pen) from each of the selected feedlot pens were systematically assigned to one of two randomly assigned anthelmintic treatments determined by processing order. The first 20 head processed were given the trade name pour-on (.22 mg/lb BW (.1mg/kg).) and the second 20 head given the generic pour-on (.22 mg/lb. BW (.1mg/kg)) (Table 1). The remainder of the pen was treated according to the feedlot operating procedures and was given the generic pour-on. The proper dose was calculated by individual body weight and was administered on the back midline from tailhead to the withers. No product was spilled during administration.

All cattle in the study received a unique ear tag for individual animal identification. A rectal fecal sample was obtained and a weight was recorded at the time of initial processing and
prior to treatment on day 0. Cattle from both treatment groups were housed in the same home pen after treatment application. On day 14 post-treatment, rectal fecal samples were taken and all cattle were weighed. Body weights were also recorded at the pre-harvest sort date of approximately 110 days on feed.

After collection, fecal samples were placed in a small plastic bag and numbered sequentially to blind laboratory personnel to treatment assignment. Samples were placed on ice and shipped overnight to a private parasitology lab for fecal egg counts. The fecal egg counts were determined using a modified Wisconsin sugar float technique with centrifugation. The reported counts given were accurate to within 10 eggs per gram (EPG) from 3 gram samples with a maximum egg count of 500 EPG.

A control group was not used because of an expected increase in average daily gains due to administration of anthelmintic products compared to an untreated control (Guichon et al. 2000, Williams et al. 1997a, Williams et al. 1999). This study was performed in a commercial field setting and the feedyard was unwilling to allow the use of untreated controls due to the possibility of lost performance on the cattle. Initial fecal egg counts of treatment groups were compared using linear and mixed models with treatment, pen and their interaction terms as predictors of net egg count difference and average daily gain using R version 2.10.1. Fecal Egg Count Reduction percentages (FECR) were calculated from net egg count reduction and used to report treatment efficacy. The FECR test has been the accepted and most used method for evaluating parasiticide effectiveness (Bliss et al. 2008, Coles et al. 1992, Taylor et al. 2002, Yazwinski et al. 2009a). In short the FECR test is a quantification of parasite eggs from the feces of a percent of the cattle in a herd. This sampling is performed before and after treatment of a paraciticide.

\[
\%\text{Reduction} = \frac{\text{Day 0 Egg Count} - \text{Day 14 Egg Count}}{\text{Day 0 Egg Count}} \times 100
\]

**Results**

There were no anthelmintic treatment by pen interactions for fecal egg count reduction or performance. Pre-treatment egg counts were not different between treatment groups (P= 0.17, Figure 2.1). There were no differences in net egg count reduction between treatments at 14 days.
post-treatment application (P= 0.15, Figure 2.1). However, cattle housed in different pens had different egg count loads prior to and after anthelmintic application. Initial parasite load within each pen was a significant factor in the decrease of fecal eggs (P< 0.01, Figure 2.3) with pens having high initial parasite loads experiencing greater reductions. Regardless of treatment, only 26% of animals had a FECR of >90% and only 35% achieved a FECR of >80% (Figure 2.2). Eighteen percent (18%) of the cattle actually had an increase in FECR 14 days post-anthelmintic treatment (Figure 2.2).

There were no differences in pre-treatment body weights (BW) between treatment groups (P=0.096; Table 2.2). Cattle treated with the generic pour-on\textsuperscript{b} (Table 2.1) had improved average daily gains (ADG) compared to cattle treated with the trade name\textsuperscript{a} (Table 2.1) product (3.90 lbs/day vs 3.74 lbs/day (1.77kg/day vs 1.70kg/day), respectively; P = 0.02; Table 2.2).

**Discussion**

In the study reported here, we did not observe a difference in post-application FECR between the original proprietary and the generic pour-on product used in this study. Contrary to previously published reports on the efficacy of generic pour-on anthelmintics (Yazwinski et al. 2004, Yazwinski et al. 2009a.), this study demonstrated an overall lack of efficacy of both generic and brand name products with 74% of animals having lower than 90% fecal egg reduction efficacy. There are several reports of ineffective control of nematode parasites with the use of Ivermectin products (Coles et al. 2006, Yazwinski et al. 2004). These reports reported FECR below 90%.

When efficacy of an anthelmintic falls below 90%, it signals product failure by either resistance or extrinsic factors. However, in this study we were unable to determine whether this was due to intrinsic product efficacy, innate resistance of parasites involved, or environmental factors such as rain or mud as these are always present within a feedlot setting. The product label indicates a reduced efficacy may occur if the hide is wet, if rain is expected within 6 hours, or if there is caked mud or manure on the hide. However, others have observed no difference between pour-on treated animals with no rain and pour-on treated animals with simulated rain (Rehbein et al. 1999, Rolfe et al. 1997). These trials observed efficacies well over 90% fecal egg reduction.

We observed a difference in ADG between the two products (p-value- 0.02). In this instance generic\textsuperscript{b} (Table 2.1) treated cattle out-performed trade name\textsuperscript{a} (Table 2.1) treated cattle
(Table 2.2). The sampling of the test groups was sufficient to detect a 5% difference in FECR between treatment groups, but the lack of FECR difference does not provide evidence for the difference in ADG observed. Other factors such as species of nematode, individual animal variation, and health may have contributed to this difference.

This trial and previous literature indicate that the use of generic products can be beneficial, but the products must be tested to prove their efficacy (Lifschitz et al. 2004, Yazwinski et al. 2004, Yazwinski et al. 2009a.). Generic anthelmintics are only required to prove the same active ingredient to be produced and must use the same carrier. In contrast, bioequivalent drugs contain the same active compound but may have other carrier formulations. In order for bioequivalent drugs to be approved they must have the outer boundaries of a 90% confidence interval around the mean for the area under the concentration curve (AUC), and maximal concentration (C max) of serum concentration between -20 and +20% of the mean value of the approved product (Brown 2001).

Acceptable decreases in fecal egg count numbers are 90%, with anything less indicating the presence of anthelmintic resistance (Bliss et al. 2008, Coles et al. 2006). Egg counts do not necessarily correlate with actual nematode numbers, because the test only measures effects of egg production by mature nematodes (Taylor et al. 2002). The gold standard for assessing the true nematode burden of an animal is quantification at necropsy (Taylor et al. 2002). However this process is not utilized commonly due to the labor, expense, and loss of animal life (Coles et al. 1992). Yazwinski has introduced several add on enhancements to the FECRT that aid in reporting a more precise estimate of efficacy of a given product (Yazwinski et al. 2009a.). The enhancements include randomization, accurate dosing of the product, individual animal identification and repeated sampling of the same animals, coproculture, and post treatment sampling done at the point of greatest egg count reduction at 10-14 days post-administration (Coles et al. 1992).

In the study reported here, animals were systematically assigned to treatment assignments based on processing order. This could have inadvertently introduced bias if there is some reason that calves processed first had a particular disadvantage or advantage compared to the calves processed last. However, this study met the other two “add-ons” of sampling with use of repeated sampling of the same animals, and sampling timed at greatest expected reduction post
treatment application. These helped gain a more accurate assessment of how the products performed at the individual animal level.

This study also brings insight to the efficacy of pour on anthelmintic products in commercial feedyards. Topical administration does not guarantee a controlled drug delivery in cattle due to physiologic licking as a second route of exposure (Laffont et al. 2003). Normal grooming of cattle makes this aspect of behavior very difficult to control. Injectable formulations should be used to decrease the chance of product loss, and give a more homogenous plasma delivery, and higher plasma levels compared to oral or intraruminal application of the product after administration (Steel 1993). This ensures uniform drug performance within a group of cattle.

**Conclusion**

Under the conditions of this study, we did not show a difference in efficacy measured by fecal egg count reduction between generic and trade name pour-on anthelmintic products. However, both treatments performed poorly in the feedlot setting based on this standard. Pen was a significant factor in the decrease of fecal eggs. Incoming nematode burdens, cattle type, and the individual response to treatment are all factors that make the pen a significant factor in the reduction gastrointestinal nematodes. The generic product did produce a better average daily gain over the trade name product, however, this was unexpected due to not having a significant difference in FECR. This trial provides evidence that FECR does not correlate well with ADG. Further research is warranted to determine a more accurate assessment to compare anthelmintic efficacy in the field.
Tables and Figures

Figure 2.1 Egg count by treatment and time
Figure 2.2 Fecal Egg Count Reduction regardless of treatment
Figure 2.3 Egg Count Per Pen and Time

Pen at pre- and post- treatment (day 0 vs day 14)
Table 2.1 Product information for anthelmintics used in the fecal egg count reduction test.

<table>
<thead>
<tr>
<th>Anthelmintic (Name (Class))</th>
<th>Dose</th>
<th>Manufacturer</th>
<th>Administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>aIvomec (Ivermectin)</td>
<td>.22mg/lbs</td>
<td>Merial</td>
<td>Topical</td>
</tr>
<tr>
<td>bVetrimec (Ivermectin)</td>
<td>.22mg/lbs</td>
<td>VetOne</td>
<td>Topical</td>
</tr>
</tbody>
</table>
Table 2.2 Performance data depicting a statistical difference in ADG

<table>
<thead>
<tr>
<th>Anthelmintic</th>
<th>In weight</th>
<th>P-Value</th>
<th>Sort weight</th>
<th>P-Value</th>
<th>ADG</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vetrimec</td>
<td>672.31</td>
<td>0.096</td>
<td>1121.14</td>
<td>0.46</td>
<td>3.89</td>
<td>0.02</td>
</tr>
<tr>
<td>Ivomec</td>
<td>680.27</td>
<td></td>
<td>1107.65</td>
<td>3.74</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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