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EFFECTS OF VARIOUS ANTHELMINTIC TREATMENTS ON FECAL EGG COUNTS, PERFORMANCE VARIABLES, BLOOD PARAMETERS, AND LACTATE DEHYDROGENASE ACTIVITY IN STOCKER CALVES

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**EFFECTS OF VARIOUS ANTHELMINTIC TREATMENTS ON FECAL EGG
COUNTS, PERFORMANCE VARIABLES, BLOOD PARAMETERS, AND
LACTATE DEHYDROGENASE ACTIVITY IN STOCKER CALVES**

A Thesis
Presented to
the Faculty of the Department of Animal Science
Murray State University
Murray, Kentucky

In Partial Fulfillment
of the Requirements for the Degree
of Master of Science in Agriculture

by Cheyenne Auburn Hooks
May 2018

I would like to dedicate my thesis to my father, Craig Hooks,
who sacrificed every day so that I would succeed.
I love you daddy.

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Abstract

Anthelmintic treatment is known to improve cattle performance; however, effects of long-acting eprinomectin (LAE) and co-treatment (Co-Trt) use has not been widely evaluated. Lactate dehydrogenase is an important cytoplasmic enzyme which can serve as an indicator of cellular damage but little information is available regarding differences in activity of calves receiving various anthelmintics. Therefore, the objective of this study was to evaluate the effects of various anthelmintic treatments on fecal egg counts (FEC), performance, blood parameters, and lactate dehydrogenase (LDH) activity of newly received stocker calves. This study consisted of 125 Angus based cross bred steers grazed during summer months of 2016 (Exp 1) and 2017 (Exp 2). Anthelmintic treatments consisted of: Control (CON), long-acting eprinomectin (LAE), dual oxfendazole and moxidectin administration (COMBO), and oxfendazole on d 0 followed by delayed moxidectin on d 45 (O+M). Fecal samples were recorded and body weight (BW), body condition scores (BCS), hair coat scores (HCS), and fly counts determined. Jugular blood was collected for determination of complete blood cell count data and serum lactate dehydrogenase activity. Data was analyzed using the MIXED procedure of SAS with preplanned orthogonal contrasts used. Body weight tended ($P=0.09$) to be greater for LAE versus Co-Trt steers in Exp 2. Greater BCS ($P<0.01$) were observed for O+M versus COMBO steers in Exp 2 and tended to be greater for LAE versus Co-Trt steers by the end of the study. Average daily gain was affected by treatment at various points throughout the study but were similar ($P=0.86$) between LAE and O+M steers overall during Exp 2. During both Exp, COMBO steers exhibited a lesser degree of shedding compared to other treatments. Fly counts were not affected by treatment in either Exp but

were affected by d ($P < 0.01$). A treatment by day interaction ($P < 0.01$) was observed in Exp 2 for RBC with effects of d ($P < 0.01$) being observed for several blood parameters. Eosinophils were higher ($P = 0.03$) in LAE versus COMBO steers in Exp 1. Serum LDH activity was lowest in O+M steers and differed ($P = 0.01$) from values observed in COMBO steers suggesting that oxidative stress may have occurred in the COMBO treated steers. Data suggests anthelmintic use can reduce FEC and may improve performance and that delayed that versus dual Co-Trt anthelmintic administration may be beneficial. Furthermore, differences may exist in LDH activity in stocker calves treated with various anthelmintics.

Keywords: Anthelmintic, bovine, lactate dehydrogenase

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Chapter 1

Justification

In the United States, approximately 40% of the cattle population currently resides in the south (USDA-NASS, 2014). Favorable environmental conditions create opportunities for internal parasites to flourish and can account for millions of dollars lost in the cattle industry each year (Kunkle et al., 2013; Rehbein et al., 2013). These losses occur from decreased weight gains and potential decreases in feedlot performance. Although anthelmintic use can be expensive for beef producers, the potential negative effects of failing to treat for internal parasites may be more expensive due to decreased rate of gain, performance, and unthriftiness (Corwin, 1997).

Over the last few years, a new anthelmintic has become available. LongRange™ is a long-acting eprinomectin that has been reported to be an effective dewormer for beef cattle with an efficacy up to 150 days for internal parasites and up to 60 days for external parasites (Soll et al., 2013). One disadvantage of this product is that it is expensive, which often discourages its use. Co-treatment application of anthelmintics may be another option for beef producers due to potentially increased efficacy from using anthelmintics with dual modes of action at a reduced cost. The cost for co-treatment

application is approximately \$5 per dose for a 550 lb steer compared to approximately \$8 for LongRange™.

While there have been several studies evaluating the effectiveness of various types of anthelmintics, little information is available concerning differences in lactate dehydrogenase (LDH) activity based on anthelmintic use. Lactate dehydrogenase is a soluble enzyme found in the cytoplasm of cells and is released into the extracellular environment due to disruption of the plasma membrane in instances of disease or injury in the body (Burd and Usategui-Gomez, 1973).

Results from this study may provide insight for Kentucky beef producers on alternatives for anthelmintic use by improving profitability and performance of cattle managed in a high stock density grazing situations. Ensuring animal health and welfare is an important concern for producers and consumers alike. This study will provide useful information concerning animal health as indicated by evaluation of LDH activity, a potential indicator of cellular damage. Furthermore, this study may be used as a tool for developing better anthelmintic practices and reducing the need for anthelmintic treatment which ultimately leading to decreased resistance to anthelmintics.

Objective

Therefore, the objective of this study was to evaluate the effects of various anthelmintic treatments on fecal egg counts (FEC), performance, blood parameters, and lactate dehydrogenase (LDH) activity of newly received stocker calves.

Chapter 2: Review of Literature

Parasite Infections

Anthelmintics are used worldwide to control external and internal parasites affecting livestock (Floate et al., 2004). Internal parasitic infections can be classified in three separate categories: the infection itself, clinical manifestation, and economic impact. Parasite infection is defined as the presence of parasites within the animal which may or may not result in clinical signs of infection. Infection is universal and at a constant equilibrium between the host animal and parasite, while clinical parasitism results when there is an adverse reaction between the host animal and the parasite (Craig, 1988). Economic losses typically occur when the level of internal parasitism escalates until it begins to affect performance of the host animal and may be affected by numerous other factors, such as quality of feed and forage, age/sex of the animal, and genetics (Craig, 1988).

Although there are many highly effective anthelmintics on the market, anthelmintics must be used correctly with consideration of the parasite/host interaction to obtain the favorable response, minimize development of resistance, and accomplish parasitic control (Vercruysse et al., 2017). Generally, anthelmintics have a wide margin

of safety when used according to manufacturer's recommendations. Anthelmintics have also been reported to have broad spectrum of activity against helminths at the adult and larval stages. Although helminth infections are currently satisfactory by chemotherapeutic control, there is increasing concern that anthelmintic resistance may develop (Vercruysse et al., 2017).

Because vast differences exist in management techniques in livestock production operations, it is not feasible to give generic recommendations for parasitic control to producers (Kumar et al., 2012).

Classes of Anthelmintics

Anthelmintics must have targeted selective toxicity (TST) to the parasite in question to work. This TST is achieved either by inhibiting metabolic processes that are vital to the parasite or by inherent pharmacokinetic properties that cause the parasite to be exposed to higher levels of anthelmintics than the host cells (Vercruysse et al., 2017). Although there are many classes of anthelmintics available, the most common dewormers used in cattle today are the macrocyclic lactones and benzimidazoles. (Vercruysse et al., 2017).

Benzimidazoles. Benzimidazoles are a large chemical family of anthelmintics used to treat nematodes and trematodes in animals but has limited action against cestodes. Commercially available benzimidazoles include: mebendazole, flubendazole, fenbendazole, oxfendazole, oxibendazole, albendazole, albendazolesulfoxide, thiabendazole, triophanate, febantel, netobimin, and triclabendazole; however, only oxfendazole is commonly used to treat internal parasitism in cattle. Due to increased

concern for anthelmintic resistance, benzimidazole use in ruminants has decreased in recent years (Vercruysse et al., 2017).

Benzimidazoles are partially soluble in water, hence, they are generally given orally as a paste, bolus, or suspension. The rate and extent of absorption from the gastrointestinal (GI) tract depend upon species, solubility, formation, operation of the esophageal groove reflex and dosage. Oxfendazole is one of the benzimidazoles that has a longer half-life, which is not as rapidly metabolized to inactive products (Vercruysse et al., 2017).

In ruminants, treating with benzimidazoles orally removes the majority of the adult GI parasites and larval stages. Systematic anthelmintic activity is greater in sheep than compared to cattle with benzimidazoles with the dosage rates being higher in cattle compared to sheep (Vercruysse et al., 2017). Macrocyclic lactones have controlled the majority of the market share as the preferred anthelmintic since the product has become available (McArthur and Reinemeyer, 2014). In 2007, macrocyclic lactones held 98% of the market share (Fort Dodge Animal Health personal communication, 2007) which had declined to 82% in 2012 due to increased use of benzimidazoles (Boehringer Ingelheim Vetmedica Inc. personal communication, 2013).

Macrocyclic lactones. The macrocyclic lactones are a chemical derivatives or products of soil microorganisms that belong to the genus *Streptomyces*. Two different forms of macrocyclic lactones are available, the avermectins and the milbemycins. Commercially available avermectins are: ivermectin, abamectin, doramectin, eprinomectin, and selamectin. Commercial products of milbemycins include: milbemycins oxime and moxidectin (Vercruysse et al., 2017).

The macrocyclic lactones are a potent anthelmintic that act against endoparasites and ectoparasites in a wide range of hosts with a single therapeutic dose acting against new nematode infections for a prolonged period of time. Macrocyclic lactones have broad anti-parasitic action and can be administered orally, parenterally, or as a pour-on. When administered as a pour-on, it is less effective than if it was administered orally or parenterally (Vercruysse et al., 2017). Regardless of the route of administration, macrocyclic lactones are well absorbed and distributed throughout the body where they concentrate in the adipose tissue (Vercruysse et al, 2017).

Because they are extremely potent, have high efficacy rates and are inexpensive to use, macrocyclic lactones have become the preferred anthelmintic for beef producers (McArthur and Reniemeyer, 2014). However, some research indicates that overuse has resulted in decreased efficacy and increased development of resistance (Anziani et al., 2004).

There is increasing concern that macrocyclic lactone residues may accumulate in the feces and soil. Furthermore, macrocyclic lactones can be highly toxic to certain species of aquatic species. However, because macrocyclic lactones have tight soil-binding properties, exposure of leaching and run-off is minimal making it highly unlikely that the use of macrocyclic lactones will have a significant ecological impact on a regional or global scale (Vercruysse et al., 2017). Furthermore, studies have shown that the half-life of macrocyclic lactones in winter environments in the northern hemisphere to range from 91-217 days and only 7-14 days during summer months (Vercruysse et al., 2017).

Imidazothiazoles. Levamisole is the most common imidazothiazole used to treat nematode infections in cattle and other livestock species but has no action against tapeworms or flukes. In ruminants, Levamisole (Ergamisol®) lacks efficacy against arrested larvae, but is highly effective against several larval stages, lungworms, and adult gastrointestinal nematodes (Vercruysse et al., 2017). Levamisole can be administered orally or subcutaneously, with equivalent efficacy using either administration. There has been some work developing a topical form for Levamisole, but it is not commonly used compared to other routes of administration (Vercruysse et al., 2017). Since imidazothiazoles are not commonly used to treat internal parasites affecting cattle in the U.S., there is little availability in the marketplace to acquire them (McArthur and Reinemeyer, 2014).

Anthelmintic Resistance

There is increasing concern about development of anthelmintic resistance in parasites affecting livestock. Resistance can be defined as a decrease in efficacy that is measurable against parasite species during stages that the parasites were previously susceptible (McArthur and Reinemeyer, 2014). Use of highly effective anthelmintics in conjunction with high stocking rates of cattle due to rotational grazing has the potential to increase profitability for beef producers. Unfortunately, many producers rely too heavily on anthelmintic use to control parasitism rather than changing management strategies and searching for alternative means of parasite control. Thus, increasing the risk of anthelmintic resistance (Gasbarre et al., 2009).

Resistance to nematodes has been shown in small ruminants, but limited research is available concerning nematodes affecting cattle. This has led to a theory that the

immune system of cattle may be more capable of handling gastrointestinal nematodes compared to small ruminants, and that anthelmintic resistance was less likely to develop due to less frequent usage compared to small ruminants (Gasbarre et al., 2009). However, as anthelmintics become a more popular means of parasite control, there have been reports of parasite resistance to anthelmintics in New Zealand (Familton et al., 2001), Europe (Stafford and Coles, 1999), South America (Anziani et al., 2001 and 2004; Fiel et al., 2001; Mejia et al., 2003; Ramos et al., 2015) with increases concern for development of resistance in the U.S. (Gasbarre et al., 2004). In fact, the first case of anthelmintic resistance of gastrointestinal nematodes in the U.S. was reported by Gasbarre et al. in 2009.

Maintaining parasite refugia in pastures is an alternative measure that can be taken to slow parasite resistance to anthelmintics. Refugia is the population of parasites that have not been exposed to anthelmintics that is capable of being ingested by the host (van Wyk and Reynecke, 2011). Refugia can be found on pasture and within untreated animals. Pastures that have been cleaned, reseeded, and experienced long periods of drought have reported low parasite refugia. Therefore, it is largely accepted that anthelmintic treatments applied when refugia is high correlate with a lower outcome of resistance, rather than when refugia is low when parasite resistance can be higher (Bartley, 2011).

Development of anthelmintic resistance of parasites can have profound impacts on livestock production, health, and welfare. Therefore, it is imperative that producers be educated about anthelmintic resistance to minimize and potential losses (Coles et al., 2004 and 2006).

Economic Impacts

Beef production in Kentucky is dominated by small family farms with producers typically reporting fewer than 50 head (USDA NASS, 2012). National cattle prices have declined over the past two years in comparison to the high of 2015. The USDA's National Agricultural Statistics Service reported January cattle prices to have dropped from \$164.00/cwt in 2015 to \$117.00/cwt in 2017 (USDA NASS, 2017). In order to ensure profitability, beef producers must seek other options for improving production and profitability without incurring additional cost.

There have been many worldwide reports stating that parasites have inflicted severe economic loss to the livestock industry (Kumar et al., 2012). In the Southeastern United States, where approximately 40% (11.8 million) of the beef cattle population resides, \$2.5 million in losses occur due to decreased weight gains (Kunkle et al., 2013; Rehbein et al., 2013). Mexico has roughly 32 million head of cattle and loses approximately \$1.4 million annually due to parasites with average losses of \$43.57 per head (Rodriguez-Vivas et al., 2015). In Mexico, the parasites causing the most significant economic losses are gastrointestinal nematodes (helminths), coccidia (*Eimeria spp.*), liver flukes (*Fasciola hepatica*), cattle ticks (*Rhipicephalus microplus*), horn flies (*Haematobia irritans*), and the stable fly (*Stomoxys calcitrans*) (Rodriguez-Vivas et al., 2015). Brazil's beef and dairy industry experience even greater losses, reporting losses of \$13.96 billion annually due to parasites affecting cattle. Similar to problematic parasites observed in the U.S. and Mexico, gastrointestinal nematodes, cattle tick, horn fly, and the stable fly have been found to significantly affect cattle performance as well as the cattle

grub (*Dermatobia hominis*) and screwworm fly (*Cochliomyia hominivorax*) (Grisi et al., 2014).

Although it can be difficult to determine the economic impact of parasites upon cattle due to differences in climatic conditions, herd size, management practices, herd health, and grazing protocol (Rodriguez-Vivas et al., 2015), the hot, moist and humid climate found in the Southeastern part of the U.S. promotes favorable conditions for the parasites to thrive (Kunkle et al., 2013; Rehbein et al., 2013). Cattle experiencing a significant parasite load can negatively impact the cattle industry due to low body weight gain resulting in increased production cost and reduced profitability for producers (Corwin, 1997). The greatest economic loss from parasites affecting cattle result from lowered milk production and reduced weight gains (Jonsson, 2006 and Rodrigues and Leite, 2013).

To fully understand the economic impact of parasitism, performance parameters must be evaluated. Corwin (1997) provided a thorough review on effect of gastrointestinal parasites of cattle performance including poor weight gain, reduced feed intake, and reduced reproductive performance and lactation. Furthermore, Hawkins (1993) reported that some internal gastrointestinal parasites can interfere with nutrient digestion and absorption of proteins. While it can be hard to determine the true economic impact of parasitism, it is widely agreed that there is need for a nationwide program that will aid producers control parasites without increasing parasite resistant to anthelmintics (Grisi et al., 2014). Although management and herd health differ between farms, better understanding and application of knowledge concerning parasitism will allow producers to make better management decisions (Hawkins, 1993).

Management Considerations

Nutritional Management. Type of diet and availability of minerals and vitamins, can directly affect an animal's ability to fight internal parasitic infection. Important vitamins needed to enhance an animal's natural immunity to parasitic infections include vitamin A, D, and the B complex vitamins while minerals such as potassium, phosphorus, and iron are required to support proper immune function (Hughes and Kelly, 2006). If young growing animals do not have the proper nutrition, their ability to withstand mild internal parasite infections may be limited due to poor immune development (Kumar et al., 2012). It may be beneficial for producers to consider age group where level of production when considering appropriate supplementation needed to withstand negative effects of parasitism (Sutherland and Scott, 2010). Furthermore, several studies have shown that protein supplementation of cattle can lead to increased resilience and better resistance against gastrointestinal nematodes (Coop and Kyriazakis, 2001; Knox et al., 2003). Since younger animals are more prone to direct damage due to parasitic infection, such as tissue damage, it is more feasible to strategically increase protein availability in growing animals (Holmes, 1993; Bown et al., 1991).

Pasture Management. It has been suggested that intensive rotational grazing schemes may reduce internal parasite numbers in cattle compared to continuous grazed pastures (Backes et al., 2016a). In this situation, large numbers of animals are grazed on small paddocks for specific periods of time then rotated to fresh paddocks based on forage availability. Ideally forages are grazed no lower than 4 to 6 inches from the ground allowing enough leaf area for plants to quickly recover from the grazing experience (Wells, 1999; Johns et al., 2004). In these situations, cattle will typically not be allowed

to return to the pasture for at least three weeks allowing sufficient time for manure piles to dry and the life cycle of parasites to be broken before returning to what's considered as a “clean” pasture (Kumar et al., 2012). Although labor intensive rotational grazing is considered more of a nutritional benefit for cattle due to increased forage production, the potential benefits of reducing parasite infection naturally cannot be overlooked (Kumar et al., 2012).

Since animals of different ages can have different levels of susceptibility to internal parasites, it may be beneficial to manage cattle in groups based on age. Parasite infestations are more commonly seen in younger animals upon weaning. The development of immunity against gastrointestinal nematodes can be acquired slowly. Immunity may be acquired up to two grazing seasons for cattle (Fox, 2018). Furthermore, multi-species grazing is another alternative that producers can utilize to improve forage quality and potentially lessen parasite infestation (Kumar et al., 2012). Whittier et al. (2003) concluded that sheep prefer to consume weeds, short tender grasses, and legumes while Kumar et al. (2012) indicated that cattle prefer to consume taller grasses. Thus, multi-species grazing may allow for reduction in parasite refugia in pastures by increasing sun exposure to the ground effectively killing parasites.

Lactate Dehydrogenase (LDH)

Molecular structure and physiological function. Cell injury can be described as disruption of normal cellular function without resulting in long-term adverse effects to the cell. However if damage is severe or irreversible, cell death may occur. Signs of cell injury include: deviations from the normal cell morphology, impaired cellular function, and the increased plasma membrane permeability (Danpure, 1984).

Lactate dehydrogenase (LDH) is a soluble cytoplasmic enzyme found in most cells throughout the body and is released into the extracellular environment when cellular damage occurs (Burd and Usategui-Gomez, 1973). Lactate dehydrogenase is the final enzyme in the glycolytic pathway responsible for converting pyruvate to lactate under anaerobic conditions reducing NAD^+ to NADH (Toyoda et al, 1985). During periods of intense exercise, LDH concentrations naturally rise due to increased production of lactic acid in the muscle as oxygen is depleted and cells begin to undergo glycolysis in anaerobic conditions. Although some species variation exists, five isoforms of LDH have been identified in mammals (Sobiech et al., 2002). Thus, determination of serum LDH activity and its isoenzymes may be a useful tool to provide preliminary health assessment for humans and animals (Bokina et al., 2008).

Isoenzymes are a group of enzymes which differ in their molecular forms, primarily the amino acid sequence of the protein, but elicit the same reaction while having specific affinity for various tissues (Hamm, 1990; Murray et al., 1994). Lactate dehydrogenase is a tetramer protein composed of two 35-kDa subunits commonly known as M (type A) and H (type B) (Market and Moller, 1959; Cahn et al., 1962; Li et al., 1983). Lactate dehydrogenase M (type A) is so named because it is primarily found in skeletal muscle and liver cells whereas LDH-H (type B) is primarily found in cardiac tissue (Kolaric et al., 1975). The five isoenzymes of LDH found in most mammalian species result from various combinations of the type A and B subunit: $\text{LDH1} = \text{B}_4$, $\text{LDH2} = \text{A}_1\text{B}_3$, $\text{LDH3} = \text{A}_2\text{B}_2$, $\text{LDH4} = \text{A}_3\text{B}_1$, and $\text{LDH5} = \text{A}_4$ (Goldberg, 1963). The isoform LDH1 is found in the greatest concentrations in cardiac muscle and kidney and in cattle and sheep, LDH1 is also found in the liver (Smith, 2009). Isoform LDH5 is primarily

found in the skeletal muscle erythrocytes (Smith, 2009). Isoenzyme LDH activity in the tissue has been reported to be 500 times greater than levels found in the serum, thus leakage from the tissue may account for higher concentrations of LDH in the serum (Lott and Nemensanszky, 1987).

Previous research. In humans, LDH and its isoenzymes are primarily used as a diagnostic aid for pathological conditions in cardiology, hematology, hepatology, and oncology (Huijgen et al., 1997). Pancreatic cancer is one of the most lethal commonly occurring cancers that may remain undiagnosed until advanced stage of development when the cancer has become resistant to treatment (David et al., 2014). Pancreatic cancer is also the fourth leading cause of death in Western countries and is projected to be the second leading cause of death within the next 10 years (Bailey et al., 2016). Identifying means of early detection of pancreatic cancer may increase survivability of patients. A study conducted by Yu et al. (2017) suggested that serum LDH levels may be associated with the overall survivability rate of pancreatic cancer patients. Findings suggest that higher levels of serum LDH were associated with lower overall survivability of pancreatic cancer patient, while lower level of serum LDH were associated with higher overall survivability (Yu et al., 2017).

Few studies have been conducted investigating the diagnostic value of using LDH activity in the diagnosis of respiratory damage in veterinary medicine (Nagy et al., 2013). However, in human medicine, several cases have been reported indicating that LDH and isoenzymes activity proved useful in determine lung damage and inflammation in various respiratory diseases (Drent et al., 1996). In the study by Nagy et al. (2013), calves treated for suspected bovine respiratory disease (BRD) exhibited significantly higher LDH

activity compared to clinically health calves. However regardless of health status, the isoenzyme LDH1, which is primarily associated with cardiac muscle tissue, was found in greater concentrations followed by LDH2, LDH3, LDH4, and LDH5 in decreasing order. Isoenzyme LDH1 represented 53.7% of total LDH activity in BRD calves in comparison to 41.1% in health calves. Nagy et al. (2013) suggested that the higher LDH1 values observed in calves experiencing lower respiratory tract distress may have originated from damage to epithelial cells lining the airways.

A study conducted by Bokina et al. (2008) investigated LDH activity, isoenzyme patterns, and hematological patterns in miniature horses and Thoroughbreds. Isoenzyme patterns indicated that LDH3 was detected in the greatest concentration followed closely by LDH1 and LDH2, with LDH5 having the lowest reported values. Bokina suggested that since LDH3 is found primarily in lung tissue, results from this study may have been due to the slightly higher than normal respiration rates observed in the horses. According to Nappert and Johnson (2001), the plasma LDH activity at resting is typically 1.5 mmol/l for equine. In the study by Bokina et al. (2008), miniature horses had slightly higher LDH levels compared to Thoroughbreds. Elevated LDH concentrations observed in miniature horses may have resulted from stress due to lack of human contact prior to start of the study or from housing location. Nogueira et al. (2002) reported increased LDH activity in horses with free access to pasture compared to horses housed in stalls which was similar to results observed by Bokina et al. (2008).

Some studies have suggested that decreased LDH activity may be associated with improved growth and performance in cattle. In a study investigating carcass quality in steers, Paria (1997) found that reduced reversed LDH activity (LDHr), meaning the

conversion of lactate to pyruvate, was associated with increased carcass quality in steers (Paria, 1997). Work by Flores et al. (2005) suggested that reduced LDHr activity was associated with increased reproductive performance of heifers. Looper et al. (2002) theorized that because LDH is the final enzyme of the glycolytic pathway, it may serve as an indicator of metabolic maturation in cattle. Decreased LDHr activity in pre-partum cows 62 days before calving resulted in taller and heavier calves at weaning (Looper et al., 2008). Furthermore, when used in conjunction with ultrasonography and evaluation of physical body measurements of the cow, decreased LDHr activity in prepartum cows may allow for earlier selection of superior calves earlier in the production cycle, possibly in-utero, increasing profitability in cow/calf operations (Looper et al., 2008).

Breed differences also exist for LDH activity in cattle (Sobiech et al., 2002, Arai et al., 2003). Although values reported were within normal ranges, serum LDH concentrations were greater in Limousin cattle compared to Holstein Friesian dairy cattle (Sobiech et al., 2002). With regard to specific isoenzyme patterns observed by Sobiech et al. (2002), LDH1 activity was lower in beef cows compared to dairy cows and that LDH4 and LDH5 activity was higher in beef cattle which is natural when considering both isoforms have higher affinity for skeletal muscle tissue. Interestingly, a study by Munoz et al. (2002) found that mares had higher LDH activity compared to stallions, which may be due to natural hormonal pre-disposition.

Early detection of mastitis in dairy cattle has the potential to save the dairy industry millions of dollars (Lightner et al., 1988; Kaneene and Hurd, 1990; Miller et al., 1993). Research by Bogin and Ziv (1973) indicated that elevated LDH activity observed in cows with mastitis originated from leukocytes in mastitic milk, as well as, mammary

epithelial and interstitial cells that become damaged during the inflammatory process. Data suggested that LDH activity in milk may provide insight into subtle changes in mammary gland function leading to improvements in preventative and treatment measures for mastitic cow (Bogin and Ziv, 1973). More recent data suggested that elevated LDH concentration in milk from subclinical mastitic cows may be due to damage and infection between the blood-milk barrier allowing LDH to be transferred from the blood into the milk (Babaei et al., 2007).

In a study conducted by Doornenbal et al. (1988), LDH concentration differed in crossbred cows according to stage of lactation. The lowest LDH concentration were observed in dry cows compared to values observed within 1 day following parturition and further increased at peak lactation 80 d postpartum (Doornenbal et al., 1988). Sobeich et al. (2002) hypothesized that the increased LDH1 activity observed in dairy versus beef cows may have been due to higher productivity and increased demand upon the liver for metabolization of nutrients associated with high levels of milk production. Similar results were observed by Owens et al. (1998) who found that LDH activity in the blood tends to increase in animals experiencing acidosis. Like Sobeich et al. (2002), Owens et. (1998) hypothesized that greater demand, or stress, upon the liver to metabolize lactic acid may be the cause of elevated LDH suggesting that LDH activity may serve as a means to identify animals at risk for developing acidosis (Bevans et al., 2005).

Elevated LDH have also been associated with of liver injury, lung damage, muscle disease, and neoplasia (Chattaerjea and Shinda, 2008). Hepatocellular injury has resulted in increased LDH activity, but unless isoenzyme analysis is performed, LDH elevations are not organ specific (Cardinet, 1997). Chronic muscle disease or injury

results in elevated LDH activity and is reported to be associated with selenium and Vitamin E deficiencies in cattle (Allen et al., 1975), sheep (Whanger et al., 1970), and swine (Ruth and Van Vleet, 1974).

Summary

Little information is available regarding LDH activity in newly received calves receiving different anthelmintic treatment. Because anthelmintic metabolization typically occurs in the liver, it is possible that oxidative cell damage may occur thus allowing LDH activity to provide more information regarding the overall health of animals. Therefore, the objective of this study was to evaluate the effects of various anthelmintic treatments on fecal egg counts (FEC), performance, blood parameters, and lactate dehydrogenase (LDH) activity of newly received stocker calves.

Chapter 3: Materials and Methods

Introduction

The goal of the beef industry is to produce high quality beef for human consumption. Raising cattle is not only a source of income, but a way of life and tradition for farmers and ranchers. Thus, it is important that we pursue all avenues that will increase profitability. One of the ways that this can be achieved is by controlling harmful internal parasites which decrease production, and ultimately decreases profitability.

There have been a number of different anthelmintics that have become available over the years. One of the most popular anthelmintics is a product called LongRange™, which is effective at treating internal parasites up to 150 days as well as external parasites for up to 60 days. However, producers are often discouraged from using this product because of its high cost. Another option for producers is co-treatment application of two anthelmintics with different modes of action. While co-treatment is possible, little information is known about differences in blood parameters and lactate dehydrogenase activity in stocker calves treated with various anthelmintics.

Lactate dehydrogenase (LDH) is an important enzyme involved in glucose metabolism and is found in the cytoplasm of cells throughout the body (Burd and Usategui-Gomez, 1973). However, oxidative stress and inflammation are known to cause

elevated levels of LDH in the blood. Low levels of LDH during gestation have been associated with increased growth and performance in calves at weaning (Looper et al., 2008), increased carcass quality in steers (Paria, 1997), and improved reproductive performance in heifers (Flores et al., 2005).

Therefore, the objectives of this study were to evaluate the effects of various anthelmintics on fecal egg counts, performance, blood parameters, and various anthelmintics in newly received stocker calves.

Materials and Methods

Two experiments were conducted in this study investigating effects of various anthelmintics on FEC, growth, performance, and blood parameters in stocker calves. All materials and methods used were conducted in accordance with the Murray State University's Animal Care and Use Committee (Experiment 1: IACUC # 2016-028; Experiment 2: IACUC # 2017-040). Two groups of animals were used, each belonging to a private producer and custom grazed at the Murray State University Beef Unit from May through August 2016 (Experiment 1) and May through September 2017 (Experiment2).

Experiment 1.

Animals and Management. Experiment 1 consisted of 66 predominantly Angus based crossbred steers. Steers were allowed a three d adjustment period upon arrival to the facility prior to allocation of treatment based on initial FEC (13.12 ± 0.08 EPG), BW (296.41 ± 23.67 kg), and BCS (5.04 ± 0.09).

All steers received vaccinations upon arrival to the beef unit. Vaccinations consisted of Draxxin (Zoetis Services LLC, Parsippany, NJ), Inforce 3 (Zoetis Services LLC, Parsippany, NJ), One Shot Ultra 8 (Zoetis Services LLC, Parsippany, NJ), Ultrabac

7/Somubac (Zoetis Services LLC, Parsippany, NJ), Multimin 90 (Multimin USA, Fort Collins, CO), and Synovex S (Zoetis Services LLC, Parsippany, NJ). Steers were given booster vaccinations 2 weeks after arrival to the beef unit. Booster vaccinations consisted of Bovi-Shield Gold 5 (Zoetis Services LLC, Parsippany, NJ) and Ultrabac 7/Somubac (Zoetis Services LLC, Parsippany, NJ).

Treatments. Treatments were applied on d 0 of the study and included the following treatment groups: Control, which received no anthelmintic treatment (CON; n = 10); long-acting eprinomectin (LongRange, Merial Inc., Duluth, GA; LAE; n = 28); and a simultaneous administration of an oral oxfendazole (Synanthic, Boehringer Ingelheim Vetmedica Inc., St Joseph, MO) and an injectable moxidectin (Cydectin injectable, Boehringer Ingelheim Inc., St Joseph, MO) combination (COMBO; n = 28). Steers were commingled and allowed to graze mixed grass pastures (0.4 – 0.8 ha) using a management intensive grazing system (47,255.4 kg/ha) with pasture rotation based on forage availability

Data Collections. Fecal samples were collected and BW, BCS, and HCS recorded on d 13, 27, 56, 90, and 101. Fecal samples were collected from each steer rectally after being restrained in a chute system. Fecal egg counts were determined using a Modified McMaster's protocol (Appendix D). Briefly, FEC were determined using a two-chambered procedure where a 4 g sample of fecal material was added to 56 mL of sucrose, mixed thoroughly and filtered through a fecal tube. Prepared samples were stored at 2°C while FEC were determined. Prior to reading samples, samples were inverted 10 times and pipetted onto the slide. Samples were allowed to sit for approximately 1 minute before being analyzed under a microscope at 10X magnification.

The number of strongyle eggs were determined by counting the number of eggs within the grid lines of both chambers of the slide and recorded by two trained observers and FEC averaged. The eggs per gram (EPG) were then calculated by multiplying the number of strongyle eggs observed by 50.

Body weight was recorded using an electronic scale (Tru-Test EZIWeigh5, Mineral Wells, Texas) and BCS was determined upon exit of the chute based on a scale from 1-9, with a score of 1 being emaciated and a score of 9 being extremely fat (Richards et al., 1986: Appendix B). Hair coat scores were also conducted and ranged from 1-5, with a score of 1 indicating complete shedding of the winter hair coat and a score of 5 indicating that the full winter coat remained (Brown et al., 2014; Appendix C). Body condition scores and HCS were conducted by the same technician throughout the study.

Twenty-nine steers were randomly selected (CON, n = 10; COMBO, n = 9; LAE, n = 10) for evaluation of blood parameters. Whole blood was collected on d 0, prior to application of treatment and again on d 27, 56, and 101 using jugular venipuncture into 5 ml vacuum tubes containing EDTA (Monoject, Covidien, Mansfield, MA) and 10 ml vacuum tubes (Monoject, Covidien, Mansfield, MA) free of anticoagulants. Blood samples were stored on ice upon collection. Samples containing EDTA were immediately transported to Breathitt Veterinary Diagnostic Center within two hours of collection for determination of CBC data. Blood parameters evaluated included red blood cells (RBC), hemoglobin (HEMO), hematocrit (HCT), white blood cells (WBC), platelets (PLA), neutrophils (NEU), lymphocytes (LYM), monocytes (MONO), eosinophils (EOS), and basophils (BASO). Samples without anticoagulant were allowed to clot at

room temperature for 30 minutes and centrifuged at 12,000 x G and serum extracted.

Serum was pipetted and stored in 1.7 ml microcentrifuge tubes at -20°C.

Individual flies were counted on both sides of steers on d 14, 31, 61, 91, and 100 to monitor external parasite load. Flies were counted individually until the number of flies reached 25, then counted in groups of 5 focusing on the head, neck, shoulder, back, middle, and rump of the animal (Steelman et al., 1991). Throughout the course of the study, fly counts were performed by the same two trained technicians from an all-terrain vehicle while steers grazed in the pasture; fly counts were then averaged.

Statistical Analysis. Statistical analysis was performed using the Mixed procedure of SAS (SAS Inst. Inc., Cary, NC) where the experimental unit were individual steers with day as the repeated measure. Effects of anthelmintic treatments were evaluated for the following main effects: FEC, BW, ADG, BCS, HCS, blood parameters, and fly counts. Two preplanned orthogonal contrasts were used to determine effects and included comparisons between: 1) CON vs Trt steers and 2) COMBO vs LAE steers. Fecal egg count data and fly counts were log transformed to the $\log_{10}(X+1)$ with geometric means reported to reduce individual variation between steers.

Experiment 2.

Animals and Management. Experiment 2 consisted of 59 Angus based crossbred steers which were grazed at the Murray State University Beef Unit in Murray, Kentucky from May through September 2017 and involved similar management practices as described in Experiment 1. Steers were allowed a three d adjustment period upon arrival to the facility prior to allocation of treatment based on initial FEC (61.6 ± 1.3 EPG), BW (284.26 ± 5.79 kg), and BCS (4.6 ± 0.09). All steers received the same vaccinations as

the steers in Experiment 1, with the exception of the antibiotic, which was Zactran (Boehringer Ingelheim, Merial, Duluth, GA). The steers were commingled and allowed to graze mixed grass pastures (0.4 – 0.8 ha) using a management intensive grazing system (42,243.46 kg/ha) with pasture rotation based on forage availability.

Treatments. Anthelmintic treatment included those described in Experiment 1 (CON, n = 14; LAE, n = 15; COMBO, n = 15) plus one additional treatment where an oral oxfendazole (Synanthic, Boehringer Ingelheim Vetmedica Inc., St Joseph, MO) was administered on d 0 followed by delayed moxidectin (Cydectin injectable, Boehringer Ingelheim Inc., St Joseph, MO) treatment on d 45 (O+M; n = 15).

Data Collections. Data collections were similar to those described in Experiment 1. Ten steers per treatment were randomly selected for determination of complete blood cell count (CBC) data and lactate dehydrogenase (LDH) activity. Fecal samples were collected and BW, BCS, and HCS determined on d 15, 31, 63, and 92 of the study. Fecal egg counts were performed as described in Experiment 1 except that 26 ml of flotation solution were used instead of 56 ml to increase sensitivity. To account for the decreased total volume, FEC were calculated by multiplying the number of strongyle eggs counted by 25.

Whole blood was collected on d 0, 32, 63, and 92 using jugular venipuncture as described previously. Samples containing EDTA were used to determine CBC data while frozen serum samples were shipped on dry ice to Cornell University's Animal Health Diagnostic Center for determination of serum LDH activity. Lastly, fly counts were performed as described previously on d 0, 15, 32, 44, 64, and 93.

Statistical Analysis. Statistical analysis was performed as indicated in Experiment 1. Experimental unit was an individual steer with day as the repeated measure. Main effects included those described in Experiment 1 plus serum LDH concentrations. Analysis also included three preplanned orthogonal contrast: 1) CON versus Trt; 2) LAE versus co-treated (Co-Trt) which consisted of COMBO and O+M treated steers; and 3) COMBO versus O+M steers.

Chapter 4: Results and Discussion

Results and Discussion

Fecal Egg Counts. *Trichostrongylus spp.*, commonly referred to as strongyle(s), are an important nematode affecting performance in cattle and was the primary internal parasite evaluated during the course of the study. Strongyle FEC differed between CON and treated steers in Exp 1 (18.91 vs 10.22 EPG; $P = 0.05$) and Exp 2 (82.24 vs 16.60 EPG; $P < 0.01$) and fewer EPG were observed in LAE versus COMBO steers (5.88 vs 14.56 EPG; $P < 0.01$) during Experiment 1.

A treatment by day interaction was found for FEC in both Experiment 1 and Experiment 2 ($P < 0.01$). In Experiment 1 (Figure 1), FEC counts were highest in COMBO treated steers on d 101 (62.57) which was similar to COMBO steers on d 90 and 56 (47.35 and 44.73) and with CON steers on d 101, 56, and 27 (39.82, 29.67, and 18.86, respectively). The lowest FEC were observed in COMBO and LAE steers on d 13 (1.56 and 2.02) which was comparable to LAE and COMBO steers on d 27 (2.91 and 3.68).

In Experiment 2 (Figure 2), FEC were highest in CON steers on d 63 (107.3 EPG) and differed from LAE, COMBO, and O+M steers on d 92 (25.77, 29.09, and 19.57, respectively), 31 (12.36, 11.39, and 26.53; respectively), and 15 (4.63, 1.0, and 1.55

EPG) and with LAE and O+M steers on d 63 (19.69 and 14.46 EPG). The lowest FEC were observed in COMBO and O+M treated steers 15 d following anthelmintic administration which was similar to FEC observed in LAE steers (1.0, 1.55, and 4.63 EPG; respectively).

Little research is available comparing the efficacy of long-acting eprinomectin and simultaneous verses co-treatment applications of oxfendazole and moxidectin. Steers were commingled due to limited availability of pastures as well as reducing variability in the data due to stress and environmental factors. Unlike studies conducted by Backes et al. (2016a) and Walker et al. (2013), steers were commingled regardless of anthelmintic treatment which may have contributed a carryover effects between treatment groups. Normal herd behaviors such as grooming and grazing in close quarters may have also contributed to carryover effects. However, Craig (1988) stated that there is no satisfactory solution to the problem of whether or not to commingle or separate by treatment group for grazing and parasite studies.

A study conducted by Walker et al. (2013), investigated various combinations of oxfendazole and moxidectin. During this study, O+M calves (d 0 oxfendazole, d 73 moxidectin) had lower FEC than the CON calves on d 14, 31, and 45 of the study and were lower than CON and moxidectin calves on d 87 after the second anthelmintic (moxidectin) was applied on d 73. There have been few studies that have administered co-treatment application of anthelmintics similar to that used in the current study. Backes et al. (2016b), conducted a grazing study comparing the effects of long-acting eprinomectin and oxfendazole/moxidectin co-treatment in heifers and found that although FEC were similar at the beginning of the study, COMBO heifers displayed higher FEC

by d 84 compared to LAE heifers. However, by conclusion of the study, (154 d), no differences were observed in FEC among control and treated heifers.

During both experiments of the current study, a treatment by day interaction was observed for FEC making it impossible to separate effects of day from treatments imposed. Environmental conditions including weather conditions, disease, and stress may have contributed to effects of day observed in this study. Rotational grazing allows for increased forage production with additional benefits including even distribution of manure (Peterson and Garrish, 1995) and reduced parasite loads (Kumar et al., 2012). Although steers were managed similarly during both experiments with regards to pastures and facilities used, pastures were rotated based on forage availability allowing for a minimum of 21 d before animals were returned to the same paddocks to graze.

Trichostrongylus spp. eggs typically hatch within one day of being excreted in the feces and undergo five stages of development to perpetuate the live cycle (Levine, 1968). In stage 1, larvae feed on microorganisms and bacteria in fecal matter and molt into stage 2 larva within one to two days. Stage three infective larvae emerge within several more days and at this point are capable of being ingested by a new host. The infectious larvae then molt into stage 4 and finally adults (stage 5 of development) which is capable of releasing eggs and repeating the life cycle again (Levine, 1968). Since the average life cycle of strongyles is approximately three weeks, it is possible that management practices such as rotational grazing may have contributed to the low FEC observed.

Lactate dehydrogenase. Lactate dehydrogenase (LDH) is a soluble enzyme found throughout the body in the cytoplasm of cells and is released into the extracellular

environment when cellular or tissue damage occurs (Burd and Usategui-Gomez, 1973). Very little information is available comparing effects of anthelmintic treatment on serum LDH activity in stocker calves. In the present study, authors planned to evaluate serum LDH activity in both experiments. Unfortunately, malfunction of a freezer resulted in loss of frozen serum samples from Experiment 1. Therefore, serum LDH activity was only evaluated during Experiment 2, upon completion of data collections.

Contrast indicated that serum LDH activity was higher in COMBO steers compared to O+M steers (1232.57 vs 1128.38; $P = 0.01$). An effect of d was observed for LDH with the greatest LDH activity being observed on d 63 of the study and differed from values observed on all other blood collection dates (Figure 4; $P < 0.01$).

Decreased levels of LDH have been shown to improve performance of cattle, specifically carcass quality in steers (Paria, 1997), reproductive performance in heifers (Flores et al., 2005), and resulted in taller and heavier calves at weaning when in evaluated in dams approximately 62 d before calving (Looper et al., 2008). Even though serum LDH activity observed during this study appears to have been affected by anthelmintic treatment and day, all values reported fell within normal reference ranges (699-1381 U/L; Appendix E). Furthermore, it is likely that LDH1 activity may have been elevated in present study due to its high affinity for lung tissue making isoenzyme evaluations a critical consideration for future research.

Blood Parameters. During Experiment 1, higher EOS concentration were observed in LAE versus COMBO steers (0.32 and 0.19; $P = 0.03$). Monocytes also tended differ (0.06) between LAE and COMBO steers (0.82 and 0.68). Monocytes and EOS are a specialized type of white blood cells (WBC). Monocytes are the predominant

type of WBC and are commonly known as scavengers aiding other WBC in removing dead or diseased cells while elevated EOS are commonly associated with increased levels of parasitism (Rothwell, 1989). Although COMBO treated steers exhibited higher FEC compared to LAE steers throughout the study, it is possible that the low levels of EOS and MONO observed in the COMBO treated steers may have been due to migration of these cells to their select target tissues. No differences were found in the following blood parameters during Experiment 1: RBC, HEMO, HCT, WBC, PLA, NEU, LYM, and BASO ($P \geq 0.15$; Table 5).

In Experiment 2, PLA tended ($P = 0.07$) to be higher in Co-Trt steers compared to LAE steers (332.20 vs 248.07). A treatment by day interaction was also found for RBC during Experiment 2 ($P < 0.01$; Figure 7). Red blood cells were highest on d 0 in LAE steers which were similar to CON and COMBO steers. The lowest RBC counts were observed on d 63 in COMBO steers which were similar to all other treatments for that day. These values were also similar to those observed from CON, LAE, and COMBO steers on d 92. No differences were observed for any other blood parameter in Experiment 2; however, effects of day were observed for a number of blood parameters during both experiments (Table 6).

During Experiment 1, effects of day was observed for RBC, HEMO, HCT, WBC, NEU, LYM, MONO, EOS, and BASO (Table 6). Values for RBC, HEMO, and HCT were highest on d 0 which was similar to d 27 and lowest on d 101 and 56 ($P < 0.01$). White blood cells were highest on d 0 which differed on d 27 and 56 and were lowest on d 101 ($P < 0.01$). Neutrophils were highest on d 0 and day 56 but were lowest on d 27 and 101 ($P < 0.01$). Lymphocytes were highest on d 0, which was similar to d 27 and 56,

and lowest on d 101 ($P < 0.01$). Monocytes were highest on d 0 and 27 and lowest on d 101 with d 56 being similar to d 0, 27, and 101 ($P < 0.01$). Eosinophils were highest on d 101 which differed from d 0 and were lowest on d 27 with d 56 being similar to both d 0 and 101 ($P < 0.01$). Basophils were highest on d 27 and 0 and lowest on d 101 and 56 ($P < 0.01$). However, PLA tended to differ with the greatest number of PLA observed on d 0 and 27 with the lowest concentrations being observed on d 101 with d 56 being similar to d 0, 27, and 101 ($P = 0.06$).

During Experiment 2, effects of day was observed for HEMO, HCT, WBC, PLA, LYM, EOS, and BASO (Table 6). Hemoglobin and HCT were highest on d 0 which differed from d 31 and were lowest on d 92 which was similar to d 63 ($P < 0.01$). White blood cell counts were highest on d 92 and 31 compared to d 0 and 63 ($P < 0.01$). Platelets were highest on d 0 with PLA being similar throughout the rest of the study ($P < 0.01$). Lymphocytes were highest on d 31 and 92 and lower on d 0 and 63 ($P < 0.01$). Eosinophils were highest on d 92 which differed from d 0 and 63 and were lowest on d 31 ($P < 0.01$). Basophils were highest on d 31 compared to d 0 and 92 and lowest on d 63 ($P < 0.01$).

Southwestern Kentucky experienced record rainfall from d 27-56 in Experiment 1. During this period, 8 of 66 steers had to be treated for suspected respiratory disease. Interestingly, 6 calves belonged to the COMBO treatment group compared to one CON and 1 LAE steers. Leading authors to speculate whether dual application of anthelmintics could have resulted in greater stress upon steers leading to decreased immune function. Furthermore, this time period corresponds to increased concentration of EOS, LYM,

NEU, and WBC but decreased concentrations of BASO, RBC, HEMO, and HCT reported.

Poor weather conditions from June through July, d 31-63 in Experiment 2, corresponds to increased incidence of suspected respiratory disease and anemia. During this time period, approximately 10 of 59 steers were treated for suspected respiratory disease. Fecal egg counts were low in O+M and LAE (14.46 and 19.69) but markedly higher in CON and COMBO steers (88.45 and 107.3). These events correspond to higher EOS concentrations, compared to values reported earlier in the study, but lower concentrations of WBC, PLA, LYM, and BASO suggesting that these specialized types of white blood cells may have exited circulation and migrated to select target tissues.

Although iron status was not evaluated nor were FAMACHA scores performed, the low values observed for HEMO and HCT lend credence to suspected anemia in Experiment 2. The highest values of LDH activity observed on d 63 furthermore suggests decreased overall health status with the highest LDH activity occurring on d 63 regardless of treatment groups.

Performance variables. Body weight was similar between treatment groups throughout the study in both Experiment 1 and Experiment 2 (Table 1; $P \geq 0.24$) except for d 92 where LAE steers tended ($P = 0.09$) to have a higher BW compared to Co-Trt steers (339.6 vs 323.6).

Body condition scores were similar among treatment groups in Experiment 1 for d 13, 56, 90, and 101 ($P \geq 0.18$; Table 2). Interestingly, CON steers (which received no anthelmintic administration) exhibited higher BCS compared to treated steers on d 27 (5.45 vs 5.02; $P = 0.02$).

Body condition was also similar among treatment groups for the majority of Experiment 2. However, by d 92, O+M steers had higher ($P \leq 0.01$) BCS compared to COMBO steers and LAE steers tended ($P = 0.07$) to have a higher BCS compared to Co-Trt steers ($P = 0.07$).

Body condition scores are a useful management tool, based on a numeric scoring system from 1 to 9, allowing producers to easily determine the nutritional needs of cattle (Richards et al., 1986). In beef cattle, BCS can provide insight into reproductive and lactation performance, health and vigor of the newborn calf, calving difficulty, postpartum period and subsequent rebreeding (Richards et al., 1986).

Body condition scores were performed by the same trained technician throughout the present study. Although background of steers used in this study are unknown, assumptions were made that steers received no vaccination or anthelmintic treatment prior to their arrival at the Murray State University Beef Unit. It is possible that the differences seen in BCS on d 27 in Experiment 1 with the CON steers having the highest BCS could have been due to previous anthelmintic administration before arrival to the MSU Beef Unit. With the possibility of previous anthelmintic administration may have contributed to the poor performance observed in treated steers on d 27.

Average daily gain (ADG) was calculated for multiple points throughout the study (Table 3). Control steers exhibited higher ADG from d 13 to 27 compared to treated steers (1.01 and 0.12; $P = 0.04$); however, ADG was similar among treatment groups between d 0 to 13, d 27 to 56, d 56 to 90, d 90 to 101, and overall ($P \geq 0.38$).

In Experiment 2, overall ADG differed between O+M and COMBO steers (1.20 and 0.77; $P = 0.01$) and tended to differ for LAE vs Co-Trt steers (1.23 vs 0.99; $P = 0.09$).

Average daily gain also differed at multiple points throughout the study. Average daily gain was greater for O+M compared to COMBO steers from d 0 to 15 (2.99 vs 1.94; $P = 0.05$). Control steers exhibited higher ($P = 0.02$) ADG compared to treated steers on d 31 to 63 for (0.09 vs 0.58) but LAE steers exhibited higher ADG compared to Co-Trt steers from d 63 to 92 (1.80 vs 1.24; $P = 0.03$).

Interestingly, ADG in Experiment 1 was lowest in the COMBO (-0.03) treated steers from d 13 to 27. In Experiment 2, while ADG was not statistically different between d 15 to 31, the COMBO treated steers did have the lowest ADG (-0.29). It is suspected that the poor ADG observed may be attributed to the simultaneous administration of both oxfendazole and moxidectin on d 0 resulting in increased stress, decreased immune function, and reduced performance.

Hair coat scores ranged from 1 to 5 in the present study with a HCS of 1 indicating a complete shedding of winter hair coat and HCS of 5 indicating the full winter hair coat remained. Interestingly, CON steers exhibited a greater degree of shedding during Experiment 1 compared to COMBO steers. Hair coat scores differed in Experiment 1 between the CON and treated steers on d 13 (2.90 vs 3.84; $P = 0.04$), d 27 (2.00 vs 3.16; $P = 0.02$) on d 56 (1.60 vs 2.54; $P = 0.03$), and on d 101 (1.30 vs 2.04). Steers treated with COMBO tended to have higher HCS compared LAE steers on d 27 (3.50 vs 2.82; $P = 0.07$), d 90 (2.89 vs 2.21; $P = 0.07$), and on d 101 (2.29 vs 1.79; $P = 0.06$).

In Experiment 2, HCS differed on d 63 between LAE and Co-Trt steers (1.60 vs 2.7; $P = 0.01$) and on d 92 between COMBO and O+M steers (2.00 vs 1.27; $P = 0.05$). Cattle that live in the Southeastern U.S. can undergo periods of heat stress due to the

warm temperatures during the summer months (Brown et al., 2014). Heat stress can cause decreased feed intake resulting in poor weight gain and death in severe circumstances (Hahn, 1994; Lefcourt and Adams 1996; Mader et al., 1997b). Although a thick coat is needed during periods of cold stress to maintain core body temperature, cattle with thick hair coats during the summer months have increased chances of developing heat stress and thus reduced performance (Brown et al., 2014; Gray et al., 2011).

Fly counts. Fly counts were performed during the morning in Experiment 1 and during the afternoon in Experiment 2. In both experiments, fly counts were determined by two independently trained observers from an all-terrain vehicle while steers grazed in a pasture setting then averaged. Fly counts were similar between all treatment groups throughout the course of the study in both Experiment 1 ($P = 0.78$; Figure 5) and Experiment 2 ($P = 0.59$; Figure 6). However, there was an effect of day observed for fly counts during both experiments ($P < 0.01$; Figure 7 and Figure 8). In Experiment 1, the lowest number of flies were observed on d 31 and the highest number of flies were observed on d 100 of the study which differed on d 14, 61, and 91. Similarly in Experiment 2, the number of flies observed was lowest during the first part of the study with no difference observed between d 0, 16, and 32. The highest number of flies were observed on d 44 which differed from d 64 with fly counts on d 93 being similar to both d 44 and 63.

Although species of flies were not determined, the primary fly species observed in Southwestern Kentucky is the horn fly (*Haematobia irritans*). Cattle are the primary host of the horn fly which are normally observed on the backs of cattle, providing them an ideal position to feed (Williams et al., 1985). Losses due to the infestation of the horn fly

can be attributed to reduced feed efficiency and weight gains in cattle (Byford et al., 1992). The horn fly remains on its host during the course of its life cycle and can feed between 20 to 40 times per day (Arthur, 1991). Therefore, it is widely accepted that the horn fly can have negative impacts on the performance of cattle (Byford et al., 1992).

In addition to potential carryover effect in FEC data, it is possible that fly counts may have been affected due to commingling of treatment groups. Although fly counts were fairly low in both experiments of the current study, there was an effect of day in both experiments. In Experiment 1 there were approximately 20 inches of rain in Murray, Kentucky from d 31 to 100 of the study (Appendix H). It is possible that the combination of high summer temperatures and high rainfall could have resulted in higher fly counts at the end of the experiment which is not unexpected considering natural fluctuations in the life cycle of horn flies (Appendix F). With the above average rainfall for the area, it is possible that moisture was maintained for a greater period of time in the manure, creating optimal conditions for fly larvae leading to higher number of flies during that period. In Experiment 2, the highest fly counts were observed on d 44, which corresponded to summer highs of 93°F (Appendix G) with average rainfall for that time of the year, approximately 3.94" (Appendix H), resulting in hot, humid conditions.

Chapter 5: Conclusion

Conclusion

Data from this study indicates that anthelmintics were effective in reducing fecal egg counts in stocker calves. Long-acting eprinomectin provided the longest continuous parasite control with similar performance observed in the delayed oxfendazole/moxidectin treatment group. Delayed administration of oxfendazole/moxidectin resulted in improved performance and provided extended parasite control compared to simultaneous oxfendazole/moxidectin treatment.

Throughout the course of the study, body weight gain and body condition scores were similar between control and calves treated with anthelmintics. Suggesting that when utilizing high density grazing schemes, anthelmintic treatment may be reduced and utilized in animals exhibiting clinical signs of parasitism. Furthermore, results suggest that blood parameters and LDH activity may differ in calves receiving various anthelmintics and that evaluation of serum LDH may provide insight into overall health status in stocker calves.

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Tables

Table 1: Effects of anthelmintic treatment on BW in newly received stocker calves.

Day	Treatments ^a					Contrast		
	CON	LAE	COMBO	O+M	SEM ^b	CON vs Trt ^c	LAE vs Co-Trt ^d	COMBO vs O+M
<i>Experiment 1</i>								
13	319.9	317.9	317.4	-	13.25	0.80	0.94	-
27	326.4	318.5	317.2	-	13.46	0.35	0.85	-
56	337.5	331.6	328.8	-	14.65	0.46	0.72	-
90	358.1	351.2	348.2	-	15.16	0.41	0.71	-
101	359.5	355.2	352.0	-	15.41	0.58	0.70	-
<i>Experiment 2</i>								
15	311.0	307.5	299.0	299.4	13.57	0.22	0.27	0.96
31	311.9	308.0	296.9	300.3	13.66	0.17	0.22	0.69
63	313.3	315.9	304.2	310.5	15.35	0.71	0.32	0.52
92	333.4	339.6	317.9	329.3	16.93	0.62	0.09	0.29

^a CON = control; LAE = long-acting eprinomectin; COMBO = moxidectin/oxfendazole combination; and O+M = oxfendazole d 0 and moxidectin d 45

^b SEM = Pooled SEM

^cTrt = All steers receiving anthelmintic treatment

^dCo-Trt = COMBO and O+M treated steers

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

Different symbols within the same row, differ by $P \leq 0.09$

Table 2: Effects of anthelmintic treatment on BCS in newly received stocker calves.

Day	Treatments ^a					Contrast		
	CON	LAE	COMBO	O+M	SEM ^b	CON vs Trt ^c	LAE vs Co-Trt ^d	COMBO vs O+M
<i>Experiment 1</i>								
13	5.2	5.1	5.0	-	0.10	0.40	0.64	-
27	5.5 ^a	5.0 ^b	5.0 ^b	-	0.12	0.02	0.80	-
56	5.8	5.6	5.4	-	0.15	0.25	0.14	-
90	5.2	5.2	4.9	-	0.14	0.71	0.14	-
101	5.8	5.7	5.6	-	0.12	0.29	0.45	-
<i>Experiment 2</i>								
15	5.2	5.0	5.0	5.2	0.10	0.20	0.40	0.15
31	5.2	5.0	5.1	5.0	0.11	0.24	0.90	0.53
63	5.4	5.2	5.2	5.5	0.16	0.67	0.38	0.23
92	5.2 ^a	5.3 ^a	4.8 ^b	5.3 ^a	0.13	0.82	0.07	< 0.01

^a CON = control; LAE = long-acting eprinomectin; COMBO = moxidectin/oxfendazole combination; and O+M = oxfendazole d 0 and moxidectin d 45

^b SEM = Pooled SEM

^cTrt = All steers receiving anthelmintic treatment

^dCo-Trt = COMBO and O+M treated steers

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

Different symbols within the same row, differ by $P \leq 0.09$

Table 3: Effects of anthelmintic treatment on ADG in newly received stocker calves.

Day	Treatments ^a					Contrast		
	CON	LAE	COMBO	O+M	SEM ^b	CON vs Trt ^c	LAE vs Co-Trt ^d	COMBO vs O+M
<i>Experiment 1</i>								
0-13	3.25	3.88	3.31	-	0.39	0.55	0.21	-
13-27	1.01 ^a	0.27 ^{a,b}	-0.03 ^b	-	0.42	0.04	0.37	-
27-56	0.84	0.92	0.88	-	0.88	0.84	0.88	-
56-90	1.34	1.27	1.25	-	0.13	0.70	0.92	-
90-101	0.27	0.81	0.77	-	0.33	0.29	0.93	-
Overall	1.28	1.31	1.18	-	0.07	0.81	0.23	-
<i>Experiment 2</i>								
0-15	2.21	2.84	1.94	2.99	0.38	0.40	0.41	0.05
15-31	0.12	0.07	-0.29	0.13	0.01	0.48	0.50	0.11
31-63	0.09 [†]	0.54 [*]	0.51 [*]	0.70 [*]	0.17	0.02	0.77	0.42
63-92	1.53	1.80	1.04	1.43	0.21	0.66	0.03	0.20
Overall	0.90 ^{b,c}	1.23 ^a	0.77 ^c	1.20 ^{a,b}	0.12	0.21	0.09	0.01

^a CON = control; LAE = long-acting eprinomectin; COMBO = moxidectin/oxfendazole combination; and O+M = oxfendazole d 0 and moxidectin d 45

^b SEM = Pooled SEM

^c Trt = All steers receiving anthelmintic treatment

^d Co-Trt = COMBO and O+M treated steers

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

Different symbols within the same row, differ by $P \leq 0.09$

Table 4: Effects of anthelmintic treatment on HCS in newly received stocker calves.

Day	Treatments ^a					Contrast		
	CON	LAE	COMBO	O+M	SEM ^b	CON vs Trt ^c	LAE vs Co-Trt ^d	COMBO vs O+M
<i>Experiment 1</i>								
13	2.90 ^b	3.57 ^{a,b}	4.11 ^a	-	0.37	0.04	0.13	-
27	2.00 ^b	2.82 ^{a,b}	3.50 ^a	-	0.32	0.02	0.07	-
56	1.60 ^b	2.29 ^{a,b}	2.79 ^a	-	0.28	0.03	0.13	-
90	1.80 [†]	2.21 ^{*,†}	2.89 [*]	-	0.32	0.11	0.07	-
101	1.30 ^b	1.79 ^{a,b}	2.29 ^a	-	0.23	0.03	0.06	-
<i>Experiment 2</i>								
15	3.14	3.27	3.33	3.60	0.43	0.61	0.70	0.66
31	2.57	2.93	3.27	2.93	0.37	0.28	0.71	0.53
63	2.00 ^b	1.60 ^c	3.07 ^a	2.33 ^{a,b}	0.33	0.39	0.01	0.12
92	1.36	1.47	2.00	1.27	0.26	0.48	0.60	0.05

^a CON = control; LAE = long-acting eprinomectin; COMBO = moxidectin/oxfendazole combination; and O+M = oxfendazole d 0 and moxidectin d 45

^b SEM = Pooled SEM

^cTrt = All steers receiving anthelmintic treatment

^dCo-Trt = COMBO and O+M treated steers

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

Different symbols within the same row, differ by $P \leq 0.09$

Table 5: Effects of anthelmintic treatment on blood parameters in newly received stocker calves.

	Treatments ^a					Contrast		
Day	CON	LAE	COMBO	O+M	SEM ^b	CON vs Trt ^c	LAE vs Co-Trt ^d	COMBO vs O+M
<i>Experiment 1</i>								
RBC	9.16	8.97	9.48	-	0.21	0.80	0.10	-
HEMO	12.38	12.35	12.20	-	0.28	0.75	0.71	-
HCT	37.51	38.08	36.62	-	1.04	0.90	0.33	-
WBC	9.81	10.50	9.70	-	0.64	0.71	0.38	-
PLA	482.21	462.28	480.85	-	30.59	0.78	0.67	-
NEU	2.93	3.03	2.85	-	0.25	0.97	0.60	-
LYM	5.66	6.18	5.85	-	0.48	0.54	0.63	-
MONO	0.77	0.82	0.68	-	0.05	0.74	0.06	-
EOS	0.29 ^{a,b}	0.32 ^a	0.19 ^b	-	0.04	0.49	0.03	-
BASO	0.15	0.14	0.13	-	0.01	0.49	0.75	-
<i>Experiment 2</i>								
RBC	9.07	9.42	9.15	9.24	0.23	0.47	0.44	0.78
HEMO	11.44	11.31	11.00	11.45	0.31	0.61	0.82	0.30
HCT	36.27	35.62	34.56	36.09	0.98	0.46	0.81	0.27
WBC	10.07	10.44	9.90	9.94	0.66	0.98	0.53	0.97
PLA	341.88	248.07	325.44	338.95	36.54	0.38	0.07	0.79

NEU	3.56	3.81	3.91	3.92	0.35	0.40	0.78	0.99
LYM	5.07	5.19	4.65	4.71	0.41	0.64	0.32	0.92
MONO	0.80	0.86	0.74	0.78	0.06	0.98	0.19	0.62
EOS	0.45	0.41	0.50	0.38	0.08	0.81	0.77	0.30
BASO	0.14	0.14	0.10	0.14	0.01	0.62	0.22	0.10

^a CON = control; LAE = long-acting eprinomectin; COMBO = moxidectin/oxfendazole combination; and O+M = oxfendazole d 0 and moxidectin d 45

^b SEM = Pooled SEM

^cTrt = All steers receiving anthelmintic treatment

^dCo-Trt = COMBO and O+M treated steers

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

Different symbols within the same row, differ by $P \leq 0.09$

Table 6: Effects of day on blood parameters in newly received stocker calves.

	Day				SEM ^a	Contrast		
	D 0	D 27	D 56	D 101		CON vs Trt ^c	LAE vs Co-Trt ^d	COMBO vs O+M
<i>Experiment 1</i>								
RBC	9.87 ^a	9.69 ^a	8.69 ^b	8.57 ^b	0.15	0.80	0.10	-
HEMO	13.19 ^a	12.90 ^a	11.63 ^b	11.52 ^b	0.19	0.75	0.71	-
HCT	39.90 ^a	39.20 ^a	35.42 ^b	35.09 ^b	0.66	0.90	0.33	-
WBC	11.19 ^a	9.67 ^b	10.39 ^b	8.77 ^c	0.44	0.71	0.38	-
PLA	530.56 [*]	488.47 [*]	456.27 ^{*,†}	425.16 [†]	30.07	0.78	0.67	-
NEU	3.48 ^a	2.52 ^b	3.13 ^a	2.61 ^b	0.20	0.97	0.60	-
LYM	6.45 ^a	5.98 ^a	6.09 ^a	5.06 ^b	0.31	0.54	0.63	-
MONO	0.82 ^a	0.80 ^a	0.73 ^{a,b}	0.66 ^b	0.04	0.74	0.06	-
EOS	0.24 ^b	0.19 ^c	0.32 ^{a,b}	0.32 ^a	0.04	0.49	0.03	-
BASO	0.16 ^a	0.17 ^a	0.12 ^b	0.12 ^b	0.01	0.49	0.75	-
<i>Experiment 2</i>								
	D 0	D 31	D 63	D 92				
RBC	9.71	9.34	8.83	8.99	0.15	0.47	0.44	0.78
HEMO	12.26 ^a	11.49 ^b	10.54 ^c	10.90 ^c	0.20	0.61	0.82	0.30
HCT	38.34 ^a	36.44 ^b	33.84 ^c	33.93 ^c	0.59	0.46	0.81	0.27
WBC	9.68 ^b	10.53 ^a	9.35 ^b	10.81 ^a	0.38	0.98	0.53	0.97

PLA	382.09 ^a	306.83 ^b	253.94 ^b	311.48 ^b	25.27	0.38	0.07	0.79
NEU	3.67	3.83	4.02	3.68	0.21	0.40	0.78	0.99
LYM	4.68 ^b	5.53 ^a	4.05 ^c	5.38 ^a	0.23	0.64	0.32	0.92
MONO	0.75	0.83	0.78	0.82	0.04	0.98	0.19	0.62
EOS	0.46 ^b	0.18 ^c	0.36 ^b	0.74 ^a	0.06	0.81	0.77	0.30
BASO	0.14 ^b	0.16 ^a	0.09 ^c	0.12 ^b	0.01	0.62	0.22	0.10

^a SEM = Pooled SEM

^cTrt = All steers receiving anthelmintic treatment

^dCo-Trt = COMBO and O+M treated steers

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

Different symbols within the same row, differ by $P \leq 0.09$

Figures

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

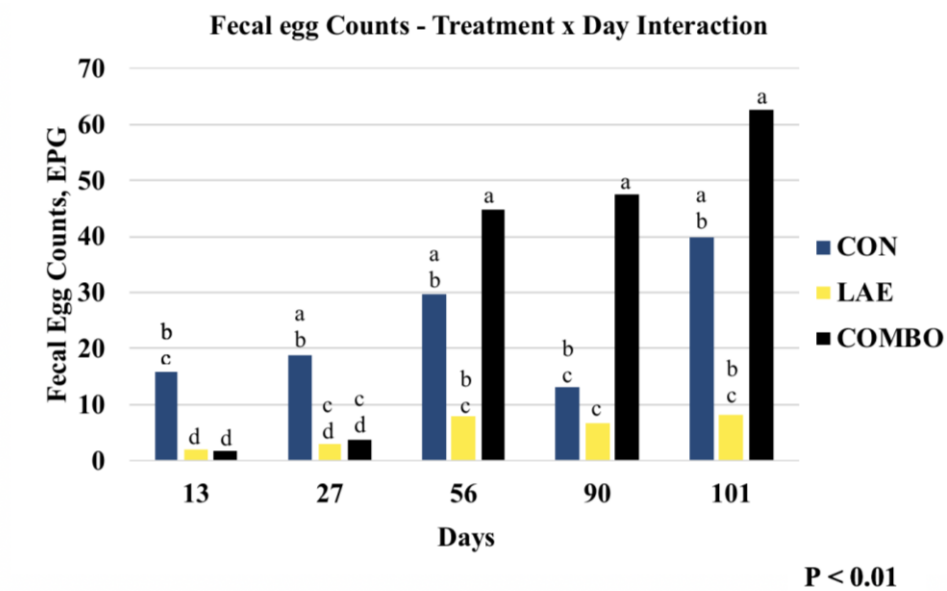


Figure 2: Experiment 2, Effect of anthelmintic treatment on fecal egg counts in newly received stocker calves.

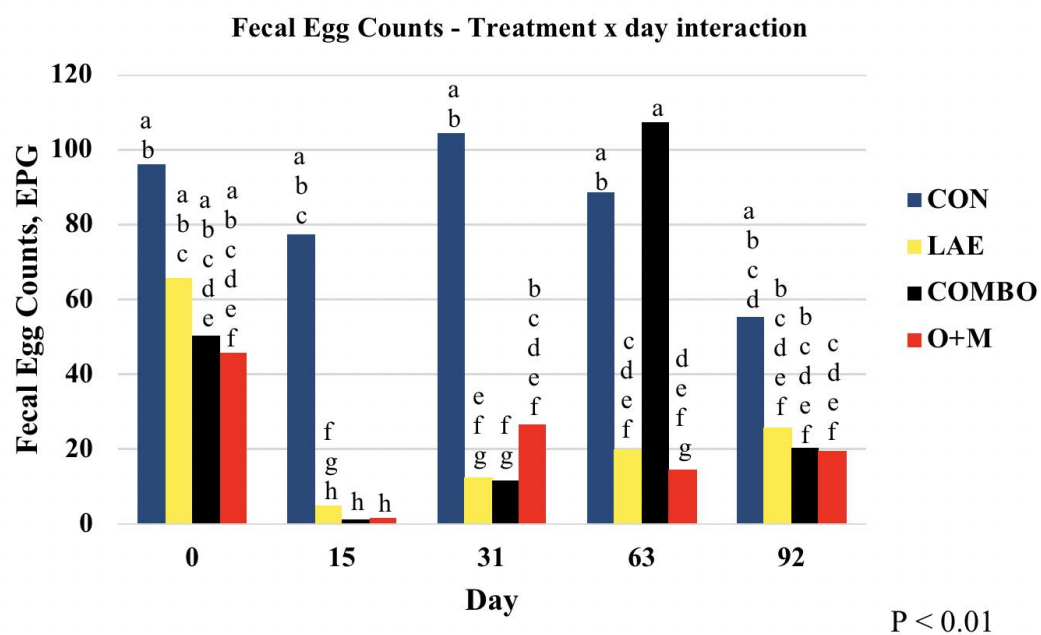


Figure 3: Experiment 2, Effect of anthelmintic treatment on LDH activity.

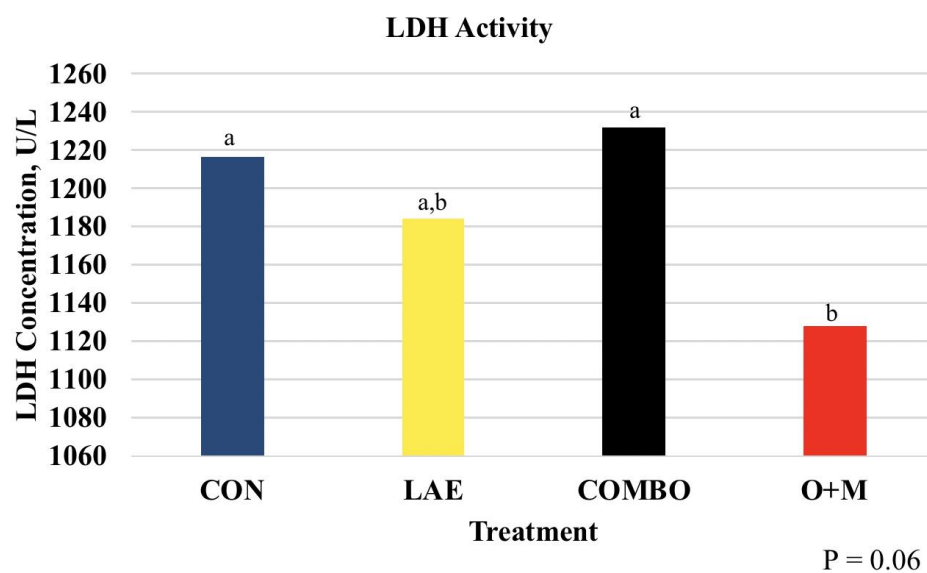


Figure 4: Experiment 2, Differences in LDH activity by day.

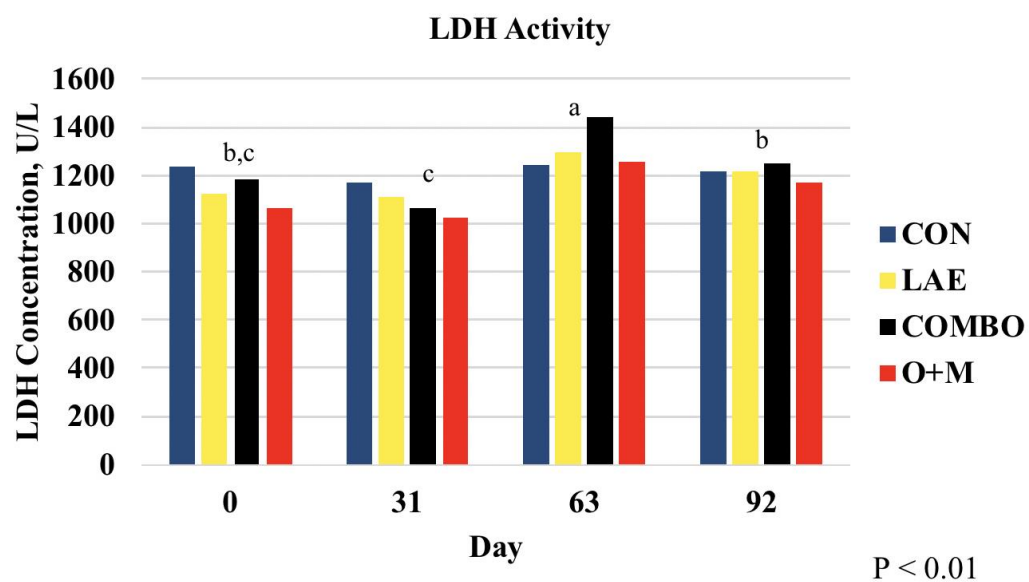


Figure 5: Experiment 1, Effect of anthelmintic treatment on fly counts in newly received stocker calves.

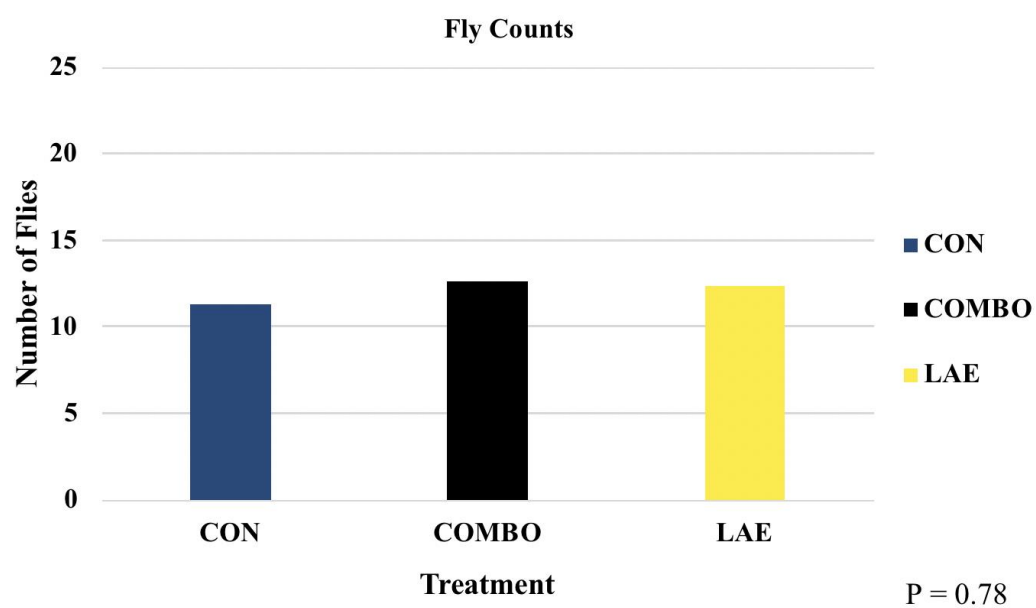


Figure 6: Experiment 2, Effect of anthelmintic treatment on fly counts in newly received stocker calves.

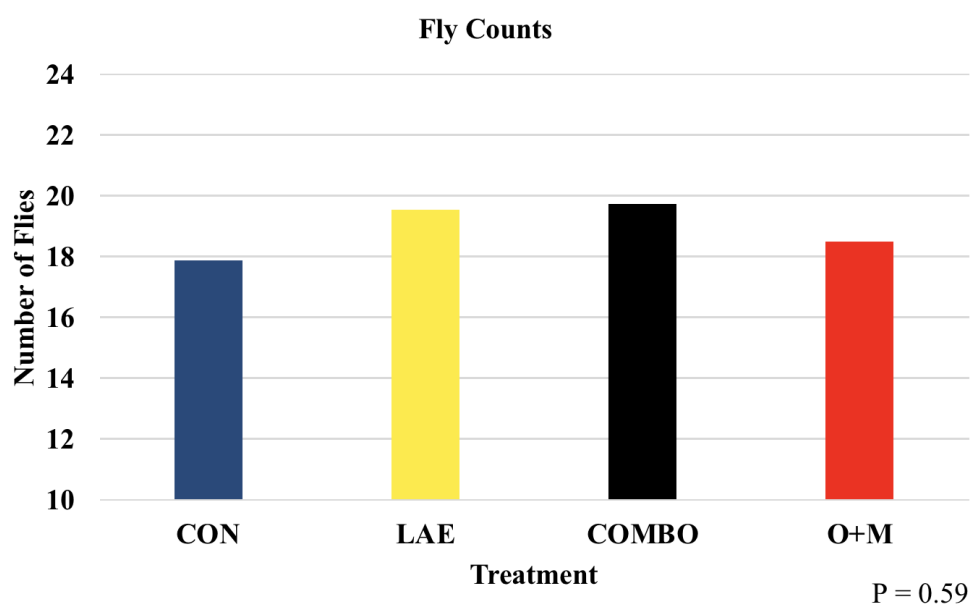


Figure 7: Experiment 1, Effect of day on fly counts in newly received stocker calves.

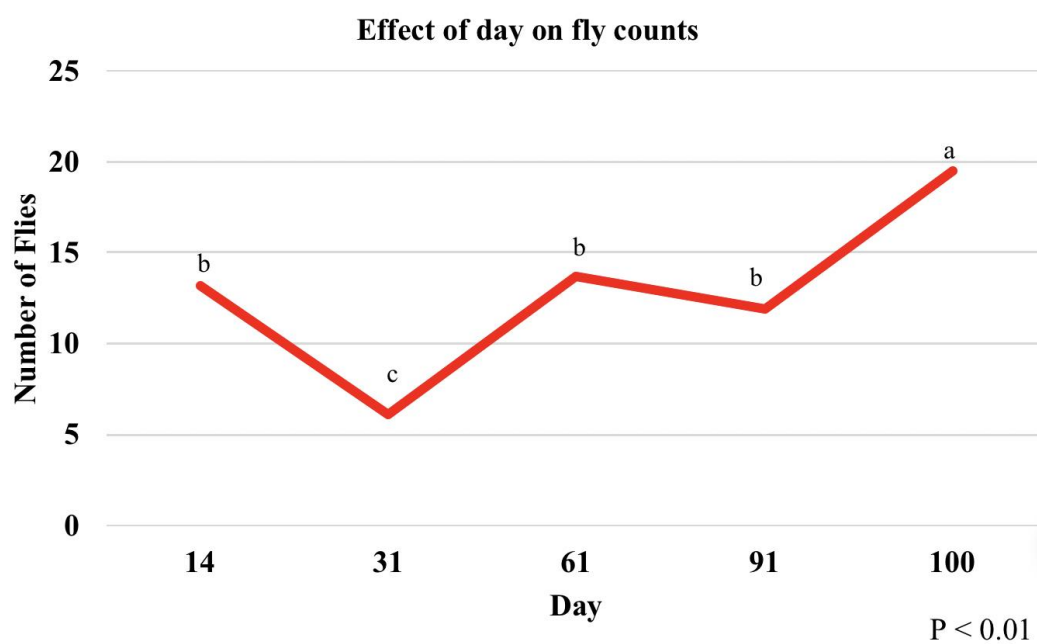


Figure 8: Experiment 2, Effect of day on fly counts in newly received stocker calves.

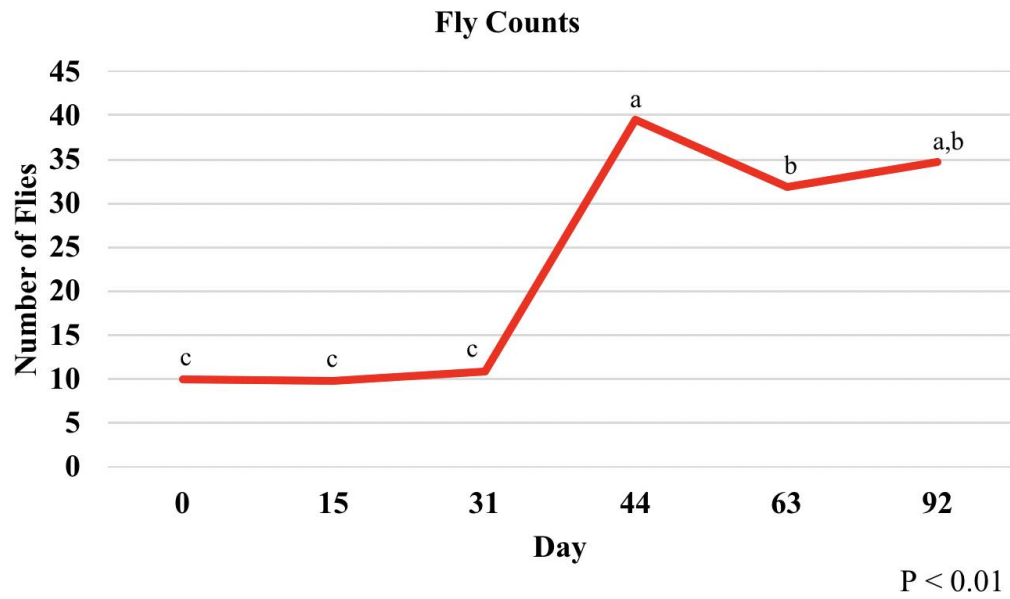
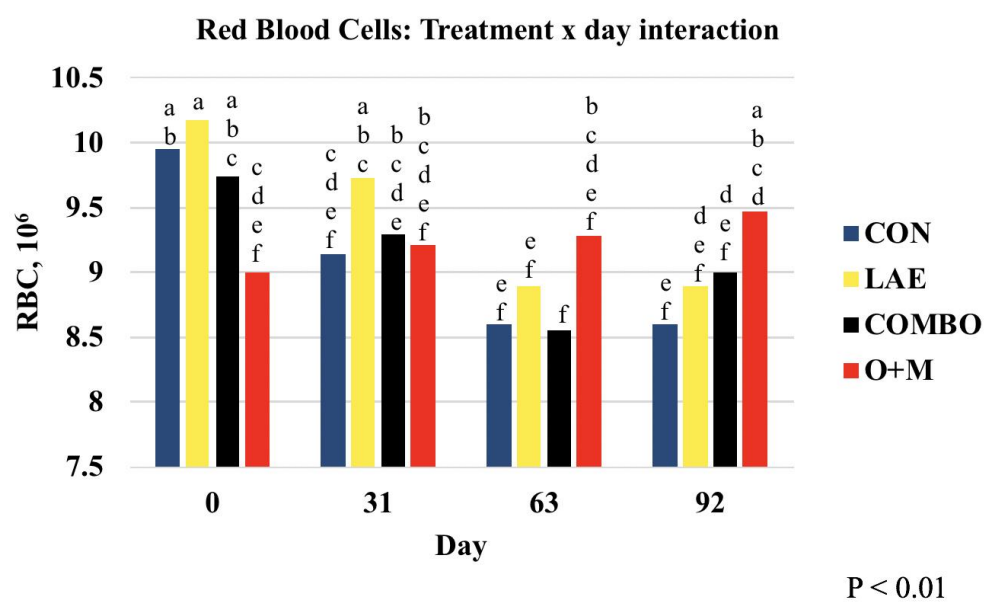


Figure 9: Experiment 2, Effect of anthelmintic treatment on RBC in newly received stocker calves.



Appendices

Appendix A: Operational Definitions

Anthelmintic – a drug used to control internal parasites affecting livestock.

Average daily gain (ADG) – changes in body weight over specific feeding period.

Body condition score (BCS) – tool used to estimate fat covering on an animal ranging on a scale of 1-9 (highly emaciated to highly obese).

Body weight (BW) – live weight of the animal.

Fecal egg counts (FEC) – the raw number of strongyle eggs observed on a microscope slide.

Fly counts – the number of flies observed on each animal.

Gastrointestinal tract (GI tract) – refers to the esophagus, stomach (reticulum, rumen, omasum, and abomasum), small intestine, and large intestine.

Hair coat score (HCS) – scores that range from 1-5 based on shedding of winter hair coat.

Lactate dehydrogenase (LDH) – an enzyme involved in glucose metabolism that can serve as an indicator of cellular or tissue damage.

Parasite – an organism that lives in or on and takes its nourishment from another organism in the intestinal tract.

Parasite resistance – development of resistance of parasites to anthelmintics.

Rotational grazing – forage management strategy used to increase forage availability and performance of grazing livestock.

Steer – castrated male bovine.

Stocker – common term to describe young cattle grazed on forages or crop residues until transitioned to feedlot facilities.

Targeted selective toxicity (TST) - drugs (anthelmintics) that are selectively toxic to internal and external parasites resulting in death of parasites.

Appendix B: Body Condition Score for Beef Cattle (Richards et al., 1986)

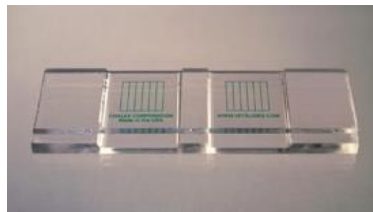
Group	BCS	Description
Thin	1	Emaciated - Cow is extremely emaciated with no palpable fat detectable over spinous processes, transverse processes, hip bones or ribs. Tail-head and ribs project quite prominently.
	2	Poor - Cow still appears somewhat emaciated but tail-head and ribs are less prominent. Individual spinous processes are still rather sharp to the touch but some tissue cover exists along the spine.
	3	Thin - Ribs are still individually identifiable but not quite as sharp to the touch. There is obvious palpable fat along the spine and over tail-head with some tissue cover over dorsal portion of ribs.
Borderline	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.
Optimal	5	Moderate - Cow has generally good overall appearance. Upon palpation, fat cover over ribs feel spongy and areas on either side of the 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 crotch.
Fat	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.

Appendix C: Hair Coat Score for Beef Cattle (Brown et al., 2014)

HCS	Description
5	Full winter coat
4	Coat exhibits initial shedding (~25%)
3	Coat halfway shed (~50%)
2	Coat is mostly shed (~75%)
1	Winter coat completely shed

Appendix D: Fecal Egg Count Procedure - Modified McMaster's Protocol (Zajac and Conboy, 2012)

1. Combine 4g of fecal material with 56mL of floatation solution for a total volume of 60mL.
2. Mix well and strain through a cheesecloth and tea strainer. To remove large pieces of debris from the mixture.
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 the slide.
4. Allow the slide to sit for at least 5 minutes before examining, allowing the floatation 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 strongyle eggs in each lane of both chambers.
6. The total egg count represents the number of eggs present in 0.3mL, which is $1/200^{\text{th}}$ of the total volume (60mL). The total egg count must be multiplied by 200 (for the fraction of the total volume) and divided by 4 (4g of feces used to make suspension) - or multiplied by 50 (for Experiment 1). The total egg count represents the number of eggs present in 0.3mL, which is $1/100^{\text{th}}$ of the total volume (30mL). The total egg count must be multiplied by 100 (for the fraction of the total volume) and divided by 4 (4g of feces used to make suspension) – or multiplied by 25 (for Experiment 2).



Appendix E: Normal Ranges for Blood Parameters and LDH Activity

Variable	Range
Red Blood Cells (RBC) – 10^6	5 - 10
Hemoglobin (HEMO) – g/dL	8 - 15
Hematocrit (HCT) - %	24 - 46
White Blood Cells (WBC) - $10^3/\mu\text{L}$	4 - 12
Platelets (PLA) - $10^3/\mu\text{L}$	100 - 800
Neutrophils (NEU) - $10^3/\mu\text{L}$	0.6 - 4.8
Lymphocytes (LYMPH) - $10^3/\mu\text{L}$	2.5 - 7.5
Monocytes (MONO) - $10^3/\mu\text{L}$	0.02 - 0.84
Eosinophils (EOS) - $10^3/\mu\text{L}$	0 - 2.4
Basophils (BASO) - $10^3/\mu\text{L}$	0 - 0.2
Lactate dehydrogenase (LDH) - U/L	699 - 1381

Blood Parameter Ranges: Murray State University Breathitt Veterinary Center
 LDH Activity Range: Cornell University Veterinary Diagnostic Center

Appendix F: Murray, KY Weather During Data Collections and Fly Counts for Experiment 1 (The Weather Company, LLC, 2018)

Experiment 1						
Day	Mean Temp. (°F)	Max. Temp. (°F)	Min. Temp. (°F)	Record High Temp (°F)	Record Low Temp (°F)	Precip. (in)
-2	77	86	68	88 (1948)	38 (1990)	0.00
-3	68	78	59	88 (2015)	35 (1966)	0.66
0	62	73	51	89 (1998)	37 (2013)	0.00
13	75	86	64	96 (1953)	42 (1961)	1.14
14	74	82	66	95 (2012)	34 (1961)	0.00
27	68	82	55	97 (1953)	53 (1960)	0.00
31	84	95	73	96 (1952)	49 (1985)	0.00
56	81	93	69	102 (1988)	58 (1960)	0.43
61	82	91	73	105 (1966)	57 (2013)	0.00
90	80	86	73	102 (1941)	54 (1967)	0.04
91	80	84	75	104 (2010)	52 (1967)	0.00
100	72	80	64	100 (1987)	50 (1950)	0.25
101	70	80	59	101 (1987)	50 (1956)	0.00

Appendix G: Murray, KY Weather During Data Collections and Fly Counts for Experiment 2 (The Weather Company, LLC, 2018)

Day	Mean Temp. (°F)	Max. Temp. (°F)	Min. Temp. (°F)	Record High Temp (°F)	Record Low Temp (°F)	Precip. (in)
-5	72	84	60	95 (1953)	45 (1984)	0.00
-4	72	82	62	94 (1951)	44 (1993)	0.08
0	72	77	66	96 (1977)	47 (1954)	1.15
15	76	87	66	99 (1953)	52 (1980)	0.00
16	78	86	69	99 (1988)	50 (1992)	0.00
31	79	89	69	104 (2012)	55 (1972)	0.00
32	77	86	68	102 (1988)	58 (1960)	0.05
44	84	93	75	102 (1980)	59 (1976)	0.00
45	83	93	73	99 (1983)	56 (1947)	0.00
63	72	75	68	100 (1951)	51 (2004)	0.02
64	74	82	66	100 (2007)	52 (1989)	0.00
92	66	75	57	104 (1954)	45 (1997)	0.41
93	61	71	51	101 (1954)	43 (1988)	0.00

Appendix H: Murray, KY Weather During the Course of the Study (The Weather Company, LLC, 2018)

Experiment 1					
Month	Max Temp. (°F)	Mean Temp. (°F)	Min. Temp. (°F)	Max Precip. (in)	Total Precip. (in)
May	86	77	68	1.14	4.77
June	95	85	77	0.54	2.10
July	93	83	73	4.08	14.29
August	95	84	75	1.45	5.80
Experiment 2					
Month	Max Temp. (°F)	Mean Temp. (°F)	Min. Temp. (°F)	Max Precip.	Total Precip.
May	86	78	71	0.58	2.55
June	91	82	73	1.18	4.77
July	95	85	77	1.29	3.94
August	91	80	73	1.23	1.71
September	91	80	75	0.46	1.49

Appendix I: IACUC Protocol, Experiment 1 (# 2016-028)



Institutional Animal Care and
Use Committee

328 Wells Hall
Murray, KY 42071
phone: 270.809.5336
fax: 270.809.3535

www.murraystate.edu

June 1, 2016

Dr. Amanda Davis
Animal/Equine Science
Murray State University
Murray, KY 42071

Dear Dr. Davis:

It is with pleasure I inform you that the Murray State University Institutional Animal Care and Use Committee (IACUC) has approved your research protocol, "Evaluation of Anthelmintics on Growth Performance and Hair Cost Scores of Stocker Calves."

The protocol timeline is approved through November 10, 2016. Please use the Animal Use Report (attached) to keep up-to-date information about the animals. At the termination of the protocol, you will need to complete the Conclusion Report (attached) and list final information concerning the animals.

The IACUC sincerely wishes you the best in your teaching pursuits. If you have any questions, please contact me at 270-809-3534.

Sincerely,

Kristi Stockdale
IACUC Coordinator

cc:
IACUC File

Appendix J: IACUC Protocol, Experiment 2 (# 2017-040)

Institutional Animal Care and
Use Committee
328 Wells Hall
Murray, KY 42071
phone: 270.809.5336
fax: 270.809.3535
www.murraystate.edu

June 26, 2017

Dr. Amanda Davis
Animal/Equine Science
Murray State University
Murray, KY 42071

Dear Dr. Davis:

It is with pleasure I inform you that the Murray State University Institutional Animal Care and Use Committee (IACUC) has approved your research protocol for the project titled, "Differences in LDH activity, blood parameters, FEC, and performance in stocker calves treated with various anthelmintics."

The protocol timeline is approved through December 1, 2017. Please use the Animal Use Report (attached) to keep up-to-date information about the animals. At the termination of the protocol, you will need to complete the Conclusion Report (attached) and list final information concerning the animals.

The IACUC sincerely wishes you the best in your teaching pursuits. If you have any questions, please contact me at 270-809-3534.

Sincerely,

A handwritten signature in blue ink that reads "Kristi Stockdale".

Kristi Stockdale
IACUC Coordinator

cc:
IACUC File