POST-WEAINING MANGMENT STRATAGIES FOR CATTLE IN THE SOUTHEAST UNITED STATES: MEASURING THE EFFICACY OF ANTHELMINTIC STRATAGIES AND THE USE OF A BLEND OF GARLIC OIL AND CINNAMALDEHYDE AS AN IONOPHORE ALTERNATIVE

By

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(Under the Direction of Robert Lawton Stewart Jr.)

ABSTRACT

Two experiments were conducted to measure the impact of different anthelmintic strategies, in addition to the use of plant extracts, on animal performance of recently weaned beef cattle in the Southeast United States. Experiment 1 studied the effects of concurrent use of oral suspended oxfendazole and intradermal eprinomectin compared to using a single anthelmintic at weaning. Concurrent anthelmintic use increased average daily gain (ADG) compared to the control group and oral suspended oxfendazole provided the greatest value when to the control group. Experiment 2 measured the effect of garlic oil and cinnamaldehyde on growth performance characteristics compared to monensin in stocker cattle. The blend did not increase ADG when compared to the control group, but the blend may have a diet dependent effect on carcass characteristics.

INDEX WORDS: Beef Cattle, Anthelmintic, Feed Additive, Plant Extract, Post-weaning

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DEDICATION

It takes a village to raise a child and I want to dedicate this work to the village that encouraged me to peruse my dreams. To my mother and father, thank you for your patience and guidance as I stubbornly choose to “Learn things the hard way”. A special thank you to my mother, who never questioned my goals and provided the love and care I needed to be confident in my pursuits. To my little brother, Seth, who was always there to support me and who motivates me to be a better person every day. To my undergraduate professors who challenged me to be a better student and to never be afraid to ask questions. To the garden club who taught me grace, and to Dr. Smith who taught me humility. To all the veterinarians and vet staff that, without question, let me “tag along” and pester them with questions. To Greg Clements, who gave me my first opportunity to work with cattle.

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TABLE OF CONTENTS

Page

LIST OF TABLES

Page

1

CHAPTER 1

INTRODUCTION

Raising beef cattle is critically intertwined with Georgia's economy and history. With every one of Georgia's 159 counties raising cattle, cattle impact both local and statewide commerce (USDA, 2018). In 2018, cattle represent an economic value of over $2 billion dollars for the state of Georgia and is the states 6th most important cash crop (USDA, 2019). Cow-calf operations, farms that produce and sell feeder calves after weaning, represent over 70% of Georgia's cattle production and cattle inventory records show that there are approximately 499,000 beef cows and heifers who calved in 2019 (USDA, 2019). Prices for calves are typically seasonal and are dictated by multiple factors such as sale method, body size, time of year, and health of calves. The weaning phase is typically a high-stress event during a calf's life. For most operations this will include additional handling, vaccination, and separation from their dam all at the same time. The stress associated with weaning leads to reduced intake, reduced production, and increased morbidity (Callaway et al., 2003). Pre-conditioning, or backgrounding, is a post-weaning management strategy that can be implemented to mitigate the negative effects of weaning. UGA's beef extension team defines pre-conditioning as a stage that involves multiple factors including: a solid nutrition program, animal health practices, socializing, dehorning, etc. (Lacy et al., 2017). Pre-condition helps producers limit the negative effects of weaning, deliver a more consistent product, and increase their calves' chance of success post sale.

Managing internal parasites is just one issue facing producers who implement a post-weaning program in the Southeast. Gastrointestinal parasites, such as roundworms, worms are a common problem impacting grazing beef cattle. Parasitism can result in visible symptoms such as: anemia, diarrhea, and rough hair coat. Sub-clinical issue may also occur which impact: milk yield, weight gain, carcass value, and fertility (Hawkins, 1993). Timing is critical for prevention with the highest risk of parasitism occurring in the spring. Parasites survive in pastures as eggs until spring, at which point they molt into their larval stage (Myers et al., 1989). Larvae infect the individual animals and disperse eggs across the pasture through fecal excretion. The seasonal nature of parasites is especially important to cattle producers in the Southeast. Around 70% of all feeder calves in the southeast are sold during the fall (Lacy et al., 2017). This means that around 70% of calves sold in the southeast are born during the early months of the year, usually between January and March. Parasites are most active in the spring and are a great risk to growing calves. Producers will implement an anthelmintic program to mitigate and limit the negative effects of parasites. Deworming programs tend to vary, poor management has led to an increase in anthelmintic-resistant parasite species (Myers et al., 1989). Increased resistance in parasitic populations has produced the need for more effective deworming programs.

Postweaning nutrition is also critical in developing productive calves. During weaning, the calf's diet will shift from the milk provided by the dam to a predominately forage based diet (Wistuba, 2019). The stress from weaning and change in diet causes a decrease in dry matter intake. Additionally, the populations of bacteria within the rumen change as the calves are introduced to a new diet, which limits efficient forage and feed utilization. A rapid change to either a high grain diet or a high forage diet may lead to issue with acidosis or bloat respectively (Chibisa et al., 2016). Producers can provide feed additives to the calves' diets to supplement or

mitigate some of the negative effects during the post-weaning phase. Ionophores are a common additive in livestock management and are known to increase propionate production, which is an essential glucogenic compound. Utilizing ionophores and other feed additives can limit the negative feed effects while also increasing feed conversion and productivity (Callaway et al., 2003).

The research conducted for this thesis addresses postweaning strategies in the southeastern United States and was conducted in two experiments. The first experiment evaluated anthelmintic programs, specifically, testing two different compounds and a combination of both. Various data points were collected and measured: fecal samples were collected to measure the different programs' efficacy at reducing parasite eggs in feces, weights were collected on d0 and d42 to measure differences in gain over a typical preconditioning phase, and rumen fluid was collected and analyzed to measure the effect of the different anthelmintic strategies on ruminal microbial populations. Results from this study will help us determine what anthelmintic program will be best suited in a backgrounding or preconditioning program. These results will also provide insight in anthelmintic efficacy and their effect on growth performance and ability to reduce fecal egg counts within the herd. Additionally, this study will help determine if there is an economic benefit to utilizing a combination of anthelmintic drugs.

The second experiment involved the use of two different feed additives in post weaned calves' diets. Three treatment groups were used in this experiment: Monensin, a mix of garlic oil and cinnamaldehyde, and a control group. Various data points were collected and analyzed including: Weights on four different days to calculate average daily gain, rumen and fecal samples to measure volatile fatty acid production differences and change in microbe populations,

and ultrasonic imaging to measure differences in fat and muscle development. Results from this study will help us determine if the mix of plant extract affects animal performance, carcass characteristics, and rumen fermentation similarly to monensin. This research may be utilized in organic or antibiotic free operations that may not be able to use compounds like monensin. This research is also relevant to producers who currently provide feed additives in their post-weaning diets by elucidating the effect of different compounds on animal performance and rumen fermentation.

Limiting the adverse effects of weaning is critical to the success and profit of cow-calf operations. These experiments will provide producers critical information that they will use to make informed decisions regarding both post-weaning animal health and nutrition. These experiments will also investigate the economic value of implementing various post weaning strategies by comparing the cost against the benefit of each strategy. In summary, this research is relevant to the agriculture industry in both practical and theoretical applications in its aim to improve animal health and performance during the post-weaning phase of the calf's lives.

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CHAPTER 2

THE REVIEW OF THE LITATURE

Challenges in Cow-Calf operations

In 2005 standardized performance analysis data collected from 394 farms across Texas, Oklahoma, and New Mexico were used to determine the most significant variables affecting profitability in cow-calf operations (Ramsey et al., 2005). Three unique models were used: 1) cost, which was defined as the pre-taxed cost before the non-calf revenue adjustment per hundredweight 2) production, which was defined as pounds weaned per exposed female, and 3) profit, which was defined as the percent return on assets. Ramsey found that large operations can mitigate operational cost by spreading expenses across a large herd (Ramsey et al., 2005). However, with 78% of cattle operations in the southeast owning 100 or fewer cows, producers must find alternative strategies to mitigate cost (Dunn, 2000).

Operational challenges can be overcome, as Ramsey at al. (2005) found that cost, production, and profitability were significantly affected by management related practices that included reproductive programs such as timed AI and pregnancy checks, animal health practices, and post-weaning strategies. In fact, producers who focused on optimal production rather than maximum production showed the greatest returns on assets (Ramsey et al., 2005). Cow-calf operations can mitigate the operational cost by increasing the value of their calf crop. In 2017, the USDA surveyed 1,414 cattle

operations from across the United States and found that calf health, and preconditioning practices were two key areas that could be improved (Martin et al., 2018). Weaning is a stressful time in a calf's life and can have detrimental impacts on growth and performance. Weaning can result in depressed intake, reduced weight gain, and increased chance of morbidity (Damian, 2013). Furthermore, calves are highly susceptive to parasitism due to their naive immune system, and if not managed properly, parasites can cause both sub-acute and acute infections that will impact both animal health and profit for the farm (Hawkins, 1993). Treating calves with alchemistic drugs increases performance and reduces morbidity, which reduces veterinary cost and produces heavier calves crop (Stromberg et al., 2012, Eppleston et al., 2016).

Producers can utilize a preconditioning phase, or backgrounding phase, to implement health management practices along with a balanced ration to improve animal health and performance at a feedlot (Stuttgen, 2013). In 2005 Dhuvetter et al. (2005) conducted a survey to measure the profitability of backgrounding cattle for a short period of time. Sales data was recoded for both non-backgrounded and backgrounded cattle and collected from a livestock exchange in Holtan, KS during 1999 to 2004. Throughout the five-year study, backgrounded cattle were, on average, 30 kg heavier than non-backgrounded cattle. Furthermore, backgrounded cattle received a premium of 0.099 $/kg when compared to non-backgrounded cattle. When associated cost of health management practices and feed for backgrounded cattle for 45 days, backgrounded cattle were valued, on average, 0.0044 $/kg more than non-backgrounded cattle. In total, when producers utilized a background phase, they earned approximately 14 dollars more per head. Two management strategies cow-calf produces can use to increase the value of their calf crop

during a background phase include utilizing feed additives and implementing a sound anthelmintic strategy.

Nutrition

The prevalence of cow-calf operations in the southeast is driven by the ability to grow forages throughout the year (Hancock et al., 2011), moreover, over 96.3% of operations in the Southeast will rely on forages to account for 50% of the herds diet (USDA, 2009). When available foraged are managed properly, producers can satisfy the nutritional demands of recently weaned calves, mitigating the cost of feed. In a two year grazing study, Curtis et al. (2008) used 75 multiparous cross-bred Gelbvieh × Angus (*Bos taurus*) cows in a three phase experiment to determine how different levels of stockpiled tall fescue (*Festuca arundinacea*) would influence pasture utilization as well as performance of lactating beef cows and their calves. During phase 1, cows would graze on tall fescue from December to February. During Phase 2, cows and calves were commingled into one group and fed stockpiled tall fescue and hay from late February until weaning, which was late April for both years. During phase 3, calves were weaned and sold; the cow group continued to be commingled and was grazed on cool-season grass-legume pastures. Tall fescue comprised approximately 85% of pasture, although orchard grass (*Dactylis glomerate L.*), Kentucky bluegrass (*Poa pratensis L.*), red clover (*Trifolium prantense L.*), and birds-foot trefoil (*Lotus corniculatus L.*) were also found in pastures and hay. Cow-pairs were stratified by cow body weight, cow age, calf age, and calf sex into one of five groups: 1) tall fescue supplemented at 2.25% of cow-calf pair bodyweight allocated daily, 2) tall fescue supplemented at 3.00% of cow-calf pair bodyweight allocated daily, 3) tall fescue supplemented at 3.75% of cow-calf pair

bodyweight allocated daily, 4) tall fescue supplemented at 4.50% of cow-calf pair bodyweight allocated daily, and 5) ad libitum cool-season, grass-legume hay. Over the entire study there was both a treatment and year effect on dry matter intake (DMI) and forage utilization ($P < 0.04$). Cow calf pairs who were allocated fescue at 4.50% of BW consumed 31% more ($P < 0.01$) DM than pairs who were allocated 2.25% of BW. Additionally, pasture utilization was, on average, 26% greater in pastures that were only supplemented with fescue at 2.25% of body weight. During phase 1 in year 1, body weight responded linearly to fescue supplementation ($P < 0.0001$), with the 2.25% treatment group losing 19 kg more than the 3.00% treatment group. During phase 1 in year 2, however, there were no differences between any of the treatments detected. Additionally, there were differences in body weight during either year detected during phase 3. Moreover, there was no difference in calf weight at weaning ($P = 0.33$). These results, demonstrate that, with proper forage management, forages alone can satisfy nutritional demands of both lactating cows and growing calves.

Feed additives

Even with intensive forage management practices, cattle can still benefit from supplemental feed additives included in their diet. Forage based diets influence rumen fermentation which leads to the increased production of acetate (Aguerre et al., 2013). Acetate, while essential for milk fat production, does not benefit growing cattle as much as increased propionate production (Gonzalez et al., 2012). Propionate is a primary precursor to glucose, and moreover, improves growth performance and increase muscle development in young calves (Aguerre et al., 2013). To increase propionate production, producers can supplement cattle with ionophores. In cattle, ionophores are utilized as a

gut modulator, changing rumen fermentation end products (Wallace et al., 1980). When used in cattle, ionophores can also increase feed efficiency by decreasing intake, while either maintaining or improving weight gain (Horton et al., 1992). There are, however, many derivatives to monensin; and in-vitro experiments demonstrated that different ionophores do not perform similarly. Hillaire et al. (1989) evaluated the effect of 19 ionophores, including the most used ionophore, monensin sodium, on in vitro fermentation. Rumen fluid was collected from cannulated sheep and wheat starch or peanut meal was as an energy source for the microbial population. All ionophores were administered at one mg per fermenter. Monensin sodium, along with the other five monensin derivatives increased propionate, on average, by 15%. Moreover, monensin greatly reduced methanogenesis, which was marked by the 41.5% increase in the CO_2 to CH_4 ratio (Hillaire et al., 1989). Other ionophores had some effect, however, not to the extent of monensin sodium.

In addition to increase propionate production and decreasing methane production, grazing cattle benefit from the added health benefits of supplemental ionophores, in addition to the increase in protein utilization and propionate production. In a study by Paisley and Horn (1998), twelve ruminally cannulated steers were treated with monensin sodium and lasalocid acid to measure the in-vivo effects of monensin and lasalocid on rumen characteristics, gas production, and bloat in beef cattle grazing on wheat pastures. Steers were randomly sorted and assigned to one of three treatment groups: 1) Control, 2) monensin at 300mg per head, 3) Lasalocid at 300mg per head. Rumen fluid was collected once a week for three weeks to measure rumen characteristics. The researchers found no differences in pH, total VFA, and ammonia concentrations between any of the treatments

($P > 0.20$). There was a tendency for steers treated with lasalocid acid to have greater acetate production than monensin ($P < 0.09$), in addition, steers who were supplemented with monensin had greater propionate production, 22.04 mmol vs 19.21 mmol, and a lower A:P ratio when compared to lasalocid acid, 2.72 mmol vs 3.18 mmol. Furthermore, the control steers tended to have more incidence of bloat than both groups of the ionophore treated steers ($P = 0.083$), and monensin treated steers had fewer incidents of bloat and decreased bloat scores when compared to lasalocid acid ($P = 0.049$). These results concur with the in-vitro results observed by Hillaire et al. (1989) and highlight both the fermentation and health benefits of monensin.

Furthermore, Packer et al. (2011) measured the effects of monensin sodium on live weight, rumen characteristics, and gas production in beef cattle grazing cool season annuals [oats (*Avena sativa*) and annual ryegrass (*Lolium perenne*)] for 91 days. Treatments included 1) supplemented daily with a control-release monensin capsule containing 32 g of monensin per capsule, or 2) untreated control group. After 91 d, steers that were treated with monensin were 11.9 kg heavier than control steers ($P < 0.05$). Moreover, the proportion of propionate was greater in monensin treated cattle, with propionate accounting for 20.52% of total VFAs compared to 18.04% of total VFAs in the non-treated group ($P < 0.01$). Additionally, acetate accounted for 63.18% of total VFAs in monensin treated cattle compared to 65.23% in non-treated animals ($P < 0.01$). The propionate to acetate ratio also differed between the two groups, with monensin decreasing the A:P ratio when compared to the non-treated group ($P < 0.01$).

Vendramini et al. (2015), who conducted two experiments comparing the effect of monensin on growth performance and rumen characteristics in heifers at different

stocking rates. Experiment one was conducted over a two-year period and included 30 Angus x Brahman cross bred heifers (*Bos Taurus x Bos indicus*) each year. Heifers were randomly assigned to 12 bahiagrass *(Paspalum notatum*) pastures and pastures were stocked with either 2 or 3 heifers to achieve two different stocking rates: 1.2 AU/ha and 1.7 AU/ha. All pastures were supplemented with 0.4 kg of a soybean hull and wheat middling concentrate mix. Pastures were assigned one of two groups, 1) supplemented with 200 mg/d of monensin, or 2) no supplement. In experiment 2, four ruminally fistulated steers, in a 4x4 Latin square design, were supplemented with 4 levels of monensin: 0 mg/d, 125 mg/d, 250 mg/d, or 375 mg/d. Steers were fed star grass hay (*Cynodon nlemfuensis*) and concentrate was added at 0.2% of body weight. During experiment 1 the researchers observed no difference between monensin and the control pastures in ADG, herbage mass, and herbage allowance ($P \geq 0.23$); however ADG and herbage allowance decreased ($P \leq 0.02$) during August in the paddocks that were stocked at a rate of 1.7 AU/ha. Additionally, DMI did not differ between the monensin and control groups ($P = 0.65$). During experiment 2 no differences were observed in forage DMI between any of the treatments ($P = 0.93$). The was a linear treatment effect on propionate ($P < 0.01$), with an increase in propionate as monensin supplementation increased and a tendency for acetate to decrease with increasing levels of monensin ($P \leq 0.09$). These results support the fermentation data observed in Packer et al. (2011), however monensin did not affect growth performance in diets that include low quality forage.

Even in concentrate-based diets, the effect of monensin on growth and intake is variable. Barreras et al. (2013) measured the impact of ionophores on performance of

heifers during the summer months in northwest Mexico. Barreras used 48 crossbred Heifers who were crossed between Hereford, Angus, and Charolais breeds. On arrival heifers were weighed and then blocked by weight and randomly sorted into one of four treatment groups: 1) control, 2) monensin at 30mg/kg, 3) lasalocid at 20mg/kg, 4) and lasalocid at 30mg/kg. All heifers were fed a steam-flaked corn diet and orts were collected and weighed each day to measure intake. There was no difference between intake across all the animals ($P > 0.10$), although lasalocid at 30mg/kg was the greatest numerically gaining 0.22 kg more than the control group. Gain to feed was greater than the control group in the lasalocid treated heifers at both concentrations ($P \leq 0.02$) but did not differ from each other; although lasalocid at 30 mg/kg produced the greatest G:F at 1.86 kg of weight to kg of feed.

Essential oils

Antibiotic use in the livestock industry has been challenged as growing concerns over antibiotic resistance has grown. In 2006 the European Union passed a ban on antibiotic use in the livestock industry, and while it was not approved, similar legislation was proposed in the United States (Calsamiglia et al. 2007). Moreover, consumer preference for antibiotic free products have opened new marketing streams that have provided organic farms, farms that do not use antibiotics, an opportunity to grow (Napolitano et al., 2010). In response, plant extracts have been investigated as a possible replacement for ionophores, specifically investigating the effect of plant extracts on rumen fermentation. Cardozo et al. (2005) measured ten different extracts in a dual-flow continuous culture over 10 days in two separate trials. The first trial measured fluid characteristics in cultures at a constant pH of 7.0 and a constant pH of 5.5. The second

trail measured fluid characteristics in pH of 5.5 with the addition of 5 N NaOH or 3 N HCL. Rumen fluid was collected from ruminally fistulated dairy heifers who were fed a consisting of 10% straw, 30% corn grain, 25% barley grain, 19% soybean mean, 14% tapioca, and a 2% mix of minerals. Tubes were filled the rumen fluid along with 0.5 g of the heifer's typical diet as the energy source for the microbiota. All plant extracts were added at a concentration of 0.3, 3, 30, and 300 mg per liter of rumen fluid and each treatment × concentration was tested in triplicate and in two periods. Treatments included: 1) Control, 2) garlic (*Allium sativa*), 3) cinnamaldehyde (*Cinnamonum cassia*), 4) yucca (*Yucca schidigera*), 5) anise (*Pimpinella anisum*), 6) oregano (*Origanum vulgare*), 7) capsicum (*Capsicum annuum*), 8) anethole, and 9) eugenol. Total VFAs, acetate proportion, and butyrate proportion decreased for all plant extracts when the pH was dropped from 7.0 to 5.5 ($P < 0.01$). Moreover, propionate production was greater when pH dropped to 5.5 ($P < 0.01$). Cinnamaldehyde, which is extracted from cinnamon, added at 0.3 and 3 mg/L increased total VFA production and acetate proportion while decreasing propionate proportion ($P < 0.05$). At 3 and 300 mg/L cinnamaldehyde decreased total VFA production and propionate proportion ($P < 0.05$). Garlic produced a negative linear effect on total VFA with 3, 30, and 300 mg/L of garlic decreasing total VFA compared to the control group ($P < 0.5$). Similarly, garlic decreased acetate proportion only at 30 mg/L ($P < 0.05$), however, propionate proportion was not affected. At a pH of 5.5, garlic increased total VFA production and acetate proportion at a concentration of 30 mg/L ($P < 0.05$). cinnamaldehyde added at 0.3, 3, and 30 mg/L increased total VFA production and propionate proportion, while decreasing acetate proportion ($P < 0.05$). cinnamaldehyde did not affect total VFA production or acetate

proportion ($P > 0.05$), but decreased propionate production at 0.3, and 30 mg/L ($P < 0.05$).

These results concur with a similar in-vitro study carried out by Busquet et al. (2005). Base on the observations in Cardozo et al. (2005), two concentrations of garlic, monensin, and a cinnamon extract called cinnamaldehyde were measured in a dual-flow continuous culture. Rumen fluid was collected from dairy heifers who were fed a 30% alfalfa and 70% concentrate diet, and the concentrate included barley grain, ground corn grain, soybean meal, and mineral. Tubes were filled with a 50:50 mix of alfalfa and the concentrate mix. The seven treatments added to the tubes include: 1) control, 2) monensin added at 1.25 mg/L, 3) monensin added at 12.5 mg/L, 4) cinnamaldehyde added at 31.2 mg/L, 5) cinnamaldehyde added at 312 mg/L, 6) garlic added at 31.2 mg/L, and 7) garlic added at 312 mg/L. This study included three replicates and tubes could ferment over nine days. Temperature (38.5°C), pH (6.4), and dilution rate was held constant throughout the study. Fermentation changes were observed during the first 48 hours. Only monensin added at 12.5 mg/L produced a greater amount of VFAs across all treatments ($P < 0.05$). Additionally, monensin at 12.5 mg/L reduced acetate production and increased propionate production when compared to all other treatments ($P < 0.05$) and was decreased by 15.3 mmol compared to the control. While garlic affected acetate production similarly to monensin, butyrate production was greatest when garlic was added at 312 mg/L ($P < 0.05$). Garlic added at 312 mg/L and cinnamaldehyde added at 31.2 mg/L produced greater propionate than all other treatment groups ($P < 0.05$) except for monensin added at 12.5 mg/L ($P > 0.05$). Moreover, garlic added at 312 mg/L and cinnamaldehyde added at 31.2 mg/L were 7.4 mmol and 4.2 mmol greater than the

control, respectively. Furthermore, Garlic added at 312 mg/L produced the lowest acetate concentration ($P < 0.05$) but did not differ from 12.5 mg/L of monensin and cinnamaldehyde added at 31.2 mg/L ($P > 0.05$). These results suggest that garlic and cinnamaldehyde can affect rumen fermentation, however, the effect of these plant extracts appears to differ from monensin.

Data in the field, however, has not demonstrated any performance benefits when supplementing with plant extracts. In 2015, Vakili et al. (2015) measured the in vivo effect of essential oils extracted from plants on dry matter intake and rumen characteristics in Holstein bull calves. In this study 12 ruminally cannulated Holstein steers were weighed and randomly assigned to one of three treatments: 1) a control, 2) thyme added to the diet at 5 g per day per calf, 3) cinnamon added to the diet at 5 g per day per calf. Steers were fed an 85:15 concentrate to forage diet that consisted of: ground barley grain, corn grain, cottonseed meal, soybean meal, and alfalfa hay. The steers were adapted to the diet over five weeks and then fed the experimental diet for 45 days. Over the 45 days, final body weight, ADG, DMI, and G:F did not differ between any the treatment groups ($P > 0.14$); however, ADG in both thyme and cinnamon steers were numerically greater at 0.12 kg/d and 0.07 kg/d ($P = 0.15$). Additionally, pH did not differ between any of the treatment groups ($P = 0.69$), with an average pH of 5.93. Treatment did influence VFA production with monensin and thyme producing 4.9 and 3.4 mol/mmol, respectively, of propionate more than the control group ($P = 0.01$).

Due to the variety of plant extracts on the market, companies have tried to combine multiple extracts to maximize the benefits of the product. To test the efficacy of one these products, Tomkins et al. (2015) compared monensin to an essential oil mix

comprised of thymol, eugenol, vanillin, limonene, and guaiacol. In this study, five ruminally fistulated Brahman (*Bos indicus*) steers were used in a 5x5 Latin square design and sorted into one of five treatment groups: 1) supplemented with the mix at a rate of 1 g/d, 2) supplemented with the mix at a rate of 2 g/d, 3) supplemented with monensin at a rate of 60 mg/d, 4) supplemented with monensin at a rate of 250 mg/d, and 5) a non-supplemented control. Steers were fed ad libitum medium quality (9.6% CP, 52.8% NDF, 30.9% ADF) Rhodes grass (*Chloria gayana*) for the entire 40 d study period. The supplemented extract mix did not affect intake when compared to control group or the group supplemented with 60 mg/d of monensin. Monensin added at 250 mg/d decreased intake by 18% when compared to the control and the plant extract mix. Monensin at 250 mg/d increased propionate compared to all other treatments and was 3.4% greater than control and 2.2% greater than the extract mix at 2 g/d. Furthermore, only Monensin at 250 mg/d decreased acetate compared to the control, with acetate proportions 4.2% lower when compared to the control group. Moreover, while monensin supplemented at 60 mg/d did not differ from monensin at 250 mg/d, only monensin at 250 mg/d decreased the A:P ratio compared to the control group. These results suggest that this mix of plant extracts did not affect behavior or rumen characteristics similarly to monensin supplemented at higher levels.

Internal Parasites

Preventing parasite infections is a major challenge for producers in the Southeast (Wagner. 2018). Parasitism may result in visible symptoms such as: anemia, diarrhea, and a rough hair coat. Additionally, sub-clinical issue may also occur which may impact milk yield, gain, carcass value, and fertility (Hawkins, 1993). Timing is critical for

prevention (Bliss et al., 2008). While parasites find it difficult to survive in dry weather, they have developed adaptations that allow them to persist in pastures as eggs until spring, at which point they are able to molt into their larval stage. Understanding the parasitic life cycle is critical for cow-calf producers in the Southeast United States as 70% of the calves born in the southeast are born in early spring (USDA, 2009). Additionally, due to their naive immune systems, young calves are highly susceptible to parasitism, and are more likely to carry high parasites loads (Nieman, 2017). Historically parasites were only treated when clinical signs were observed (Ranjan et al., 1992). Opinions began to shift after research found that treating sub-clinical parasitism increased animal performance, eventually anthelmintic use became widely adopted within the cattle industry. The use of anthelmintic drug, without a sound strategy, has produced a growing concern of parasites developing resistance to anthelmintic (Gasbarre, 2014).

The parasite burden on any given operation is largely driven by the specific species present, the number of adult parasites in the system, the host immune system, along with the rate at which adult parasites are able to produce eggs (Gordon et al., 1970; Thomas, 1982). Understanding this dynamic system is critical to mitigating the impact parasites have on animal health and the profitability of the farm. Parasites can infect a wide variety of species. Worse, depending on the environment, parasites can easily spread from animal to animal, or even animal to humans (Molento et al., 2016). Defining the epidemiological state of parasites is complicated and requires an understanding of a variety of dynamic factors. Not only are sub-clinical infections difficult to diagnose, there are a variety of host-parasites interactions, pasture management techniques, and environmental factors that all must be taken into account when implementing preventive

parasite options (Yazwinski et all., 2006). Anthelmintic are an option, but with the recent rise in anthelmintic resistance, it is important to implement effective, sustainable management programs.

Life cycle of internal parasites

Common parasites impacting beef cattle who graze on pastures include roundworms and coccidia. Other parasites such as lungworms, flukeworms, and tapeworms are also clinically important, and may have detrimental impacts on animal performance (Corwin, 1997). The life cycle of internal parasites is short, approximately 16 to 28 days, and is specific to the species. Parasites have four development stages throughout their life that may take place in the primary host, intermediate host, or the pasture (Gordan et al., 1970). Grazing animals who are infected with parasites will spread infected eggs throughout the pastures. Eventually, the eggs will develop in to stage one larva, known as L1. Larva will continue to develop and molt into stage two larva, known as L2 (Gordon, et al., 1970). Both L1 and L2 larva will remain in the feces and feed on bacteria until they molt into stage 3 larva, or L3 (Strickland, 1992). Once the parasite reaches L3 it becomes infectious and begins to translocate from feces to moist blades of grass where they will be consumed by grazing animals (Strickland, 2009). Once the parasite enters the host, it will latch onto tissue and survive by feeding off the metabolites carried in the host blood (Gordan et al., 1970). The parasites will eventually mature, reaching the adult stage, or L4. In L4, sexual differentiation occurs, and females will begin to produce eggs that will be expelled from the animal in the feces. During the dry, hot months, or the extreme cold months, eggs can survive in pasture utilizing different strategies. During the summer months the larva will enter an inhibited L3 stage, in which

it remains in a protective sheath, preventing the parasite from drying out (Craig, 2018). When the temperatures fall below 4°C, larval development slows with little risk of drying out. Long term persistence can even remain in stored hay and silage for months after harvest (Njau et al., 1991; Craig, 2018).

Roundworms

Roundworms are a gastrointestinal parasite that typically burrow into the abomasal wall of the host, feeding on metabolites within blood. The most common species of roundworms include *Haemonchus contortus*, *Osteragia osteragi*, *Cooperia oncophora*, and Trichstrongylus axei (Porter et al., 1942). *Haemonchus contortus* is responsible for significant mortality and economic loss in the small ruminant world (Mushonga et al., 2018), but it is less impactful in cattle. In cattle, *Cooperia oncophora* and *Osteragia osteragi* are often the roundworm of interest. *Cooperia oncophore* is found in the small intestine can damage the lining of the intestine (Nielsen, 2009). Sufficient intestinal damage may result in gut leakage and loss of protein. *Ostertagia Sp*. is considered the most economically impactful parasite in cattle (Hawkins, 1993). *Ostertagia Sp.* infect cattle two different ways, type I, which typically affects young animals, and type II which affects mature cattle (Bansal et al., 2012). During a type I infection larva will not enter an arrest stage, progressing through their development normally (Bansal et al., 2012). Type II infections will enter an arrest stage and will not begin to develop until the animal's immune system is weakened (Bansal et al., 2012). Known to reduce feed intake, *Ostertagia Sp*. interacts with the host animal through intestinal hormones by significantly increasing host gastrin and pepsinogen levels (Hawkins, 1993; Fox et al., 2006). *Ostertagia* infections may also decrease nitrogen

digestibility, changing normal mineral blood concentrations, and cause severe diarrhea (Porter et al., 1942; Fox et al., 2006).

A statewide survey was conducted 1993 across the state of Kentucky. Fecal samples were collected from 1765 cattle across 11 counties and were analyzed (Lyons et al., 1995). Anthelmintic use was discontinued on each from four to eight months before the study and anthelmintic included: oxfendazole on 10 farms, fenbendazole and ivermectin on one farm, and levamisole on three farms. Fecal egg counts were measured using the floatation method with a saturated zinc sulfate solution. Additionally, the presence or absence of specific type of eggs or larvae was measured in fecal samples from all cattle in the study. From this data researchers were able to make two clear observations. First, calves were more susceptible to parasitism than mature animals. Second, roundworms, specifically *Trichostrongylus*, were the most prevalent parasite in the animals with 93% of infected animals containing roundworms. This study suggests that roundworms are the greatest threat to the health and performance of growing calves.

Coccidia

Coccidia is another parasite that resides in the gastrointestinal track of its host. Unlike roundworms, coccidia is a protozoon and is a major risk to young calves (Reddy et al., 2015). It is said that almost all cattle are infected with coccidia, however, not all animals will suffer from coccidiosis (Mundt et al., 2005). Once infected, coccidia promotes physiological damage to intestinal lining and intestinal mucosa (Stockdale, 1977a). Coccidia does not cause significant cases of mortality, however, it is economically important due to its subclinical complications, symptoms that include diarrhea, reduced intake, and reduced gain (Reddy et al., 2015).

Lungworms

Lungworms, like their name implies, reside in the bronchial tubes of the lungs (Cockcroft, 2015). In the L3 stage, lungworms will penetrate the intestinal lining and will use lymph glands to work their way to the lungs of cattle. Lungworm infections will cause local inflammation and may lead to long coughing spells along with shortness of breath. Lungworms are especially dangerous to calves in the Southeast. BRD is a debilitating issue affecting cattle who are transported off the farm and is a leading cause for increased morbidity and mortality in feedlots. Evidence suggests that lungworms may increase the risk of BRD and may even exacerbate the symptoms of BRD (Cockcroft, 2015).

Tapeworms

Tapeworms are a gastrointestinal worm that can be found in the intestinal lining along with other tissues like muscle and liver (Porter et al., 1942). Tapeworms are unique from other GIN because adult tapeworms are not found in cattle, they are found in humans (Khaniki et al., 2010). Within the host, tapeworms will grow in segments, with the terminal segments containing eggs that are then released into the intestine. Humans can acquire tapeworms from cattle by consuming contaminated product that has been prepared improperly (Khaniki et al., 2010). Tapeworm eggs must use an intermediate host to survive in the environment. Tapeworms typically utilize grass mites, who will eat tapeworm eggs and the tapeworm will develop within the mite. The biggest symptom of a tapeworm infection is the cyst in the liver, lungs, or any tissue the larvae burrows into.

Liver Fluke

Flukes, much like tapeworms, require an intermediate hose to survive during the early developmental stage. During the larva stage the fluke worm will infect snails, where it will reproduce and expel eggs back into the pasture (Gadberry et al., 2012). Once consumed by cattle, fluke worms will migrate to the liver, where they reside in the bile ducts. In the liver, the parasites will cause localized inflammation and fibrosis of the liver tissue (Gadberry et al., 2012). Liver damage caused by parasites may impact the packers' profit. Livers account for 30% of profit of the packing industry (Nagaraja et al., 2007)

Anthelmintic

Anthelmintic is a class of drugs that are used to treat and prevent parasitic infections (Holden-Dye et al., 2014). There are several anthelmintic drugs designed to combat parasites, however, anthelmintic drugs generally have a specific mode of action and only a few anthelmintic classes are considered to be broad-spectrum and can treat multiple different phyla of parasites (Hu et al, 2013). Broad-spectrum options are often preferred in livestock production due to their ability to target a large array of species, while remaining relatively harmless to the animal (Hu et al, 2013). The two most common anthelmintic used in agriculture are macrocyclic lactones, benzimidazoles.

Macrocyclic lactones were originally discovered as a natural product that was produced by *Streptomyces avermitilis*, a bacteria strain that resides in grass. Macrocyclic lactones do not kill parasites, instead they are antagonist to nACh and GABA-gated chloride channels (Krause et al., 1998; Pemberton et al., 2001). Once stimulated, the neuropeptide channels allow an influx of ions into the nerve cells, leaving the parasite

paralyzed (Pemberton et al., 2001). The popularity of macrocyclic lactones is largely due to their high specificity to invertebrates and effectiveness when administered in low doses (Haber et al., 1991). Additionally, while there are different forms of macrocyclic lactones on the market, there is little evidence of toxicity in low doses (Yang, 2012).

Benzimidazoles have a unique mode of action, disrupting the parasite's cytoskeleton and microtubules, impairing critical functions like movement and reproduction (Holden-Dye et al., 2014). Benzimidazoles are more effective in ruminants, as the rumen will slow the rate of passage. However, there is low absorption within the digestive tract. Optimal administration is dependent on animal weight and target parasite, with either a single large does or multiple small doses serving as an effective treatment (Page, 2008). Like Macrocyclic lactones, Benzimidazoles are highly specific with low toxicity in mammals, making them a popular option for livestock parasite prevention (Jaeger et al., 2017).

Anthelmintic strategies have a positive production impact on herd health and herd performance (Miller, 1993). Utilizing anthelmintic as a preventive management tool may limit infections across the herd. Anthelmintic have been shown to: 1) Reduce the parasite load within the animal 2) increase animal performance 3) increase reproductive success 4) produce a positive economic impact (Andresen et al., 2018).

Fecal egg counts & performance benefits

The economic impact of parasites on the beef industry is difficult to quantify. However, the estimated cost of parasites, was estimated to be $2.5 billion annually in 2013 (Walker et al., 2013). Consequences of clinical cases of parasitism may require

veterinary care or end in mortality, both of which impact the potential profit of the animal. High parasitic loads depress DMI, and impact protein absorption, both which impact the animal's overall ability to utilize energy (Hawkins, 1993).

The development of broad spectrum anthelmintic changed the way livestock producers managed parasites within their herd. Instead of exclusively treating infected animals, producers were able to implement preventive programs that would limit or even prevent infections (Gasbarre et al., 2015). Preventing parasitic infections improves the growth performance of the herd, leading to long term production and economic gains for the farm. Stromberg et al. (2006) measured the benefits of fenbendazole on eggs per gram (EPG) reduction and animal performance over two years in a pure-bred shorthorn herd. In year 1, 60 cows and 12 bred heifers were allocated to the study; and in year 2, 61 cows and four bred heifers were allocated to the study. Cattle were stratified by age and randomly assigned to one of two groups: 1) treated with oral suspension fenbendazole administered at 5 mg per kg of body weight, 2) untreated, control group. Over the two years, untreated cows gained, on average, 18.3 kg more than the treated cow ($P = 0.001$). Additionally, body condition scores (BCS) for untreated cows were greater than treated cows at weaning ($P = 0.0218$). Deworming did improve reproduction, with on average a 12.1% increase in pregnancies in the treated group compared to the untreated group ($P = 0.0357$). Unlike the cow group, treated calves gained 18.6 kg more than untreated calves ($P < 0.001$). Moreover, ADG in the treated calves was 0.13 kg greater than the untreated calves. Furthermore, EPG counts were significantly lower in both calves and cows after the first treatment in May ($P < 0.0001$).

Rehbein et al. (2013) has observed similar results when administering eprinomectin, classified a macrocyclic lactone, in five studies across three countries. Of the five studies conducted, three were conducted in the United States (studies 3,4 & 5), one study was conducted in Germany (study 2), and one study was conducted in the UK (study 1). Breeds included in this study were: Holstein, Limousin, Pinzgauer, and cross-bred beef cattle (not specified) were included in this study. Animals in all studies were grazed for 120 days on a naturally infected pasture. Studies one through four were conducted using a randomized block design based on pre-treatment, d 0, body weights. Treatment groups were arranged so both groups included 15 animals each and animals were randomly sorted into one of the two groups: 1) control, or 2) injectable extended release eprinomectin 1 mL per 50 kg of body weight. Animals were randomly assigned to either the control group, or eprinomectin group. All animals were euthanized 27-30 days after the removal from the pasture and parasites were counted via necropsy. The extended release eprinomectin was ≥ 94% reduction for all post treatment time points. When analyzing disappearance of specific parasites, treated cattle had fewer strongylid eggs recovered in feces than the feces from the control animals, 102 EPG compared to 0, respectively ($P < 0.05$). At necropsy, treated cattle had fewer nematodes than the control cattle ($P < 0.05$). Additionally, treated cattle, on average gained between 4.8 to 41.0 kg more than their respective control ($P < 0.05$).

Andresen et al. (2018) compared the effects of extended release eprinomectin to ivermectin on the performance and reproductive success of a cow calf herd. In this study, 119 fall-calving cows were separated into two different pastures by age, with pasture 1 including first calving heifers and pasture 2 including mature cows. Cows/heifers on each

pasture were then weighed and assigned one of two treatments: 1) short term injectable ivermectin, and 2) injectable extended released eprinomectin. Body weight in the heifers tended to be greater when treated with eprinomectin ($P \leq 0.10$) and ADG was greater in ivermectin treated heifers ($P < 0.01$). In the cows, eprinomectin treated cows weighed 77.1 kg more than the ivermectin cows ($P < 0.01$). Moreover, while ADG decreased in all groups, the lowest ADG was observed in the ivermectin cows. When compared to ivermectin, cows that were treated with extended release eprinomectin tended to have greater pregnancy rates, 96.9% compared to 88.6% ($P = 0.15$). Furthermore, heifers treated with extend-release eprinomectin calved 21 days earlier in the season ($P = 0.02$).

Anthelmintic resistance

The World Association for the Advancement of Veterinary Parasitology guidelines defined anthelmintic resistance when efficacy is below 95% (Geary et al., 2012). Fourteen years later Mason and Mckay (2006) measured the effect on anthelmintic resistance across 5 farms in New Zealand. Herds on all farms were suffering from clinical signs of parasitism. Cattle on each farm were blocked by weight and 47 to 81 calves per farm were randomly sorted into one of four groups: 1) pour on levamisole administered at 10 mg per kg, 2) pour on ivermectin administered at 0.5 mg per kg, 3) pour on eprinomectin administered at 0.5 mg per kg, and 4) a combination of levamisole and ivermectin delivered at the rates described previously. Fecal samples were collected before the treatment and 7, 14, 21, and 28 days after treatment. Feces were analyzed using the modified McMasters method to determine EPG count. On four out of five farms tested, EPG in cattle treated with levamisole were increased in day 28. Additionally, the response to specific parasites varied based on compound, with eprinomectin effective

against *Ostertagia spp*., but not effective against *C. oncophora*. Only the combination application of ivermectin and levamisole was effective against all genera of internal parasites on all farms. Body weight did not differ between treatment on day 14 and 28 after treatment ($P = 0.503$).

With increasing reports of anthelmintic resistance across the world, Geurden et al. (2015) conducted a study to measure the efficacy of ivermectin and moxidectin across Europe. In this study, 753 animals, from 40 farms across Italy, Germany, France, and the UK were included, and farms were selected based on historic use of macrocyclic lactones with animals that contained at least 20-50 EPG. On each farm, 20 to 50 animals were blocked based on EPG count and within each block animals were randomly assigned one of two treatment groups: 1) injectable ivermectin at 0.2 mg per kg, or 2) injectable moxidectin at 0.2 mg per kg. Fecal samples were collected at d 0 prior to treatment and again 14 days later. Feces were analyzed using the modified McMasters method to determine EPG count. Additionally, effective reduction was based on the WAAVP guideline of 95% reduction. In Germany, moxidectin was considered effective on four out of twelve farms and ivermectin was effective on two of twelve farms. In Italy, moxidectin was determined to be effective in nine out ten farms tested and ivermectin was effective on seven out of ten farms. In the UK moxidectin was determined to be effective in four out of ten farms and ivermectin was effective in three out of ten farms. Finally, in France, moxidectin was determined to be effective in three out of four farms and ivermectin was effective in four out ten farms. Out of 40 farms sampled, reduced efficacy to moxidectin was found on 20 farms, and reduced efficacy to ivermectin was found on 24 farms. Moreover, both moxidectin and ivermectin resistance was confirmed

on five different farms, 12.5%, and 7.5% of farms were confirmed to have resistant parasites to both drugs.

Resistance is even being reported in the United States. In 2007 the United States Department of Agriculture started a nationwide survey called the National Animal Health Monitoring System beef study (Gasbarre et al., 2015). 72 producers from 19 states were included in the study. Each producer was required to ensure that cattle were not treated for 45 prior to the start of the study and were instructed to treat with the same anthelmintic they have historically used. Feces were collected prior treatment, and again fourteen days later. Samples were taken from 20 weaned calves from each farm and calves were randomly sampled from the same group on both days. Samples were analyzed using the modified Wisconsin technique to determine EPG count. Reduced efficacy was defined as less than a 90% reduction following the anthelmintic treatment. The 90% cut off, while lower than the WAAVP guidelines, was based on the work of Levecke et al. (2012), who found that a 90% cut off limit was sufficient at detecting reduced efficacy in samples sizes with fifteen or more animals. When tested, more than 1/3 of all operations did not meet the 90% fecal egg count reduction test cut off value. Moreover, all operations that did not meet the 90% cut off value used a macrocyclic lactone, both in a pour-on and injectable form. The most observed parasite post-treatment was *Cooperia spp.*, which was observed in 95% of post-treatment samples that contained parasite eggs. *Ostertagia ostertagi* and *haemonchus contortus* were also observed in 40% and 50% respectively, of post treatment samples. While there were only five farms that used a benzimidazole instead of a macrocyclic lactone, all five farms observed reductions greater than 90%.

CONCLUSION

Post-weaning management is critical to mitigate depressed production and morbidity. Literature has shown that supplementing with protein and feed additives have the potential to provide both nutritional and health benefits, especially during the weaning phase of production. While ionophores have been thoroughly researched and are widely used, plant extracts are still relatively new and require further research to prove efficacy in a production setting. Furthermore, properly managing parasites has also shown to increase animal performance and reduce health issues. Despite the growing observations of anthelmintic resistance on farms, new anthelmintic development is slow. Management practices may be required to bridge the gap until a new option reaches the market (Kaplan et al., 2012). Improved anthelmintic strategies will be required to combat increasing threat of anthelmintic resistance. The research that follows includes two experiments to address these issues.

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CHAPTER 3

POST-WEANING ANTHELMINTIC STRATAGIES: PERFORMANCE BENEFITS AND EFFICACY OF UTLIIZING A COMBINATION OF TWO DIFFERENT CLASSES OF ANTHELMINTICS

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Abstract

The effect of three different anthelmintic strategies on growth performance and eggs per gram (EPG) count during a 42d backgrounding trail on four UGA research farms: JPC, Calhoun, Eatonton, and Alapaha. On each farm newly weaned calves were stratified by weight, sex, and age and assigned one of four treatment groups: 1) Oral Suspension Oxfendazole (ORAL), 2) transdermal Eprinomectin (POUR), 3) Both anthelmintic drugs (BOTH), and 4) a control group (CON) whom received nothing. On d0 body weights were recorded, fecal samples were collected, and animals were placed into their treatment groups; on d14 fecal samples were collected to measure EPG difference. In addition, on one farm rumen fluid was collected on d0 and d6 from n=20 animals, or five animals from each group to measure volatile fatty acid changes in the rumen. There was a location effect ($P < 0.001$). Accounting for the difference in weight, from d0 to d42, body weight greater in the ORAL and BOTH groups compared to the CON group ($P = 0.008$), with ORAL and BOTH groups gaining five and four more kg respectively, compared to the CON group. ADG responded similarly ($P = 0.008$), with total ORAL gaining 0.43 kg per day and BOTH gaining 0.42 kg per day, compared to the CON group who gained 0.33 kg per day. When analyzing fecal egg counts, there was not a location effect. There was a treatment effect, with all three treatments producing a greater EPG reduction when compared to the CON ($P < 0.001$). While not statistically significant, BOTH and ORAL produced a numerically better reduction rate than pour on, with BOTH at 98.8% reduction and ORAL at 88.3% reduction, compared to pour on at 71.3% reduction ($P > 0.09$). The performance and EPG reduction advantages indicate

that using both Oxfendazole and Eprinomectin in tandem is an effective strategy for backgrounding cattle.

Key words: beef, background, anthelmintic, parasites

Introduction

Gastrointestinal parasites are a tremendous burden on the beef cattle industry, especially in the Southeastern United States. In the U.S. internal parasites cost the industry over 3 billion dollars annually (Derouen et al., 2009). Parasitism can impact production by depressing weight gain, reducing feed intake, reducing reproductive success, and depressing lactation (Andresen et al., 2018). The development of broad spectrum anthelmintic changed the way livestock producers managed parasites within their herd. Instead of exclusively treating infected animals, producers were able to implement preventive programs that would limit or even prevent infections (Gasbarre et al., 2015). In 2008, the USDA surveyed 24 states that incorporates over 79.6% of cow-calf operations. Among the operations surveyed, 86.8% of the operations dewormed their cows and 54.1% of operations deworm calves at weaning (APHIS, 2009).

Developing deworming strategies, however, can be challenging, as there are a variety of anthelmintic options on the market. The popularity of broad-spectrum anthelmintic use, combined with the variety of deworming strategies, has increased the environmental pressure on parasites, increasing resistance to broad-spectrum anthelmintic drugs. Resistance to anthelmintic drugs has been observed both in the U.S. and other countries (Geurden at al., 2015). Developing new anthelmintic drugs is one solution, however it is estimated to cost over 400$ million to development a new anthelmintic drug

for livestock use (Abongwa et al., 2017). Furthermore, the development process is time consuming, and companies may not be incentivized to develop new products with the current options on the market.

Presently, the most popular anthelmintic strategy is to routinely utilize one single type of anthelmintic drug (Nielsen, 2009). Utilizing one anthelmintic, while not ideal, does limit parasites from becoming resistant to multiple anthelmintic drugs, however, it can also depress the benefits of anthelmintic application. Anthelmintic rotation is an effective strategy at limiting anthelmintic resistance (Leathwick, 2013). Alternatively, concurrent application of two different classes of anthelmintic drugs has shown to be effective, even in herds with known resistance (Leathwick, 2013; Edmond et al, 2018). A cow/calf operation can take advantage of these alternative strategies while mitigating the chance of developing on farm resistance to multiple anthelmintic drugs. Effective strategies can potentially increase revenue during a background phase by increasing average weight gain and increasing the value of the calf crop compared to untreated calves. Despite the potential benefits of utilizing alternative strategies in a cow-calf operation, little research has measured the efficacy and effect on performance in a cow-calf operation. Therefore, the objective of this study was to evaluate the efficacy and animal performance of oxfendazole, eprinomectin, and a combination of both during a 42-day background phase.

Materials and Methods

All practices and procedures used in this study were examined and approved by the University of Georgia Animal Care and Use Committee.

Animal and Diet Management

This Study utilized four cattle herds at four University of Georgia research units and included the Eatonton Beef Research Unit (Eatonton), Northwest Georgia Research and Education Center (Calhoun), Alapaha Range Station (Alapaha), and the J. Phil Campbell Sr. Research and Education Center (Watkinsville; JPC). At each location, calves were weighed at weaning and stratified by weight, age, and sex into groups and within groups animals were randomly assigned into one of four treatments: 1) oxfendazole (Synanthic®, Boehringer Ingelheim, Duluth, GA), 2) transdermal eprinomectin (Eprinix®, Boehringer Ingelheim, Duluth, GA), 3) Both (BOTH), and 4) control (CON) group whom received nothing. Anthelmintic was applied per manufacture recommendation, the transdermal eprinomectin was administered at 1ml per 10kg and oxfendazole was administered orally at 1ml per 50kgs.

At Eatonton, 123 nine to ten-month-old spring born calves were weaned in September 2019 (272.8 kg ± 40.8). At weaning, calves were vaccinated with Triangle 4, Type II BVD, and Ultra Vac 8 (Fort Dodge Animal Health, Overland Park, KS). During the 42d trail, calves were backgrounded on stockpiled fescue (*Festuca arundinacea*) and supplemented with 50:50 mix of corn gluten and soybean hull pellets at an approximate rate of 3.6 kg per head per day. In previous years, the herd, including bulls, cows, and calves were dewormed with transdermal ivermectin (Pfizer, New York, NY).

At Calhoun, 86 six to seven-month-old spring born calves were weaned in September 2019 (255.9 kg ± 41.6). At weaning all calves were vaccinated with Bovishield Gold and Ultra Choice 7 (Zoetis, Florham Park, NJ). During the 42d trail, Calves were backgrounded on fescue (*Festuca grundinacea*) and annual ryegrass (*Lolium multiflorum*) pastures, along with Russel Bermudagrass (*Cynodon dactylon*) hay and baleage. Calves were also supplemented with a 50:50 corn gluten feed and soybean hull mix delivered at 3.6 kg a head per day. In previous years, the entire herd, including bulls, cows, and calves were treated with transdermal Dectomax (Zoetis, Florham Park, NJ), and injectable Cydectin (Bayer, Shawnee Mission, KS).

At Alapaha, 109 five to eight-month-old spring born calves were weaned in September 2019 (260.7 kg ± 36.7). At weaning, calves were vaccinated with Bovishield Gold and Ultra Choice 7 (Zoetis, Florham Park, NJ). During the 42d trail, calves grazed on crabgrass (*Digitaria sanguinalis*) pastures and were fed Tifton 85 bermudagrass hay (*Cynodon dactylon*). All calves were supplemented with a ration that consisted of 33% whole corn, 33% corn gluten pellet, 33% soybean hull pellet, and 1% limestone which was fed at 2.27 kg per head per day. The past two years, anthelmintic drugs were not used as a prophylactic, only treating cattle that demonstrated observable clinical signs of parasite infections. The heard was also annually screened for parasites by a veterinarian using fecal egg count measurements. Historically, the herd, including bulls, cows, and calves were treated with oral suspension fenbendazole (Merck, Kenilworth, NJ) and transdermal Cydectin (Bayer, Shawnee Mission, KS).

At JPC 74 eight to ten-month-old spring born calves were weaned in September 2019 (278.9 kg ± 24.3). At weaning the steers were vaccinated with pyramid 5+ (Fort

Dodge Animal Health, Overland Park, KS) and Vison 7 with spur (Merck, Kenilworth, NJ); the heifers were vaccinated with Express 5 VL5 (Boehringer Ingelheim, Duluth, GA) and Vision 7 with spur (Merck, Kenilworth, NJ). During the 42d trail calves were fed with ad libitum alfalfa silage and dry hay and were supplemented with a 70:30 ground corn and cotton seed mix at a rate of 2.27 kg per head per day. In previous years, the herd, including bulls, cows, and calves were dosed with orally suspended oxfendazole.

Animal performance and Economics

Weights were collected at each location prior to treatment on d0 and on the final day d42. Additionally, the value of each calf at the end of the trail was determined using a conservative value of 2.60$ per kg gained over the 42d period. The net value of each calf was calculated by subtracting the cost of the treatment from the value added over the 42d period. The cost of each treatment was: Eprinomectin at $3.00 per head, oxfendazole at $1.20 per head, both treatments at $4.20 per head, and control the control group did not have a cost.

Anthelmintic efficacy

Fecal samples were collected at each location prior to treatment on d0, and again on d14, to evaluate differences in eggs per gram (EPG). Fecal samples were collected into 50ml conical tubes and transported to the laboratory on ice. Fecal samples were analyzed using the modified McMasters fecal egg count procedure at the University of Georgia veterinary diagnostic lab, Athens, GA .Fecal egg reductions were calculated using a critical test, which determines efficacy based on eggs eliminated in feces,

compared to the preferred control test, which compares specific parasite populations between necropsied animals that were either treated or untreated (Wood, 1995). The pre-treatment and post-treatment fecal egg counts were used to calculate the efficacy for each of the treatment groups using the following formula:

$$\%\ Reduction = 100\ x\ \frac{(pre - treatment\ count) - (Post - treatment\ count)}{pre - treatment\ count}$$

Volatile Fatty Acid analysis

Rumen fluid was collected by esophageal tubing from 20 animals on d0 and d6 at the Eatonton location. Rumen fluid was collected into 50 mL conical tubes and were stored on ice at 0°C when samples were transported to the lab. Upon arrival, samples were stored in a -80°C freezer until samples were analyzed. Concentrations of VFAs were determined using an ethyl acetate extraction processed described by Lourenço et al. (2020). In summary, 5 mL of rumen fluid was centrifuged for 10 min at 10,000 x g at 4°C, and 2.5 mL of the supernatant was transferred to another centrifuge tube. Then, 1 mL of an internal standard, was added and samples were vortexed and frozen overnight. Samples were thawed and centrifuged for 10 min at 10,000 x g at 4°C. One mL of supernatant was transferred into a vial and mixed with 2 mL of ethyl acetate, and the vial was vortexed and left to sperate for 5 mins. Half a mL of the ethyl acetate fraction was transferred to another vial to be analyzed by gas chromatography (Shimadzu GZ-2010 Plus: Shimadzu Corporation, Kyoto, Japan) using a flame ionization detector and a capillary column (Zebron ZB-FFAP GC Cap. Column 20 m x 0.32 mm x 0.25 um; Phenomenex Inc., Torrance, CA). Column temperature was initially set to 110°C and

gradually increased to 200°C. Injector and detector temperatures were set at 250°C and 350°C, respectively.

Statistical Analysis

Data was analyzed in SAS 9.4 (SAS Inst. Inc., Cary, NC) using a randomized complete block design with location, weight, age, and sex used as blocking factors. Means were separated using least square means. Pairwise comparisons were computed using the LSMEANS student's t test. Animal was defined as the experimental unit and was used to determine differences across all four treatments. The By statement was utilized to compare means within a treatment across time, and among treatments for a given sample. Differences were considered significant at $P = 0.05$ and tendencies were considered at $P < 0.10$.

Results and Discussion

Anthelmintic efficacy

All Fecal analysis data are presented by location in Table 3.1. and by treatment in Table 3.2. On d0 there was a location difference in fecal egg counts ($P < 0.05$), although there was not a treatment or treatment by location effect ($P > 0.743$). On d0 both JPC and Eatonton locations had lower fecal egg counts compared to Calhoun but did not differ from Alapaha (Table 3.1.). On d14 there was a treatment ($P < 0.001$) and location ($P = 0.039$) effect along with a tendency for treatment × location ($P = 0.076$) effect. On d14, both Eatonton and Alapaha had fewer EPG than Calhoun, and did not differ from JPC (Table 3.1.). Additionally, on d14, EPG did not differ between JPC and Calhoun (Table

3.1.). On d14 EPG in CON was greater than all other treatments (Table 3.2.). Using both eprinomectin and oxfendazole in combination produced the fewest EPG on d14 (Table 3.2.). Moreover, EPG in BOTH did not differ from ORAL but was less than POUR (Table 3.2.). Additionally, POUR did not differ from ORAL (Table 2.)

There was not a location effect ($P = 0.272$), but there was a treatment ($P < 0.001$) and a location × treatment effect ($P = 0.045$) on fecal egg count reduction. This was a result of the control groups at both Eatonton and JPC, who increased EPG count by 60.1% and 139.7%, receptively, from d0 to d14. As such, this discussion will focus on the comparison of treatments.

All three anthelmintic strategies decreased fecal egg count reduction compared to the control group ($P < 0.001$). Furthermore, BOTH produced > 95% fecal egg reduction across all locations. These results demonstrate that the concurrent application of both drugs was effective, based on the World Association for the Advancement of Veterinary Parasitology (WAAVP), which sets the minimum limit for an effective anthelmintic at 95% or greater reduction in EPG (Wood, 1995). Regardless, eprinomectin failed reach 95% EPG reduction, reducing EPG by only 71.3% (Table 3.2.), which is below the effective rating set by the WAAVP, in addition to highlighting possible resistance to transdermal oxfendazole at both Calhoun and Eatonton locations. Every location has historically used a pour on macrocyclic lactone to on the herd. These results support Edmounds et. al. (2018) who measured concurrent application of macrocyclic lactones along with benzimidazole in a herd with macrocyclic lactone resistant parasites. Fecal egg counts confirmed resistance to injectable doramectin and extended release

eprinomectin, but also found 100% reduction in animals who were treated with both eprinomectin and albendazole (Edmond et al., 2018).

Developing resistance to anthelmintic drugs can reduces the genetic fitness of the parasite. Moreover, the fitness cost to developing resistance to multiple drugs is additive and can reduce the chance for the resistant populations to persist in the environment (Leathwick, 2013). When genetic fitness was accounted for, models demonstrated that using a combination of anthelmintic drugs was more effective than using a single anthelmintic over 40 years, especially in a naïve population or when refugia was maintained in the herd (Leathwick, 2013). The results support the use of a combination of anthelmintic drugs as an effective deworming strategy.

Animal Performance

Performance and economic data are presented by treatment in Table 3.3. below. On d0 and d42 there was only a location effect ($P < 0.001$). Unlike the other three locations, JPC calves were weaned in November. Weaning at a later date allowed the calves to gain more weight before they were placed on the trial, which led to greater d0 weights. On d42 JPC calves were the lightest, and this was also caused by the later weaning time as JPC weaned in the winter months when there is less available forage for the calves to graze on. There was both a treatment ($P = 0.007$) and location effect ($P < 0.001$) for weight gained and ADG. Calhoun animals gained the most weight and had the greatest ADG compared to the other three locations (Table 3.3.). Moreover, cattle in either the ORAL and BOTH treatment groups had greater weight gained and ADG compared to the control group (Table 3.3.). The POUR group was not statistically different from the control group (Table 3.3.). These results are similar to Walker et al.

(2013) who measured the effect of oxfendazole, moxidectin, and a combination of the two given at separate times on the performance of beef calves weaned during the summer. Like the results observes in this study, using a combination of both oxfendazole and moxidectin produced significantly greater ADG when compared to the control and moxidectin. Moreover, there was no difference detected in ADG between the oxfendazole group and the combination group. These results suggest that when efficacy of an anthelmintic drug decreases, utilizing a different class of anthelmintic drug or a combination of anthelmintic drugs improves growth performance in weaned calves, in addition to improving anthelmintic efficacy.

Additionally, the lower performance at the JPC location is most likely attributed to the time in which the calves entered the background phase, compared to the other three locations. Winter weight loss is a concern for producers and may be caused by a variety of factors (Kelin et al., 2011). Weaning during the winter months is exceptionally challenging on calves. When weaned in the winter, the calves' may have limited available forages for gazing. In this study winter weaning resulted in an average loss 9.76 kgs at the JPC location.

Economic Results

Value-added results match weight gain results, and there was a treatment ($P < 0.001$) and location ($P = 0.007$) effect for value added by weight. Additionally, when accounting for the cost of the anthelmintic doses, there was a treatment effect ($P = 0.033$). Accounting for the cost of the treatment, ORAL added the greatest value compared to other three treatments, adding $11.80 per head compared to the control calves (Table 3.3.). Additionally, when accounting for the cost of the anthelmintic, there

was no difference between BOTH, POUR, and CON for value added (Table 3.3.). These results suggest that, while more labor intensive, there is economic value to utilizing oral suspension benzimidazole in farms that have historically used transdermal macrocyclic lactones.

Volatile Fatty Acids

VFA data collected at Eatonton is reported by time in Table 3.4. below.

Plant extracts have been used as anthelmintic treatments in small ruminants (Kim et al, 2014). Studies have found significantly less rumen ammonia and significantly greater rumen VFA concentrations when anthelmintic plant treatments were added to the diet of goats (Frutos et al., 2008; Osoro et al., 2007). However, in this experiment, regardless of anthelmintic strategy, total VFA concentrations were not affected by treatment, time, or treatment × time ($P > 0.172$). Propionate and acetate to propionate ration did differ by time ($P < 0.0350$), however, there was not a treatment effect ($P > 0.237$). The lack of difference is most likely due to the mode of action of both anthelmintic drugs. The mode of action of both oxfendazole and eprinomectin is highly selective, either binding to the nicotinic acetylcholine receptors within the parasites muscles, or antagonist to highly specific glutamate-gated chlorine channels in the neurons and muscles of nematodes (Abongwa et al., 2017; Wolstenholme et al., 2006). These results suggest that neither transdermal eprinomectin, nor oral suspension oxfendazole influenced the rumen microbe population in beef calves.

Conclusion

New strategies must be implemented to limit anthelmintic resistance and maximize the benefits of anthelmintic use. This research has shown that using a combination of two different classes of anthelmintic is an effective strategy at reducing parasite infections. Furthermore, when the efficacy of a class of anthelmintic drugs has decreased, using a different anthelmintic drug can also be an effective at reducing parasite infections. Moreover, both strategies provided an economic and performance benefit that could provide an increase to the profits of a cow-calf operation during a background phase. However, future research will be required to measure the effect of using a combination of anthelmintic drugs on on-site development of resistance.

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Table 3.1. Fecal Eggs per gram (EPG) presented by location from weaned calves at four locations prior to (D0) and 14 d after (D14) receiving one of four anthelmintic treatments. Treatments included: 1) Orally suspended Oxfendazole, 2) Transdermal Eprinomectin, 3) Both, 4) no anthelmintic. Oxfendazole was administered at 1ml per 50kg and eprinomectin was administered at 1ml per 10kg

	Location					
	Eatonton	Alapaha	Calhoun	JPC	SEM	P-value
D0, EPG	210[b]	397[ab]	432[a]	233[b]	75.8	0.040
D14, EPG	60[b]	33[b]	156[a]	111[ab]	35.0	0.039
Absolute Reduction, EPG	150[b]	364[a]	276[ab]	122[b]	71.1	0.031
EPG Reduced, %	71.4	91.7	63.9	52.4	18.6	0.272

[ab] Means within a row without a common superscript differ ($P < 0.05$).

Table 3.2. Fecal Eggs per gram (EPG) presented by treatment from weaned calves at four locations prior to (D0) and 14 d after (D14) receiving one of four anthelmintic treatments. Treatments included: 1) Orally suspended Oxfendazole (Oral), 2) Transdermal Eprinomectin (Pour), 3) Both (Both), 4) no anthelmintic (Control). Oxfendazole was administered at 1ml per 50kg and eprinomectin was administered at 1ml per 10kg

	Treatment					
	Control	Both	Oral	Pour	SEM	P-value
D0, EPG	259	346	349	318	75.0	0.743
D14, EPG	225[a]	4[c]	41[bc]	91[b]	34.6	< 0.001
Absolute Reduction, EPG	34[b]	342[a]	308[a]	227[a]	70.3	0.002
EPG Reduced, %	13.1[b]	98.8[a]	88.3[a]	71.3[a]	18.6	< 0.001

[ab] Means within a row without a common superscript differ (P < 0.05).

Table 3.3. Individual Treatment Performance data presented by treatment. Data collected from weaned calves prior to (D0) and again on (D42) receiving one of four anthelmintic treatments. Treatments included: 1) Orally suspended Oxfendazole (Oral), 2) Transdermal Eprinomectin (Pour), 3) Both (Both), 4) no anthelmintic (Control). Oxfendazole was administered at 1ml per 50kg and eprinomectin was administered at 1ml per 10kg

	Treatment					
	Control	ORAL	POUR	BOTH	SEM	P-Value
Initial Weight, kg	265	266	261	263	3.88	0.741
Final Weight, kg	279	285	277	281	3.94	0.549
Weight Difference, kg	14[b]	19[a]	16[ab]	18[a]	0.957	0.007
ADG, kg	0.333[b]	0.433[a]	0.381[ab]	0.417[a]	0.022	0.007
[1]Value of Weight gained, $	$36.40[b]	$49.40[a]	$41.60[ab]	$46.80[a]	2.52	0.007
[2]Value of added with treatment cost included, $	$36.40[b]	$48.20[a]	$38.60[b]	$42.60[ab]	2.52	0.033

[ab] Means within a row without a common superscript differ ($P < 0.05$).

[1]Value was calculated using a conservative value of $2.60 per kg gained.

[2]Calulated using the average cost of each treatment: Drench = 1.20$, Pour on= 3.00$, Both = 4.20$

Table 3.4. Volatile Fatty acid data collected from rumen fluid samples collected at Eatonton and presented by time. Rumen fluid samples were collected from weaned calves prior to (D0) and six days after (D6) receiving one of four anthelmintic treatments. Treatments included: 1) Orally suspended Oxfendazole (Oral), 2) Transdermal Eprinomectin (Pour), 3) Both (Both), 4) no anthelmintic (Control). Oxfendazole was administered at 1ml per 50kg and eprinomectin was administered at 1ml per 10kg

	D0						D6					
	CON	BOTH	ORAL	POUR	SEM	P-Value	CON	BOTH	ORAL	POUR	SEM	P-Value
Volatile Fatty Acids												
Acetate	45.0	40.0	42.7	47.6	3.75	0.452	48.5	50.1	41.2	49.5	4.04	0.381
Propionate	8.57	7.21	8.21	9.14	0.78	0.284	10.2	10.4	8.86	12.0	0.645	0.445
Butyrate	5.30	4.66	5.11	6.38	0.621	0.201	5.71	5.73	3.75	5.93	0.655	0.087
Isobutyrate	0.403	0.396	0.409	0.430	0.029	0.810	0.441	0.480	0.455	0.447	0.074	0.975
Valerate	0.348	0.277	0.352	0.401	0.036	0.098	0.421	0.420	0.271	0.428	0.056	0.159
Isovalerate	0.665	0.602	0.623	0.727	0.055	0.328	0.673	0.725	0.582	0.602	0.107	0.725
Caproate	0.167	0.125	0.182	0.199	0.031	0.309	0.073	0.086	0.021	0.050	0.021	0.151
Total	60.4	53.3	57.6	64.8	5.15	0.372	65.98	67.94	55.19	68.93	35.78	0.317
A:P	5.27	5.56	5.25	5.24	0.161	0.372	4.78	4.87	5.32	4.22	0.508	0.470

[ab] Means within a row without a common superscript differ ($P < 0.05$).

CHAPTER 4

POST-WEANING NUTRITION STRATAGIES: EVALUATION OF PLANT EXTRACTS AND MONENSIN ON PERFORMANCE AND VOLITLE FATTY ACIDS

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Abstract

In a two-year study across two locations, 81 calves (initial BW = 287.8 ± 27.2 kg) were supplemented for 84 d (Year 1, Blairsville), 82 d (Year 2, Alapaha), 108 d (Year 2, Blairsville), and 82 d (Year 2, Alapaha) to evaluate the performance, carcass growth characteristics, and rumen fermentation effects of cinnamon and garlic extracts (SPM) compared to monensin (MON) and control animals (CON). Animals were stratified by weight into one of three treatments. Each treatment was separated into 3 different pens, with 9 animals per pen. Supplements were mixed into minerals and top dressed on top of feed supplements. Body weight was recorded at the start, mid-point, and endpoint of each year and ribeye area, intramuscular fat, and 12th rib fat thickness was measured via ultrasound at the start and endpoint of each study. Rumen fluid was collected at the mid-point and endpoint (Year 1, Blairsville) and the start and mid-point (Year 2, Alapaha). Animal performance data showed that all animals increased body weight over time ($P < 0.0001$). Additionally, there was a location effect ($P < 0.001$) for weight gain with calves at Blairsville gaining more weight than calves at Alapaha. Throughout the entire study, MON supplementation increased weight gain compared to SPM and CON supplemented calves ($P < 0.040$). Furthermore, after the midpoint, ADG was greater in the MON group, compared to the SPM and CON groups ($P < 0.025$). Ultrasound data showed that all predicted carcass traits (REA, IMF, FT) increased over time ($P < 0.001$). Additionally, there was not a treatment effect ($P = 0.689$), but there was a location ($P < 0.001$), with Blairsville cattle having greater values in all carcass traits. At Blairsville, MON supplemented animals developed the greatest REA ($P = 0.018$) compared to the SPM and CON animals; however, SPM supplemented animals developed the greatest

percent of IMF ($P = 0.007$) compared to the MON and SPM animals. REA and IMF did not differ at Alapaha, although, FT was lowest in MON supplemented animals compared to the SPM and CON group. VFA analysis showed that location had an effect on all VFAs measured ($P < 0.031$) with the exception of the branched chain fatty acids isovalerate and iso-butyrate ($P > 0.118$). Moreover, total VFA production was greater at Alapaha than Blairsville ($P = 0.030$). At Alapaha, the A:P ration was significantly lower in the MON group compared to the SPM group ($P < 0.003$) but did not differ at Blairsville ($P > 0.466$). Time also had an effect at both locations ($P < 0.001$) with total VFAs increasing at Blairsville and decreasing at Alapaha. Data from this study suggest that this mix of cinnamon and garlic extract does not perform similarly to monensin. However, the plant extracts did improve IMF in calves on a corn silage diet. Increased IMF can lead to improved quality grade at slaughter and increase the value of the animal.

Key words: Plant extract, Ionophore, monensin, garlic, cinnamon, secondary plant metabolites, essential oils, organosulfur compounds.

Introduction

Ionophores are a commonly used feed technology in the cattle industry and have been since the approval of monensin in 1975 (Osweiler, 2011). Ionophores, classified as an antibiotic, are also used as a growth promoter, gut modulator, and a prophylactic supplement (Wallace, 1995). Ionophores interacts with the cellular membrane of gram-positive bacteria, disrupting the normal ion concentration gradient of the bacteria, limiting the bacteria from performing normal metabolic functions (Bell et al., 2015). The specificity for gram-positive bacteria creates a flora shift within the rumen, affecting normal volatile fatty acid production, increasing propionate production, and decreasing

acetate production (Bell et al., 2015). Several ionophores are commercially available such as lasalocid, salinomycin, and most recently, laidlomycin propionate, however monensin is the most commonly used. Ionophore's decrease methane production in high starch diets while also increasing average daily gain, feed efficiency, and apparent digestibility (Duffield et al., 2015). Moreover, monensin can provide similar benefits to grazing cattle, either increasing feed efficiency or depressing intake, while either maintaining or improving weight gain; although, these benefits are typically reduced when supplemented into a diet with poor nutritive quality (Vendramini et al., 2015). Furthermore, ionophores, depending on diet, can mitigate acidosis in high grain diets in addition to preventing bloat (Bell et al., 2015).

Regardless of their benefits, there are many concerns regarding antibiotic use in agriculture. Bacteria can develop resistance to antibiotics, rendering the drug ineffective (McEwen, 2007). Resistance to ionophores has been observed in cattle operations, regardless if the cattle were supplemented with ionophores or not (Russell, 2003). Moreover, research has found no evidence that ionophore usage is a risk to current human pharmaceuticals options (Callaway. 2003). Despite this, Europe banned the use of ionophores in 2006 (Florez-Caudrado, 2018), and a similar proposal was considered in the U.S. but was ultimately not included in the Animal Drug Availability Act (Allen et al., 2013). Despite the lack of regulatory limitations, consumer concerns over antibiotic use in agriculture has increased the prevalence of organic and all-natural marketing streams. Producers who elect to sell their animals through these marketing streams can increase the profit of their operation, however, they cannot use antibiotic like ionophores

(Umberger et al., 2009). The European ban, along with consumer awareness, has motivated researchers to look for alternatives to ionophores.

Secondary plant metabolite such as phenols, terpenes, and organosulfur compounds, are synthesized by plants as protection from environmental predators like competing plants and scavenging animals (Pagare et al., 2015). Plant metabolites have historically been utilized as pharmaceuticals due to their antifungal and antimicrobial activity (Voda et al., 2003). Recently, SPM have been investigated in livestock due their antioxidant activity and gut health benefits in human health (Beretta et al., 2017). Like ionophores, secondary plant metabolites like essential oils and organosulfur compounds modify ruminal fermentation end-products, decrease rumen protein degradation, and reduce methane (Cardoza et al., 2004). Moreover, in lactating dairy cows, a mixture of secondary plant compounds incorporating cinnamaldehyde and diallyl disulfide provided health benefits, including increased insulin concentration, decreased total cholesterol, and increased non-esterified fatty acids (Gorgulu et al, 2012). Despite the potential benefits of SPM, there is little researching evaluating the benefits of EO and OS in stocker cattle in the southeast. Therefore, the objective of this study was to evaluate the impact of a blend of EO and OS on animal performance and volatile fatty acid profile compared to monensin in stocker cattle in the Southeast United States.

Methods and Materials

All practices and procedures used in this study were examined and approved by the University of Georgia Animal and Care and Use Committee. The research was conducted during the fall and early winter at two locations and included the Georgia Mountain Research and Education Center (Blairsville), in 2017-2018 (year 1) and 2018-

19 (year 2), and the University of Georgia Alapaha Range Grazing Unit (Alapaha) in 2018-2019 (year 1) and 2019-2020 (year 2).

Animal and Diet Management

In Blairsville, 81 spring born steers (304 ± 27.3 kg) were used in year 1; and 81 spring born steers (319.2 ± 35.3 kg) were used in year two. Prior to the study, animals weaned in early September at the Eatonton Beef Research Unit in Eatonton, Ga. Weaned steers were vaccinated with Triangle 4, Type II BVD, and Ultra Vac 8 (Fort Dodge Animal Health, Overland Park, KS) and dewormed with transdermal ivermectin (Pfizer, New York, NY). Post-weaning, calves were backgrounded for approximately 45-60 days on stockpiled fescue (*Festuca arundinacea*) and supplemented daily with a 50:50 mix of corn gluten and soy pellets and fed at approximately 3.6 kg per head per day. In late October, steers were transported to the Blairsville location and weighed on arrival. Steers were given 28 days to acclimate prior to the start of the study. During the project, the steers were fed a corn silage-based diet, supplemented with dried distillers' grains at 1.81 kg per head per day.

In Alapaha, 81 steers (260.7 ± 17.1 kg) were used in year 1, and 81 steers (263.3 ± 39.7) were used in year 2. Prior to the study, steer calves were weaned at the Alapaha Range Grazing Unit in early September in both years. At weaning, calves were vaccinated with Bovishield Gold and Ultra Choice 7 (Zoetis, Florham Park, NJ). In year 1, calves were dewormed with either transdermal Cydectin (Bayer, Shawnee Mission, KS), or oral suspended fenbendazole (Merck, Kenilworth, NJ). In year 2, calves participated in a previous study and were either: not dewormed, dewormed with transdermal Eprinix® (Boehringer Ingelheim, Duluth, GA), orally suspended Synanthic®

(Boehringer Ingelheim, Duluth, GA), or both anthelmintic options. During both years, in late October, calves were backgrounded at Alapaha for approximately 42 to 60 days. During backgrounding, calves grazed on crabgrass (*Digitaria sanguinalis*) and were fed Tifton 85 bermudagrass hay (*Cynodon dactylon*) along with a 3:3:3 mix incorporating corn gluten pellets and soy pellets at 2.27 kg per head per day. During the trial, calves were fed bermudagrass hay, along with the 3:3:3 mix at approximately 3.0 kg per head per day.

At each location, steer calves were stratified by weight into nine different pens. Pens were randomly assigned to one of three treatments, such that there were three pens per treatment. Treatments include: 1) no supplementation (CON); 2) supplemented with the secondary plant metabolites, Garlic oil and Cinnamaldehyde (Cinnagar®) at a rate of 200 mg per head per day (SPM); and 3) supplemented with monensin (Rumensin®) at rate of 120 mg per head per day (MON). Animals had free access to water and feed was offered to groups once per day and the supplement was mixed into minerals top dressed on the feed. The mineral mix in this experiment is represented in Table 4.1 and the chemical composition of the base diets at Blairsville and Alapaha are represented in Table 4.2 below.

Animal Performance

To evaluate animal performance, calves were weighed at the beginning, middle, and end of the study at each location except for year 1 at Alapaha, in which only the initial and end weights were evaluated. Additionally, for both years at each location the Ribeye area (REA), Intramuscular fat (IMF), and 12th rib fat thickness (FT) were measured on calves on d 0 and the final day.

Ultrasound Data

Ultrasound measurements for REA, FT, and IMF were collected by a trained technician from the University of Georgia Meat Science Technology Center. The ultrasound system included an Aloka 500V equipped with a 17 cm-3.5 MHz transducer (Aloka Inc. Tokyo Japan). Ultrasound images were captured and measured using Beef Image Analysis (BIA) Feedlot software (designer Genes Technologies Inc, Harrison, AR). The ultrasound location was located on the right side, between the shoulder and 14th rib. Ribeye area and FT were collected between the 12th and 13th rib juncture, perpendicular to spinal column. IMF images were collected parallel to the spine and perpendicular to the 11th, 12th, and the 14th ribs. Prior to imaging, the site was clipped with a razor and a blow dryer was utilized to clean the area of loose air and debris. Vegetable oil, along with a waveguide (Designer Genes Technologies INC., Harrison, AR) was used to enhance the sensitivity of the measurements.

Volatile Fatty Acid analysis

Rumen fluid was collected by esophageal tubing from 26 animals in year 1 at Blairsville, 9 animals from CON and MON groups and 8 animals from the SPM group. In year 2 at Alapaha, rumen fluid was collected by esophageal tubing from 27 animals, 9 animals from each treatment group. Rumen fluid at Blairsville was collected from the animals at the midpoint, and again at the conclusion of the study. Rumen fluid at Alapaha was collected at the start of the study, and again at the midpoint. Rumen fluid was collected directly into 50 mL conical tubes and stored on ice at 0°C while samples were delivered to the lab. Upon arrival, samples were stored in a -80°C freezer until further processing. Concentrations of VFA in calves' ruminal fluid were determined in a water-

based solution using ethyl acetate extraction as described by Lourenço et al. (2020). In summary, ruminal fluid samples were removed from the freezer and allowed to thaw at 20°C. Five milliliters of rumen fluid were centrifuged for 10 min at 10,000 x g at 4°C, and 2.5 mL of the supernatant was transferred to another centrifuge tube and were frozen overnight in a -20°C freezer. Samples were then thawed at 20°C and centrifuged for 10 min at 10,000 x g at 4°C. One mL of supernatant was transferred into a vial and mixed with 2 mL of ethyl acetate, and the vial was vortexed and left to separate for 5 mins. 0.5 mL of the ethyl acetate fraction was then transferred to another vial and analyzed by gas chromatography (Shimadzu GZ-2010 Plus: Shimadzu Corporation, Kyoto, Japan) using a flame ionization detector and a capillary column (Zebron ZB-FFAP GC Cap. Column 20 m x 0.32 mm x 0.25 um; Phenomenex Inc., Torrance, CA). Column temperature was initially set to 110°C and gradually increased to 200°C. Injector and detector temperatures were set at 250°C and 350°C, respectively.

Statistical Analysis

Data was analyzed as a mix model in SAS 9.4 (SAS Inst. Inc., Cary, NC) and means were separated (LSMEANS). Pen was defined as the experimental unit, and animal was used as the observational unit used to determine differences across the three treatments. Animal in pen was used as the random error term and was used to test scores of variations. The By statement was utilized to compared means within a treatment across time and location among treatments. Analysis of variance was performed using treatment, year, and location as fixed effects, along with the interaction terms for these effects.

For VFA concentrations, ANOVA were constructed using treatment and sample time as factors, as well as treatment × sample time interaction. Additionally, for the mid

sample at both locations, ANOVA were constructed using treatment and location as factors, as well as treatment × location interaction. Contrasts were calculated using Tukey's pairwise comparison test, and results were considered significant at $P \leq 0.05$ and tendencies were considered at $P > 0.05$ and $P \leq 0.10$.

Results and Discussion

Performance Traits

There was year ($P < 0.001$) and location ($P < 0.001$) effect on final body weight, but there was no treatment affect ($P = 0.298$). Blairsville cattle were heavier on the final day compared to the Alapaha cattle. Moreover, at both locations, cattle were heavier in YR 2 than in YR 1. For total ADG there was a treatment ($P = 0.040$), location ($P < 0.001$), year ($P < 0.001$), treatment × year ($P = 0.002$), and treatment × location ($P < 0.050$). Cattle at Blairsville had greater ADG than cattle at Alapaha. Moreover, at Blairsville, ADG was grater in YR 2 compared to YR 1; while at Alapaha, ADG was greater in YR 1 than YR 2. Therefore, all animal performance data is presented and discussed by year within location and are located in Table 4.3 and Table 4.4.

Final body weight for YR1 and YR 2 in Blairsville did not differ (Table 4.3.), however, at Alapaha, year 2 cattle finished heavier than year 1 (Table 4.4.). In Blairsville, there was no difference between any of the treatment groups during YR 1 and YR 2 (Table 4.3.). In Alapaha, final body weight did not differ between any of the treatment groups during YR 1 and YR 2 (Table 4.4.). In Alapaha, only the SPM group differed from YR 1 to YR 2 with the YR 2 SPM group weighing more than YR 1 ($P = 0.007$). Moreover, cattle supplemented with MON had greater total ADG during the entire study

when compared to the CON group (P = 0.013). Moreover, total ADG in cattle supplemented with SPM tended to be less than MON (P = 0.094) but did not differ from the CON group (P = 0.923). In Blairsville, during YR 1, MON was greater than CON and SPM, while CON and SPM did not differ from each other (Table 4.3.). In YR 2, MON was greater than CON but did not differ from SPM, and CON and SPM did not differ (Table 4.3.). Furthermore, in YR 1, ADG of MON was greater than CON and SPM during both period 1 and period (Table 4.3.). In YR 2, there were no differences in ADG during period 1, while both MON and SPM were greater in period 2 (Table 4.3.). In Alapaha, there was no difference between any treatments during YR 1 or YR 2 (Table 4.4.). Additionally, in YR 2, both SPM and CON were greater than MON during period 1, while RUM and SPM were greater than CON in period 2 (Table 4.4.).

Previous research has been inconsistent, as there appears to be a relationship with diet and the effect of either additive. In Blairsville, cattle were fed a corn-silage diet that provided greater energy than the forage-based diet fed in Alapaha. In contrast to the current study, Ornaghi et al. (2017), found garlic and cinnamon extract improved growth performance in young beef bulls fed a cracked corn-based diet. Both cinnamon and garlic extract increased weight compared to the control group. In contrast to Ornahi et al. (2017), Yang et al. (2014) did not find any difference in body weight or ADG in beef steers fed a barley grain-based diets supplemented with either monensin or cinnamon extracts. In the current study, SPM did not increase ADG or final weight compared to MON or CON. Even at Blairsville, were the diet included corn-silage, ADG in SPM was less than MON (Table 4.3.). Calsamiglia et al., (2007) reported that an acidic rumen can enhance the effect of essential oils, which may be the cause for the difference between

Ornaghi et al. (2017) and the current study. In Blairsville, however, cattle were fed a diet based on corn silage, which, like high concentrate diets, decrease rumen pH (Graf et al., 2005).

This study, however, is concurrent with two previous studies measuring the effect of garlic and cinnamon extract on grazing beef cattle (Beck et al., 2017; Moriel et al., 2018). In stocker steers grazing on cool-season annual grasses, Beck et al. (2017) did not observe any body weight or ADG difference between control cattle and cattle supplemented with the garlic and cinnamon extract mix. Moreover, when Moriel et al. (2018) supplemented weaned heifers with a garlic and cinnamon extract mix over 72 days, there were no differences in performance between control calves and treated calves. This suggests that the essential oil mixture of cinnamon and garlic extracts do not affect growth performance of grazing cattle.

Volatile Fatty Acid

There was a location effect on all VFAs measured ($P < 0.002$) except for the branched chain fatty acids isovalerate and iso-butyrate ($P > 0.118$) when comparing the midpoint samples of both locations. Total VFA production and the acetate to propionate ratio was greater at Alapaha than Blairsville ($P < 0.001$). Moreover, there was a time effect ($P < 0.001$) for all VFAs measured Alapaha, and all VFAs measured except for valerate and caproate at Blairsville. Therefore, data is presented and discussed by location and located in Table 4.5 and Table 4.6.

At Alapaha, VFA production was measured at the start of study and again at the midpoint. There was treatment effect for the A:P ratio, along with a treatment × time

effect for: Acetate, propionate, total VFA production, and the A:P ratio (Table 5.). Total VFAs decreased from the start of the study to the midpoint ($P < 0.001$). At the midpoint, the CON group produced more VFAs than the SPM supplemented cattle and did not differ from the MON group (Table 4.5.). Additionally, at the midpoint, total VFA production was not different between the MON treated group and the SPM treated group ($P = 0.128$). Both the SPM and CON produced greater a A:P ration than MON, while not differing from each other (Table 4.5.). At Blairsville, VFAs were measured from the midpoint of the trial and at the end of the trial. There was a treatment effect for acetate, butyrate, and valerate. Total VFA's increased from the midpoint to the end of the study while the A:P ratio decreased (Table 4.6.). Acetate production in CON and SPM groups did not differ and both treatments produced greater acetate than MON (Table 4.6.). Moreover, CON produced greater butyrate than MON, while SPM did not differ from CON or MON (Table 4.6.).

Similar to the performance data, these results are inconsistent with previous studies. Diet directly influences fermentation in the rumen as ionophores like monensin shift the microbial populations within the rumen, increasing propionate producing bacteria via increased abundance of members of *Succinivibrio* and *Selenomonas* genus (Callaway et al., 1997). The diet at Blairsville was based on corn silage and contained more starch than the diet at Alapaha. Monensin is more effective at increasing propionate in high concentrate diets when compared to forage-based diets (Ramanzin et al., 1997). At Blairsville and Alapaha, monensin supplementation decreased the A:P ratio, and propionate concentration was greater when compared to the SPM and CON groups. Moreover, in a corn-silage diet, cinnamon extract is known to produce greater total VFA

production when compared to monensin (Khorrami et al., 2015). This study found that, at Blairsville, SPM supplementation produced greater total VFA when compared to the MON group. Additionally, these results are concurrent with Packer et al. (2011). Contradictory to previous studies, however, SPM did not increase butyrate production, compared to the CON group ($P = 0.1400$), which was observed in Holstein calves fed a concentrate diet and were supplemented with a cinnamon extract based product (Shen et al., 2017). Moreover, both CON and MON groups produced greater butyrate concentrations compared to the SPM group ($P < 0.009$). Additionally, several studies, both in-vivo and in-vitro, have found that cinnamon extracts did not affect butyrate concentrations (Busquet, 2006, Yang, 2010).

These results suggest that the blend of cinnamon and garlic extract is effective at modifying the rumen fermentation characteristics of beef steers on both a forage-based and corn-silage based diets. The mix of extracts used in this study, however, did not affect VFA production similarly to monensin. Unlike monensin, the SPM supplement did not increase propionate production, or increase the A:P ratio. Furthermore, in high starch diet, the plant extract mix has shown to increase total VFA production when compared to monensin.

Ultrasound Data

All predicted carcass traits (REA, IMF, FT) increased over time ($P < 0.001$). Additionally, there was a location effect ($P < 0.001$), with Blairsville cattle having greater values in all carcass traits. Therefore, data for REA, IMF, and FT is discussed and presented by location and located in Table 4.7.

At Alapaha, there was a tendency for year to affect REA on the final date (P = 0.0819), and there was a treatment × year effect for change in REA (P = 0.004). In YR 2, SPM developed greater REA than MON (P < 0.01). In Blairsville, there was a treatment effect for final REA measurements (P = 0.021) along with a treatment (P = 0.009), year (P < 0.001), and a treatment × year (P = 0.009) effect for increase in REA. In YR 1, REA in MON was increased by 5.34 and 3.93 cm^2 compared to CON and SPM, respectively (Table 4.7.). Moreover, REA in CON did not differ from SPM calves (Table 4.7.). In YR 2, REA in CON increased 3.02cm^2 more than SPM but did not differ from the MON group (Table 4.7.). Additionally, REA in MON supplementation tended to be greater than SPM calves by 2.21 cm^2 (Table 4.7.).

At Alapaha, there was only a time affected IMF with YR 1 developing greater IMF than YR 2 (P = 0.003). At Blairsville, SPM developed 0.471% more IMF than MON (Table 4.7.), and did not differ from CON. Additionally, CON developed 0.284% more IMF than MON (Table 4.7.). Furthermore, in YR 1, SPM developed the greater IMF than all treatment × year combinations (P < 0.013) excluding YR 1 CON, in which there was a tendency for SPM to develop greater IMF (P = 0.077).

There was a treatment (P = 0.028) and year (P < 0.001) effect for FT at Alapaha. Both CON and SPM developed greater FT than MON (Table 4.7.) and did not differ from each other. Moreover, Cattle in YR 2 developed greater FT than cattle in YR 1. At Blairsville, there was no difference between treatments in FT development.

The results in this study highlight the interaction between supplement and diet. At Blairsville, where calves were provided a diet with high available energy, monensin supplemented cattle produced the largest REA. Monensin supplementation can increase

protein utilization, which is likely the cause of increased REA. Furthermore, cattle who were supplemented with monensin produced the greatest percent of propionate ($P <$ 0.014). Swyers et al. (2014) suggested that monensin supplementation, specifically by increasing propionate production, can lead to increased IMF development. These results, however, contradict Swyer et al. (2014), as the monensin supplemented cattle developed less IMF than the SPM treated cattle and did not differ from the CON group. Additionally, while Yang et al. (2010) did not observe any difference in carcass characteristics when supplementing up to 1600 mg per steer per day of cinnamon extract, at Blairsville, SPM supplemented cattle developed the greatest IMF. Unlike Yang et al. (2010), this study utilized a combination of two extracts, incorporating garlic extract along with cinnamon extract. Furthermore, these results support Ornaghi et al. (2017), in which feedlot cattle were fed concentrate based diets for 187 d with either no supplementation, two different concentrations of garlic extract, or two different concentrations of cinnamon extract. At the end of the study, no differences were observed in any carcass characteristics compared to control animals. Over twenty-five plant extracts have recently been investigated (Calsamiglia et al. 2007), although little research has measured the effect of combining cinnamon and garlic extract on carcass characteristics. More research is needed to fully elucidate the effect of the garlic and cinnamon extract mix on carcass characteristics.

Conclusion and implications

New marketing programs, along with increased regulatory pressure to decrease antibiotic use in livestock has pushed the industry to seek ionophore alternatives. Research suggests that plant extracts produce unique metabolic and physiological effects

within the animal and could serve as possible replacements. The results of this study suggest that a combination of cinnamon and garlic extracts do not perform as an ionophore alternative. These results do suggest that the cinnamon and garlic mix do have an interaction with the rumen microbial population when calves are fed a diet high in available energy. The plant extract additive did increase intramuscular fat by almost 1%, which could lead to significant improvement in the quality grade of animal, although the interaction between IMF and the plant extract is not clearly understood. Further research is required to better understand both the microbial and biochemical effect of cinnamon and garlic extracts in cattle.

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Table 4.1. Mineral composition provided at each location for the control group (CON), the plant extracts supplemented group (SPM), and the monensin supplemented group (MON).

Mineral	CON	SPM	MON
Calcium (Ca), %	22	22	30.5
Phosphorus (P), %	2.50	2.50	0
Sodium Chloride (NaCl), %	20	20	8.9
Magnesium (Mg), %	1.00	1.00	0
Copper (Cu), ppm	2,000	2,000	2,000
Selenium (Se), ppm	26.4	26.4	26.50
Zinc (Zn), ppm	7,500	7,500	7,500
Vitamin A, IU/kg	136,363	136,364	136,364
Vitamin D_3, IU/kg	9,091	9,091	9,091
Vitamin E, IU/kg	91	91	91
Thiamine, IU/kg	0	0	91
Plant Blend[1], mg/kg	0	1764	0
Monensin Sodium, mg/kg	0	0	1058

[1]Plant blend consisted of Garlic oil extracted from garlic and cinnamaldehyde extracted from cinnamon

Table 4.2. Chemical composition of Corn Silage fed at Blairsville and Tifton 85 bermudagrass fed at Alapaha as the base diets for weaned calves.

	Feedstuff	
Chemical composition, % of DM	Corn Silage[1]	Tifton 85[2]
Crude Protein	8.6	7.04
Neutral Detergent Fiber	51.2	71.74
Acid Detergent Fiber	29.63	37.3
Lignin	3.69	3.81
Non-fiber Carbohydrates	36.67	14.25
Calcium	0.21	0.3
Phosphorous	0.22	0.24
Magnesium	0.14	0.25
Potassium	1.03	2.42

[1]Corn Silage was the primary feed stuff fed to all cattle at the Ga mountain research center throughout the trial.

[2]Tifton 85 bermudagrass was the primary feed stuff fed to all cattle at Alapaha grazing range unit throughout the trail.

Table 4.3. Growth performance data presented by year[1] for beef cattle at Blairsville supplemented with either: Cinnamaldehyde and Garlic oil supplemented at 200 mg/head/day (SPM), Monensin at 120 mg/head/day (MON), or no supplementation (CON).

	Year 1[1]					Year 2[1]				
	SPM	MON	CON	SEM	P-Value	SPM	MON	CON	SEM	P-Value
Weight, kg										
Initial	309	307	308	5.45	0.987	319	319	320	6.89	0.998
Midpoint	365	374	368	6.01	0.563	386	391	386	7.71	0.848
Final	412	428	412	6.26	0.136	423	428	415	7.70	0.532
Gain	104	119	104	3.13	0.001	104[ab]	108[a]	96[b]	2.97	0.023
ADG, kg/d										
Period 1[2]	1.32[b]	1.51[a]	1.38	0.05	0.013	1.02	1.10	1.03	0.04	0.270
Period 2[3]	1.15[b]	1.31[a]	1.09[b]	0.04	0.001	0.86[a]	0.85[a]	0.69[b]	0.04	0.001
Total	1.23[b]	1.42[a]	1.24[b]	0.04	0.001	0.96[ab]	1.00[a]	0.89[b]	0.03	0.023

[ab] Means within a row without a common superscript differ ($P < 0.05$).

[1] Year 1 = 2017-18, Year 2 = 2018-19

[2]Period 1, YR1 = D0 – D56; YR2 = D0 – D65

[3]Period 2, YR1 = D56 – D96; YR2 = D65 – D108

Table 4.4. Growth performance data presented by year[1] for beef cattle at Alapaha supplemented with either: Cinnamaldehyde and Garlic oil supplemented at 200 mg/head/day (SPM), Monensin at 120 mg/head/day (MON), or no supplementation (CON).

	Year 1[1]					Year 2[1]				
	SPM	MON	CON	SEM	P-Value	SPM	MON	CON	SEM	P-Value
Weight, kg										
Initial	260	261	261	3.32	0.948	263	264	263	7.74	0.996
Midpoint	---	---	---	---	---	314	306	316	8.55	0.776
Final	314	321	318	4.16	0.531	340	333	333	8.40	0.855
Gain	54	60	57	2.74	0.391	77	70	71	2.62	0.141
ADG, kg/d										
Period 1[2]	---	---	---			0.914[a]	0.760[b]	0.900[a]	0.04	0.008
Period 2[3]	---	---	---			0.978[ab]	1.04[a]	0.804[b]	0.06	0.025
Total	0.664	0.729	0.692	0.003	0.391	0.934	0.848	0.867	0.03	0.141

[ab] Means within a row without a common superscript differ ($P < 0.05$).

[1] Year 1 = 2018-19, Year 2 = 2019-20

[2]Period 1 for YR1 & YR2 = D0 – D56

[3]Period 2 for YR1 & YR2 = D56 = D84

Table 4.5. Volatile fatty acid results by treatment for beef cattle at Alapaha supplemented with either: Cinnamaldehyde and Garlic oil supplemented at 200 mg/head/day (SPM), Monensin at 120 mg/head/day (MON), or no supplementation (CON). [1]

	Initial					Midpoint				
	SPM	MON	CON	SEM	P-Value	SPM	MON	CON	SEM	P-Value
Volatile Fatty Acids, mmol										
Acetate	37.3	37.8	39.8	1.73	0.588	27.0	32.2	36.2	2.52	0.054
Propionate	7.83	8.41	8.23	0.460	0.656	5.01[b]	7.15[a]	7.08[a]	0.645	0.045
Butyrate	5.29	4.91	5.14	0.301	0.677	3.57	3.86	4.07	0.377	0.641
Isobutyrate	0.472	0.482	0.473	0.025	0.949	0.431	0.445	0.432	0.034	0.951
Valerate	0.332	0.327	0.364	0.019	0.358	0.243	0.272	0.310	0.023	0.142
Isovalerate	0.681	0.656	0.712	0.041	0.620	0.593	0.557	0.614	0.006	0.761
Caproate	0.093	0.093	0.105	0.009	0.543	0.059	0.080	0.081	0.014	0.483
Total	52.0	52.7	54.8	2.47	0.699	36.9[b]	44.6[ab]	48.8[a]	3.56	0.078
A:P	5.00[a]	4.53[b]	4.94[a]	0.106	0.005	5.43[a]	4.56[b]	5.24[a]	0.159	0.001

[ab] Means within a row without a common superscript differ ($P < 0.05$).

[1]SPM = Extract blend supplemented at 200 mg/d, MON = monensin supplemented at 120 mg/d

Table 4.6. Volatile Fatty acid results by treatment for beef cattle at Blairsville supplemented with either: Cinnamaldehyde and Garlic oil supplemented at 200 mg/head/day (SPM), Monensin at 120 mg/head/day (MON), or no supplementation (CON). [1]

	Midpoint					Final				
	SPM	MON	CON	SEM	P-Value	SPM	MON	CON	SEM	P-Value
Volatile Fatty Acids, mmol										
Acetate	26.7[ab]	24.2[b]	31.2[a]	1.97	0.035	30.1	24.9	33.6	3.31	0.145
Propionate	6.03	5.98	7.18	0.630	0.283	7.53	6.41	8.54	1.08	0.329
Butyrate	2.39[ab]	1.96[b]	2.99[a]	0.280	0.029	2.74	1.92	3.45	0.514	0.094
Isobutyrate	0.487	0.512	0.542	0.033	0.472	0.479	0.437	0.500	0.053	0.658
Valerate	0.229	0.223	0.295	0.023	0.046	0.238	0.192	0.310	0.043	0.119
Isovalerate	0.636	0.776	0.732	0.048	0.111	0.553	0.587	0.596	0.070	0.897
Caproate	0	0	0.001	0.088	0.876	0	0	0.020	0.009	0.174
Total, mmol	36.4[ab]	33.7[b]	42.9[a]	2.89	0.058	41.6	34.4	47.1	4.98	0.169
A:P	4.69	4.24	4.64	0.176	0.121	4.10	3.93	4.16	0.198	0.651

[ab] Means within a row without a common superscript differ ($P < 0.05$).

[1]SPM = Extract blend supplemented at 200 mg/d, MON = monensin supplemented at 120 mg/d

Table 4.7. Ultrasound data presented for beef cattle at both Blairsville and Alapaha supplemented with either: Cinnamaldehyde and Garlic oil supplemented at 200 mg/head/day (SPM), Monensin at 120 mg/head/day (MON), or no supplementation (CON). [1]

	Blairsville					Alapaha				
	SPM	MON	CON	SEM	P-value	SPM	MON	CON	SEM	P-value
REA, cm^2										
On-Test	8.10	8.21	8.26	0.141	0.713	7.42	7.48	7.23	0.151	0.479
Off-Test	10.1[b]	10.7[a]	10.4[ab]	0.144	0.021	9.56	9.47	9.38	0.157	0.706
Delta	2.03[b]	2.49[a]	2.15[b]	0.119	0.018	2.15	1.99	2.15	0.109	0.501
Intra-Muscular Fat, %										
On-Test	4.22[a]	4.12[ab]	3.84[b]	0.115	0.056	3.18[b]	3.40[ab]	3.66[a]	0.112	0.010
Off-Test	5.12[a]	4.55[b]	4.56[b]	0.162	0.019	3.31[b]	3.48[ab]	3.76[a]	0.104	0.010
Delta	0.903[a]	0.043[b]	0.716[ab]	0.104	0.007	0.134	0.085	0.094	0.636	0.846
Fat Thickness, cm										
On-Test	0.161	0.165	0.153	0.007	0.456	0.118	0.131	0.132	0.006	0.151
Off-Test	0.234	0.244	0.242	0.011	0.790	0.161	0.165	0.153	0.007	0.456
Delta	0.073	0.079	0.089	0.104	0.210	0.047[a]	0.030[b]	0.048[a]	0.006	0.047

[ab] Means within a row without a common superscript differ ($P < 0.05$).

[1]SPM = Extract blend supplemented at 200 mg/d, MON = monensin supplemented at 120 mg/d

CHAPTER 5

CONCLUSION AND IMPLICATIONS

Weaning stress can negatively impact animal performance and animal health. To mitigate the impact of weaning, producers can utilize a background phase to implement management practices that will increase the value of the calf crop. Two specific areas a producer can target during a background phase is reducing parasitism and improving post-weaning nutrition. Parasites can be managed with the use of anthelmintic drugs; however, new strategies are required to limit anthelmintic resistance and maintain efficacy. Furthermore, producers can improve performance with the use of ionophores, although growing antibiotic resistance has increased the demand for alternatives to antibiotic feed additives.

The objective of the research in this thesis was to investigate potential strategies that will benefit cow-calf producers in the Southeast. Thus, the research presented herein is divided into two experiments. The first experiment examined the efficacy of concurrent application of two different anthelmintic drugs, intradermal eprinomectin and orally suspended oxfendazole, compared to using one anthelmintic. The second experiment investigated the effect of a supplemental blend of garlic oil and cinnamaldehyde on animal performance and rumen fermentation compared to a control group and monensin.

In experiment 1,392 calves at four different locations were sorted into four treatment groups and dosed at weaning. Locations included the Eatonton Beef Research Unit, Northwest Georgia Research Unit, Alapaha Range Station, and the J. Phil Campbell Sr. Research and Education Center. Treatments included orally suspended Oxfendazole (Synanthic®, ORAL), transdermal eprinomectin (Eprinix®, POUR), both anthelmintics (BOTH), and a control group (CON). Fecal samples were collected on d0 and d14 to measure EPG and calculate efficacy, and weights were collected on d0 and d42 to measure animal performance during the 42d experiment. Additionally, rumen fluid was collected on d0 and d6 from 20 calves, five from each treatment, at Eatonton to measure rumen fermentation.

Using the two anthelmintic dugs concurrently was the most effective treatment, reducing EPG count by 98.8% on d14. Furthermore, neither POUR or ORAL reached 95% reduction, which would not be considered effective treatments based on the WAAVP guidelines. Additionally, the three anthelmintic treatments did not affect rumen fermentation. Calves treated with oxfendazole or both anthelmintic gained more weight that the control group ($P = 0.0077$) increasing ADG by 0.08 and 0.1 kg/day respectively. When accounting for the cost of the treatment, calves treated with oxfendazole were more valuable compared to the control calves, worth $11.20 more than calves who were not treated with any anthelmintic.

Experiment 2 was conducted at two locations which included the Georgia Mountain Research Center (2017-18 and 2018-19) and the Alapaha Grazing Range Unit (2018-19 and 2019-20). Each year 81 recently weaned calves were sorted into three treatment groups: 1) supplemented with monensin at 120 mg per head per day, 2) supplemented with blend of garlic oil and cinnamaldehyde at 200 mg per head per day, or

3) a control group. Weights were collected on d0, d56, and d82 to measure animal performance except for year 1 at Alapaha, which weights were only recorded on d0 and d82. Ultrasound was also used on d0 and d82 to measure carcass characteristics over the course of the study. Additionally, rumen fluid was collected from 26 animals in year 1 at Blairsville on d56 and d82, and 27 animals from year 2 at Alapaha on d0 and d56.

The garlic oil and cinnamaldehyde blend did not improve performance when compared to the control group ($P > 0.5$). At Blairsville, cattle supplemented with monensin had the greatest ADG out of all three treatments ($P < 0.023$). Additionally, cattle supplemented with monensin developed the largest REA when compared to the blend and the control cattle ($P = 0.018$). Moreover, cattle supplemented with the extract blend developed greater intra-muscular fat than monensin ($P < 0.02$) and tended to be greater than the control group ($P = 0.058$). Furthermore, total VFA production increased from the d56 to d82, although t there were differences between treatments. At Alapaha, supplementation did not improve total ADG during either year. Additionally, fat thickness in the extract blend did not differ from the control group, although both the blend and the control group producer greater fat thickness than the monensin supplemented cattle ($P = 0.0471$). Furthermore, total VFA production decreased from d0 to d52 with monensin producing the smallest acetate to propionate ratio ($P = 0.0019$).

The research in this thesis demonstrates that proper post-weaning management can increase the value of the calf crop. When utilizing an anthelmintic program, it is important to ensure the anthelmintic drug is an effective option. In this research, there was not a statistical difference in value between the control group or intradermal eprinomectin. The orally suspended oxfendazole, however, returned $11.20 more per

animal when compared to the control group, and utilizing both treatments concurrently reduced EPG by 98.8%. Additionally, while the blend of garlic oil and cinnamaldehyde does not affect animal performance, carcass characteristics, or rumen fermentation similarly to monensin; the plant extract blend, however, did have an effect on IMF when supplemented in a high energy diet like the corn-silage diet fed at Blairsville. Increased IMF can lead to higher quality carcasses, and increased value when marketed properly.

Further research is needed for both experiments in this thesis. While concurrent application of two different anthelmintic drugs was effective, the long-term impact of using two anthelmintic on resistance is necessary. Additionally, further research is needed to elucidate the effect of the garlic oil and cinnamaldehyde blend on the rumen microbial population, along with the biochemical mechanism of the blend on intra-muscular fat.