

COMPARISON OF VARIOUS ECTOPARASITE
CONTROL STRATEGIES AND VIRAL VACCINE
REGIMENS IN FEEDLOT CATTLE: IMPACTS ON
PERFORMANCE, HEALTH, PARASITE BURDEN,
ACUTE PHASE PROTEINS, AND ANTIBODY TITERS

By

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Bachelor of Science in Animal Science

Oklahoma State University

Stillwater, Oklahoma

May, 2017

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
July, 2021

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ACKNOWLEDGEMENTS

I would like to thank Dr. Blake Wilson for serving as my major advisor and committee chair, thank you for the guidance and help along the way. Dr. Wilson reluctantly accepted me as a graduate student but continued to support me, which I sincerely appreciate. I'd also like to thank Dr. Beck and Dr. Talley for serving on my committee and the continued support. I appreciate you all for investing the abundant amount of time and effort put towards my education and endless questions.

Secondly, thank you to the graduate students that helped me along the way. I am forever grateful for the connections and friendships I made while pursuing my degree. I was lucky enough to leave with numerous memories and lessons learned from my fellow students. Thank you for being there with me through late night study sessions, long mornings at the feedlot, and more.

Thank you to my friends outside of the graduate program. Even from hours away, I could always count on them bringing a smile to my face!

Most importantly, thank you to my family for the never-ending support. Thank you to my parents, Kyle and Heather, and my grandparents, Larry and Jane, for being there every step of the way. I would not have succeeded without the encouragement and love I received. A special thank you to my dad raising me to pursue what I wanted, while staying hardheaded, keeping me grounded in my pursuits, and instilling the "don't let school get in the way of you education" mindset. Love you all!

Thank you ALL. I couldn't have done it without you!

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Date of Degree: JULY, 2021

Title of Study: COMPARISON OF VARIOUS ECTOPARASITE CONTROL STRATEGIES AND VIRAL VACCINE REGIMENS IN FEEDLOT CATTLE: IMPACTS ON PERFORMANCE, HEALTH, PARASITE BURDEN, ACUTE PHASE PROTEINS, AND ANTIBODY TITERS

Major Field: ANIMAL SCIENCE

Abstract: Two experiments were conducted to compare various ectoparasite control strategies and viral vaccine regimens in feedlot cattle to determine the impacts on performance, health, parasite burden, acute phase proteins, and antibody titers. In experiment 1, angus bulls and steers ($n = 100$; bulls = 64, steers = 36) were blocked by sex and body weight (BW) and assigned to 1 of 4 experimental treatments: control (CON; no fly control), abamectin and piperonyl butoxide insecticide tags (FT), permethrin and piperonyl butoxide pour on (PO), or a garlic-powder top dress (GR) administered at $0.28 \text{ g} \cdot \text{animal}^{-1} \cdot \text{d}^{-1}$. No differences were observed in final BW, average daily gain (ADG), dry matter intake (DMI), or gain to feed (G:F) overall ($P \geq 0.30$). There was a tendency ($P \leq 0.10$) for GR to have decreased BW on d 28 and decreased ADG from d 0 to 28. Fly abundance tended to differ on wk 1 and wk 6 ($P \leq 0.10$). In experiment 2, angus or crossbred calves ($n = 406$) were assigned to 1 of 2 experimental treatments: a modified-live virus (MLV) vaccine (Titanium 5, Elanco Animal Health, Greenville, IN) or an inactivated (INA) vaccine (ViraShield 6; Elanco Animal Health). No treatment effect ($P \geq 0.21$) was observed for BW from d 0 to 56. A treatment effect ($P < 0.01$) was observed from d 0 to 56 for ADG with MLV being greater than INA. Dry matter intake was greater for MLV than INA from d 28 to 41 and overall (d 0 to 56; $P \leq 0.01$). Gain:feed did not differ between treatments ($P \geq 0.22$). However, a treatment effect ($P < 0.001$) was observed for BVDV 1a titers, where INA had greater titers than MLV. A day \times treatment interaction ($P = 0.03$) was observed for BVDV 1b, titers for both treatments increased from d 0 to 14, while INA increased more rapidly. From d 15 to 56 INA BVDV 1b titers remained fairly constant while MLV decreased from d 15 to 28, increased rapidly from d 29 to 42, and then decreased through d 56. No treatment \times day interaction was observed ($P > 0.73$) for serum amyloid A (SAA). However, a main effect of day ($P = 0.01$) and a tendency for main effect of treatment ($P = 0.07$) were observed. No treatment \times day interaction and no main effect of treatment ($P \geq 0.72$) were observed for haptoglobin. However, a main effect of day ($P = 0.002$) was observed, where Hp concentrations increased from d 0 to 14 then decreased from d 14 to 56. Overall, morbidity, clinical severity score, and rectal temperatures did not differ between treatments ($P \geq 0.22$).

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CHAPTER I

REVIEW OF LITERATURE

Introduction

The stable fly, *Stomoxys calcitrans* (Linnaeus), is an important ectoparasite within the cattle industry (Berry and Campbell, 1983; Campbell et al., 1987). The estimated economic impact of stable flies on the U.S. cattle industry has increased from \$152 million in the early 30's (Bishop et al., 1938) to a current estimate of \$2.2 billion (Taylor et al., 2012). The obligate bloodsucking fly feeds on livestock, companion animals, and humans. Stable flies cause a disturbance in animals' feed intake due to the painful action of mouth parts which results in defensive behaviors such as head tossing, leg stomping, skin twitching, and tail swishing (Baldacchino et al., 2013). Inducing defensive behaviors result in a loss of energy and increased stress levels. These nuisances have an effect on cattle in all sectors of the beef industry causing a decrease in efficiency and production (Taylor et al., 2012). The stable fly can also mechanically transmit pathogens, such as bovine anaplasmosis, by blood-contaminated mouthparts (Baldacchino et al., 2013). Stable flies thrive in confined livestock operations, because soiled feed and manure accumulate to create preferred breeding habitats.

Meanwhile, bovine respiratory disease (BRD) continues to be the most economically important disease in cattle, specifically within the U.S. feedlot industry. This disease is

responsible for 75% of morbidity and 50 to 70% of mortality in feedlots (Brooks et al., 2011). The BRD complex results in an estimate loss of \$1 billion annually for the U.S. cattle industry (Griffin et al., 1997).

This economic loss is associated with cattle death, treatment costs, and decreased animal performance (Wilson et al., 2012; Beck et al., 2019). Griffin et al. (1997) estimated that producers spend over \$1 billion annually on BRD preventatives and treatments. The BRD complex is initiated by several factors including viruses, bacteria, and stress (Powell et al., 2013). The classical development of BRD involves a primary infection with a primary respiratory virus and a compromised respiratory immune system. Respiratory disease can be induced by several viruses including infectious bovine rhinotracheitis (IBR), bovine viral diarrhea virus (BVDV), parainfluenza type 3 (PI3), and bovine respiratory syncytial virus (BRSV) and several bacteria including *Mannheimia haemolytica* (MH; previously known as *Pasteurella haemolytica*), *Pasteurella multocida* (PM), *Haemophilus somnus* (HS), and *Mycoplasma bovis* (MB) bacteria (Powell et al., 2013). These viruses and bacteria are a threat to animals when immunity is suppressed due to stress factors which can include weaning, castration, dehorning, poor nutrition, and improper handling (Powell et al., 2013). Therefore, the prevention and treatment of BRD continues to be an important focus of the feedlot industry. In large feedlots ($\geq 1,000$ hd of cattle), 93.8 percent vaccinate against viral and bacterial agents that induce BRD during processing at arrival (USDA NAHMS, 2013). These BRD vaccines often include BVDV, IBR, PI3, and BRSV (USDA NAHMS, 2013).

Data also suggest that fly control strategies have been ineffective in reducing stable fly abundance on feedlot cattle. Although advancements have been made in vaccines to decrease the impact of BRD, the prevalence of BRD has not been significantly reduced in recent years (Wilson et al., 2012). These data suggest that BRD prevention strategies have been ineffective in reducing BRD in feedlot cattle. The objectives of the experiments presented in this thesis were to: 1)

Evaluate the effects fly control strategies on stable fly abundance, fly avoidance behaviors, and animal performance in feedlot bulls and steers; 2) Evaluate the effects of modified-live vs inactivated vaccines viral vaccines on performance, health, acute phase proteins, and BVDV titers in receiving feedlot calves. The ultimate goal of these experiments would be to expand on the current knowledge regarding fly control strategies and BRD prevention to improve upon the management of cattle by reducing the overall economic impact stable flies and BRD have on the beef industry.

The Stable Fly

Biology of the Stable Fly

The stable fly, *Stomoxys calcitrans* (Linnaeus), is a biting fly in the Muscidae family and in the fly order Diptera. These pests are primarily known for the painful bite on several different hosts such as; livestock, humans, and companion animals. The stable fly completes a metamorphosis cycle; egg, larva, pupae, and adult. Once an oviposition substrate is located, eggs are deposited by the female. Stable fly eggs are deposited in numbers of approx. 20 – 100 (Showler and Osbrink, 2015). During the life cycle of a female stable fly, 60-800 eggs can be produced throughout 4 to 5 occasions of depositing eggs. Bishopp et al. (1931) reported that eggs are placed in a moist substrate, and hatch within 1 to 3 d. Larvae then bury themselves further into the oviposition substrate where proper food conditions are available and pupate after 11 to 30 days (Bishopp et al., 1931). An adult stable fly is then produced within 6 to 20 d. Depending on different variables, the adult female lifespan is 72 d and male 94 d, in laboratory conditions. According to Showler and Osbrink (2015), the longevity for field conditions is a substantially shorter lifespan, approximately < 2 wk. Showler and Osbrink (2015) reported that once adults, the female flies are able to begin laying eggs in 5 to 8 d. The breeding capabilities of both male and female stable flies are dependent on ingestion of a blood (Showler and Osbrink, 2015). Adult

male and female flies' probe with mouthparts under animal skin in order to ingest 7 to 31 mg of blood during a feeding, lasting approximately 4 min per feeding (Berry and Campbell, 1983).

Breeding Habitats of the Stable Fly

The preferred breeding and larval habitats of stable flies has been thoroughly evaluated to determine the overall developmental habitats. Ideal conditions for stable fly development include decaying plant matter mixed with manure and old hay feedings combined with spilled grain and manure (Talley et al., 2009; Cook et al., 2018). Stable flies are primarily an issue in confined operations because of the abundance of breeding sites. Meyer and Peterson (1983) evaluated 16 different breeding sites of stable flies. When comparing the 16 different sites, in 4 different small feedlots (100 to 800 head), 26% of stable flies were found in fence line soiled manure that accumulated and remained undisturbed. In agreement with this research, when comparing stable fly population variation between feedlots, Berry and Campbell (1983) suggested that the larger counts of stable flies could be due to rotting silage and accumulation of manure.

Stable Fly Economic Impact on Performance

The impact of stable fly infestations to the U.S. cattle industry has been investigated to evaluate the extent of production loss. The production losses occur due to pain and irritation caused by stable fly feeding. In a series of 5 experiments, Campbell et al. (1987) compared the effects of stable flies on weight gain and feed efficiency of feedlot cattle. In the first experiments over the course of 2 yr, different fly levels were used to determine stable fly effects on performance. In yr 1, 40 heifers were assigned to 1 of 2 treatments: 0 stable flies •⁻¹ foreleg•⁻¹ animal or 50 stable flies•⁻¹ foreleg•⁻¹ animal. In yr 2, 40 heifers were assigned to 1 of 2 treatments: 0 stable flies•⁻¹ foreleg•⁻¹ animal or 100 stable flies•⁻¹ foreleg•⁻¹ animal. Animals were placed in fly-screens and exposed to treatment fly loads. Animals were weighed at d 0, 30, 60, and 90. In the first experiment, no differences in performance were reported. In the second experiment,

animals had a decreased performance when exposed to 100 stable flies \bullet^{-1} foreleg \bullet^{-1} animal.

Campbell et al. (1977) incorporated Britey et al. (1975) method for production cost to determine production loss in fly infested (50 or 100) calves versus fly-free calves. Using this method, to produce an equivalent weight gain for fly infested animals compared to fly-free animals, the feed cost increased by \$8.44.

After the initial 2 yr Campbell et al. (1977), McNeal and Campbell (1981) determined an economic threshold of 5 stable flies/foreleg in integrated pest management programs. Therefore, Campbell et al. (1977) evaluated the economic threshold in a field study. Four fly-screens were placed over 4 different pens, fly pupae were placed in 2 pens, while the other 2 remained fly-free. The original Campbell et al. (1977) stable fly numbers (50 flies \bullet^{-1} foreleg \bullet^{-1} animal and 100 flies \bullet^{-1} foreleg \bullet^{-1} animal) were reduced and the average numbers of stable flies were 2, 5, and 7 stable flies \bullet^{-1} foreleg \bullet^{-1} animal. Lower numbers of stable flies numerically reduced weight gains and feed efficiency; however, the differences were not significant. Campbell et al. (1987) recorded 5 flies \bullet^{-1} foreleg \bullet^{-1} animal would result in a 3.85% reduction in weight gain. Overall, the percent reduction in weight gain would require an increased day on feed. Comparatively to the diet the animals were fed, a stable fly population of 5 flies \bullet^{-1} foreleg \bullet^{-1} animal would result in a loss of \$8.51 per animal. Campbell et al. (1977, 1987) reported overall an average 14 stable flies \bullet^{-1} foreleg \bullet^{-1} animal reporting a reduced average daily gain (ADG) of 7%. Compared to heifers that remained fly-free, feed conversion ratio was increased by 9%, indicating that exposure to stable flies requires more feed to gain equivalent weight. Over a 2-yr study, production losses were compared by Berry and Campbell, (1983) in 14 feedlots in yr 1 and 13 feedlots in yr 2. In yr 1, the average seasonal losses were 2.30 kg per animal, meanwhile in the following yr 2, the losses increased to 3.51 kg per animal (Berry and Campbell, 1983). Additionally, research has shown that stable flies cause bunching of animals which also induces heat stress which effects weight gain and feed efficiency. The 4-screen closed method used by Campbell et al. (1977) was adapted

by Wieman et al. (1992) to record the effects of stable flies on heat stress and performance in feeder cattle. Over the course of 2 yr, 1985 and 1986, studies were conducted in self-contained feedlot pens. The 4 treatments were no bunching-no flies (NB-NF), flies-no bunching (F-NB), no flies-bunching, (NF-B), and flies-bunching (F-B). Wieman et al. (1992) reported that when animals were bunched together in pens and exposed to stable flies performance decreased. Meanwhile, cattle with no bunching and no fly exposure had a greater performance. While direct effect of the stable fly bites reduced the feed efficiency and rate of gain (Campbell et al., 1977; Campbell et al., 1987; Berry and Campbell, 1983) of cattle, Berry and Campbell (1983) reported that when subject to stable fly attacks, cattle stood in compact groups and the bunching behavior increased thermal stress.

Literature has reported that breed may cause a variance in fly abundance and the impacts on performance. Performance was evaluated on Brahman-crossbred and English \times exotic feeder heifers exposed to low (4 ± 2 flies \cdot^{-1} foreleg \cdot^{-1} animal), medium (12 ± 3 flies \cdot^{-1} foreleg \cdot^{-1} animal), and high (32 ± 13 flies \cdot^{-1} foreleg \cdot^{-1} animal) stable fly abundance. Over a 2-yr study, Catangui et al. (1993) released known numbers of adult stable flies into 4 screened feedlot pens, while 4 adjacent unscreened pens were biweekly sprayed with 1% dichlorvos to control naturally occurring stable flies. Animals were exposed to the treatment and level of fly exposures for 28 d. In yr 1, English \times exotic heifers ADG was reduced at low, medium, and high stable flies. The magnitude in reduction of ADG (0.22 kg/d) was similar for all 3 levels. Catangui et al. (1993) suggested the reductions were similar because animal max irritation was met when exposed to the low level of flies, therefore the medium and high levels of flies did not reduce ADG. Conflicting results were recorded with the Brahman-crossbred heifers, ADG was not affected by any of the tested levels.

In yr 2, Catangui et al. (1993) recorded a reduction in ADG for both breeds of 0.16 kg/d at the high level of stable flies (32 ± 13 flies \cdot^{-1} foreleg \cdot^{-1} animal). Within the 2-yr experiments,

breed and age were also considered. The ADG of Brahman-crossbred heifers was approximately 13% below the English × exotic heifers in the first experiment and 17% below the English × exotic heifers in the second experiment, with or without stable flies.

Stable fly impacts on performance in feeder heifers were determined with long term exposure. Catangui et al. (1995) used the previous method of Catangui et al. (1992) on 4 groups of mixed breed heifers were randomly assigned into 4 screened pens, 2 groups received stable fly treatment of 13.7 ± 0.6 flies•⁻¹ foreleg•⁻¹ min for 112 d, and the 2 control groups remained fly-free. From d 1 to 84, ADG continued to decline for both breeds from d 29 to d 84. However, ADG was not affected from d 85 to d 112 for either breed. Average daily gain declined over time when closer to maturity (Berg and Butterfield, 1976). English × exotic heifers gained 18% more than Brahman-crossbred heifers (Catangui et al., 1993).

Catangui et al. (1997) conducted an experiment using 8 different replicated studies to calculate the economic injury level in relation to stable flies on performance in feeder heifers. A negative exponential equation was adapted and altered from Jones and Bliss (1980) using the quantitative relationship between stable fly level and reduction in ADG. Catangui et al. (1993) recorded ADG can be reduced but only to a maximum level of stable flies, which reflected the low, medium, and maximum level of stable flies and a constant 11% regardless of abundance recorded (Catangui et al., 1993). The negative exponential curve used exemplifies that there is a maximum reduction in weight gain of cattle, therefore economic injury level can be calculated. 1) The gain threshold (GT) is calculated by fly control cost/animal divided by market price at slaughter. 2) The gain threshold is then divided by ADG and made into a percentage (GTP). 3) Economic injury threshold is then determined by the following equation:

$$\{ \ln [1 - (GTP \div 16.7083\%)] \} \div \{ -0.0627\% / \text{flies} \cdot^{-1} \text{ foreleg} \cdot^{-1} \text{ animal} \cdot^{-1} \text{ min} \} =$$

maximum number of stable fly to avoid economic loss

Catangui et al. (1997) recorded the economic injury threshold can be calculated in feeder cattle to determine loss from stable flies which was supported by previous research (Catangui et al., 1993). Taylor et al. (2012) recorded animals with high numbers of flies consumed, on average, as much feed as pens with lower numbers of flies or no flies. Taylor et al. (2012) developed a model based off of yield-loss functions related to stable fly infestations to estimate economic impact of stable flies on cattle. Economic impact in this experiment was determined from previous research and formulated to predict differences in yield-loss functions and metabolic equivalence. Conversely, Taylor et al. (2012) used 5 confined feedlot experiments and calculated dry matter intake (DMI), ADG, and gain to feed (G:F). Average daily gain, absolute differences (Δ ADG; treated and untreated herds) and relative differences (% ADG; % fly-free ADG) were calculated for replicate pens with the same experimental treatments (fly infested versus fly-free). Fly infested treatments had a 0.12 kg/d decrease in DMI compared to fly-free treatments. The absolute yield-loss function determined 5 and 10 stable flies reduced ADG by 7.1 and 10.5 kg/animal total over a 90-d fly season. Metabolic equivalence, the relation between decreases in productivity of metabolic energy and corresponding leg counts was also analyzed. Over the course of 5 mo the median monthly leg counts were 6.6, 5.4, 2.9, 0.6, and 0.6 which reported a live weight loss annually of 8 kg in feeder cattle.

Fly Abundance and Fly Avoidance Behaviors

While many studies evaluate stable flies impact on performance, few evaluate fly avoidance behaviors in relation to performance. Dougherty et al. (1993) determined how stable flies effect beef cattle while grazing by using 3 treatments, in the presence of natural fly populations, in enclosures that exclude natural fly populations, and enclosed screens with 2500 stable flies. Twelve 4-yr-old cows were used and 24 circular plots were the experimental units. Cows were individually taken to an assigned circular plot at 1000 h and measurements were recorded for 1 h. Measurements recorded were fly populations, behavior measurements (head,

ear, skin, tail, and leg movements), height of forage in circular plot before and after grazing period, and prehension. Animals enclosed with 2500 stable flies had 91.5% more flies than the natural fly population treatment. Cows grazing plots with natural fly populations had an average of 9.2 flies/animal with 6.5 flies on the legs, which supports that 70.7% of flies in the natural population were stable flies. Cows without the natural fly population exhibited little fly-induced behavior (0.44 movements) while cows grazing with natural fly populations exhibited more movements (13 movements). However, cows exposed to stable flies in the enclosed area exhibited 82.8% more stable-fly induced behavior movements than the cows exposed to natural fly populations during grazing periods. The rate of forage intake did not differ between treatments, however, cows exposed to stable flies did not visit the feeding stations as much as the other 2 treatments, with cows exposed to natural fly populations visited the feeding stations an average of 3.9 times, cows without flies 3.41 times, and cows exposed to flies 2.79 times. Overall, cows in this experiment that were exposed to stable flies exhibited many fly avoidance behaviors and had decreased feeding time. Dougherty et al. (1993) concluded that stable fly induced behavior movements are energy-consuming muscle movements that also disrupt grazing time.

Mullens et al. (2006) evaluated behavioral responses of dairy cattle to the stable fly in an open field environment. Although controlled studies have been conducted using exclusively stable flies, Mullens et al. (2006) conducted an experiment where stable flies were the dominant fly species in a confined dairy. Four groups of dairy cows ($n = 77$ to 123) were used in the trial, cows were grouped depending on the stage of lactation. Fly abundance was recorded for 3 wk during a pre-treatment period to record baseline fly abundance. Groups 2 and 4 were sprayed with 0.1% permethrin twice per wk from wk 4 of the trial to wk 12. Using Dougherty et al. (1993) determined fly avoidance behaviors, behaviors were recorded twice per d from Monday to Friday. Fly avoidance behaviors were not recorded on d with high winds. Mullens et al. (2006) reported

an extreme relationship between all 4 fly avoidance behaviors and stable flies. Fly avoidance behaviors do not occur in fly-free cattle. The authors suggest front leg stamps are a behavioral gauge for the stable fly. Also, once fly avoidance behaviors begin, they continue for a period of time, with skin twitches still occurring when flies are absent. However, fly abundance was not high enough to detect the economic effects (Mullens et al., 2006).

Fly Control Methods

Stable flies are difficult to manage, the fly briefly visits its host for a blood meal which makes chemical control difficult, while breeding and larval sites are widespread (Cook, 2020). Historically, insecticides have been used to control adult stable fly numbers (Marçon et al., 1997), but repeated applications are required to provide seasonal control which can become costly for the producer (Campbell and Hermanussen, 1971). Stable fly resistance to insecticides such as dieldrin, toxaphene, permethrin, and pyrethroid has been recorded (Mount et al., 1965; Cilek and Greene, 1994). Control techniques used for larval stages such as insect growth regulators (IGR) are less useful in stable flies since manure mixed with hay, grain, and rotten soil are preferred developmental sites.

Sanitation

Sanitation is the most effective method to control stable fly numbers in confined feedlots (Cilek and Green, 1994), but is not considered cost effective and is labor intensive. The removal of accumulated manure decreases the breeding sites. Skoda et al. (1991) conducted a 3-yr study to determine developmental places of immature stable flies in a feedlot using different management practices. The 3 different practices were labeled as: 1) Minimum management: manure was removed once (or less) annually, 2) intermediate management: feedlot was cleaned once annually and insecticides were used, and 3) intense management: feedlot was cleaned as needed and insecticides for control were administered on a schedule. In yr 1, only 1 feedlot with minimum

management was monitored. On each sampling date, stable fly numbers were recorded on 20 cattle. Within feedlot pen, 5 different samples were taken from the feed apron, back fence, dividing pen fences, mound, and the general lot including potholes. Immatures collected were held for 2 wk for allowed emergence of adult stable flies. Using the number of immatures, a correlation analysis was conducted to compare number of adults. Feedlots were sampled every other wk. In yr 3, 9 feedlots were used (3 feedlots / level of management). In yr 1, 85% of immature stable fly collected were from the feed apron and mound, while the side fence and general lot produced low numbers of immatures. In yr 2, the feedlot with minimum management had the highest number of immatures, the feedlot with intermediate management had a moderate decrease in immatures, while the feedlot with intense management had the lowest number of immatures. Similar to yr 1 experiment, > 80% of immatures were collected from the feed apron. The results from yr 3 experiment contradict yr 1 and 2, with stable fly abundance increased throughout the season regardless of management protocol. Regardless, the authors conclude that by removing or dispersing waste surrounding the feed apron, stable fly populations may be reduced.

Thomas et al. (1996) conducted a 2-yr experiment to determine sanitation influence on stable fly populations in 4 feedlots assigned to 1 of 2 treatments, sanitation (cleaned) or no sanitation (uncleaned). The sanitation feedlot was completely cleared of manure, bedding material, and excess feed from areas inside the feedlot pens such the feed apron, and along the feed bunk every 2 wk. Additionally, the area surrounding feedlot pens were cleaned. Populations of adult stable flies were counted weekly on 20 cattle. For yr 1, the feedlots that were cleaned had 50.9% fewer flies than the feedlots that uncleaned feedlots. For yr 2, the feedlots cleaned had 36.2% fewer flies than the uncleaned feedlots. In conclusion, Thomas et al. (1996) determined sanitation as an effective method to control stable fly populations that is cost effective to the producer. However, determination of cost effectiveness may be determined by size of feedlot, as

feedlots used in Thomas et al. (1996) did not exceed 400 animals either yr. In support of previous literature, sanitation is an effective method to control stable fly abundance in confined feeding operations. However, the cost effectiveness of this method may be determined on size of feedlot and available labor.

Synthetic Chemical Control

Insecticides and pesticides are the most common form of control for adult stable flies in confined feeding operations. Although, over the years, insecticide and pesticide resistance has increased in stable flies. Schmidt et al. (1976) conducted an experiment comparing 1 standard synthetic pyrethroid and 2 commercially-prepared synthetic pyrethroids to control stable flies by using spot tests and large cage tests. For the spot tests, 1 of 3 synthetic pyrethroids were added to a small area of the steer and stable flies were released for feeding. The synthetic pyrethroids used in the spot test were then sprayed on 3 steers which were then placed in individual large cages. One steer was not sprayed with synthetic pyrethroid and placed in an individual large cage. Once steers were in individual cages, 100 starved stable flies were released. After allowing stable flies to feed for 2 h, stable flies were recaptured and then determined if flies fed on the steers and final mortality time was recorded. Three steers were then sprayed with different percentages (0.1%, 0.05%, 0.025%) of synthetic pyrethroids and animals were exposed to flies for 7, 10, to 21 d. A fourth test was done using a standard synthetic pyrethroid compared to 2 commercially-prepared synthetic pyrethroids. Overall, the synthetic pyrethroids controlled stable flies for 8 d post treatment in the spot tests and controlled stable flies 7 – 10 d post treatment in the large cages. The authors conclude the results in this trial reflect a short-term efficacy (Schmidt et al., 1976). Schmidt et al. (1976) results are supported by Blackman and Hodson (1977) who investigated the activity of permethrin (pyrethroid insecticide) against stable flies in fly chambers and field studies. Four Friesian steers were used for the fly chamber experiment. Animals were sprayed with 1 L permethrin 3 times weekly. Once treatment was applied, steers were placed in fly

chambers with 100 starved stable flies. Ten Shetland ponies were used in the field trial, 5 animals were assigned to 1 of 2 treatments: 500 mL of permethrin application or no treatment. Fly populations were recorded at intervals after treatment application. In the fly chamber experiment, the authors determined 67% chemical protection from stable flies was considered acceptable. The stably fly repellency tested in the fly chamber was high for 2 d ($> 75\%$), but by d 7 fell to a low level ($< 45\%$). In the field study, the control of stable flies was acceptable for 10 d, then protection began to decline (Blackman and Hodson, 1977).

Plastic ear tags have been impregnated with different insecticides to decrease stable fly populations on cattle. Block and Lewis (1986) conducted an experiment to evaluate the efficacy of insecticide- impregnated ear tags for the control of livestock biting flies. In this experiment, horn, house, face, and stable fly populations were recorded. Twenty Holstein cows were placed into 10 blocks (2 cows/block) and assigned 1 of 2 treatments, tagged or not tagged. The cows were open to a pasture setting, but were brought into the barn for milking and feeding. Fly counts were recorded twice weekly from early June to late September. Overall, horn flies made up 85.2% of all flies observed. Horn flies had a 99.9% reduction in tagged cows compared to untagged from wk 1 to 14, the reduction dropped to 63.5% in wk 15 and to 0% by wk 16. Stable flies were noted to feed below the hooves, which lead Block and Lewis (1986) to assume ear tags were ineffective to those areas. In fact, stable fly abundance was highest on tagged animals. The authors assumed stable flies attacked animals when competition is reduced. Regardless, stable fly abundance was not reduced by the fly tags.

Contrary to Block and Lewis (1986) that saw no efficacy of impregnated ear tags on stable flies, Hogsette and Ruff (1986) compared 2 different impregnated ear tags and ear tapes, which resulted in decreased stable fly numbers. Two experiments were conducted comparing 2 insecticide pyrethroid ear tags and permethrin cattle ear tape. In experiment 1, 140 lactating cows received 2 ear tags (1 / ear), 140 received 2 ear tapes (1 / ear), and 25 dairy heifers were used as

the negative control. Both were applied to animals at the beginning of May. Pre-treatment fly counts were recorded and post-treatment fly counts were recorded 24 h after treatment application, then once weekly. In experiment 2, beef yearlings and cows were assigned to 1 of 5 different treatments; 2 different proprietary formulations of 1 pyrethroid (treatments A and B), a commercial tag (C), 8% pyrethroid tag (D), and a control group (E). Fly counts were made 24 h pre-treatment, 24, 48, 72 h post treatment, and then weekly. Experiment 1 had 100% efficacy in horn fly reduction from wk 0 to wk 13, but stable flies did not decrease until wk 2 and 3. The extended time required to reduce stable fly populations were assumed to be due to high daily stable fly replacement, as there were several potential breeding sites. Stable fly numbers decreased throughout the experiment and remained low (0-1 fly/animal) for 10 wk. Unlike experiment 1, experiment 2 was conducted later in the fall and immediate on horn fly decrease was not seen until wk 7, and stable fly numbers were too low to analyze.

Natural Insecticides

Numerous experiments have determined an increase in inefficacy of insecticides as a method off fly control, Cook (2020) reported in a review of literature. Therefore, research has been conducted recently to analyze the insecticidal activity of botanical compounds (Birrenkott et al., 2000; Prowse et al., 2006). Birrenkott et al. (2000) recorded that garlic juice has insecticidal repellent properties on northern fowl mite (NFM) infested hens. Thirty hens were administered 1 of 2 treatments: sprayed vent and abdomen with tap water (control) or 10% garlic juice solution. Prior to treatment application, NFM infestations were scored from 0 to 4, in increments of 0.5, according to the presence and concentration of NFM. Respective treatments were applied every 7 d for 3 wk. The hens sprayed with 10% garlic juice had a decrease of NFM when analyzed on wk 4 and wk 8. Birrenkott et al. (2000) concluded that garlic juice did show toxicity on the pest, NFM.

Prowse et al. (2006) conducted an experiment to determine garlic juices insecticidal activity on 3 life stages (egg, larvae, adult) of 2 dipteran pests, the cabbage root fly, *Delia radicum* (L.), and the house fly, *Musca domestica* (L.). Seven treatments were examined, 6 concentrations of garlic juice (0%, 0.25%, 0.5%, 1%, 2%, and 5%) and a commercially available organophosphate pesticide. Fly species were tested separately. Eggs were exposed to 1 mL of assigned treatment on filter paper in a petri dish for 7 d. After the 7-d period, adult flies and mortality were recorded. Larvae and adults (7-10 d old) were then exposed to 0.8 mL of assigned treatment. After preliminary studies, the cabbage fly concentrations were reduced to 0%, 0.2%, 0.4%, 0.6%, 0.8%, 1% and 2%. Larvae and adults were enclosed in sample tubes lined with filter paper that had been dampened with treatment solution. Percentage mortality was calculated after 24 h and 48 h exposure for the larvae and 24 h for the adults. The cabbage fly egg had an increase in average mortality when compared to the control (0% treatment), and 5% garlic juice concentration had an increase in mortality compared to the organophosphate pesticide. The cabbage fly larvae had increased mortality only after 48 h exposure to the 5% garlic juice compared to the control, however, no garlic juice treatment had a higher mortality rate than the OP. Adult cabbage flies treatments greater than 0.2% garlic juice had an increased mortality compared to the control, whereas mean mortalities were not different for concentrations of greater than 0.8% compared to the OP. The house fly egg exposure to concentrations of greater than 0.5% increased average mortality compared to the control, however, no garlic juice concentration had comparable mortality levels to the OP. The house fly larval mortality was low in all treatments after 24 h, although larvae in the 5% garlic juice and OP treatments had a higher mortality rate than the other treatments. After 48 h mortality was higher for concentrations of greater than 2% compared to the control, but was not statistically different compared to the OP. Overall, garlic juice, dependent on concentration, did have a toxic effect on both the cabbage and house fly. Prowse et al. (2006) suggests garlic products in the field should be targeted at the egg

and adult life stages. Therefore, garlic juice may be an appropriate replacement for traditional insecticides.

Research is limited on the efficacy of garlic in reducing fly abundance in cattle. Recently, Durunna et al. (2020) evaluated garlic powder in trace mineral salt and the effects on fly abundance in pastured cattle. Over a 2-yr experiment, 3 groups of cows were allocated to 1 of 2 treatments: trace-mineral salt (TMS) or TMS infused with garlic powder (GPTMS). Durunna et al. (2020) recorded fly abundance, fly avoidance behaviors, and TMS intake. A treatment effect was observed, where GPTSM animals had 47% less fly abundance than TMS. Similarly, animals receiving GPTMS had 42% fewer fly avoidance behaviors. However, performance and garlic powder dose were not recorded.

Viral and Bacterial Pathogens in Bovine Respiratory Disease

Bovine respiratory disease is initiated by viruses, bacteria, and stress (Powell et al., 2013). Viruses involved in BRD are: infectious bovine rhinotracheitis (IBR), bovine viral diarrhea (BVD), parainfluenza type 3 (PI3), and bovine respiratory syncytial virus (BRSV); and bacteria: MH (previously known as *Pasteurella haemolytica*), PM, HS, and MB (Powell et al., 2013). These viruses and bacteria are a threat to animals when immunity is suppressed due to stress factors which can include weaning, castration, dehorning, poor nutrition, and handling (Powell et al., 2013). Therefore, weaned animals shipped to feedlot operations are impacted by BRD, which is the main cause of morbidity and mortality in feedlots (Griffin et al., 1997).

Infectious Bovine Rhinotracheitis Virus

Bovine herpes virus-1, which is the causative agent of IBR virus, infects cattle and induces upper respiratory disorders and suppressed immunity (Jones and Chowdhury, 2007). The bovine herpes virus subtype 1 is the primary respiratory form and is most commonly found in feedlots. Clinical IBR symptoms include high temperatures, anorexia, coughing, nasal discharge,

excessive salivation, and dyspnea if the larynx becomes occluded (Jones and Chowdhury, 2007). Infectious bovine rhinotracheitis induced immune suppression can also lead to bacterial infections. These secondary bacterial infections can result in pneumonia. Although IBR is not always associated with BRD, it is understood that IBR has the ability to reactivate from a dormant infection to initiate BRD (Jones and Chowdhury, 2007). Acute IBR infection can lead to increased production of the virus secretion in oral, nasal, and ocular cavities. In addition, IBR can cause bronchoconstriction, resulting in secretions being trapped in lower airways, which could impair lung defense mechanisms and increase bacterial growth (Cusack et al., 2003). Overall, IBR induces a suppressed immune system that can result in severe pneumonia due to secondary bacterial infections (Cusack et al., 2003).

Bovine Viral Diarrhea Virus

The relationship between BRD and BVDV has been extensively examined (Fulton et al., 2000; Speer et al., 2001). The development of BRD due to BVDV is dependent several factors such as the presence of secondary pathogens, BVDV strain (type 1 or type 2), infection type (acute or persistent), and time of BVDV exposure, i.e., fetal or postnatal time (Ridpath, 2010). Bovine virus diarrhea viruses have been classified into prevalent subtypes, BVDV 1a, BVDV 1b, and BVDV 2, and are respiratory pathogens that infect phagocytes such as the bronchoalveolar macrophages (Ellis, 2001). Biotypes of the virus come in 2 forms, cytopathic which induces cellular degradation and noncytopathic that does not induce cell degradation (Potgieter et al., 1997). Animals can be persistently infected (PI) with BVDV when exposed to BVDV in utero and continually shed the virus to other cattle populations (Ridpath, 2010). Although PI animals represent < 0.5% of the population of cattle entering feedlots in the U.S., PI animals also represent approximately 5% of mortalities. Calves that are PI are sources of cytopathic and noncytopathic types of BVDV and can develop fatal mucosal disease (Taylor et al., 1994). However, the spread of BVDV is not limited to PI animals, but can also be spread by acutely

infected animals, therefore both fetal exposure and acute postnatal BVDV infections are contributors to BRD (Ridpath, 2010).

In addition to the immunosuppressive effect of acute BVDV infection, BVDV can also impair humoral antibody production, result in reduction of monocyte chemotaxis, and weaken the antibacterial system in leukocytes (Cusack et al., 2003). Increased colonization of other pathogens in the lungs is a result of these mechanisms, which results in aggravation to the pulmonary tissues. Martin et al. (1999) reported that out of 700 calves in feedlots, 24% had positive BVDV titers from previous exposure at arrival and were more likely to receive treatment for BRD compared to calves with no BVDV titers upon arrival. In this experiment, 50% of unvaccinated calves seroconverted to BVDV throughout the trial and likelihood of being treated for BRD was increased compared to calves that received vaccination upon arrival (Martin et al., 1999). Martin et al. (1999) also concluded that BVDV was consistently related to an increased risk of BRD and decreased weight gains.

Bovine Parainfluenza 3 Virus

Bovine parainfluenza 3 is affiliated with acute and chronic pneumonia in cattle, and infection is often correlated with BHV1 and BVDV viruses (Hodgins et al., 2002). After infection occurs, PI3 replication transpires in the upper and lower epithelial cells of the respiratory tract (Cusack et al., 2003). Although replication occurs in epithelial cells, occurrence of respiratory tissue damage is primarily in the lower respiratory tract which result in bronchitis, bronchiolitis, and alveolitis (Cusack et al., 2003). In addition, infection of PI3 in alveolar macrophages impairs pulmonary defense mechanisms which ultimately impairs mucociliary escalator function and depresses cellular immune responses caused by PI3, resulting in secondary bacterial pneumonia (Cusack et al., 2003).

Bovine Respiratory Syncytial Virus

Bovine respiratory syncytial virus is a common pathogen involved in bovine respiratory disease, and when interacted with bacterial agents, establishes pneumonia in cattle (Hodgins et al., 2002). Similar to BHV-1 and PI3, infection of BRSV destructs the ciliated respiratory epithelium, which infects alveolar macrophages, causing depressed cellular immunity (Cusack et al., 2003). Bovine respiratory syncytial virus can infect ciliated and non-ciliated epithelial cells, resulting in necrotizing bronchiolitis and interstitial pneumonia (Hodgins et al., 2002). The destruction of ciliated epithelium prevents pulmonary clearance, which causes secondary bacterial infections in cattle (Cusack et al., 2003).

Mannheimia haemolytica

Mannheimia haemolytica (formerly known as *Pasteurella haemolytica*) is normal bacterial flora that is located in the upper respiratory tract, nasopharynx, and tonsillar crypts of healthy calves (Griffin et al., 2010). It is understood that MH exists in the host animal while healthy, but stress of co-morbidity can change these commensal conditions (Griffin et al., 2010). Once the relationship is disrupted, MH has shown to be responsible for characteristic BRD infection, resulting in tissue damage in the lung. Once MH is established in the lung, pulmonary invasion of MH can cause severe pneumonic damage due to the increase of virulence factors such as vascular damage, excess fibrin effusion, and neutrophil infiltration that causes lung injury, adhesions used for colonization, a lipopolysaccharide complex causing hemorrhaging, edema, and inflammation (Hodgins et al., 2002; Griffin et al., 2010).

Prevention of Bovine Respiratory Disease

Management

Bovine respiratory disease is complex and has many risk factors, along with the viral and bacterial agents involved in development (Hodgins et al., 2002). Therefore, management protocols should be in place to decrease the development of BRD. Effective management

strategies can be applied in the early stages of a calf's life as a form of prevention of BRD, but due to the U.S. cattle marketing system, some risk factors are unavoidable (Peel, 2020). Calves marketed through the Southern Great Plains and Southeastern U.S. are not often not appropriately weaned or vaccinated prior to market. Cattle are commingled with other animals that have an unknown history of disease or vaccinations (Wilson et al., 2012). Once animals are marketed, animals are typically transported long distances, which increases stress and exposure to extreme temperature fluctuations. Calves marketed through cattle auctions are often defined as high-risk. High-risk cattle are lighter-weight, unlikely to be vaccinated, and have increased stress levels at feedlot arrival (Griffin et al., 2010). Meanwhile, low-risk calves are heavier-weight and are more likely to be vaccinated before feedlot arrival. Low-risk calves are more likely to have been through a preconditioning phase prior to market. Preconditioning of calves determined to improve health outcomes in the feedlot by reducing BRD incidence (Hay et al., 2016).

Regardless of vaccination history, the majority of cattle are vaccinated upon arrival to the feedlot to help manage BRD pathogens (USDA NAHMS, 2013). By administering an efficacious vaccine to a clinically healthy, unstressed, immunocompetent calf, BRD can be reduced and optimal vaccine response can be achieved (Edwards et al., 2010). In U.S. feedlots, 96.6% vaccinate for BVDV, 93.7% vaccinate for IBR, 85.1% vaccinate for PI3, and 89.5% vaccinate for BRSV (USDA NAHMS, 2013). In addition to viral vaccines, 2 out of 3 feedlots vaccinated for bacterial agents commonly associated with BRD: HS, MH, and PM. Although a majority of feedlots vaccinate against viral and some bacterial agents, vaccination as a control of BRD is controversial (Ellis, 2001). Vaccination efficacy is dependent on animals' stress levels, timing of vaccination, number of vaccinations, and type of vaccine administered.

Vaccination administration timing has been extensively researched, but results are variable. Richeson et al. (2008) determined that improvement in immune response could occur when initial vaccination is delayed by 14 d. However, a second experiment conducted resulted in

no effect of vaccination timing on performance in receiving calves (Richeson et al. 2009).

Although stress is thought to reduce vaccine efficacy, stress and BRD interactions are influenced by the type of vaccination (MLV vs. INA), the type of antigen in vaccines, and overall immunocompetence of animals when stressed due to natural factors and genetics of the animal (Richeson and Falkner, 2020).

Impacts of Bovine Respiratory Disease on Animal Health

Bovine respiratory disease accounts for the majority of morbidity and mortality in feedlots (USDA NAHMS, 2013). Loneragan et al. (2001) reported that BRD was the cause of 57.1% of mortality that occurred in feedlots. In a meta-analysis, Theurer et al. (2015) reviewed 31 studies that comprised 88 experiments that analyzed the effectiveness of commercially available vaccines effects on BRD. The effectiveness of MLV vs INA vaccines for BHV-1, BVDV, and BRSV were evaluated. Modified-live BHV-1 vaccine was evaluated for protection on beef and dairy calves in 10 trials. Overall, morbidity risk was decreased in animals that received vaccines in 1 of the 10 trials. No difference in morbidity was determined in the other 9 trials. When evaluating INA BHV-1 vaccines, only 2 studies were used. One of the 2 trials reported a decreased morbidity risk, with the remaining trial having no difference in morbidity. Eleven trials evaluated MLV BVDV vaccine. Six of the 11 had a decreased morbidity and the remaining 5 had no differences in morbidity. Additionally, 4 trials challenged MLV BVDV vaccinated and nonvaccinated calves with BVDV. One of the 4 trials had a decreased mortality risk in the vaccinated calves compared to nonvaccinated calves. Only 2 trials evaluated INA BVDV vaccines compared to controls. No difference in morbidity was reported in either trial. Less research is available concerning INA vaccines compared to MLV, therefore making them difficult to compare.

West et al. (1999) analyzed the efficacy of MLV vaccines in experimentally infected calves. Twenty-seven neonatal dairy calves were assigned to 1 of 4 treatments: no vaccine (group 1; $n = 9$), vaccinated twice IM at 3 wk intervals with MLV vaccine containing BRSV, BVH-1, PI3, and BVDV (group 2; $n = 6$), single vaccination with MLV containing BRSV, BVH-1, PI3, and BVDV (group 3; $n = 6$), and single vaccination with an MLV vaccine containing BRSV, BHV-1, PI3, and BVDV with an adjuvant (group 4; $n = 6$). All calves received initial vaccination at 2 – 4 wk of age, while group 2 received a revaccination 3 wk later. Calves were kept in individual pens until 7 d prior to challenge, then were then placed into 4 large pens and randomly commingled. Three wk following the last vaccination, calves were challenged using lung wash from an infected calf with BRSV. The lung wash was delivered using an ultrasonic nebulizer (Ultra-Neb 99, Devilbiss, Somerset, PA) and a face mask on d 0 (d of challenge). The experiment lasted for 8 d following challenge, then calves were euthanized. Animals receive clinical assessments daily by a veterinarian blinded to treatments. Scores were assigned to individual animals for heart rate, respiratory rate and effort, cough, nasal discharge, and depression. Cumulative clinic scores (CCS) were used for analysis. By the CCS method, all calves that did not receive vaccination presented moderate to severe clinical respiratory disease signs. A reduced number of calves in vaccine treatments presented clinical disease signs, although authors did not specify the number of calves. However, CCS treatment effects were different between unvaccinated animals (group 1; control) and animals that received 2 vaccinations of MLV vaccine (group 2). Group 2 did not have an increase in CCS scores over time, unlike the other groups. Overall, West et al. (1999) presented a reduction in clinical disease when calves were vaccinated vs no vaccine.

In a review of literature, Larson and Step (2012) determine evidence-based effectiveness of BRD vaccinations in feedlot cattle. Data were extracted from 22 trials examining naturally occurring respiratory disease in feedlot cattle. The 22 trials tested the effectiveness of vaccination

against 1 or more of the bacterial pathogens. For all 22 trials, a cumulative incidence of morbidity was determined due to BRD. Commercially available vaccines effectiveness of MH was evaluated in naturally occurring BRD, whereas 2 of the 15 experiments reported a decrease in BRD morbidity when vaccinates were compared to nonvaccinated or controls. However, 3 of the 15 recorded and increased risk of BRD morbidity. Larson and Step (2012) use the same model and compare studies using commercially available MH vaccines and the effects on pathogen-challenged feedlot cattle. Five trials were conducted to evaluate vaccines and mortality risk and lung lesion severity. Increased survival post challenge was reported in all trials. Overall, Larson and Step (2012) determine that summary data indicated potential benefit for vaccination against MH and PM. However, the data indicated no benefit for vaccination against HS. The authors determine the data does not provide consistent effectiveness of vaccination in feedlot cattle against MH, PM, or HS.

Impacts of Bovine Respiratory Disease on Animal Performance

Animal performance has been proven to decrease when infected with BRD, but the extent of the effect is controversial. Bryant et al. (2008) compared the effect of 3 different MLV vaccines on steer performance in a commercial feedlot setting. Feeder steers ($n = 3,147$; initial BW = 255 kg) were purchased from several different auction markets and delivered to a feedlot over the course of 1 mo. Animals were assigned to 1 of 3 MLV commercial vaccines: PYR5 (Pyramid[®] 5; Fort Dodge Animal Health, Overland Park, KS) containing IBR, BVDV types 1 and 2, PI3, and BRSV, BOV5 (Bovi-Shield Gold[®] 5; Pfizer Animal Health, New York, NY) containing IBR, BVDV type 1 and 2, and BRSV, or BOV3 (Bovi-Shield Gold[®] IBR-BVD; Pfizer Animal Health) containing IBR, BVDV type 1 and 2. All animals received the respective treatment upon arrival at feedlot during initial processing and were given a MH toxoid at initial processing as well. All cattle were revaccinated following initial vaccination (76 to 126 d following) with the same vaccine, a MLV IBR-BVDV type 1 and 2. Due to increased morbidity

and mortality, 3 replicates of cattle were revaccinated prior to a terminal implant. Initial and Final BW were recorded over the entirety of the trial, 234 d. Steers administered PYR5 had a better feed conversion compared to animals administered BOV3. However, there were no differences in performance between BOV and BOV3. The DMI and ADG did not differ among treatments from initial to final BW.

Duff et al. (2000) performed 2 experiments to determine the effect of 2 different MLV vaccines and vaccine timing on performance of receiving calves in a feedlot setting. In experiment 1, steers ($n = 120$; initial BW = 166 kg) and heifers ($n = 108$; initial BW = 192 kg) were assigned to 1 of 3 treatments: no IBR-PI3 vaccine (control), an intranasal (IN) MLV IBR-PI3 vaccine, or an intramuscular (IM) MLV IBR-PI3 vaccine. The receiving period lasted a total of 28 d. Cattle administered the MLV IBR PI3 vaccine IN had a greater ADG compared to IM treatment over the 28 d experiment. However, neither IN or IM differed from the control for ADG over the 28 d experiment. No differences were recorded for DMI between treatments over the day of the experiment, however, the F:G ratio was increased for the IM group compared to IN group. The second experiment conducted by Duff et al. (2000), steers ($n = 102$; initial BW = 207 kg) were purchased from an auction market. Steers were administered 1 of 4 experimental treatments: 1) no vaccine (control); 2) no vaccine at processing; 3) an IM MLV vaccine containing IBR, PI3, BVDV, and BRSV administered on d 7 (CON/IM), an IN MLV IBR-PI3 vaccine administered at processing, and revaccination on d 7 with an IM MLV vaccine containing IBR, PI3, BVDV, and BRSV (IN/IM); 4) and an IM MLV vaccine containing IBR, PI3, BVDV, and BRSV administered at processing and d 7 (IM/IM). Calves ($n = 8$ to 9) were placed in 4 pens / treatment. Over the 28 d experiment, performance did not differ between treatments ($P > 0.10$) other than F:G ($P < 0.10$) being improved for calves receiving vaccination compared to control calves. In experiment 1, the authors concluded there may be an advantage in using an IN vaccine when compared to an IM vaccine. The advantage could be due to rapid onset of protection when

administering an IN vaccine. The authors also debated the anecdotal information between IN and IM, suggesting that IM vaccines could increase body temperature. However, rectal temperatures were not recorded and therefore results do not support or refute the assumption. In experiment 2, the authors suggest that management program makes little difference on performance.

Hudson et al. (2020) compared respiratory vaccine antigen type and stress factors effects on performance over a 56-d receiving trial in feedlot calves. Previously unvaccinated beef steers ($n = 48$; initial BW = 226 kg) were assigned to 1 of 4 experimental treatments: 1) non-stress control with INA vaccine; 2) non-stress control with MLV; 3) stress model implementation with INA; 4) and stress model implementation with MLV. Animals administered INA were revaccinated 14 d after initial vaccination, whereas MLV animals were not. Vaccine type affected DMI and ADG, calves administered INA had a greater ADG from d 0 to 56 compared to calves administered MLV. The authors suggested the decrease in ADG reduction for MLV was influenced by differences in inflammation. However, BW and DMI intake did not differ between treatments.

Serology

Viral Neutralizing Antibody Titers

Rodning et al. (2010) evaluated the effects of 3 commercial vaccines (2 MLV, 1 INA) on 80 heifers exposed to PI ($n = 3$; persistently infected with BVDV type 1a, 1b, and 2) animals. Heifers were assigned to 1 of 4 treatments: 1) received no vaccination (control); 2) was administered FP5 MLV vaccine (Bovi-Shield Gold[®] FP5; Pfizer Animal Health); 3) was administered PYR5 MLV vaccine (Pyramid[®] 5; Fort Dodge Animal Health); and 4) was administered the INA vaccine (Vira Shield[®] 6; Novartis Animal Health, Larchwood, IA). Both MLV vaccines contained BVDV type 1a and 2, whereas INA contained BVDV type 1a, noncytopathic type 1, and noncytopathic 2. Cattle receiving FP%, PYR%, and INA were

vaccinated with the respective treatment at weaning, 28 d post-weaning, and received a booster 56 d following weaning. The challenge period took place 68 to 126 d following AI, on heifers that were confirmed bred ($n = 70$). Persistently infected animals were housed with heifers until calving. Roding et al. (2010) reported animals that did not receive vaccination became viremic after exposure to PI animals. The control heifers developed BVDV-neutralizing antibodies and calves were born PI animals. Eleven of the vaccinated heifers became viremic and two of the vaccinated heifers gave birth to PI calves. Overall, vaccination with a total of 4 doses provided protection against BVDV and giving birth to PI calves. The 4 vaccinations administered between weaning and calving provided 100% protection for treatment groups administered MLV vaccines and 89% protection for groups administered INA vaccines. A difference in virus isolation was observed between treatments, with the virus being isolated from more heifers in control and INA compared to MLV groups from d 0 to 28. The authors suggest this indicates an improved protection for animals administered MLV. Regardless, vaccination proved protection regardless of vaccine type when compared to control.

In an experiment comparing respiratory vaccine antigen type (MLV vs INA) effects on immune responses, Hudson et al. (2020) evaluated BVDV neutralizing antibody titers in serum. Both vaccines were administered on d 0 per label recommendations, then INA was booster vaccinated on d 14. Each treatment groups were housed in 3 consecutive pens with an empty pen between treatment groups. All calves in the experiment were seronegative to BVDV on d - 37. A vaccine type \times time interaction was observed for BVDV 1a, the MLV had increased BVDV 1a neutralizing antibody titers from d 14 to 56. The authors suggest the trends for vaccine antigens were typical vaccine responses, where antibodies are detected from d 7 and 14, before peaking d 28 and 42 following initial vaccination.

Acute Phase Proteins

Acute phase proteins (APP) are biomarkers of infection and inflammation in livestock. Acute phase proteins are the large proteins released into the blood stream during the onset of disease, inflammation, or traumatic injury (Joshi et al., 2018). Serum amyloid A (SAA) and haptoglobin (Hp) are 2 important APP in cattle. Acute phase proteins are produced in response to endogenous release of glucocorticoids and pro-inflammatory cytokines in the liver (Takashashi et al., 2007). Bacterial infections influence an acute-phase response when increased production of APP occur (Ulutas et al., 2011). The APP can increase and decrease during acute-phase response to infection.

Joshi et al. (2018) analyzed HP and SAA as biomarker candidates of naturally occurring BRD in dairy calves. Two groups were used for the experiment, the control group had no history of BRD, and an infected group with calves suffering from natural infection of BRD. The calves (dairy and buffalo calves) used for this trial ranged from 2 wk to 6 mo of age. Clinical scores were determined on d 0. A physical examination was performed, and clinical signs associated with BRD were recorded: cough, nasal or ocular discharge, dyspnea, tachypnea, anorexia, and depression. If two or more clinical signs were present, rectal temperature was measured and a clinical score (CS) was given. Clinical scores ≥ 5 were considered morbid and were put into the infected group. Serum was harvested from all calves on d 0, 5, and 10, for quantitative determination of APP. Calves positive for BRD (CS ≥ 5) had higher SAA and Hp concentrations when compared to healthy calves on d 0 (10.2 vs 138.2 ng/mL). However, changes occurred in APPs on d 5 and 10, as the abnormal values decreased towards baseline values. The authors report 14 times in Hp serum concentrations in calves suffering from BRD, which indicates an occurrence of acute-phase response to naturally occurring BRD. However, only 3 times increase was recorded in SAA concentrations. The authors relate the increase of SAA to the role of SAA in host immunity, which bind Gram-negative bacteria and allows destruction by phagocytic cells (Nikunen et al., 2006). Haptoglobin and SAA curves differed with Hp increasing immediately

with onset of BRD while SAA increases were delayed. In this experiment, Hp was more sensitive compared to SAA in the field conditions. Haptoglobin increased rapidly when mild to moderate BRD infection took place. Whereas, SAA required more stimulation for a longer period before concentrations increased. Overall, it was concluded that clinical severity of BRD was associated with Hp and SAA alteration in serum concentrations. Haptoglobin is highly sensitive and may be capable of diagnosing BRD during early onset of BRD, even in mild cases (Joshi et al., 2018).

Summary of Literature

Stable flies remain a significant ectoparasite in feedlots, accounting for decreased performance and efficiency. Annual losses to the U.S. cattle industry due to stable flies are estimated to be \$2.2 billion. Although there are multiple commercial fly control methods available, the synthetic chemical insecticides being administered today appear to be becoming less effective at decreasing stable fly abundance. There are reports that suggest garlic has insecticide capabilities, but the published research supporting the use of garlic as a fly control method is limited. The use of garlic and the associated potential insecticide capabilities have been researched in laboratory trials but have yet to be evaluated in field trials. There is no research to the authors' knowledge evaluating garlic as a means of fly control in a feedlot setting. Therefore, experiments comparing commercially available fly control methods with garlic in a feedlot setting is needed to fill a void in the published research.

Bovine respiratory disease is a complicated illness that remains the most costly problem for the feedlot industry, resulting in excess of \$1 billion in losses annually (Peel, 2020). Bovine respiratory disease reduces animal performance through increased feedlot morbidity and mortality. Several risk factors can increase BRD incidence including: viruses, bacteria, and immune suppression due to stress (Loneragan et al., 2001). Preventative measures and antimicrobial treatments have been extensively researched over the years. However, over the last

several decades, no reduction in the incidence of BRD has occurred (Gifford et al., 2012; Theurer et al., 2021). Vaccines are a common BRD prevention method incorporated into feedlot protocols. While the vaccine antigen type, timing of vaccination, and number of vaccinations administered has been evaluated in attempt to decrease BRD incidence, the results are inconsistent as other factors such as animals' previous immune status and stress level vary dramatically. While some experiments support that vaccinating for pathogens involved in BRD positively impacts animal health and performance, other research has proven inconclusive or even counterproductive. As such, further research should be conducted on commercially available vaccines to improve upon inconsistent literature.

CHAPTER II

EFFECTS OF FLY CONTROL STRATEGIES ON STABLE FLY ABUNDANCE, FLY AVOIDANCE BEHAVIORS, AND ANIMAL PERFORMANCE IN FEEDLOT BULLS AND STEERS

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ABSTRACT: The stable fly, one of the most economically important ectoparasites in the cattle industry, causes decrease performance of cattle in confined feeding operations and pasture settings. The objective of this experiment was to examine the effects of commercial insecticide and garlic fly control strategies on stable fly abundance, fly avoidance behaviors (i.e., foot stomping, head tossing, tail switching, and twitching of the skin), and animal performance in a feedlot setting. Angus bulls ($n = 64$; BW = 281 ± 36.2 kg) and steers ($n = 36$; BW = 475 ± 40.4 kg) were blocked by sex and BW and assigned to 1 of 4 experimental treatments in a randomized complete block design (7 pens/treatment; 4 bulls/pen or 3 steers/pen). Treatments included: 1) a negative control (CON; no fly control); 2) abamectin and piperonyl butoxide insecticide tags (FT; XP820[®], Y-Text Corporation, Cody, WY); 3) permethrin and piperonyl butoxide pour on (PO; Permethrin[®] CDS, Bayer Animal Health, Shawnee Mission, KS); 4) garlic-powder top dressed on feed (GR) administered at $0.28 \text{ g} \cdot \text{animal}^{-1} \cdot \text{d}^{-1}$. The experiment was conducted from June 2019

to September 2019. Stable fly abundance and fly avoidance behaviors were recorded by trained personnel once / wk on days with similar temperatures, humidity, wind speed, and without precipitation between 0700 h and 1200 h. There was no treatment \times week interaction for stable fly abundance or fly avoidance behavior, however both were affected by week ($P < 0.001$). There were no differences in final BW ($P = 0.34$), overall ADG ($P = 0.30$), overall DMI ($P = 0.53$), or overall G:F ($P = 0.39$). There was a tendency ($P \leq 0.10$) for GR to have decreased BW on d 28 and decreased ADG from d 0 to 28 compared to FT and PO. There was also a tendency ($P = 0.09$) for decreased DMI from d 0 to 56 for GR compared to CON and FT. Overall fly abundance was less than expected (mean = 1 to 17 flies/animal) throughout the experiment and animal performance was unaffected by fly control strategy.

Key Words: fly control, garlic, insecticide fly tag, insecticide pour on, stable fly

INTRODUCTION

The stable fly, *Stomoxys calcitrans* (L.), is one of the most economically important ectoparasites within the cattle industry. The stable fly feeds on blood from livestock, companion animals, and humans. Stable flies can decrease cattle performance in both confined feeding operations (Campbell et al., 1977; Campbell et al., 1987; Wieman et al., 1992) and pasture settings (Talley et al., 2009). Campbell et al. (1987) determined that the decreased performance occurs due to the stable fly's painful bite which can interfere with animal feeding behavior, thus decreasing dry matter intake (DMI), average daily gain (ADG), and body weight (BW). The stable fly causes defense behaviors including foot stomping, head tossing, tail switching, and twitching of the skin (Dougherty et al., 1993). In addition to potential decreases in performance, the stable fly is also a mechanical vector for pathogens such as anaplasmosis (Dikmans et al., 1950) and other viruses and bacteria (Baldacchino et al., 2013). The negative economic impact of stable flies as a result of decreased cattle performance can be substantial, resulting in estimated

annual losses to U. S. cattle industry increasing from \$152 million (Bishopp et al., 1938) to \$2.2 billion (Taylor et al., 2012).

Historically, applying synthetic repellents or insecticides has been a common practice to decrease adult stable fly numbers (Marçon et al., 1997). There are several methods and variations of repellents and insecticides including: insecticide fly tags, insecticide pour-on products, and the release of pupal parasitoids. While these methods have previously been effective, the susceptibility of stable flies to insecticides such as dieldrin, toxaphene, permethrin, and pyrethroid has continued to decrease (Mount et al., 1965; Cliek and Green, 1994). Therefore, there is an increased need to find alternative methods of fly control that are effective.

Newly developed commercial products are being marketed with natural ingredients such as essential oils, garlic oil, and garlic powder as methods of fly control. However, research on the efficacy of these potential natural insecticides in a feedlot setting is limited. Durunna et al. (2020) determined that adding garlic powder to trace mineral salt may reduce Diptera flies on pastured cattle. However, to the authors knowledge, no research has been conducted comparing garlic powder supplementation in feedlot diets with other commercially available insecticides. The objective of this experiment was to examine the effects of commercial insecticide and feed through garlic fly control strategies on fly abundance, fly avoidance behaviors (i.e., foot stomping, head tossing, tail switching, and twitching of the skin), and animal performance in a feedlot.

MATERIALS AND METHODS

All procedures were approved by the Institutional Animal Care and Use Committee at Oklahoma State University (Animal Care and Use Protocol number: AG-19-8).

Experimental Design and Animals

Angus bulls ($n = 64$; initial BW = 281 ± 36.2 kg) and steers ($n = 36$; initial BW = 475 ± 40.4 kg) were transported to the Willard Sparks Beef Research Center (WSBRC) in Stillwater, OK. The bulls originated from a single-source ranch in Southeastern, Oklahoma. The steers originated from the Oklahoma State University Field Research Service Unit west of Stillwater, OK. Bulls and steers arrived on separate dates and were processed separately. Steers arrived on d - 21 (12 d prior to bulls, 21 d prior to treatment application). Steers were implanted (Revalor 200; Merck Animal Health, Madison, NJ), vaccinated against clostridial (Vision with SPUR; Merck Animal Health) and viral and bacterial respiratory pathogens (Titanium 5 + PH-M; Elanco Animal Health, Greenfield, IL), and administered an anthelmintic (Safeguard; Merck Animal Health). Steers were randomly assigned to 12.2×30.5 m soil surfaced feedlot pens with a shared 76-L concrete water tank between 2 adjacent pens (model J 360-F; Johnson Concrete, Hastings, NE) and with a 12.2-m concrete feed bunk and fence-line automatic water basin. Steers were fed the receiving (RCV) diet until d -7. Upon arrival, bulls were weighed, identified, and were administered an anthelmintic (Safeguard; Merck Animal Health). Bulls were then held with *ad libitum* access to prairie hay and water for 24 to 48 h. On d - 7, bulls and steers were individually weighed, sorted, and placed into experimental pens. Bulls and steers were housed in twenty-eight 4.57×13.24 m partially covered feedlot pens with a shared water source between 2 adjacent pens. Bulls and steers were housed in the assigned pens for 7 d prior to the beginning of the experiment to allow adjustment to the new pens and to document baseline fly abundance and fly avoidance behavior prior to treatment application.

Experimental Treatments

Within each block, a group of 4 bulls or 3 steers were assigned randomly to pens. Pens were previously assigned randomly to and 1 of 4 experimental treatments. Animals were weighed and treatments were applied on d 0. Experimental treatments included 1) a negative control (CON) which received no fly control treatment; 2) abamectin and piperonyl butoxide insecticide

tags (FT; XP820[®], Y-Tex Corporation, Cody, WY) applied to the back of both ears on an animal; 3) permethrin and piperonyl butoxide pour on (PO; Permethrin[®] CDS, Bayer Animal Health, Shawnee Mission, KS) applied according manufacturer label rates down the center of the animal's back every 28 d of trial or 4) garlic-powder (GR) administered at 0.28 g •⁻¹ animal •⁻¹ d top dressed onto feed at morning feeding (0700 h).

Feed Management

All bulls were fed a common growing diet with 28% roughage (Table 2.1) for the entirety of the trial. Steers were transitioned to a finishing diet over a 15-d period using a step-up program (Table 2.1). Bunks were read at approximately 0530 h every morning to determine the quantity of feed remaining from the previous day's feed call. The feed call was adjusted daily so that there was no overabundance of feed left in the bunk. Diet samples were collected twice weekly and dry matter (DM) was calculated after samples were dried in an oven at 60°C for a minimum of 48 h. A monthly composite was created after DM was calculated and stored in a freezer until data analysis could be completed. Feed refusals were collected from feed bunks when cattle were weighed. Dry matter intake for each feed period was adjusted by subtracting the appropriate feed refusal amount that was weighed back.

Cattle Health

Three steers, 1 each from GR, FT and PO treatments, were removed from the trial for reasons unrelated to the dietary treatments. The PO steer was euthanized due to a broken leg, the FT steer was euthanized due to a dislocated hip, and the GR steer was removed the last wk of the trial due to a chronic infection.

Performance Data Collection and Calculations

Individual BW was collected for all bulls and steers on d 0, 28, 56, 84, and 98. All BW recorded were adjusted with a 4% pencil shrink ($BW \times 0.96$). Individual ADG was calculated by dividing individual pencil shrunk weight gain in kg by day on feed for each period. Pen ADG was then calculated by averaging the individual ADG for each animal for that period. Dry matter intake was calculated as total DMI for each pen divided by the number of head days for each pen. Gain to feed ratio was calculated by dividing the mean ADG by the DMI of each period.

Fly Abundance and Behavior Monitoring Collection

The experiment began in early June and continued through the middle of September (14 wk). Stable fly abundance and fly avoidance behaviors were visually observed once per wk by the same trained observer. Stable fly abundance on the front legs comprises approximately 45% of the total abundance on the animal (Lysyk, 1995), so only the leg counts were recorded in the current study. Observations were made between 0700 h and 1200 h weekly on days with similar temperatures, humidity, wind speed, and without precipitation based on Oklahoma Mesonet data. Data were collected from the Mesonet Stillwater site (site 89) located 8 km from WSBRC. Fly counts were made while standing in the pen and slowly approaching an animal. Once animal was calm and visible, stable fly abundance was recorded by counting the number of flies on the forelegs of an individual animal, using 7×35 binoculars as needed. Stable fly counts were recorded utilizing a tally counter and a countdown timer (Taylor et al., 2012) for a 30 sec period. After stable fly abundance was recorded on all individual animals in the pen, fly avoidance behaviors were monitored by the same observer for a 1 min period. The same tally counter and countdown timer that were used to record fly abundance were used for fly avoidance behaviors. Behavioral responses such as leg stamping, tail flicking, subcutaneous skin twitches (panniculus reflex), and head tosses were recorded as fly avoidance behavior responses. All animals in a pen were visually monitored and fly avoidance behaviors were documented on a per pen basis. Counting methods are described in greater detail by Berry and Campbell (1983).

Economic Injury Level Calculation

Economic injury level (EIL) was calculated with methods described in detail in Catangui et al. (1997). In brief, the quantitative relationship can be calculated using the negative exponential equation where X is defined as the stable fly level and Y is described as the reduction in ADG (Gomes et al., 1953; SAS institute, 1989): $Y = B_0 (1 - e^{-B_1 X})$, where B_0 is the upper asymptote, B_1 is the slope, and e is the natural logarithms base. Using the estimates of B_0 (b_0) and B_1 (b_1), the economic injury level of stable flies is calculated as: $GTP = b_0 [1 - e^{-b_1 (EIL)}]$ or logarithmic form, $EIL = \{\ln [1 - (GTP \div b_0)]\} \div \{-b_1\}$, where GTP is the gain threshold proportionally expressed of the expected ADG (Control cost \div market value), b_0 is the maximum reduction that may occur in ADG, and b_1 is estimated reduction in ADG of animal per stable fly.

Statistical Analysis

This experiment used a randomized complete block design with animals being blocked by sex and BW. Performance was analyzed using the MIXED procedure of SAS 9.4 (SAS Institute Inc; Cary, NC) with treatment as a fixed effect and block as a random effect. For all data measurements, pen served as the experimental unit ($n = 28$). Fly abundance data were analyzed using the MIXED procedure of SAS 9.4 (SAS Institute Inc). Behavioral data were analyzed using the GLIMMIX procedure of SAS 9.4 and the model statement included treatment, week, and treatment \times week. Time was considered the repeated measure and pen served as the experimental unit was determined when $P \leq 0.05$ and tendencies were considered when $P > 0.05$ and $P \leq 0.10$.

RESULTS AND DISCUSSION

Performance

Performance data are reported in Table 2.2. As expected, no differences in BW were observed between treatment groups on d 0 ($P = 0.87$). However, BW tended to differ on d 28 ($P =$

0.09), where BW tended to be greater for FT and PO than GR. There was no treatment effect on BW for any other period ($P \geq 0.10$).

Average daily gain also tended to differ between treatments from d 0 to 28 ($P = 0.10$). The CON tended to have a lower ADG than FT by 12.8% and the FT tended to have a greater ADG than GR by 17.9%. The PO also tended to have a greater ADG than GR by 14.8%. The tendency for decreased ADG from d 0 to 28 for the GR compared to PO and FT likely resulted in the tendency for a difference in BW on d 28 observed across treatments. No differences in ADG were observed among treatment groups from d 0 to final ($P = 0.30$).

Dry matter intake tended to be greater in CON than GR by 6.0%. Dry matter intake also tended to be greater in FT than GR by 5.2%. The BW and ADG and subsequent BW reduction of cattle consuming the GR treatment recorded on d 28 presumably resulted from decreased DMI observed from d 0 to 56, although d 56 BW did not differ. The tendency for a difference in BW on d 28 for GR could have occurred due to the introduction of garlic powder into the diet and animals adjusting to the smell or flavor. While a tendency was observed in DMI in the GR treatment from d 0 to 56, there was no affect ($P = 0.53$) on overall DMI indicating that the animals in the GR treatment adjusted to the flavor or smell.

The performance results in the current experiment are similar to those reported by Durunna et al. (2020) when comparing trace mineral salt combined with various levels of garlic powder and garlic oil (2.5% or 5% garlic powder, 0.3% garlic-oil-based premix, or 0% garlic product). Durunna et al. (2020) reported no difference in BW or DMI when garlic products were included in trace mineral salt.

Fly Abundance and Fly Avoidance Behavior

In cattle, fly abundance and fly avoidance behavior can decrease overall performance due to decreased animal feeding time, induced irritation reactions, and bunching due to fly attack

which causes heat stress (Campbell et al., 1977; Campbell et al., 1983; Bristow et al., 2006). By recording fly abundance and fly avoidance behaviors weekly, the influence of stable fly abundance on the animals and the resulting fly avoidance responses were analyzed.

Stable fly abundance data are reported in Table 2.3. Stable fly abundance averaged 5 stable flies per foreleg and ranged from 2 to 9 stable flies per foreleg over the 14 wk experiment. As expected, fly abundance did not differ between treatments on wk 0 ($P = 0.45$), prior to initial treatment application. There was a tendency for a difference in fly abundance on wk 1 ($P = 0.08$), with stable fly abundance being lower for the GR treatment by 21.7% compared to the PO treatment and 26.4% compared to the CON treatment. There were no differences in fly abundance between treatments from wk 2 to 5 ($P \geq 0.28$). Stable fly abundance tended to differ on wk 6 ($P = 0.09$), with the FT treatment having a lower fly abundance by 26.9% compared to CON and 37.1% compared to PO. There were no other differences in stable fly abundance for the remainder of the experiment from wk 7 to 14 ($P \geq 0.18$).

Influence of age on stable fly abundance data is reported in Table 2.4. Stable fly abundance was greater than the predetermined economic threshold from McNeal and Campbell (1981) of 5 stable flies per foreleg in the first 4 wk of the current experiment. Therefore, Table 2.4 reports only the first 4 wk of the experiment. Prior to treatment application on wk 0, a treatment \times age interaction was observed ($P = 0.03$) with steers having greater stable fly abundance than bulls. There was no treatment \times age interaction ($P = 0.65$) on wk 1, however, main effects of treatment ($P = 0.08$) and age ($P = 0.06$) were observed, where in wk 1, steers had a greater stable fly abundance compared to bulls. No treatment \times age interaction ($P = 0.33$) was observed for week 2. No main effect of treatment ($P = 0.67$) was observed on week 2. However, a main effect of age ($P = 0.01$) was observed, with steers having a greater stable fly abundance than bulls. This is expected since the bulls were smaller when compared to the heavier larger framed steers. Lehane (1991) stated smaller animals were attacked less by flying insects because of the

effectiveness of the defensive behaviors. A tendency for treatment \times age interaction ($P = 0.09$) was observed during week 3. No main effect of treatment ($P = 0.86$) or age ($P = 0.15$) was observed during week 3. There was no main effect of treatment ($P = 0.19$), age ($P = 0.19$) or treatment \times age interaction ($P = 0.13$) observed for week 4.

The limited efficacy of the fly control methods evaluated in the current experiment is supported by Blackman and Hodson (1977) who investigated the activity of permethrin on stable flies in a field study using 4 Shetland ponies and applying 500 mL permethrin to compare fly abundance of treated versus untreated animals. Blackman and Hodson (1977) reported adequate control ($> 67\%$ repellency) for a maximum of 10 d. Additionally, Guglielmone et al. (1999) compared a 5% cypermethrin combined with either 5% piperonyl butoxide or 10% piperonyl butoxide as a pour-on to determine horn fly (Diptera order) resistance using Holstein cows in a separated paddock setting. Guglielmone et al. (1999) reported a rapid decreasing efficacy of the mixtures, reporting that the pour-on had decent efficacy on fly abundance for a maximum 14 d. It is important to remember that the PO treatment was applied every 28 d after initial application in the present study. The results from the present study supports Blackman and Hodson (1977) and Guglielmone et al. (1999), of decreasing efficacy decreases of the PO solution, as fly abundance tended to be greater in the PO compared to the GR, only 6 d after initial application in wk 1, and PO fly abundance was higher than the other 3 treatments 13 d after application in wk 6.

In addition to poor PO and FT efficacy, the inconsistent results of the present study are similar to those from Berry and Campbell (1983) and Campbell and Hermanussen (1971) who reported that insecticide ear tags and insecticide sprays do not provide efficient control on stable flies. The lack of control is presumably due to the primary feeding site of the stable fly, the animals' forelegs, which is both not directly treated or possibly removed due to animals standing in water or mud.

The current experiment was conducted from June to September 2019, since this is when fly activity is typically at the highest during the yr (Campbell et al., 1977). Across all treatments, stable fly numbers decreased by 65.3% from wk 4 to 5 and remained low for the rest of the trial (Table 2.3). Average temperatures and wind speeds were recorded throughout the duration of the experiment. The reduction of stable flies could be due to various factors, including an increase in temperature. Stable fly numbers numerically decreased once max temperatures reached 30 °C (Table 2.5). Skoda et al. (1991) reported decreasing stable fly abundance during the mid-to-late summer (end of July- September) which is in agreement with the results of the present experiment. According to Showler and Osbrink (2015), stable flies are active where sunlight shines on the host, but at higher temperatures the flies are more active on the shaded parts of the animals or resting on surrounding areas (i.e., bunks, walls, pipes). Berry and Campbell (1983) and Showler and Osbrink (2015) reported that high winds (km / h) can have a direct effect on decreasing immature and adult stable fly abundance as well.

Although sanitation was not a treatment analyzed in the present study, the frequency of pen cleanings is a factor that should be taken into consideration when assessing fly abundance. The pads of pens were scraped of excess manure every 28 d, and any remaining feed was frequently removed from the bunk as a requirement of facility management protocols enforced. Additionally, pens were cleaned of excess manure and old feed before animals were initially placed into assigned pens. The initial cleaning could have had an impact on base fly counts (d – 7) done prior to treatment application. The decrease of total stable fly abundance in wk 5 could be due to the pen cleaning 8 d prior to wk 5 fly abundance count. Although insecticides are historically the most common fly control practice (Marçon et al., 1997), removing the accumulated manure, feed, and hay from confined pens is the most effective method to control stable fly numbers, however, this method is both expensive and labor intensive (Cilek and Green, 1994; Greene et al., 1989), and therefore not commonly used as a control method. Controlling

stable fly abundance by sanitation also poses a challenge due to numerous larval development sites. Meyer and Peterson (1983) collected stable fly immatures from 16 potential breeding sites in 5 different feedlots (100 to 800 hd per feedlot) from late May through October to further identify major stable fly breeding sites. Fence lines contained 26% of the sampled immature stable fly population followed by empty lots, haylage, and spilled feed which accounted for an additional 41.9% of stable fly immatures. Taking into consideration the Meyer and Peterson (1983) results, it could be suggested that pen cleaning had an impact on stable fly reduction in the current experiment.

Animals display fly avoidance behaviors when attempting dislodge stable flies (Table 2.6) therefore, skin twitches, tail flicks, head tosses, and stomps were recorded (Dougherty et al., 1993). Over the 14 wk experiment, there were no differences in the fly avoidance behavior response across treatments ($P \geq 0.10$). Although there was no difference in fly avoidance behaviors when comparing treatments, avoidance behavior responses were correlated with stable fly abundance (Fig. 1; $R^2 = 0.7121$). The results from the present experiment are similar to Dougherty et al. (1993), who reported behavior responses in cows were correlated to stable fly abundance. Dougherty et al. (1993) compared 3 different levels of stable flies on cows by recording fly populations, fly avoidance behaviors, forage DMI, and prehension. The 3 different levels were no flies, natural populations of flies (average 9.2 flies / animal), and exposure to 2500 stable flies in an enclosure. Cows were taken to individual circular plots and analyzed for 1 h / d. Cows without flies exhibited little to no-fly avoidance behaviors of < 0.5 movements on average per grazing period, cows exposed to natural fly populations exhibited an average of 13 movements, and cows exposed to 2500 stable flies exhibited 76 movements. Additionally, cows exposed to the highest number of stable flies visited the feeding stations less often than the other 2 treatments. Overall, Dougherty et al. (1993) concluded that stable flies are the driving factor of the fly avoidance behaviors that result in energy consuming muscle movements and reduced

feeding time. Similar to Dougherty et al, (1993), Mullens et al. (2006) conducted a field trial comparing stable fly abundance in a confined dairy operation where stable flies were the dominant fly species. Mullens et al. (2006) reported that fly avoidance behaviors occurred during the presence of stable flies. The authors reported all 4 fly avoidance behaviors were induced by stable flies, with leg stomping being the most common.

Baldacchina et al. (2013) reported the painful bite of a stable fly can increase energy exertion, reduce time feeding, and increase the stress levels of animals which can negatively impact animal performance. Since there were no differences in fly avoidance behaviors across all 4 treatments, the present study can neither support or refute previous research regarding stable fly abundance and fly avoidance behaviors in relation to the fly control methods evaluated in the current settings.

Economic Injury Level

Economic injury level is defined as the lowest fly population that will cause sufficient economic injury to justify the cost of control measures (Octavio et al., 1999). In this experiment, cost of fly control methods were documented to determine EIL. The cost of fly control methods were recorded as cost \cdot^{-1} animal \cdot^{-1} d over the 98 d experiment: the CON had no cost, insecticide FT cost \$0.05 USD \cdot^{-1} animal \cdot^{-1} d, PO cost \$0.02 USD \cdot^{-1} animal \cdot^{-1} d, and GR cost \$0.14 USD \cdot^{-1} animal \cdot^{-1} d. Using the EIL method described by Cantangui et al. (1997), EIL can be calculated using the overall market weight of animals. The market price was dependent upon sex and BW; therefore, the average market price was determined to be \$1.00 /kg⁻¹ for both sexes. The FT treatment exceeded (3.0 stable fly \cdot^{-1} foreleg \cdot^{-1} animal) the EIL 8 of the 14 wk, the PO treatment exceeded (1.1 stable fly \cdot^{-1} foreleg \cdot^{-1} animal) the EIL all 14 wk, and the GR treatment never exceeded (10.5 stable fly \cdot^{-1} foreleg \cdot^{-1} animal) EIL. Economic injury level indicates the maximum level of stable fly \cdot^{-1} foreleg \cdot^{-1} animal before economic production loss, but the results of the

present study do not support these findings due to no production losses being incurred across treatments. Although, ADG (Table 2.2) was not affected in the current experiment (Catangui et al., 1997). The results in the present study indicate the CON had no economic loss compared to other fly control treatments. Therefore, commercial, and natural fly control strategies may not be economically stable in conditions with low fly abundance similar to the current experiment.

CONCLUSION

Although tendencies were observed among BW, ADG, DMI, and fly abundance in the current experiment, it does not appear that fly control methods were consistently effective throughout the 14 wk experiment. Additionally, fly abundance did not have an impact on overall performance for any of the treatments. Although fly avoidance behaviors were not different between treatments, stable fly abundance had a direct correlation with fly avoidance behaviors. Additional studies should be conducted to investigate the potential of natural fly control strategies compared to commercial products to understand impacts on animal performance and health when fly abundance is managed with various ectoparasite control methods and greater fly burdens. Overall, this experiment suggests that fly control strategies did not impact fly abundance or fly avoidance behaviors. Additionally, the fly abundance and fly avoidance behaviors in the current experiment had no impact on the performance of bulls and steers in a feedlot.

ACKNOWLEDGEMENTS

The authors wish to thank the employees of the Willard Sparks Beef Research Center for assisting with this experiment. The authors also wish to thank Dr. Justin Talley for supplying the products used for the current experiment.

Table 2.1: Ingredients and nutrient composition of diets¹

Ingredient, % of DM ²	Receiving	Step 1	Step 2	Step 3	Step 4
Rolled corn	15.0	42.9	50	60.7	60.7
Prairie hay	28.0	22.8	15.6	8.2	8.2
Sweet bran ³	51.9	29.1	29.2	20.9	20.9
Liquid supplement ⁴	-	-	-	5.3	5.3
Dry supplement ⁵	5.1	5.2	5.2	4.9	4.9
<u>Nutrient Composition, DM basis</u>					
Dry matter, %	70.6	75.5	77.1	75.8	75.0
Crude protein, %	16.7	14.9	13.6	13.5	13.5
Acid detergent fiber, %	24.1	19.1	13.9	10.4	10.0
TDN ⁶ , %	68.7	73.9	83.9	87.6	88.0
NE _m ⁷ , Mcal/kg	0.33	0.36	0.43	0.45	0.45
NE _g ⁸ , Mcal/kg	0.20	0.24	0.29	0.31	0.31
Ca, %	0.59	0.56	0.52	0.53	0.59
P, %	0.66	0.49	0.50	0.47	0.45
Mg, %	0.33	0.23	0.21	0.20	0.21
K, %	1.15	0.74	0.66	0.76	0.74

¹Diet analyzed by Servi-Tech Laboratories; Dodge City, KS

²Dry matter

³Cargill Inc, Dalhart, TX

⁴Liquid supplement was formulated to contain (% DM basis): 45.86% corn steep, 36.17% cane molasses, 6.00% hydrolyzed vegetable oil, 5.46% 80/20 vegetable oil blend, 5.20% water, 1.23% urea (55% solution), and 0.10% xanthan gum

⁵Dry supplement was formulated to contain (% DM basis): 40.0% ground corn, 29.6% limestone, 20.0% wheat middlings, 7.0% urea, 1.0 % salt, 0.53% magnesium oxide, 0.51% zinc sulfate, 0.17% manganese oxide, 0.13% copper sulfate, 0.08% selenium premix (0.6%), 0.0037% cobalt carbonate, 0.32% vitamin A (30,000 IU/g), 0.10% vitamin E (500 IU/g), 0.009% vitamin D (30,000 IU/g), 0.20% tylosin (Tylan-40; Elanco Animal Health, Greenfield, IN) and 0.33% monensin (Rumensin- 90; Elanco Animal Health)

⁶Total digestible nutrients

⁷Net energy maintenance

⁷Net energy maintenance

⁸Net energy gain

Table 2.2: Effects of fly control strategies on the performance of feedlot bulls and steers

Item	Treatment ¹				SEM ²	P-value
	CON	GR	PO	FT		
BW ³ , kg						
d 0	351	348	349	349	40.0	0.87
d 28	399 ^{ab}	394 ^b	402 ^a	404 ^a	40.5	0.09
d 56	454	447	452	452	40.2	0.33
d 84	495	489	493	494	39.0	0.34
Final	522	516	524	524	38.5	0.34
ADG ⁴ , kg						
d 0 to 28	1.71 ^{bc}	1.61 ^c	1.89 ^{ab}	1.96 ^a	0.107	0.10
d 29 to 56	1.94	1.90	1.81	1.73	0.109	0.40
d 57 to 84	1.47	1.52	1.44	1.49	0.102	0.91
d 85 to final	1.98	1.93	2.27	2.18	0.161	0.40
d 0 to final	1.74	1.71	1.79	1.79	0.043	0.30
DMI ⁵ , kg/d						
d 0 to 28	11.3	10.6	11.2	11.4	0.94	0.18
d 29 to 56	11.9	11.1	11.1	11.5	0.71	0.15
d 57 to 84	12.0	12.2	11.8	11.9	0.63	0.86
d 85 to final	12.9	12.1	12.1	12.3	0.50	0.54
d 0 to final	11.9	11.4	11.5	11.7	0.67	0.53
G:F ⁶						
d 0 to 28	0.156	0.160	0.173	0.178	0.0138	0.22
d 29 to 56	0.165	0.174	0.168	0.157	0.0136	0.48
d 57 to 84	0.127	0.129	0.124	0.128	0.0125	0.98
d 85 to final	0.156	0.161	0.189	0.180	0.0153	0.28
d 0 to final	0.151	0.154	0.160	0.157	0.0103	0.39

¹ Treatments included: (CON) = No fly control, (GR) = 0.028 kg •⁻¹ animal •⁻¹ d of garlic (Regal garlic powder; WebstaurantStore, Lititz, PA) added to diet; (PO) = 2.0 mL per 45.35 kg of 7.4% permethrin and 7.4% piperonyl butoxide administered every 28 d (Permethrin CDS Pour-on™; Bayer Corporation, Whippany, NJ.); (FT) = 2 tags per animal of 8% abamectin and 20% piperonyl butoxide (XP820 Insecticide Cattle Ear tag™; Y-Text Corporation, Cody, WY)

² *n* = 28 pens; 16 bull pens and 12 steer pens

³ Body weight adjusted by a 4% calculated pencil shrink

⁴ Average daily gain calculated as shrunk body weight, kg per days on feed for each period

⁵ Dry matter intake equals total feed consumed by the pen for the period divided by animal number of head in the pen and days in the period

⁶ Gain to feed calculated as ADG / DMI for each period

^{a,b,c} Means with different superscripts tended to differ by $P \leq 0.10$

Table 2.3: Effects of treatments on average stable fly abundance per head by observation week

Wk	Average stable fly per animal ¹				SEM ²	P-value
	CON	GR	PO	FT		
0	9.5	10.0	9.4	10.2	0.97	0.45
1	12.4	9.1	11.6	10.8	1.20	0.08
2	9.6	8.3	9.1	8.7	0.98	0.63
3	9.7	9.8	10.2	10.0	1.23	0.98
4	8.2	6.9	7.3	6.3	1.04	0.28
5	2.0	2.6	2.8	2.6	0.54	0.49
6	2.9	2.8	3.4	2.1	0.54	0.09
7	1.7	1.4	1.5	1.6	0.30	0.91
8	1.4	1.4	1.6	1.3	0.34	0.85
9	1.5	1.2	1.8	0.9	0.28	0.18
10	4.0	4.1	3.4	4.2	0.81	0.45
11	1.1	1.7	1.8	1.7	0.43	0.53
12	1.6	1.3	1.3	1.3	0.28	0.80
13	3.8	3.5	2.9	3.6	0.75	0.76
14	3.7	3.3	3.5	3.1	0.48	0.80

¹ Treatments included: (CON) = No fly control; (GR) = 0.028 kg •⁻¹ animal •⁻¹ d of garlic (Regal garlic powder; WebstaurantStore, Lititz, PA) added to diet; (PO) = 2.0 mL per 45.35 kg of 7.4% permethrin and 7.4% piperonyl butoxide administered every 28 d (Permethrin CDS Pour-on™; Bayer Corporation, Whippany, NJ.); (FT) = 2 tags per animal of 8% abamectin and 20% piperonyl butoxide (XP820 Insecticide Cattle Ear tag™; Y-TeX Corporation, Cody, WY)

²n = 28 pens; 16 bull pens and 12 steer pens

Table 2.4: Influence of age on stable fly abundance by observation week.

Wk ³	Age ¹		Treatment ³	P-value	
	Bulls ²	Steers ²		Age	Treatment × Age
0	8.7 ± 0.78	11.8 ± 0.90	0.23	0.03	0.03
1	9.5 ± 0.93	12.9 ± 1.1	0.08	0.06	0.65
2	7.6 ± 0.50	10.7 ± 0.58	0.67	0.01	0.33
3	8.7 ± 1.1	11.5 ± 1.2	0.86	0.15	0.09
4	6.14 ± 1.0	8.6 ± 11.2	0.19	0.19	0.13

¹Average number of stable flies per animal

²Young receiving bulls, finishing steers n = 28 pens; 16 bull pens and 12 steer pens

³Treatments included: (CON) = No fly control, (GR) = 0.028 kg •⁻¹ animal •⁻¹ d of garlic (Regal garlic powder; WebstaurantStore, Lititz, PA) added to diet; (PO) = 2.0 mL per 45.35 kg of 7.4% permethrin and 7.4% piperonyl butoxide administered every 28 d (Permethrin CDS Pour-on™; Bayer Corporation, Whippany, NJ.); (FT) = 2 tags per animal of 8% abamectin and 20% piperonyl butoxide (XP820 Insecticide Cattle Ear tag™; Y-Tex Corporation, Cody, WY)

Table 2.5: Weather data and stable fly abundance summarized by observation week.

Wk	Temperature, °C ¹			Wind (km/h) ³	Leg counts ²
	Avg	Min	Max		Max
0	24.1	18.7	29.7	8.2	14.67
1	21.8	14.9	27.9	12.3	18.67
2	25.4	20.4	30.8	10.3	14.33
3	26.0	20.4	31.4	9.7	18.50
4	26.6	21.8	32.4	10.0	13.33
5	27.3	20.9	33.6	8.3	7.00
6	29.3	23.4	35.3	12.3	7.00
7	25.4	17.9	32.2	10.4	3.00
8	28.2	22.3	34.3	11.7	3.25
9	27.7	22.8	33.7	8.0	3.50
10	29.2	23.6	35.8	10.7	7.00
11	27.6	22.2	33.5	11.3	5.00
12	24.5	19.8	30.0	10.2	3.00
13	26.7	19.6	33.7	5.7	9.00
14	26.4	21.8	32.0	11.9	6.67

¹Temperatures (°C) and mean wind speed (km/h) data were collected by the Oklahoma Mesonet (Stillwater, Oklahoma site)

²Mean number of flies per front leg

³Total number of stable flies across all treatments

Table 2.6: Effects of treatments on fly avoidance behaviors by observation week

Wk	Treatments ¹				SEM ²	P-value
	CON	GR	PO	FT		
0	7.8	8.6	8.4	9.0	0.73	0.28
1	6.7	5.9	6.0	6.2	0.45	0.45
2	5.5	5.0	5.3	4.8	0.49	0.47
3	4.1	4.3	4.6	3.8	0.34	0.31
4	3.3	3.0	3.3	3.1	0.43	0.86
5	3.0	2.3	2.8	2.4	0.35	0.50
6	2.8	3.0	2.7	2.4	0.32	0.64
7	1.9	2.4	1.6	2.0	0.31	0.23
8	1.8	1.1	1.2	1.8	0.28	0.12
9	1.6	1.5	1.4	1.7	0.27	0.83
10	2.2	2.3	2.3	2.1	0.40	0.91
11	1.2	1.8	2.3	1.9	0.37	0.26
12	1.8	1.9	2.2	1.6	0.34	0.70
13	2.6	2.4	2.4	2.5	0.39	0.98
14	2.7	2.5	2.4	2.5	0.27	0.94

¹ Treatments included: (CON) = No fly control; (GR) = 0.028 kg •⁻¹ animal •⁻¹ d of garlic (Regal garlic powder; WebstaurantStore, Lititz, PA) added to diet; (PO) = 2.0 mL per 45.35 kg of 7.4% permethrin and 7.4% piperonyl butoxide administered every 28 d (Permethrin CDS Pour-on™; Bayer Corporation, Whippany, NJ.); (FT) = 2 tags per animal of 8% abamectin and 20% piperonyl butoxide (XP820 Insecticide Cattle Ear tag™; Y-TeX Corporation, Cody, WY)

²n = 28 pens; 16 bull pens and 12 steer pens

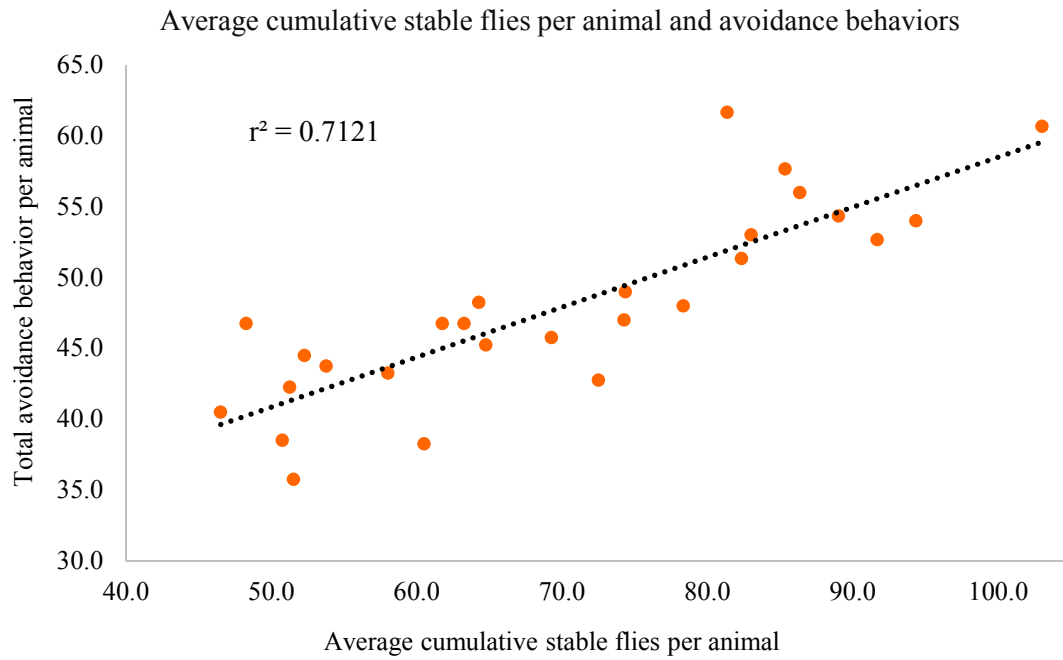


Figure 2.1: Weekly herd mean fly avoidance behaviors as a function of mean numbers of stable flies per foreleg on bulls and steers on a feedlot for the duration of a 14-wk stable fly season.

CHAPTER III

COMPARING THE EFFECTS OF MODIFIED-LIVE VERSUS INACTIVATED VIRAL VACCINES ON PERFORMANCE, HEALTH, ACUTE PHASE PROTEINS, AND BOVINE VIRAL DIARRHEA VIRUS TITERS IN RECEIVING FEEDLOT CALVES

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ABSTRACT: Newly received calves are susceptible to bovine respiratory disease (**BRD**), which has a negative effect on health and performance in the feedlot. The objective of this experiment was to evaluate the effects of modified-live (**MLV**) vs inactivated (**INA**) viral vaccines during the receiving period on performance, health, acute phase proteins [serum amyloid A (**SAA**) and haptoglobin (**Hp**)], and bovine viral diarrhea virus (**BVDV**) antibody titers in calves. Heifers and steers from 2 different sources (Block 1; $n = 194$, initial body weight (**BW**) = 233 ± 72 kg; Block 2; $n = 212$, $BW = 184 \pm 96$ kg) were administered 1 of 2 experimental treatments upon arrival at the feedlot and on d 28 a MLV vaccine containing infectious bovine rhinotracheitis (**IBR**), BVDV type 1 and 2, parainfluenza 3 (**PI3**), and bovine respiratory syncytial (**BRSV**) virus (Titanium 5; Elanco Animal Health, Greenfield, IL) or an INA vaccine containing IBR, BVDV type 1 and 2, PI3, and BRSV (ViraShield 6; Elanco Animal Health) in a split plot experimental design with vaccine treatment in the main plot (pen) and sex in the split

plot. All calves received a MLV vaccine previously at 3 to 4 mo of age containing IBR, BVDV type 1 and 2, PI3, and BRSV. No treatment \times day interaction was observed for BW or ADG ($P \geq 0.17$). A main effect of treatment ($P < 0.01$) was observed for ADG with MLV having greater ADG than INA from d 0 to 56. Dry matter intake did not differ from d 0 to 28 but was greater for MLV than INA from d 28 to 41 and overall (d 0 to 56; $P < 0.01$). Gain:feed did not differ between treatments from d 0 to 56 ($P \geq 0.22$). There was no treatment \times day interaction for BVDV 1a antibody titers ($P = 0.72$). However, INA had greater ($P < 0.003$) BVDV 1a titers than MLV. There was also a time effect ($P < 0.01$) observed, where BVDV 1a titers increased from d 0 to 14, decreased from d 14 to 28, increased from d 28 to 42, then plateaued from d 42 to 56. A treatment \times day interaction ($P = 0.01$) was observed for BVDV 1b, where INA titers for both treatments were greater for INA on d 14 and 28 but plateaued from MLV on d 42 or 56. No treatment \times day interaction ($P = 0.22$) or effect of treatment ($P = 0.21$) was observed for BVDV 2 antibody titers. However, a main effect of time ($P < 0.001$) was observed with BVDV 2 titers, with titers increasing from d 0 to 14, decreasing from d 14 to 28, increasing again following revaccination, and decreasing from d 42 to 56. No treatment \times day interaction ($P > 0.80$) or main effect of treatment ($P = 0.72$) was observed for Hp. However, a main effect of time ($P = 0.002$) was observed where Hp increased to d 14, then decreased through d 56. No treatment \times day interaction was observed for SAA ($P > 0.73$). However, a tendency for main effect of treatment ($P = 0.07$) and a main effect of time ($P = 0.01$) and were observed. The MLV treatment tended to be greater in SAA than INA. Serum amyloid A decreased to d 28, then increased from d 28 to 42 while MLV increased greater than INA, SAA decreased for both treatments from d 42 to 56. No differences were observed between treatments for morbidity, clinical severity scores, or rectal temperatures ($P \geq 0.22$).

INTRODUCTION

Bovine respiratory disease (**BRD**) continues to have a severe economic impact on beef calves entering feedlots, resulting in annual losses of \$1 billion to the U.S. cattle industry (Powell et al., 2013). The economic loss from BRD results from mortalities, increased labor, antimicrobial treatments, and decreased performance of calves infected with BRD (Wilson et al., 2012; Beck et al., 2019). Bovine respiratory disease is the most common feedlot illness, directly affecting 16.2% of cattle placed on feed (USDA NAHMS, 2013). The disease is responsible for 50 to 70% of all feedlot mortality and 75% of feedlot morbidity (Brooks et al., 2011). Bovine respiratory disease is a consequence of stressors, environmental factors, immunity, and pathogen introduction (Loneragan et al., 2001; Taylor et al., 2010). Therefore, prevention and treatment of BRD is still a major concern for the beef industry.

Vaccination is a commonly used preventative measure taken to decrease morbidity and mortality due to BRD, however, literature supporting the efficacy of vaccination is inconclusive (Chamorro et al., 2020). Most feedlots (>85%) vaccinate receiving cattle for bovine viral diarrhea virus (**BVDV**), infectious bovine rhinotracheitis (**IBR**), parainfluenza 3 (**PI3**), and bovine respiratory syncytial (**BRSV**) virus (USDA NAHMS, 2013). Immunosuppression and BRD interactions are extremely complicated and dependent on multiple factors, one of which includes the type of vaccine, modified live virus (**MLV**) versus inactivated (**INA**) virus (Richeson and Falkner, 2020). Modified-live virus vaccines activate T lymphocytes by stimulating humoral and cell-mediated immune responses in preparation of natural pathogen exposure (West et al., 1999). Meanwhile, INA antigens produce B cells and antibodies against the specific antigen presented by stimulating a humoral response (Edwards et al., 2010). Additionally, in order to produce an adequate immune response, INA vaccines require a booster vaccination as less robust cell-mediated immunity occurs with a single vaccination (Chamorro and Palomares, 2020). Thus, the objective of the current experiment was to compare the effect of MLV vs. INA viral vaccines on performance, health, acute phase proteins, and BVDV titers in receiving feedlot calves.

MATERIALS AND METHODS

All procedures were approved by the Oklahoma State University Institutional Animal Care and Use Committee (Animal Care and Use Protocols # AG-15-21 and AG-19-8).

Pre-Experiment Animal Management and Experimental Treatments

Angus heifers and steers ($n = 194$; Block 1; initial BW = 233 ± 96 kg) were transported approximately 409 km from the Oklahoma State University (OSU) Field and Research Service Unit (FRSU) in Valliant, OK to the Willard Sparks Beef Research Center (WSBRC) in Stillwater, OK on October 6th, 2020. A second set of crossbred steers and heifers ($n = 212$; Block 2; initial body weight [BW] = 184 ± 72 kg) were transported approximately 510 km from a single-source ranch in Glen Rose, TX to the WSBRC on October 14th, 2020. Prior to the initiation of the experiment, both groups were vaccinated with MLV containing infectious bovine rhinotracheitis (IBR), bovine viral diarrhea virus (BVDV) type 1 and 2, parainfluenza 3 (PI3), and bovine respiratory syncytial virus (BRSV) vaccine (Titanium 5; Elanco Animal Health, Greenfield, IN) at 3 to 4 mo of age. Prior to shipment, heifers and steers were weighed and individual BW are recorded. Upon arrival at the WSBRC, calves were held overnight in dry lot pens with *ad libitum* access to fresh water and prairie hay. Animals within block were randomly assigned to mixed sex pens. Pens were randomly assigned 1 of 2 experimental treatments in a split plot design: 1) a MLV vaccine containing IBR, BVDV 1 and 2, PI3, and BRSV (Titanium 5; Elanco Animal Health) or 2) an inactivated vaccine (INA) containing inactivated viruses IBR, BVDV 1 and 2, PI3, and BRSV (ViraShield 6; Elanco Animal Health). Heifers and steers were blocked by source and treatment (10 pens / source; 5 pens / treatment within each block). The number of each sex per block varied, however, heifers and steers per pen in each block were equivalent. Six animals (steers, $n = 3$; heifers, $n = 3$) were pre-selected as a representative sample of the pen for subsequent blood collection for serum analysis. At approximately 0500 h the following morning,

calves were individually weighed and administered a *Clostridium chauvoei* (Blackleg), *septicum* (Malignant edema), *novyi* (Black disease), *sordellii* and *perfringens* Types C & D (Enterotoxemia), and *Moraxella bovis* (Pinkeye, or infectious bovine keratoconjunctivitis) vaccine (20/20 Vision[®] with SPUR[®]; Merck Animal Health, Madison, NJ); *Mannheimia haemolytica* (NUPLURA[™] PH; Elanco Animal Health); an oral anthelmintic (Safeguard; Merck Animal Health), and administered the experimental vaccine treatments. Arrival castrate status was determined (bull or steer) and bull calves ($n = 21$) were surgically castrated using a Newberry Knife to incise the scrotum, followed by castration by emasculaton. Newly castrated steers were equally distributed across replicates and treatments during initial processing. All animals received a booster vaccination on d 28 with the respective treatment that was administered at processing. Calves ($n = 20 \pm 3$ per pen) were housed in twenty 12.2×30.5 m soil surfaced feedlot pens with a shared 76-L concrete water tank between 2 adjacent pens (model J 360-F; Johnson Concrete, Hastings, NE) with a 12.2-m concrete feed bunk.

Feed Management

All calves were fed a common receiving diet (**RCV**) for the entire trial (Table 3.1). Bunks were read at approximately 0530 h every morning to determine the quantity of feed remaining from the previous day's feed call. The delivered feed was adjusted daily so that there was no overabundance of feed left in the bunk, but also to ensure that animals had near *ad libitum* access to feed. Diet samples were collected twice weekly, and DM was calculated after samples were dried in an oven at 60°C for a minimum of 48 h. A monthly composite was created after DM was calculated and stored in a freezer until analysis. At the end of the experiment, sub samples of each monthly composite were taken to make a composite of the diet over the duration of the experiment. Feed refusals were weighed back prior to feeding on weigh days or if excessive orts remained in the bunk. Refusal samples were dried to determine DM content and were subtracted from DM delivered to calculate DMI.

Assessment of Signs of Bovine Respiratory Disease and Antimicrobial Administration

During the receiving period, calves were observed for health status as described by Wilson et al. (2015). In brief, trained personnel blinded to experimental treatments visually monitored the calves for clinical signs of BRD at 0700 h daily. The evaluators used the depression, appetite, respiratory, and temperature (DART) system with modifications described by Step et al. (2008) and Wilson et al. (2015). Calves were pulled according to WSBRC protocol for clinical signs of BRD, including, but not limited to, signs of abnormal appetite, depression, cough, nasal or ocular discharge, and obvious BW loss. The evaluators assigned a clinical severity score from 0 to 4. A score of 0 was assigned to an animal that appeared clinically normal. A score of 1 was assigned to an animal with minor clinical signs, 2 for moderate clinical signs, 3 for severe clinical signs, and 4 for a morbid animal. Animals assigned a clinical severity score of 3 or 4 received immediate attention from facility personnel. Animals receiving a clinical severity score of 1 to 4 were pulled from a respective pen, walked to the chute, and had a rectal temperature obtained and recorded (GL M-500; GLA Agricultural Electronics, San Luis Obispo, CA). To receive antimicrobial treatment, an animal had to exhibit a clinical severity score of 1 or 2 and have a rectal temperature of $\geq 40^{\circ}\text{C}$ or exhibit a clinical severity score of 3 or 4 regardless of rectal temperature. Animals that received a clinical severity score < 3 but did not have a rectal temperature $\geq 40^{\circ}\text{C}$ did not receive antimicrobial treatment and were returned to the respective pen and considered a “pull”. Animals were pulled from respective pens up to 5 times. A maximum of 3 antimicrobial treatments were administered to an animal during the experiment. All antimicrobials were administered subcutaneously, per manufacturer’s label directions while following National Cattlemen’s Beef Association Beef Quality Assurance Guidelines (NCBA, 2001). The first antibiotic administered was tilmicosin 300 mg/mL (Micotil; Elanco Animal Health) administered at 10 mg / kg BW. A 7-d moratorium was observed after tilmicosin administration before the second antimicrobial treatment could be administered. If animal were

pulled for a second time and met treatment criteria, florfenicol, 300 mg/mL (Nuflor; Merck Animal Health) was administered at 40 mg / kg BW with a 4-d moratorium following administration. If treatment criteria were met a third time, enrofloxacin 100 mg/ mL (Baytril; Bayer Animal Health, Shawnee Mission, KS) was administered at 7.5 mg / kg of BW. Further information explaining antimicrobial administration can be referenced to in Wilson et al. (2015).

Performance Data Collection

Individual BW were collected on d 0, 14, 28, 42, and 56. Individual ADG was calculated by dividing BW gain, by days on feed for each period. Dry matter intake was calculated from total DMI for the pen for that period divided by the number of animals and the days on feed in that period. Gain to feed ratio was calculated by dividing the average ADG for the pen by the average daily DMI for the pen for each respective period.

Serum Collection and Preparation

On d 0, 14, 28, 42 and 56, two 10-mL blood samples were collected via jugular venipuncture (BD Vacutainer; Franklin Lakes, NJ) from the same 6 pre-selected animals per pen. Whole blood was allowed to clot for an average of 2 to 4 h prior to centrifuging. Blood tubes were centrifuged at $3,000 \times g$ for 20 min at 4°C (Sorvall RC6; Thermo Scientific, Waltham, MA). Following centrifuging, individual animal serum was then aliquoted to 2 mL microcentrifuge tubes and stored at -80 °C until subsequent analysis.

Serum was required for multiple analyses in this experiment including: BVDV serum neutralization panel (BVDV 1a, BVDV 1b, and BVDV 2), haptoglobin (**Hp**), and serum amyloid A (**SAA**). Serum samples were collected from individual pre-selected animals per pen therefore, individual animal serum samples were thawed at 4°C to allow for the creation of a pen composite sample. Immediately after thawing, individual animal serum sample was pipetted at 0.25µL and aliquoted into a 2 mL microcentrifuge tube to make a composite tube for each respective pen.

Serum was then again stored at -80°C until subsequent analysis. Serum analysis was then conducted using the pen composite sample.

Bovine Viral Diarrhea Virus Neutralizing Antibody Titers

Serum samples were shipped on dry ice via overnight parcel service to the Texas Veterinary Medical Diagnostic Laboratory (Canyon, TX). Serum samples were analyzed for serum neutralizing antibody titer concentrations for BVDV type 1a, BVDV type 1b, and BVDV type 2 using the virus neutralization (VN) assay. The VN antibody titer is reported as the reciprocal of the highest dilution of serum that neutralizes the infectivity of the virus (i.e., endpoint dilution 1:128 = antibody titer of 128). Values reported < 4 (no detectable antibody at the lowest readable dilution) were considered negative for seroconversion. Samples with serum neutralization value ≥ 4 were considered positive for seroconversion to BVDV. For the titer level analysis, reported values were \log_2 transformed for normality prior to statistical evaluation.

Haptoglobin

Serum samples were thawed at 4°C immediately before Hp analysis. Haptoglobin concentrations were measured in serum samples using a 2-side enzyme linked immunoassay (ELISA) test kit (Aviva Systems Biology; San Diego, CA) according to manufacturer's instructions. Pen composite samples were diluted 1/50 in diluent and were pipetted into a 96 well pre-coated ELISA micro plate. The plate was then incubated at 37°C for 15 min. Wells were washed $4 \times$ with 200 μl wash solution. Sera were diluted with 100 μl of Horseradish Peroxidase Conjugated Antibody in a stabilizing buffer diluted at a dilution of 1/100. The plate was then incubated in the dark at 37°C for another 15 min. Wells were then washed $4 \times$ using 200 μl wash solution. Sera were diluted in 100 μl 3,3',5,5'-tetramethylbenzidine and hydrogen peroxide in citric acid buffer at pH 3.3. The plate was then incubated in the dark at 37°C for 10 min. After incubation, 100 μl of STOP solution was dispensed to stop further color development. The

absorbance was read at 450 nm using OPTIMA plate reader. Haptoglobin concentrations were expressed as ng/mL.

Serum amyloid A

Serum samples were thawed at 4°C immediately before SAA analysis. Serum amyloid A was measured in serum samples using a solid phase sandwich ELISA test kit (Fisher Scientific; Atlanta, GA) according to manufacturer's instructions. Serum samples were diluted 1/1500 in diluent. Fifty µl of Anti-SAA / Horseradish Peroxidase Conjugated Antibody was pipetted into a to 96-well pre-coated ELISA micro plate. Then, 50 µl of diluted samples were pipetted into the 96-well plate. The plate was then incubated at 37°C for 1 h. Wells were washed 4 × with 200 µl wash buffer. Sera were diluted in 100 µl 3,3',5,5'-tetramethylbenzidine substrate. The plate was then incubated at room temperature for 15 min. After incubation, 100 µl of STOP solution was dispensed. The absorbance was read at 450 nm using OPTIMA plate reader. Serum amyloid A concentrations were expressed as ng/mL.

Statistical Analysis

The experiment was organized in a generalized randomized complete block design with a split-plot arrangement. Individual animal BW was the observational unit for BW and ADG and the experimental unit for the split plot (sex). Pen was considered the experimental unit ($n = 20$) for BW, ADG, DMI, and G:F of the main plot, serum neutralization, SAA, Hp, and morbidity. Animal performance, feed intake, feed efficiency, acute phase proteins, and BVDV neutralizing titers were analyzed using the MIXED procedure of SAS 9.4 (SAS Institute Inc., Cary, NC). Animal BW and ADG were analyzed as a split-plot design with treatment as the main plot and sex as the split plot. Animal individual weights were the fixed effects of the model and included sex, treatment, and sex × treatment interaction. Pen within block was used as the random statement. Dry matter intake and G:F were analyzed based on pen averages with block in the

random statement. All data from dead animals were excluded from the analyses (deaths and removals out data). The fixed effects of treatment, day, and treatment \times day and the random effect of block were used to analyze acute phase proteins and BVDV antibody titers. Pen within block was used in the random statement. Day of sampling was included as a repeated measure. Morbidity data and data from animals pulled that received no treatment were analyzed using the GLIMMIX procedure of SAS 9.4 (SAS Institute Inc.) as categorical data and rectal temperature was analyzed as a continuous variable. Significance was declared when $P \leq 0.05$ and tendencies were considered when $P > 0.05$ and $P \leq 0.10$.

RESULTS AND DISCUSSION

Animal Performance

Body weight and ADG data are presented in Table 3.2. Body weight and ADG were generally less for heifers than steers ($P \leq 0.04$) except ADG from d 0 to 13 and d 28 to 41 when they did not differ ($P \geq 0.39$). There was no sex \times treatment interaction ($P \geq 0.19$) for BW or ADG observed from d 0 to 56 so data are presented for the main effect of vaccine treatment. Also, there was also no main effect of treatment ($P \geq 0.21$) detected for BW from d 0 to 56. However, a main effect of treatment ($P < 0.01$) was observed for ADG from d 0 to 56, with MLV having a greater ADG than INA. Dry matter intake and G:F data are presented in Table 3.3. Dry matter intake did not differ from d 0 to 13 or d 14 to 27 ($P \geq 0.22$). However, DMI was greater for the MLV following revaccination treatment from d 28 to 41 ($P < 0.01$) and overall (d 0 to final; $P < 0.01$). Feed efficiency did not differ throughout the trial ($P \geq 0.22$).

Previous research has reported that vaccination against BRD pathogens can impair performance for a brief period. Rodrigues et al. (2015) compared heifers that received BRD vaccination versus nonvaccinated heifers and reported reduced feed intake for heifers that received vaccination for d 1 and 2 following the initial vaccination. Similarly, Richeson et al.

(2008) compared MLV vaccination on arrival versus a delayed vaccination, where the animals that received MLV on arrival had a lower ADG from d 0 to 14 compared to the animals that had not yet received an initial MLV vaccine. The ADG remained greater for the animals that received the delayed vaccination through d 42 of the experiment (Richeson et al., 2008). Conversely, Bryant et al. (2008) reported no differences in feed intake when comparing 3 MLV vaccines in feeder steers. Additionally, when comparing respiratory vaccine antigen type (MLV vs INA) on 48 calves, when both initial vaccinations were administered on d 0 and INA revaccinated on d 14, Hudson et al. (2020) reported that ADG was greater for MLV compared to INA from d 28 to 35, before transitioning to INA having a greater ADG than MLV from d 35 to 49. Contrary to Hudson et al. (2020), the present experiment reported MLV having a greater ADG than INA overall (d 0 to 56). When evaluating the impacts of various vaccination strategies on performance, the results of the aforementioned experiments reported impacts for ADG (Richeson et al., 2008; Hudson et al., 2020). However, data in these experiments do not support or refute the results of the aforementioned reports. Collectively, it can be concluded that despite transitional changes in ADG, vaccination antigen type has the potential improve performance for MLV compared to INA throughout the feeding period, although these results are inconclusive.

Animal Health

The data for BRD treatments are presented in Table 3.4. No mortalities took place over the 56-d trial. Initial, secondary, and tertiary treatment percentages for BRD did not differ between INA and MLV ($P \geq 0.33$) through the 56-d experiment. Additionally, clinical severity scores and rectal temperatures did not differ between treatments throughout the 56-d receiving period ($P \geq 0.22$).

The data for animals that did not receive antimicrobial treatment but received a clinical severity score are presented in Table 3.5. Percentage of calves pulled but did not receive

antimicrobial treatment and rectal temperatures (1st pull to 5th pull) did not differ between INA and MLV ($P \geq 0.15$). There was a tendency for difference ($P = 0.08$) observed for 1st pull severity score, with MLV mean severity score being greater than INA but no other differences were recorded for subsequent pulls.

Duff et al. (2000) compared an intranasal vs intramuscular MLV vaccine and reported no differences in morbidity between groups. However, Stilwell et al. (2016) reported that the use of a quadrivalent vaccine containing INA IBR, MLV BRSV, INA BVDV, MLV PI3 (Risposal 4[®]; Pfizer, Porto Salvo, Portugal) versus a control group that received no vaccination. The vaccinated group received a booster vaccination 21 – 27 d following initial vaccination. Stilwell et al. (2016) reported a reduced incidence of BRD morbidity and mortality in calves that received vaccination versus the control. Chamorro and Palomares (2020) did a comprehensive review of published literature to compare the effectiveness of MLV and INA vaccines for providing clinical protection against BRD. In experiments analyzing the natural occurrence of BRD, two studies determined that calves vaccinated with two doses of INA vaccine had no effect on the natural occurrence of BRD. Meanwhile, one study reported an increased natural occurrence of BRD in calves vaccinated with an INA vaccine (Chamorro and Palomares, 2020). Additionally, Chamorro and Palomares (2020) evaluated 3 studies, 2 using MLV and 1 using INA vaccines, on animals challenged with BVDV. For all studies, animals were exposed 30 to 45 d following vaccination. Reduction of BRD-associated morbidity and mortality did not occur in the 2 experiments when animals were vaccinated with a MLV vaccine (Chamorro and Palmoraes, 2020). Meanwhile, the experiment that vaccinated with the INA vaccine had a reduction of clinical severity signs when animals received 2 doses of inactivated vaccine. Nevertheless, the outcomes of the current experiment support those from Duff et al. (2000), who reported a similar outcome during a receiving period with no differences in morbidity. Based on the results of this experiment, on-arrival MLV vs INA vaccine did not appear advantageous for morbidity prevention.

Bovine Viral Diarrhea Virus Viral Neutralizing Antibody Titers

The data for BVDV 1a viral neutralizing antibody titers are presented in Figure 3.1. No treatment \times day interaction was observed for BVDV 1a viral neutralizing antibody titers in serum ($P = 0.72$). However, a main effect of treatment ($P \leq 0.01$) was observed with INA having greater BVDV 1a antibody titers than MLV throughout the 56 d experiment. Also, a main effect of day ($P \leq 0.001$) was observed, where BVDV 1a titers increased from d 0 to 14, decreased from d 14 to 28, increased again from d 28 to 42, then plateaued from d 42 to 56.

The data for BVDV 1b viral neutralizing antibody titers are presented in Figure 3.2. A treatment \times day interaction was observed for BVDV 1b viral neutralizing antibody titers in serum ($P = 0.01$). After arrival vaccination (d 0), BVDV 1b titers increased for INA from d 0 to 14, with INA animals presenting greater ($P \leq 0.01$) titers than MLV (166 vs 88) respectively on d 14. Titers for INA remained greater than MLV on d 28 ($P \leq 0.01$). Titers in MLV then increased following revaccination, not differing ($P \geq 0.29$) from INA on d 42 or 56.

The data for BVDV 2 viral neutralizing antibody titers are present in Figure 3.3. No treatment \times day interaction or main effect of vaccine treatment or were observed for BVDV 2 viral neutralizing antibody titers in serum ($P \geq 0.22$). However, a day effect ($P < 0.001$) was observed for BVDV 2 titers. Titers increased for both treatments from d 0 to 14, following arrival vaccination on d 0. Titers then decreased from d 14 to 28, before increasing from d 28 to 42, following the booster vaccination on d 28.

Experiments involving MLV vs INA vaccines and the effects of BVDV antibody titers of newly received cattle report conflicting results. In 1 trial, Hudson et al. (2020) compared MLV vs INA vaccines on newly received feedlot calves and analyzed BVDV 1a titers. The authors observed a treatment \times day interaction for BVDV 1a titers with the MLV treatment having greater antibody titers compared to the INA treatment. Additionally, the INA vaccinated cattle

received a booster vaccination on d 14, while the MLV treatment group did not. The authors concluded that MLV had a greater antibody response to BVDV than INA. This response is similar to that reported by Richeson et al. (2009), who presented an increase in BVDV 1a titer concentrations on d 14 when cattle were vaccinated on arrival. In the current experiment, BVDV 1a titers increased again from d 28 to 42, following revaccination, before plateauing from d 42 to 56. Step et al. (2009) determined BVDV 1a and BVDV 2a serum neutralizing antibody titers on steers that were administered a single MLV vaccination or revaccination on d 0 and d 60 of receiving. Similar to the present experiment, the authors determined that all cattle had serum neutralizing antibody titers against BVDV 1a and BVDV 2a on d 60. The authors assumed that the MLV vaccine administered resulted in the protection against BVDV. While vaccination stimulates antibody production, exposure to disease stimulates production as well (O'Connor et al., 2001), therefore, the results reported are difficult to interpret in field research settings.

Acute Phase Proteins

Acute phase proteins, Hp and SAA, are considered indicators of inflammation in cattle (Boosman et al., 1989; Gruys et al., 1993; Alsemgeest et al., 1994; Baumann et al., 1994). Proinflammatory cytokines that are secreted by leukocytes stimulate the hepatic synthesis of acute phase proteins, changing the acute phase protein concentration in serum (Peterson et al., 2004). The data for Hp are presented in Figure 3.4. No treatment \times day interaction ($P = 0.80$) or main effect of vaccine treatment ($P = 0.72$) were observed for Hp concentrations. However, a day effect ($P < 0.01$) was observed for Hp concentrations, where Hp increased from d 0 to 14, then decreased on d 14, 28, 42, and 56. Joshi et al. (2018) suggested that in naturally occurring BRD in calves, Hp may be considered as an ideal biomarker. Joshi et al. (2018) gave clinical severity scores to 24 calves based on clinical signs associated with BRD (anorexia, depression, cough, nasal discharge and ocular discharge). Calves were split into 1 of 2 groups, healthy ($n = 12$; clinical severity score < 5) calves, and infected calves ($n = 12$; clinical severity score ≥ 5). Joshi

et al. (2018) recorded that as clinical severity score increased an increase was observed in Hp concentrations.

Arthington et al. (2013) conducted two 21 d experiments to investigate the influence of vaccination on the acute phase protein reaction of calves that received viral vaccinations. Animals were randomly assigned to 1 of 3 treatments: no vaccine (control), MH vaccine, or *Clostridia* vaccine. Steers were utilized in experiment 1 (approximately 8 mo of age), while heifers were utilized in experiment 2 (approximately 12 mo of age). In both experiments, treatments were administered at time of weaning. Blood samples were collected and processed for both experiments on d 0, 3, 9, 12, and 15 to assess the effect of the respective treatment on the acute phase protein reaction. The Hp response on d 14 was possibly influenced by inflammatory effects of transportation, weaning and vaccination on d 0. However, there was no response d 42 following the revaccination administered on d 28. Arthington et al. (2013), who determined an increase in Hp when animals were vaccinated with 1 of 3 treatments, may support the results of the current study. Both treatments were administered on d 0 to newly weaned steers. The Hp concentrations were $2.5 \times$ greater by d 3 for steers administered MH vaccine compared to the *Clostridia* vaccine and the control. In the present study, both treatment groups were administered a MH vaccine on d 0. Therefore, the MH may have increased Hp concentrations from d 0 to 14, but revaccination of experimental treatments had no effect on Hp concentrations, resulting in the decrease from d 14 to 56. Richeson et al. (2016) also saw an increase in Hp following vaccination on d 0 when animals were administered a respiratory vaccine containing IBR, IP3, BVDV type 1 and 2, and BRSV with a MH toxoid.

The data for serum amyloid A (SAA) are presented in Figure 3.5. No treatment \times day interaction was observed for SAA concentrations ($P = 0.72$). A tendency for treatment effect ($P = 0.07$) was observed for SAA concentrations with MLV tending to be greater than INA. Additionally, a day effect ($P = 0.01$) was observed for SAA concentrations as concentrations

decreased from d 0 to 28, then increased from d 28 to 42, before decreasing again from d 42 to final. According to Joshi et al. (2018), SAA requires more stimulation for a longer period of time to increase serum concentrations. When observing SAA concentrations in calves with naturally occurring BRD, a moderate and gradual increase was recorded (Joshi et al., 2018). The authors hypothesized the increase in SAA could be attributed to host immunity, where as SAA binds to gram-negative bacteria following by the destruction by phagocytic cells (Joshi et al., 2018). Normal SAA levels in healthy cattle range between 0.3 and 48.59 $\mu\text{g}\cdot\text{ml}^{-1}$ while animals with chronic inflammatory diseases had SAA levels reported between 17.1 and 298.2 $\mu\text{g}\cdot\text{ml}^{-1}$ (Tourlomoussis et al., 2004; Takahashi et al., 2007; Wiese et al., 2017). Compared to these normal ranges, SAA levels in this experiment were high.

CONCLUSION

In conclusion, the experiment suggests that animals administered a MLV vaccine could have improved ADG and DMI compared to animals administered an INA vaccine when calves were previously vaccinated with a MLV vaccine at 3 to 4 mo of age. However, the data revealed alterations in neutralizing antibody response from implementation of vaccination with MLV or INA vaccine. Inactivated vaccine resulted a greater BVDV 1a neutralizing antibody response and a tendency for a greater BVDV 1b neutralizing antibody response when compared to MLV. The BVDV 1a and 1b neutralizing antibody response could indicate a more intense response to BVDV 1a and 1b pathogens. Morbidity associated with BRD did not differ between vaccine treatments. Because no differences in morbidity were detected between the treatments, and gain was slightly improved for the MLV treatment, results of the current study suggest an advantage to administration of a MLV vaccine at weaning in calves previously vaccinated with an MLV.

ACKNOWLEDGEMENTS

The authors wish to thank the employees of the Willard Sparks Beef Research Center for assisting with this experiment. This experiment was funded in part by Elanco Animal Health.

Table 3.1: Ingredient and nutrient composition of diet¹

Ingredient, % of DM	RCV
Prairie hay	28.4
Rolled corn	15.0
Sweet Bran ²	51.4
Dry supplement ³	5.2
<u>Nutrient Composition, DM basis</u>	
Dry matter, %	70.6
Crude protein, %	16.7
Acid detergent fiber, %	24.1
TDN ⁴ , %	68.7
NE _m ⁵ , Mcal/kg	0.33
NE _g ⁶ , Mcal/kg	0.20
Ca, %	0.59
P, %	0.66
Mg, %	0.33
K, %	1.15

¹Diet was analyzed by Servi-Tech Laboratories; Dodge City, KS

²Sweet Bran (Cargill Inc., Dalhart, TX)

³Dry supplement was formulated to contain (% DM basis) 40.0% ground corn, 29.6% limestone, 20.0% wheat middlings, 7.0% urea, 1.0 % salt, 0.53% magnesium oxide, 0.51% zinc sulfate, 0.17% manganese oxide, 0.13% copper sulfate, 0.08% selenium premix (0.6%), 0.0037% cobalt carbonate, 0.32% vitamin A (30,000 IU/g), 0.10% vitamin E (500 IU/g), 0.009% vitamin D (30,000 IU/g), 0.20 % tylosin (Tylan-40, Elanco Animal Health, Greenfield IN) and 0.33% monensin (Rumensin- 90; Elanco Animal Health)

⁴Total digestible nutrients

⁵Net energy maintenance

⁶Net energy gain

Table 3.2: Effects of modified-live vs inactivated vaccine on BW and ADG of feedlot heifers and steers.

Item	Treatment ¹		SEM ²	P-value		
	INA	MLV		Trt	Sex	Sex × Trt
BW ³ , kg						
d 0	207	209	3.8	0.69	0.03	0.17
d 14	216	217	4.0	0.84	0.02	0.23
d 28	238	240	4.3	0.68	<0.01	0.31
d 42	256	260	4.2	0.43	<0.01	0.38
d 56	277	283	4.5	0.21	<0.01	0.31
ADG ⁴ , kg/day						
d 0 to 13	0.66	0.60	0.09	0.51	0.39	0.55
d 14 to 27	1.57	1.63	0.11	0.56	0.04	0.63
d 28 to 41	1.27	1.39	0.06	0.15	0.41	0.49
d 42 to 56	1.51	1.67	0.11	0.14	<0.01	0.19
d 0 to 56	1.25	1.33	0.03	<0.01	<0.01	0.68

¹ Treatments included a modified-live virus vaccine (MLV) containing infectious bovine rhinotracheitis (IBR), bovine viral diarrhoea virus (BVDV) type 1 and 2, parainfluenza 3 (PI3), and bovine respiratory syncytial virus (BRSV) vaccine (Titanium 5; Elanco Animal Health, Greenville, IN) or an inactivated (INA) vaccine containing IBR, BVDV type 1 and 2, PI3, and BRSV (ViraShield 6; Elanco Animal Health) administered upon arrival at the Willard Sparks Beef Research Center (d 0) and 28 days after arrival. All calves received MLV vaccine (Titanium 5; Elanco Animal Health) at the ranch of origin at approximately 3 to 4 mo of age.

²*n* = 20 pens, 10 pens in each block and 5 pens for each treatment within each block

³Body weight in kg

⁴Average daily gain, kg/day

Table 3.3: Effects of modified-live vs inactivated vaccine on DMI and G:F of feedlot heifers and steers.

Item	Treatment ¹		SEM ²	P-value
	INA	MLV		
DMI ³ , kg/d				
d 0 to 13	3.5	3.5	0.32	0.64
d 14 to 27	6.5	6.6	0.81	0.22
d 28 to 41	7.2	7.8	0.86	<0.01
d 42 to 56	8.5	8.6	0.96	0.25
d 0 to 56	6.4	6.6	0.73	<0.01
G:F ⁴				
d 0 to 13	0.186	0.172	0.0163	0.53
d 14 to 27	0.244	0.249	0.0114	0.73
d 28 to 41	0.182	0.180	0.0258	0.90
d 42 to 56	0.176	0.191	0.0420	0.22
d 0 to 56	0.196	0.200	0.0068	0.37

¹Treatments included a modified-live virus vaccine (MLV) containing infectious bovine rhinotracheitis (IBR), bovine viral diarrhea virus (BVDV) type 1 and 2, parainfluenza 3 (PI3), and bovine respiratory syncytial virus (BRSV) vaccine (Titanium 5; Elanco Animal Health, Greenville, IN) or inactivated (INA) vaccine containing IBR, BVDV type 1 and 2, PI3, and BRSV (ViraShield 6; Elanco Animal Health) at arrival at the Willard Sparks Beef Research Center (d 0) and 28 days after arrival. All calves received MLV vaccine (Titanium 5; Elanco Animal Health) at the ranch of origin at approximately 3 to 4 mo of age

² $n=20$ pens, 10 pens in each block and 5 pens for each treatment within each block

³Dry matter intake (DMI) equals total feed consumed by the pen for the period divided by animal days

⁴Gain to feed calculated as ADG / DMI for each period

Table 3.4: Modified-live vs inactivated vaccine effects on animals treated for bovine respiratory disease, clinical severity scores, and rectal temperatures on feedlot heifers and steers

Item	Treatment		SEM ²	P-value
	INA	MLV		
Treated ³ , %				
1 st treat ⁴	15.4	14.5	3.50	0.79
2 nd treat ⁵	1.5	2.0	1.31	0.74
3 rd treat ⁶	0.0	0.4	0.50	0.33
Clinical Severity score ⁷				
1 st treat	1.19	1.07	0.102	0.22
2 nd treat	1.67	1.50	0.441	0.72
Rectal temperature, ⁸ °C				
1 st treat	40.3	40.3	0.16	0.74
2 nd treat	40.1	40.1	0.11	0.68
Days to treat				
1 st treat	24.7	20.1	2.66	0.24

¹Treatments included a modified live virus vaccine (MLV) containing infectious bovine rhinotracheitis (IBR), bovine viral diarrhea virus (BVDV) type 1 and 2, parainfluenza 3 (PI3), and bovine respiratory syncytial virus (BRSV) vaccine (Titanium 5; Elanco Animal Health) or inactivated multivalent vaccine (INA) for IBR, BVDV type 1 and 2, PI3, and BRSV (ViraShield 6; Elanco Animal Health). All calves received MLV vaccine (Titanium 5; Elanco Animal Health) at the ranch of origin at approximately 3 to 4 mo of age.

²n= 20 pens

³Animals that received an antimicrobial for BRD. To receive antimicrobial treatment, animals had to have a clinical severity score of 1 or 2 and a rectal temperature of $\geq 40^{\circ}\text{C}$ or a clinical severity score of 3 or 4 regardless of rectal temperature.

⁴Animals received tilmicosin (Micotil; Elanco Animal Health) with a 7-d moratorium.

⁵Animals received florfenicol (Nuflor; Merck Animal Health) with a 4-d moratorium.

⁶Animals received enrofloxacin (Baytril; Bayer Animal Health).

⁷DART system. Subjective clinical severity score (1 = mild clinical signs, 2 = moderate clinical signs, 3 = severe clinical signs, and 4 = extreme clinical signs or a moribund animal) assigned by trained personnel.

⁸Rectal temperature at the time of BRD treatment

Table 3.5: Modified-live vs inactivated vaccine effects on animals pulled, clinical severity scores, and rectal temperatures on feedlot heifers and steers.

Item	Treatment ¹		SEM ²	P-value
	INA	MLV		
Pulled ³ , %				
1 st pull	31.1	30.1	4.5	0.83
2 nd pull	12.5	8.2	3.0	0.15
3 rd pull	3.0	2.9	1.7	0.96
4 th pull	1.0	0.5	0.9	0.54
5 th pull	1.0	0.0	0.7	0.15
Clinical severity score ⁴				
1 st pull	1.03	1.12	0.047	0.08
2 nd pull	1.08	1.06	0.084	0.77
3 rd pull	1.00	1.17	0.166	0.34
Rectal temperature, ⁵ °C				
1 st pull	39.2	39.2	0.14	0.79
2 nd pull	39.2	39.0	0.25	0.16
3 rd pull	39.3	39.1	0.31	0.25
4 th pull	39.8	39.1	0.60	0.29

¹Treatments included a modified live virus vaccine (MLV) containing infectious bovine rhinotracheitis (IBR), bovine viral diarrhea virus (BVDV) type 1 and 2, parainfluenza 3 (PI3), and bovine respiratory syncytial virus (BRSV) vaccine (Titanium 5; Elanco Animal Health) or inactivated multivalent vaccine (INA) for IBR, BVDV type 1 and 2, PI3, and BRSV (ViraShield 6; Elanco Animal Health). All calves received MLV vaccine (Titanium 5; Elanco Animal Health) at the ranch of origin at approximately 3 to 4 mo of age.

² $n = 20$ pens

³Animals were pulled for receiving a clinical severity score but received no antimicrobial treatment for BRD due to having a rectal temperature < 40°C and a clinical severity score less than 3 or 4.

⁴DART system. Subjective clinical severity score (1 = mild clinical signs, 2 = moderate clinical signs, 3 = severe clinical signs, and 4 = extreme clinical signs or a moribund animal) assigned by trained personnel.

⁵Rectal temperature °C at the time of pull.

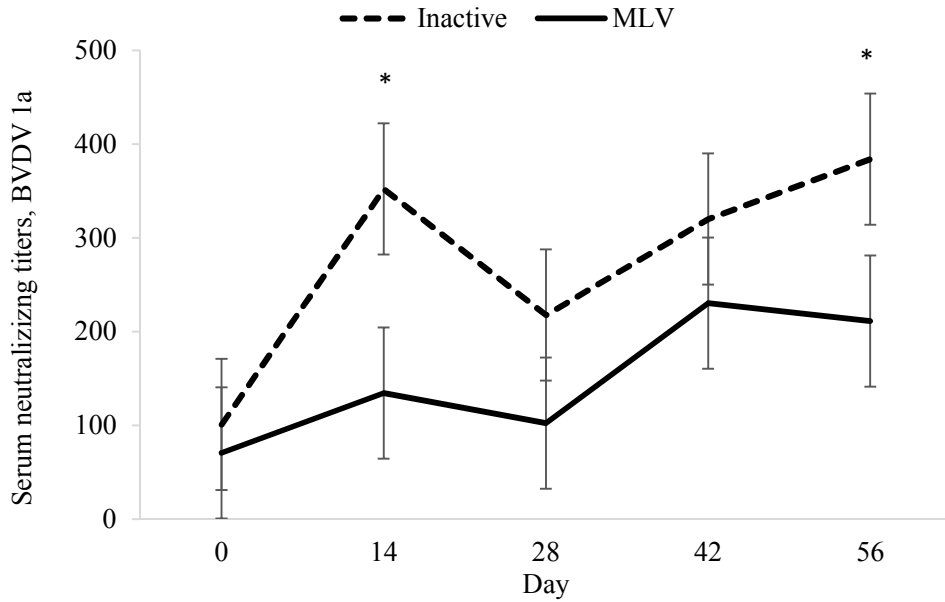


Figure 3.1: Effects of multivalent modified-live virus vaccine (MLV; Titanium 5; Elanco Animal Health) or inactivated multivalent vaccine (INA; ViraShield 6; Elanco Animal Health) containing IBR, BVDV type 1 and 2, PI3, and BRSV on BVDV 1a viral neutralizing antibody titers in serum after vaccine administration on d 0 and booster on d 28. No treatment \times day interaction was observed ($P = 0.72$). A main effect of treatment ($P = 0.003$) and day ($P < 0.001$) were observed.

* Indicate treatment differences ($P \leq 0.05$)

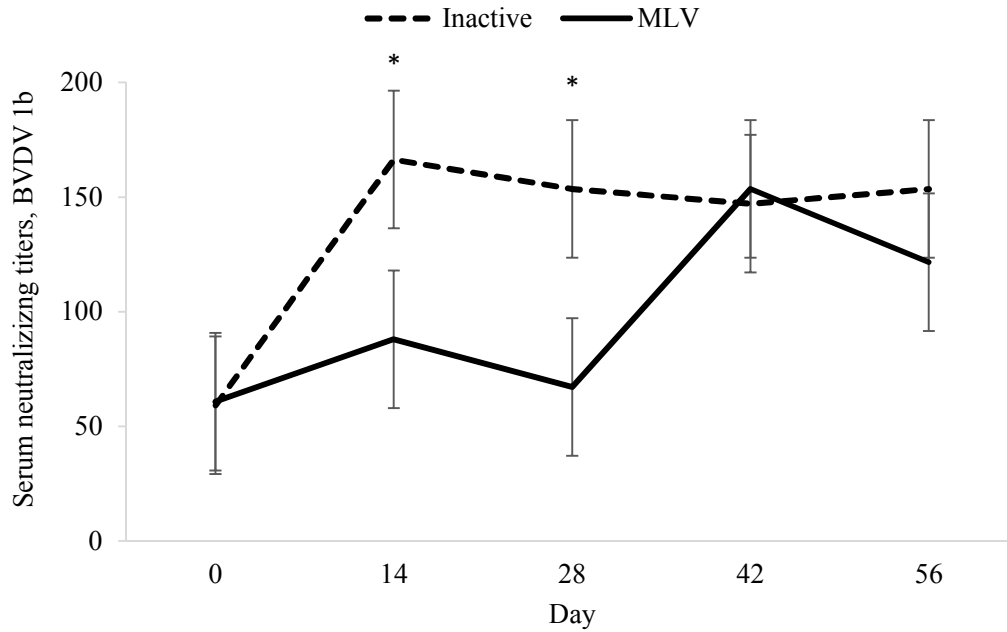


Figure 3.2: Effects of multivalent modified-live virus vaccine (MLV; Titanium 5; Elanco Animal Health) or inactivated multivalent vaccine (INA; ViraShield 6; Elanco Animal Health) containing IBR, BVDV type 1 and 2, PI3, and BRSV on BVDV 1a viral neutralizing antibody titers in serum after vaccine administration on d 0 and booster on d 28. A treatment \times day interaction ($P = 0.01$) was observed. A tendency for a main effect of treatment ($P = 0.07$) was detected, while a main effect of day ($P \leq 0.01$) was observed.

* Indicate treatment differences ($P \leq 0.05$)

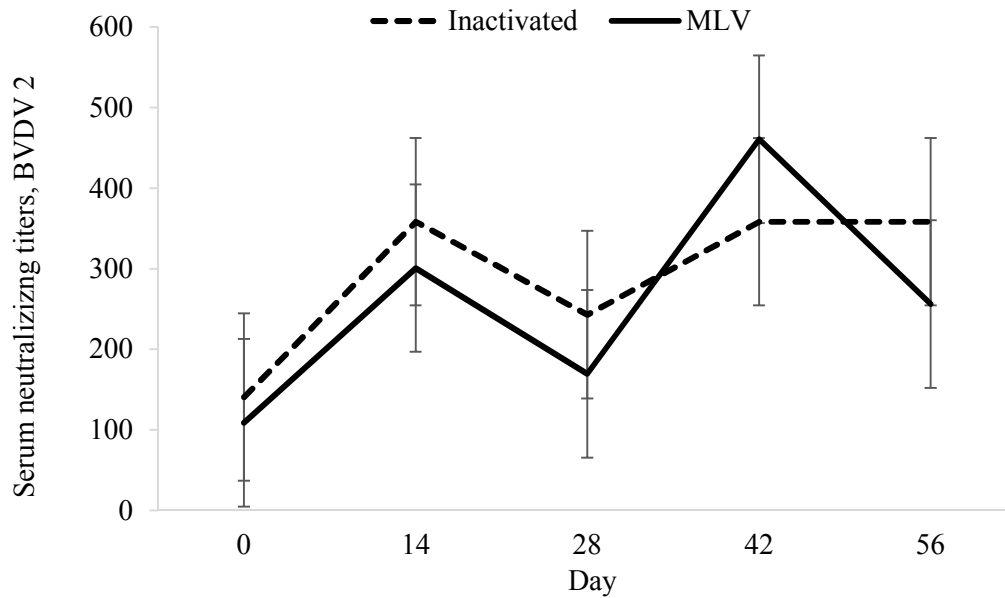


Figure 3.3: Effects of multivalent modified-live virus vaccine (MLV; Titanium 5; Elanco Animal Health) or inactivated multivalent vaccine (INA; ViraShield 6; Elanco Animal Health) containing IBR, BVDV type 1 and 2, PI3, and BRSV on BVDV 1a viral neutralizing antibody titers in serum after vaccine administration on d 0 and booster on d 28. No treatment \times day interaction ($P = 0.22$) or main effect of treatment ($P = 0.21$) were observed. However, main effect of day ($P < 0.001$) was observed.

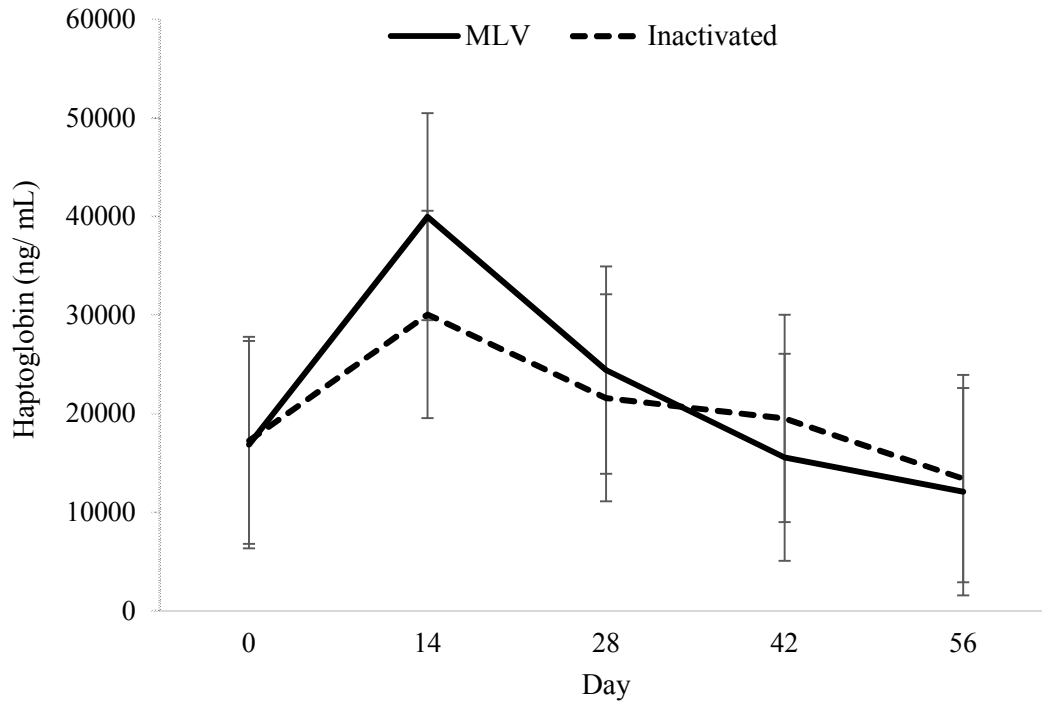


Figure 3.4: Effects of multivalent modified-live virus vaccine (MLV; Titanium 5; Elanco Animal Health) or inactivated multivalent vaccine (INA; ViraShield 6; Elanco Animal Health) containing IBR, BVDV type 1 and 2, PI3, and BRSV on serum variations of acute phase proteins haptoglobin (Hp) in calves after vaccine administration on d 0 and booster on d 28. No treatment \times day interaction ($P = 0.80$) or main effect of treatment ($P = 0.72$) were observed. However, a main effect of day ($P = 0.002$) was observed.

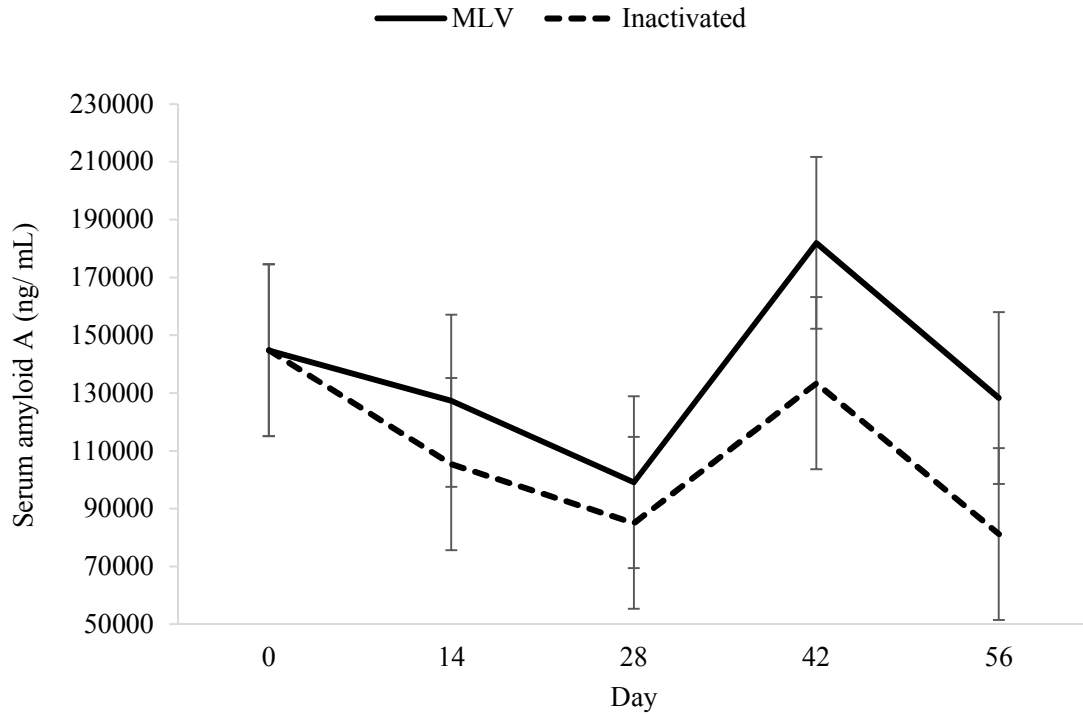


Figure 3.5: Effects of multivalent modified-live virus vaccine (MLV; Titanium 5; Elanco Animal Health) or inactivated multivalent vaccine (INA; ViraShield 6; Elanco Animal Health) containing IBR, BVDV type 1 and 2, PI3, and BRSV on serum variations of acute phase proteins serum amyloid A (SAA) in calves after vaccine administration on d 0 and booster on d 28. No treatment \times day interaction was observed ($P = 0.72$). A tendency for a main effect of treatment ($P = 0.07$) and a main effect of day ($P = 0.01$) were observed.

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