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Effects of anthelmintic treatments on performance indicators in stocker calves

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Effects of anthelmintic treatments on performance indicators in stocker calves

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Effects of anthelmintic treatments on performance indicators in stocker calves

Submitted in partial fulfillment
of the requirements
for the Murray State University Honors Diploma

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Table of Contents

Abstract

The objective of this study was to evaluate the effects of various anthelmintic treatments on fecal egg counts (FEC), body condition score (BCS), average daily gain (ADG), fly counts, and hematology parameters in newly received stocker calves. Upon arrival at the Murray State University Beef Unit, steers (n = 30) were allowed a one-week adjustment period before allocation to treatment. Steers were randomly allocated to treatment based on initial body weight (BW; 297.77 kg ± 16.53) and strongyle FEC (11.64 eggs/gram; EPG). Treatments included: control (n = 10; no anthelmintic treatment; CON); moxidectin/oxfendazole combination (n = 10; COMBO); and long-acting eprinomectin (n = 10; LAE). Steers were co-mingled and allowed to graze mixed-grass pastures with pasture rotation based on forage availability. Body weight, fecal and blood samples were collected on d 0, 27, 56, and 101, and the following hematology parameters were evaluated: white blood cells (WBC), platelets (PLA), neutrophils (NEU), lymphocytes (LYM), monocytes (MON), basophils (BASO), and eosinophils (EOS). Fly counts were estimated on d 0, 31, 61, and 100. Data was analyzed using the PROC MIXED procedure of SAS with individual steer as the experimental unit and d as a repeated measure. Two preplanned orthogonal contrasts were used to determine effects and included comparisons between: 1) CON vs treated steers, and 2) COMBO vs LAE steers. Strongyle EPG were similar between CON and treated steers ($P > 0.1$), but EPG were higher ($P = 0.02$) for COMBO vs LAE steers (22.97 vs 5.61 EPG, respectively). A treatment by d interaction was found for EPG ($P = 0.02$). Body weight and ADG were similar ($P > 0.1$) between treatments; however, ADG from d 27 to 56 was greater ($P = 0.04$) for LAE vs COMBO steers (1.29 vs 0.54) and BW tended to be greater for LAE steers on d 56 compared to COMBO steers (338.45 vs 320.61 kg; $P = 0.08$). An effect of d ($P < 0.01$) was observed for fly counts and the following hematology parameters:

WBC, NEU, LYM, MON, BASO, and EOS. Eosinophils were greater ($P = 0.03$) in LAE vs COMBO steers (0.32 vs 0.19, respectively) while MON tended to be greater ($P = 0.06$) in LAE and COMBO steers (0.82 vs 0.65, respectively). Data suggests that anthelmintic use may have affected FEC, ADG, and hematology parameters in newly received stocker calves.

Chapter 1: Literature Review

Overview of the United States Beef Industry

The size of United States' beef industry has seemingly been in decline for the last ten years. From the 2007 Census of Agriculture to the 2012 Census, the number of beef cattle farms has decreased by over 37,000 farms (~ 5%) and the number of beef cattle has decreased by nearly 4 million head (~ 12%). However, data suggests that the percentage of beef cattle for slaughter supplied by small farms (containing fewer than 50 head) is growing within the beef industry, particularly between 2007 and 2012. The 2007 Census indicated 765,000 farms reported inventories of cattle and calves. Of these, 79.4% were small farms supplying 28.7% of beef cattle for slaughter. At the time of the 2012 Census, the number of beef farms declined by approximately 30,000 farms compared to the 2007 Census. Of these, 81.6% are considered to be small farms that supply approximately 29.8% of all beef for slaughter (USDA NASS, 2012).

Performance Indicators in Stocker Calves

Beef producers have several ways to monitor the performance of their cattle. Body condition score and fly counts are readily available tools capable of being utilized from the field or chute system with little additional labor, equipment, or calculation. Fecal egg count, average daily gain, and hematology parameters are also valuable tools that can provide useful information to producers. However, these tools require training and access to laboratory equipment.

Body Condition Score. Body condition scores (BCS) serve as an indicator of fatness in domestic livestock. Body condition scores are based on assessment of fat on the back, tail-head, pins, hooks, ribs, and brisket areas of the cow and is a valuable method to evaluate carcass quality (Little et al., 2002). Scores are observed on a scale of 1 to 9, with 1 being emaciated and

9 being extremely fat (Appendix 2; Richards et al., 1986). Animals with a score of 5 look average – not too thin or too fat. Animals with a score of 3 or below have little to no fat and are scored on the degree of muscle loss. USDA Utility and Commercial grade cows generally have enough intramuscular fat (marbling) and muscling to be marketed for rib and loin cuts. The ability for a carcass to be marketed outside the ground beef trade greatly enhances the value of the carcass and helps avoid price discounts. Price discounts are typically applied to carcasses with a BCS over 6 (where there is too much external fat) and under 4 (where there is not enough muscle or fat; Little et al., 2002).

Fly Count. Horn flies (*Haematobia irritans*), a major blood-sucking parasite, are regularly observed around cattle herds in North America. Horn flies can feed up to 40 times per day (Mays et al., 2014), causing stress and annoyance that can decrease performance (DeRouen et al., 2009). Losses due to horn fly infestations are estimated at $700 million annually, but an effective fly control program can provide a 15 – 30 pound weight increase in stocker calves (Arthur, 1991).

Horn flies tend to congregate around the face, back, and top of the shoulder and spend the majority of their life cycle around cattle (Mays et al., 2014). Female flies leave the cow to lay eggs in fresh manure, producing up to 400 eggs in her lifetime. Eggs typically hatch in less than one day, with larvae remaining in the manure as they feed and develop. When young flies emerge from the manure, they can travel up to 7 miles in search of a host to feed. During warm summer months, the complete life cycle can last as little as 10 days, resulting in 10 generations of flies per year (Arthur, 1991).

Fecal Egg Count. Fecal egg counts (FEC) are used to estimate the extent of parasite contamination or infestation of a pasture and to determine the effectiveness of an anthelmintic treatment. Fecal egg counts can give producers a general idea of internal parasite load in their animals (Zajac and Conboy, 2012); low parasite loads are associated with higher performance due to less stress on the body. A common method for determining FEC is the modified McMasters protocol (Appendix 1) in which fecal samples are collected via rectal grab while cattle are restrained in the chute system. While it can be an important tool for determining the overall health of an animal, determining FEC is typically not practical or feasible for many producers unless evidence suggests severe anemia or poor performance.

Average Daily Gain. Determination of average daily gain (ADG) is a valuable tool available to producers and is a major indicator of performance in beef cattle. Average daily gain is typically calculated by subtracting the beginning body weight (BW) from the ending BW and dividing that value by the number of days in the feeding period. One study analyzing the profitability of cow-calf operations assumed a 0.757 kg daily gain over a 180 day feeding period to produce a USDA Utility (BCS 5 to 6) carcass (Little et al., 2002). Earlier research has shown that about 80% of cattle raised for slaughter should be able to reach this BCS (Schnell et al., 1997; Rasby et al., 2000).

Hematology Parameters. Complete blood count (CBC) data can provide valuable information regarding the overall health of animals, allowing for improved diagnosis and prognosis of diseases (Roland et al., 2014). Important hematology variables evaluated include: white blood cell (WBC) count, monocytes (MON), neutrophils (NEU), eosinophils (EOS), basophils (BASO), lymphocytes (LYM), and platelets (PLA). Like FEC, CBC data is typically not feasible for most producers to monitor the health of their stock unless evidence indicates incidence of severe illness or disease.

White Blood Cells. Roland, et al., (2014) provided a comprehensive review of the use of hematology measurements as a diagnostic tool in bovine medicine. White blood cells play a crucial role in immune defense by fighting infection triggered by an immune response. Elevated number of WBC, a condition known as leukocytosis, is associated with prolonged periods of stress, infection, endocrine conditions, and central nervous disorders. Whereas leukopenia, a depression in WBC, often occurs with metabolic disorders, liver disease, infectious disease, and bacterial septicemia.

Phagocytes are a specialized type of WBC produced in the bone marrow that function to engulf, ingest, and destroy invading microorganisms. There are three types of phagocytes that are of particular interest in bovine hematology: MON, NEU, and BASO. Monocytes circulate in the blood ranging from a few hours to several days before entering the tissue and becoming macrophages while NEU circulate for only a few hours before entering the tissue. Monocyte levels are variable and cannot be the sole determining factor for infection. Increased levels of MON (monocytosis) have been observed during periods of stress, healing of acute and chronic infections, and corticosteroid therapy. Depressed levels of MON (monocytopenia) is associated with endotoxemia and inflammation, but has not been proven to have much clinical relevance. Neutrophilia, higher than normal levels of NEU, is most commonly caused by chronic inflammation and stress, but can also be seen with endometritis, ketosis, and indigestion. Depression of NEU count (neutropenia) occurs in the first few days of severe inflammation, sepsis, and viral infection (Roland et al., 2014).

Eosinophils regulate the inflammatory process by chemically reacting to histamines. Eosinophilia, defined as a peripheral EOS count greater than 450 cells per microliter, is associated with parasitic infestation, allergic inflammation, infections, and drug reactions

(Fulkerson and Rothenberg, 2013; Roland et al., 2014); while a condition resulting from below normal numbers of EOS, known as eosinopenia, is associated with the early phases of infectious disease. Basophils have similar inflammatory modulation abilities, but are not true phagocytes. An increase in BASO, basophilia, has been linked to hyperlipidemia and parasitic infections (Roland et al., 2014).

Lymphocytes, another type of white blood cell, are responsible for hormonal and cellular immunity. Like phagocytes, LYM are produced in the bone marrow and are released into the body. Lymphocytes that migrate to the thymus to become T cells, which support immunologic functions. Lymphocytes that become B cells migrate directly to organs and are responsible for antibody production (Kahn and Line, 2010). Lymphocytosis, a condition resulting due to elevated LYM, can occur during healing of infection. Lymphocytopenia, a reduction in lymphocytes, can result due to periods of elevated stress, viral or bacterial infection, and application of corticosteroids (Roland et al., 2014).

Platelets. Platelets are responsible for clotting when blood vessels hemorrhage. They are also the source of phospholipids, which is needed for coagulation. Mature PLA contain adenosine triphosphate (ATP), adenosine diphosphate (ADP), calcium, serotonin, glycogen, and other compounds required by the body. When vessels hemorrhage, PLA adhere to the tear through von Willebrand's factor, undergo a change in shape, and release ADP to supply energy for healing. Platelets accumulate to form a hemostatic plug, stop the bleeding, and allow the tissue to heal (Kahn and Line, 2010).

Parasite Control

It is estimated that beef cattle producers spend upwards of $2.5 billion annually on pharmaceutical means of parasite control (Williams and Loyacano, 2001). Of all farms in the

United States regardless of size, 86.8% deworm their cattle using anthelmintics, whereas 82.4% of small farms use recommended anthelmintic practices (USDA NAHMS, 2010).

Anthelmintics are separated into classes based on their mode of action. To be effective, anthelmintics must be selectively toxic to certain parasites. Selective toxicity is achieved through inhibiting vital metabolic processes in the parasite that do not severely damage the host or by inherent properties of the anthelmintic that cause the parasite to be exposed to higher concentrations of the anthelmintic than the host cells.

Parasites must maintain a feeding site, actively ingest sustenance to maintain a stable energy state, maintain proper neuromuscular coordination, and maintain homeostasis despite immune reactions of the host to survive. To interrupt these activities and eventually kill the parasite, different classes of anthelmintics target the integrity of parasitic cells and neuromuscular coordination of the parasite causing paralysis and eventual expulsion or digestion (Kahn and Line, 2010). Benzimidazoles and salicylanilides attack the integrity of the parasite's cells; imidazoles, macrocyclic lactones and tetrahydropyrimidines interfere with neuromuscular coordination (Kahn and Line, 2010).

Macrocyclic Lactones (Avermectins and Milbemycins). According to the USDA's 2007-08 National Animal Health Monitoring System (NAHMS) Beef Cow-calf Studies, the macrocyclic lactone class of anthelmintic is the most popular among all beef cattle operations, being used by 87.5% of all operations and 83.1% of small farms (2010).

Macrocyclic lactones (avermectins and milbemycins) are derived from soil microorganisms belonging to the genus *Streptomyces*. Commercially available avermectins and milbemycins include ivermectin, doramectin, eprinomectin, milbemycin oxime, and moxidectin. Even at a low dose, macrocyclic lactones are potent and active against a wide variety of gastrointestinal parasites with efficacy ratings greater than 98% (Kahn and Line, 2010). Macrocyclic lactones have an average efficacy period of 14 to 28 days, while a single dose of a non-long-acting moxidectin has a slightly longer efficacy period (14 to 42 days; Prichard et al., 2012). Regardless of the route of administration (orally, subcutaneously, pour-on, etc.), macrocyclic lactones spread quickly throughout the body and concentrate in adipose tissue (Kahn and Line, 2010). Pour-on formulations are more convenient for use on cattle, but experience greater variability in efficacy compared to oral or subcutaneous administration (Kahn and Line, 2010). Due to their high potency and excretion through milk, all macrocyclic lactones except for moxidectin and eprinomectin are not recommended for use in dairy cows (Kahn and Line, 2010).

A special class of eprinomectin, known as a long-acting eprinomectin (LAE), has generated much interest throughout the beef industry in the past few years due to an increased efficacy period up to 150 days. One study estimated its efficacy at over 99% for all parasite species investigated (Pitt et al., 1997). This anthelmintic has a unique extended-release formula resulting in dual efficacy peaks. The first peak occurs within the first few days following administration while the second peak occurs approximately 90 to 120 d post administration. The convenience of a single injection every five months is a strong driver for the use of a long-acting eprinomectin. The extended efficacy period lessens the animal handling needed and, therefore, reduces stress on the animals (Forbes, 2013). However, the cost of LAE is 2 to 3 times higher per dose compared to other anthelmintic treatments thus can be a major deterrent for many producers.

Benzimidazoles. The benzimidazole class of anthelmintics is the second most popular among all operations, including small farms (19.3% and 20.8% of operations, respectively;

USDA NAHMS, 2010). Commercially available benzimidazoles include albendazole, flubendazole and oxfendazole. These products have the longest half-life and, therefore, longest efficacy period. Oral treatment, which is the most effective in ruminants, removes the majority of adult and larval gastrointestinal parasites. One study found that treatment with oxfendazole and an oxfendazole-moxidectin combination saw a 99.99% FEC reduction within two weeks (Walker et al., 2013).

Other Classes of Anthelmintics. Other classes of anthelmintics include imidazothiazoles, salicylanilides, and tetrahydropyrimidines. Imidazothiazoles, like levamisole, are highly effective against common gastrointestinal nematodes and lungworms, but are not effective against flukes and tapeworms. This class of anthelmintic can be administered orally or subcutaneously with equal efficacy, and topical treatments have been developed (Kahn and Line, 2010). Members of the salicylanilide class of anthelmintics include brotianide, closanel, and oxyclozanide. This class is given orally and effective primarily against the adult stages of liver flukes (Kahn and Line, 2010). The tetrahydropyrimidines class of anthelmintics includes pyrantel. Monogastric animals, such as canine and swine, absorb this class of anthelmintic better than ruminants, but tetrahydropyrimidines can be used to treat gastrointestinal parasites in cattle. However, this class is not recommended for use in debilitated animals (Kahn and Line, 2010).

Research Proposal

Control of internal parasites has proven to be a challenge for beef producers and costs the beef industry upwards of $2.5 billion annually (Williams and Loyacano, 2001). Although the use of LAE has proven to be an effective means of reducing a variety of parasites affecting cattle (Kahn and Line, 2010), the higher cost of this anthelmintic may discourage its use, especially among small producers. Very little information is available concerning the effectiveness of simultaneous treatment of moxidectin/oxfendazole combination (COMBO) in beef cattle. Thus, the objective of this study was to evaluate the effects of various anthelmintic treatments on fecal egg counts (FEC), body condition score (BCS), average daily gain (ADG), fly counts, and hematology parameters in newly received stocker calves.

Chapter 2: Effects of anthelmintic treatments on performance indicators in stocker calves

Abstract

The objective of this study was to evaluate the effects of various anthelmintic treatments on fecal egg counts (FEC), body condition score (BCS), average daily gain (ADG), fly counts, and hematology parameters in newly received stocker calves. Upon arrival at the Murray State University Beef Unit, steers (n = 30) were allowed a one-week adjustment period before allocation to treatment. Steers were randomly allocated to treatment based on initial body weight (BW; 297.77 kg ± 16.53) and FEC (11.64 eggs/gram; EPG). Treatments included: control (n = 10; no anthelmintic treatment; CON); moxidectin/oxfendazole combination (n = 10; COMBO); and long-acting eprinomectin (n = 10; LAE). Steers were co-mingled and allowed to graze mixed-grass pastures with pasture rotation based on forage availability. Body weight, fecal and blood samples were collected on d 0, 27, 56, and 101, and the following hematology parameters were evaluated: white blood cells (WBC), platelets (PLA), neutrophils (NEU), lymphocytes (LYM), monocytes (MON), basophils (BASO), and eosinophils (EOS). Fly counts were estimated on d 0, 31, 61, and 100. Data was analyzed using the PROC MIXED procedure of SAS with individual steer as the experimental unit and d as a repeated measure. Two preplanned orthogonal contrasts were used to determine effects and included comparisons between: 1) CON vs treated steers, and 2) COMBO vs LAE steers. Strongyle EPG were similar between CON and treated steers ($P > 0.1$), but EPG were higher ($P = 0.02$) for COMBO vs LAE steers (22.97 vs 5.61 EPG, respectively). A treatment by d interaction was found for EPG ($P = 0.02$). Body weight and ADG were similar ($P > 0.1$) between treatments; however, ADG from d 27 to 56 was greater ($P = 0.04$) for LAE vs COMBO steers (1.29 vs 0.54) and BW tended to be greater for LAE steers on d 56 compared to COMBO steers (338.45 vs 320.61 kg; $P = 0.08$). An effect of d

($P < 0.01$) was observed for fly counts and the following hematology parameters: WBC, NEU, LYM, MON, BASO, and EOS. Eosinophils were greater ($P = 0.03$) in LAE vs COMBO steers (0.32 vs 0.19, respectively) while MON tended to be greater ($P = 0.06$) in LAE and COMBO steers (0.82 vs 0.65, respectively). Data suggests that anthelmintic use may have affected FEC, ADG, and hematology parameters in newly received stocker calves.

Introduction

Gastrointestinal and external parasites, such as strongyles and horn flies, can significantly impact the health and productivity of cattle. It is estimated that beef producers spend approximately $2.5 billion annually on pharmaceutical means of internal and external parasite control (Williams and Loyacano, 2011). The most popular anthelmintic classes among small producers (less than 50 cows) are macrocyclic lactones and benzimidazoles (USDA NAHMS, 2010). Macrocyclic lactones like moxidectin and eprinomectin have a 98% or higher efficacy rate against gastrointestinal parasites (Kahn and Line, 2010). Efficacy of long-acting eprinomectin have been reported for up to 150 days versus 6 – 8 weeks with other anthelmintics (Forbes, 2013). Whether they are administered orally, subcutaneously, or as a pour-on, macrocyclic lactones circulate quickly through the body. Benzimidazoles, such as oxfendazole, have the longest half-life, and therefore efficacy period, of any anthelmintic except eprinomectin (Kahn and Line, 2010).

Few studies are available comparing effectiveness of oxfendazole/moxidectin treatment combination to other types of anthelmintics. Simultaneous treatments of oxfendazole and moxidectin may provide increased parasite treatment due to their specific modes of action. Moxidectin is in the milbemycin chemical class of anthelmintics, which shares the distinctive mode of action characteristic of macrocyclic lactones, and has proven to be an effective anthelmintic by interfering with neurotransmission in parasites. Moxidectin binds to glutamate-gated chloride ion channels, which are critical to the function of invertebrate nerve and muscle cells (Cydectin, Boehringer Ingelheim, St. Joseph, MO). Synanthic is of the benzimidazole class of anthelmintics, which prevents tubulin polymerization in parasites. Thus, benzimidazoles cause inhibition of parasitic cellular transport and energy metabolism (Synanthic, Boehringer Ingelheim, St. Joseph, MO). While simultaneous treatment of these two types of anthelmintics is expected to have increased efficacy on intestinal parasites in cattle, their combination treatment is safe for cattle due to their distinctly different modes of action. One study found that treatment with oxfendazole and an oxfendazole-moxidectin combination saw a 99.99% FEC reduction within two weeks (Walker et al., 2013). Backes et al. (2016) evaluated effects of anthelmintic treatment, either LAE or COMBO, on reproductive performance in Angus based crossbred heifers. Data suggested that similar numbers of LAE and COMBO heifers were considered cyclic before start of the breeding season, displayed estrus in response to synchronization, and conceived to either AI or natural service ($P > 0.16$). However, overall pregnancy rates tended to be greater for LAE compared to COMBO treated heifers (88% vs 71%, respectively; $P = 0.10$). Furthermore, evidence suggests that BW and BCS may have been similar between treatments in fall born beef heifers treated with either LAE or COMBO treatments (Backes et al., 2016). The objective of this study was to evaluate the effects of various anthelmintic treatments on fecal egg counts (FEC), body condition score (BCS), average daily gain (ADG), fly counts, and hematology parameters in newly received stocker calves.

Materials and Methods

Animals. Sixty-six steers belonging to a private producer were custom grazed at the Murray State University Beef Unit from May to August 2016. Steers were grazed on mixed-grass pastures, in a high-density grazing situation with pasture rotation occurring based on forage availability, and with ad libitum access to water. Pastures ranged from 0.40 to 0.81 ha with an average stocking density of 47,255.4 kg/ha.

Animals used in this study were a subset of individuals participating in a larger study. Thirty steers were randomly selected upon allocation to respective anthelmintic treatment groups for determination of hematology variables. Materials and Methods and Results sections herein will pertain only to those animals in which hematology parameters were evaluated. All treatments and procedures were approved by the Murray State University Institutional Animal Care and Use Committee (IACUC).

Treatment Protocol and Data Collection. Upon arrival to the facility, steers were allowed a one-week dietary adjustment period before allocation to treatment. Three days prior to the start of the study, steers were randomly assigned to treatment groups based on initial FEC expressed in eggs per gram (EPG = 11.64 ± 1.43) and body weight (BW = 297.77 kg ± 16.53).

Blood samples were collected, body condition scores (BCS) determined, and treatments applied on d 0 of the study. Treatments consisted of one control group in which no anthelmintic was administered (n = 10; CON) and two anthelmintic treatment groups. Steers belonging to the COMBO treatment group (n = 10) received a combination of oxfendazole (Synanthic) and moxidectin (Cydectin) while those belonging to the LAE treatment group (n = 10) received a long-acting eprinomectin (LongRange). Synanthic was administered orally at the rate of 4.5 mg/kg BW. Cydectin and LongRange were administered by subcutaneous injection in front of the shoulder at 0.2 mg/kg BW and 1 mL/50kg BW, respectively.

Body weight, fecal samples, blood samples, and BCS were again collected on d 27, 56, and 101 of the study. Fly counts were estimated on d 0, 31, 61, and 100 of the study. Body weight was measured upon the steers' entrance to the chute via an electronic scale. Fecal samples were collected via rectal grab in sterile palpation sleeves and identified by animal ID number while the steer was restrained in the chute. Samples were cooled in a refrigerator until evaluated using a modified McMasters protocol (Appendix 1). Strongyle EPG were determined by two trained independent observers, and average EPG was calculated by multiplying the average number of strongyles present in the viewing field of the slide by 50 (Zajac and Conboy, 2012). Jugular blood samples were collected by venipuncture and placed in 5 mL EDTA treated vacutainer tubes. The samples were stored on ice and transported to Breathitt Veterinary Center (Murray State University, Hutson School of Agriculture, Hopkinsville, KY) for determination of complete blood count (CBC) data including the following: white blood cells (WBC), platelets (PLA), neutrophils (NEU), lymphocytes (LYM), monocytes (MON), basophils (BASO), and eosinophils (EOS).

Body condition scores were determined by a single trained observer upon the steers' exit from the chute using the protocol developed by Richards et al. (1986; Appendix 2). Fly counts were observed concurrently by two trained observers. Cattle were evaluated from an all-terrain vehicle in a pasture setting between 0630 and 0830, and binoculars were used if cattle were more than 5 m away from the vehicle (Gerhardt and Shrode, 1990). Flies were counted on individual steers on the face, back, and top of the shoulders until the number exceeded 20; then flies were counted in groups of five (Steelman et al., 1991). Individual fly counts were averaged between observers and log transformed for statistical analysis.

Average daily gain was calculated for each steer for the periods between d 0 to 27, d 27 to 56, d 56 to 101, and an overall ADG from d 0 to 101. Gain was calculated by subtracting the beginning BW from ending BW for each period and then divided by the number of days in that particular period.

Statistical Analysis. One steer belonging to the COMBO treatment group was removed from this study due to injury unrelated to the study design or treatments implemented. Data collections for this steer were not included in the statistical analysis. Statistical analysis was performed using SAS (SAS Inst. Inc., Cary, NC). Individual steer was assigned as the experimental unit and d was the repeated measure. The PROC MIXED procedure was used for determining effects of anthelmintic treatment on FEC, BW, ADG, BCS, CBC data, and fly counts. Two preplanned orthogonal contrasts were used to determine effects and included comparisons between: 1) CON vs treated (COMBO and LAE) steers and 2) COMBO vs LAE steers. Fly counts and EPG data was log transformed to the $\log_{10}(X+1)$ with geometric means reported. Effects of anthelmintic treatment were evaluated within day for fly counts.

Results and Analysis

Statistical analysis was performed comparing main effects of treatment and day on FEC, performance indicators, and hematology parameters in newly received stocker calves.

Fecal Egg Counts. Fecal egg counts, measured as eggs per gram (EPG), were similar between control and treated steers ($P = 0.19$); however, strongyle EPG were greater in COMBO steers compared to LAE steers (22.97 vs 5.61 EPG, respectively; $P = 0.02$). A treatment by d interaction was found for EPG ($P = 0.02$; Figure 1). The greatest FEC were observed in COMBO treated steers on d 101 (113.53 EPG) and were similar to COMBO steers on d 56 as well as CON steers on d 101 and 56 (62.06 and 39.82 EPG, respectively; $P \geq 0.13$). Fecal egg counts observed in CON steers on d 56 were similar to d 0 and 27 and FEC in COMBO steers on d 0 and LAE steers on d 0 and 56 ($P \geq 0.11$). Fecal egg counts were the lowest in LAE steers on d 27 and were similar to those observed in COMBO steers on d 27 and LAE steers on d 101 (1.92, 3.19, and 5.50 EPG; $P \geq 0.16$) but differed from those observed in LAE steers on d 56 (7.31 EPG, respectively; $P = 0.08$).

Performance Variables. Body weight was similar between CON steers compared to COMBO and LAE steers throughout the study (Table 1; $P \geq 0.14$). However, orthogonal contrasts indicated that BW tended to differ between LAE and COMBO steers on d 56 of the study. Body weight was higher in LAE steers on d 56 compared to COMBO steers (338.45 versus 320.61 kg, respectively; $P = 0.08$).

Body condition scores were similar between treatments at the start of this study for CON, COMBO, and LAE steers (5.1, 4.9, and 4.9, respectively; $P > 0.11$). However, type of anthelmintic treatment appeared to affect BCS in CON versus treated calves (5.5 vs 5.2, respectively; $P = 0.03$) and were different between CON and COMBO steers (5.5 versus 5.0, respectively; $P = 0.03$). However, BCS were similar between CON and LAE steers (5.5 and 5.2, respectively; $P = 0.11$) and between LAE and COMBO (5.2 and 5.0, respectively; $P = 0.47$).

Average daily gain was similar between CON, COMBO, and LAE steers from day 0 to 27 ($P = 0.24$), day 27 to 56 ($P = 0.12$), day 56 to 101 ($P = 0.28$), and overall ADG from d 0 to 101 ($P = 0.40$; Table 2). However, ADG did differ between LAE versus COMBO steers from d 27 to 56. Average daily gain from d 27 to 56 was 1.29 versus 0.54 lbs/day for LAE and COMBO steers, respectively ($P = 0.04$). Interestingly, four of the ten steers in the COMBO treatment group and one steer from the CON treatment group were treated for suspected respiratory disease between d 27 to 56 of the study, which may explain the decreased performance observed in COMBO; however, no LAE steers displayed signs of respiratory disease.

Fly Count. Fly counts were similar between treatments throughout the study ($P = 0.99$). However, an effect of day was observed (Figure 2). The greatest number of flies were observed at the start of the study (d 0) with the fewest observed on d 31 (23.75 vs 5.61; $P = 0.08$). Within treatment (Table 3), the greatest number of flies were observed on d 0 of the study in the CON calves and were similar to those observed on d 100 (21.31 and 19.62, respectively; $P = 0.79$), but tended to differ from those on d 61 (12.30, respectively; $P = 0.09$). The fewest number of flies were observed in CON steers on day 31 (7.46, respectively; $P < 0.00$).

As in the CON steers, the greatest number of flies were observed on COMBO steers on day 0 of the study (33.46, respectively); unlike CON steers, fly counts differed on each day throughout the study with the fewest flies occurring on d 31 ($P \leq 0.05$). Curiously, the greatest number of flies were observed on LAE steers on d 100 of the study and were similar to d 0 and 61 (20.10, 18.81, and 18.49, respectively; $P = 0.84$) but differed on d 31 (5.10, respectively; $P < 0.00$).

Hematology Parameters. Anthelmintic treatments had no effect on hematology parameters of WBC, PLA, NEU, LYM, or BASO (Table 4) but tended to affect EOS values. Blood EOS values are related to the inflammatory response and have been associated with increased parasite loads (Fulkerson and Rothenberg, 2013). Interestingly, the highest concentrations of EOS were observed in LAE steers, while COMBO treated steers displayed the lowest EOS concentrations (0.32 versus 0.19 $10^3/\mu L$, respectively; $P = 0.03$). However, CON steers were similar to both LAE and COMBO treated steers (0.29 $10^3/\mu L$, respectively; $P > 0.10$). Monocytes were similar between CON and treatment steers (0.77 versus 0.74, respectively; $P = 0.74$); however, MON tended to be greater in LAE versus COMBO steers (0.82 versus 0.68, respectively; $P = 0.06$).

Discussion and Conclusions

Little research is available comparing the efficacy of long-acting eprinomectin and moxidectin/oxfendazole combination. Similar to Backes et al. (2016), this study saw treated steers having similar BW, ADG, and BCS to CON steers and found FEC to be lowest in LAE steers at the end of the study. However, COMBO steers had almost triple the EPG of CON steers at the conclusion of this study, which is in contrast to Backes et al. (2016).

It is possible that comingling treatment groups in this study could have induced carryover effects between treatment groups. Due to limited availability of pastures suitable for grazing, steers were commingled without regard to treatment group. Although commingling treatments reduces variability in the data due to stress factors and environmental effects, it is possible that commingling could have caused treatment carryover effects through normal herd behaviors (licking, grooming, grazing in close quarters, etc.) with the greatest impact occurring in the FEC and fly count data.

Overall, no differences were observed between CON and treated steers for FEC (P = 0.19); however, strongyle EPG were higher in the COMBO steers compared to LAE steers (P = 0.02). The greatest carryover effect is expected to occur from the LAE treatment group to CON, as LAE is noted for both internal and external parasite control. It is possible that carryover effect could, in part, explain why the lowest fly counts were observed in CON steers on d 61 of the study. Furthermore, administration of anthelmintics may have reduced the number of fly larvae present in the feces enough to decrease the number of flies observed on CON steers.

In addition to commingling of treatment groups, the type of rotational grazing method used in this study may have affected fly counts. In typical rotational grazing situations, paddocks are grazed based on forage availability followed by a period of rest. For example, in a grazing situation utilizing 15 paddocks, each paddock is to be grazed for one week with cattle rotated to a fresh paddock. This would allow 14 weeks rest before cattle would graze the same paddock (Barger, 1999). The 14-week rest period greatly exceeds the average life cycle of horn flies, which Summerlin et al. (1984) estimated to be approximately 26 d. Using this system of rotational grazing would potentially decrease the number of flies present on the day of observation. Also, FEC in this study may have been affected by the method of rotational grazing used. Peterson and Gerrish (1995) observed that rotational grazing ensures more even distribution of manure in pastures, which would reduce the risk of ingesting strongyle eggs previously shed in the feces. Therefore, it is not surprising that since little differences were observed in FEC and fly counts, few differences were observed in hematology parameters evaluated. Although differences were observed in EOS, all values observed in this study fall within normal hematology ranges reported for bovine (Appendix 4).

Weather may have impacted several variables in this study as well. Horn fly numbers often decline during hot and dry conditions due to drying of manure patties before larvae completely develop (Boxler, 2015). The average actual temperature for the weeks leading up to d 0 and 31 fly count observations were approximately 2 °C higher compared to normal high temperatures based on historical data (NOAA NESDIS, 2016). In addition, the actual rainfall for the same periods were lower than historical rainfall (Appendix 3; NOAA NESDIS, 2010). Higher than average temperatures and lower than average rainfall could have reduced the number of flies available for observation on d 0 and 31. In addition, rainfall from d 27 – 56 was a total of 9.95 inches, 5.42 inches higher than normal (NOAA NESDIS, 2010). Higher than average rainfall could contribute to higher fly counts; maintaining moisture in manure patties long after it would normally dry out would prevent the death of fly larvae.

Body weight and ADG was similar between treatments throughout the study with the exception of LAE versus COMBO treated steers on d 56; however, CON steers exhibited the highest BCS compared to either treatment group. Curiously, four of the ten calves in the COMBO treatment group were treated for suspected respiratory disease by d 56 of the study compared to only one CON steer and no LAE steers. Although hematology variables were considered normal, the lower EOS concentrations observed in COMBO steers may indicate greater stress and could have contributed to decreased performance observed in COMBO steers on d 56 of the study.

Overall, data suggest that anthelmintic treatment may have affected FEC, ADG, and hematology parameters in newly received stocker calves; however, further study is needed to compare effectiveness of long-acting eprinomectin and moxidectin/oxfendazole combination in high-density grazing management schemes.

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Tables

Table 1: Effect of anthelmintic treatment on body weight[a] (kg) of newly received stocker calves.

	Treatment			
Day	CON (n=10)	COMBO (n = 9)	LAE (n = 10)	P-Value
0	300.73	296.67	295.82	0.79
27	326.63	313.44	319.29	0.43
56	337.46	320.61	338.45	0.14
101	359.46	346.67	357.82	0.44

[a] Least square means.

Table 2: Effect of anthelmintic treatment on average daily gain of newly received stocker calves.

	Treatment			
Period	CON (n=10)	COMBO (n = 9)	LAE (n = 10)	P-Value
0 - 27	2.09	1.37	1.83	0.24
27 - 56	0.84	0.54	1.29	0.12
56 - 101	1.08	1.27	0.94	0.28
Overall	1.28	1.09	1.35	0.40

Values represented as least square means.

Table 3: Effect of anthelmintic treatment on fly counts by day in newly received stocker calves.

	Day of the Study			
Treatment	0	31	61	100
CON (n = 10)	21.31[a]	7.46[b]	12.30[a,b]	19.62[a]
COMBO (n = 9)	33.46[a]	4.63[d]	14.59[c]	17.30 [b]
LAE (n = 10)	18.81[a]	5.10[b]	18.49[a]	20.10[a]

Values presented as least square means.

Different letters within the same row differ by $P \leq 0.05$.

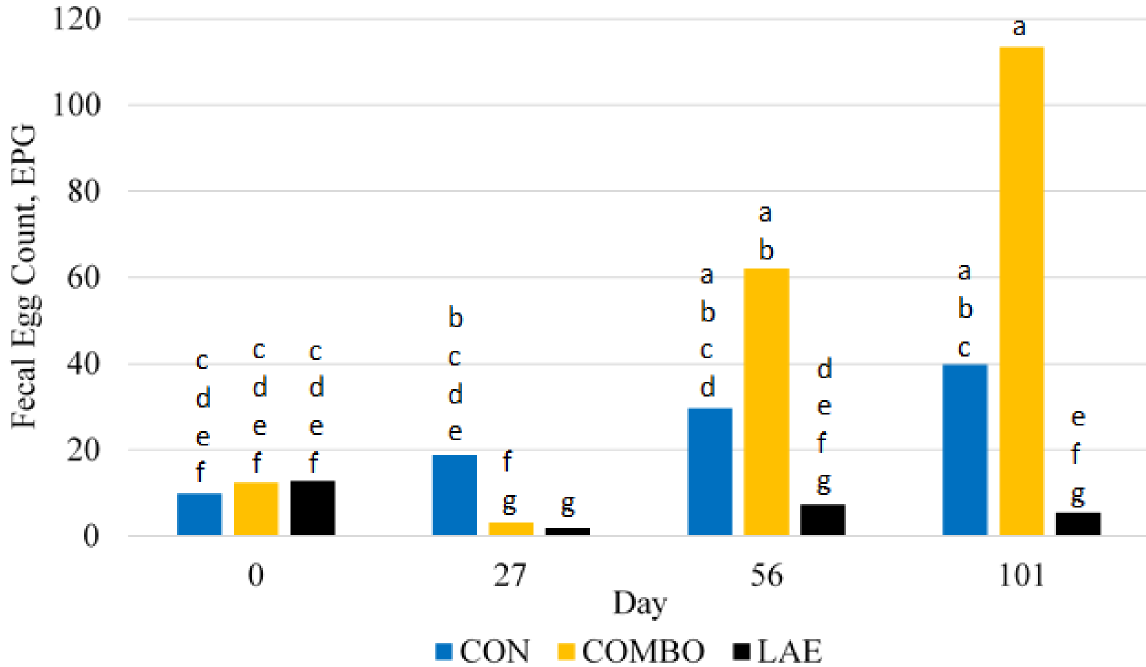
Table 4: Effect of anthelmintic treatment on hematology parameters in newly received stocker calves.

	Treatment			
Parameter	CON (n = 10)	COMBO (n = 9)	LAE (n = 10)	P-Value
WBC ($10^3/\mu L$)	9.81[a]	9.70[a]	10.50[a]	0.63
PLA ($10^3/\mu L$)	482.21[a]	480.85[a]	462.28[a]	0.87
NEU ($10^3/\mu L$)	2.93[a]	2.85[a]	3.03[a]	0.87
LYM ($10^3/\mu L$)	5.66[a]	5.85[a]	6.18[a]	0.73
MON ($10^3/\mu L$)	0.77[a]	0.68[a]	0.82[a]	0.17
BASO ($10^3/\mu L$)	0.15[a]	0.13[a]	0.14[a]	0.75
EOS ($10^3/\mu L$)	0.29[a,b]	0.19[b]	0.32[a]	0.03

Different superscripts within the same row differ by $P \leq 0.05$.

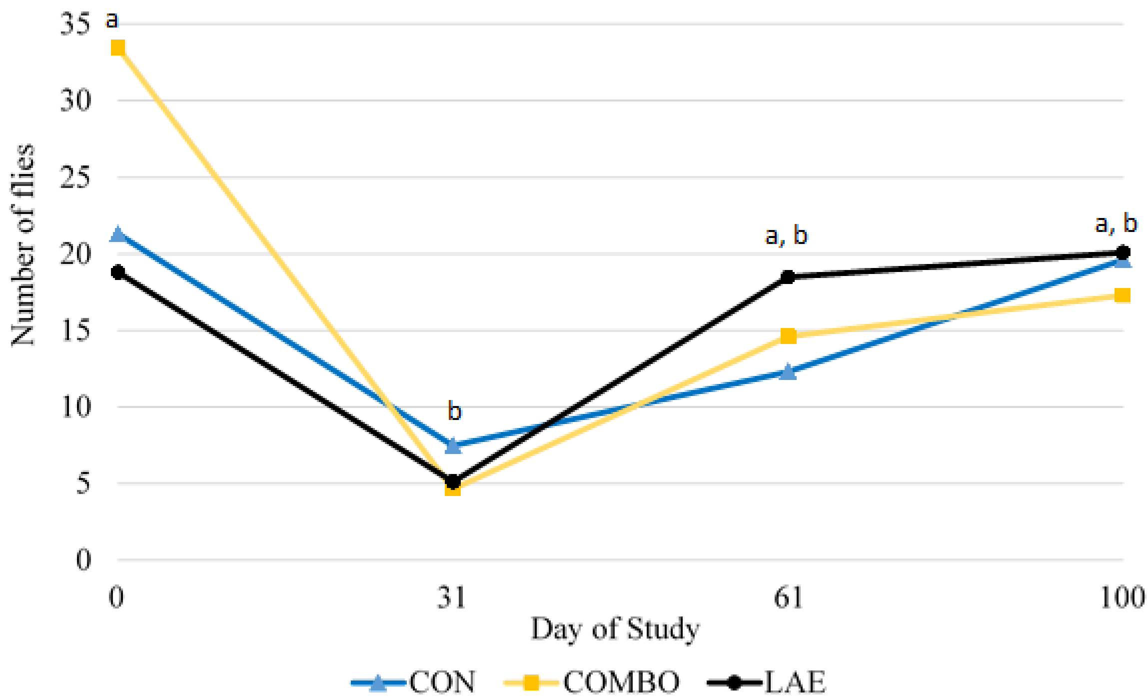
Figures

Figure 1: Effect of anthelmintic treatment on fecal egg counts in newly received stocker calves.



Different letters differ by P ≤ 0.05.

Figure 2: Effect of anthelmintic treatment on fly counts in newly received stocker calves by day.



Different letters tend to differ by P = 0.09.

Appendices

Appendix 1: Fecal Egg Count: Modified McMasters Protocol

1. Combine 4g of fecal material with 56mL of flotation solution for a total volume of 60mL.
2. Mix well. Strain through cheesecloth if large pieces of debris are present.
3. Immediately fill each chamber of the McMaster slide with the mixture using a disposable transfer pipette. The entire chamber must be filled to ensure an accurate reading. If large air bubbles are present, remove the fluid and refill.
4. Allow slide to sit for at least 5 minutes before examining, allowing the flotation process to occur.
5. Examine the slide under 10x magnification, focusing on the top layer containing air bubbles. At this level, the lines of the grid will also be in focus. Count strongyles eggs in each lane of both chambers.
6. The total egg count represents the number of eggs present in 0.3mL, which is 1/200th of the total volume (60mL). The total egg count must be multiplied by 200 (for the fraction of total volume) and divided by 4 (4g of feces used to make suspension) – or multiplied by 50.

Zajac, A. M. and G. A. Conboy. 2012. Veterinary Clinical Parasitology. 8th ed. Wiley-Blackwell, West Sussex, UK.

Appendix 2: Body Condition Score

1 = Emaciated – cow is extremely emaciated with no palpable fat detectable over spinous processes, transverse processes, hip bones or ribs. Tail-head and ribs project quite prominently.

2 = Poor – cow still appears somewhat emaciated but tail-head and ribs are less prominent. Individual spinous processes are still rather sharp to the touch but some tissue cover exists along the spine.

3 = Thin – ribs are still individually but not quite as sharp to the touch. There is obvious palpable fat along spine and over tail-head with some tissue over dorsal portion of ribs.

4 = Borderline – individual ribs are no longer visually obvious. The spinous processes can be identified individually on palpation but feel rounded rather than sharp. Some fat cover over ribs, transverse processes and hip bones.

5 = Moderate – cow has generally good overall appearance. Upon palpation, fat cover over rips feel spongy and areas on either side of tail-head now have palpable fat cover.

6 = High Moderate – firm pressure now needs to be applied to feel spinous processes. A high degree of fat is palpable over ribs and around tail-head.

7 = Good – cow appears fleshy and obviously carries considerable fat. Very spongy fat cover over ribs and around tail-head. In fact "rounds" or "pones" beginning to be obvious. Some fat around vulva and in crotch.

8 = Fat – cow very fleshy and over-conditioned. Spinous processes almost impossible to palpate. Cow has large fat deposits over ribs, around tail-head and below vulva. "Rounds" or "pones" are obvious.

9 = Extremely Fat – cow obviously extremely wasty and patchy and looks blocky. Tail-head and hips buried in fatty tissue and “rounds” or “pones” of fat are protruding. Bone structure no longer visible and barely palpable. Animal’s motility may even be impaired by large fatty deposits.

Richards, M. W., J. C. Spitzer, and M. B. Warner. 1986. Effect of varying levels of postpartum nutrition and body condition at calving on subsequent reproductive performance in beef cattle. J. Anim. Sci. 62:300-306.

Appendix 3: Actual and Historical Weather Data for the Week Preceding Fly Count Observations

Day	Actual High Temperature[1] (°C)	Historical High Temperature[1] (°C)	Actual Rainfall[2] (in)	Historical Normal Rainfall[3] (in)
0	26.2	24.7	1.03	1.29
31	31.2	29.1	0.0	1.14
61	32.0	31.7	1.88	0.96
100	28.7	31.7	2.17	0.78

[1] Calculated by averaging maximum temperature for the 7 days before collection date, using the same date period for historical.

[2] Calculated by adding rainfall per day for the 7-day period before collection date.

[3] Calculated by multiplying average rainfall per day by 7.

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Appendix 4: Normal Hematology Ranges for Cattle

Variable ($10^3/\mu L$)	Range
White Blood Cells (WBC)	4.0 - 12.0
Platelets (PLA)	100 - 800
Neutrophils (NEU)	0.6 - 4.0
Lymphocytes (LYM)	2.5 - 7.5
Monocytes (MON)	0.0 - 0.9
Basophils (BASO)	0.0 - 0.2
Eosinophils (EOS)	0.0 - 2.4

Merck Veterinary Manual. 2015. Hematologic Reference Ranges. http://www.merckvetmanual.com/mvm/appendixes/reference_guides/hematologic_reference_ranges.html. (Accessed 8 November 2016.)