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INFLUENCE OF HORN FLIES AND BREED TYPE ON MILK PRODUCTION, CALF PRODUCTION TRAITS, PASTURE BEHAVIOR, AND TEMPERAMENT MEASUREMENTS IN BEEF CATTLE

INFLUENCE OF HORN FLIES AND BREED TYPE ON MILK PRODUCTION, CALF PRODUCTION TRAITS, PASTURE BEHAVIOR, AND TEMPERAMENT MEASUREMENTS IN BEEF CATTLE

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy in Animal Science

By

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May 2013
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ABSTRACT

An experiment conducted in El Reno, Oklahoma during the fly season (May – September/October) evaluated the effects of horn flies on milk production, calf performance, and pasture behavior and temperament measurements of beef cow calf pairs. Cows (n = 53) sired by Bonsmara (BONS; n = 7), Brangus (BRAN; n = 13), Charolais (CHAR; n = 8), Gelbvieh (GELV; n = 5), Hereford (HERF; n = 12), and Romosinuano (ROMO; n = 8) from Brangus dams and their Angus sired calves (n = 51) were used in the study. Horn fly counts (HFC) and milk yield and quality estimates were collected every 28 d from May to October. Pasture behavior (grazing, standing or lying) was recorded monthly twice a day (AM and PM). Exit velocity (EV) and chute score (CS) were obtained from cows and calves monthly. Monthly HFC differed ($P < 0.0001$), with populations lesser in May (94±42 flies) and greater in August (503±41 flies). The regression coefficients for milk yield on log HFC were not consistent across sire breed ($P < 0.05$), with milk yield reduced 0.99 and 0.64 kg/d per unit increase in log HFC in GELV and BONS. The regression coefficients of preweaning ADG on log HFC depended on sire breed ($P < 0.10$), with results indicating preweaning ADG reduced by 0.19 kg/d per one unit increase in HFC in BONS calves ($P < 0.05$), but not other breeds. A one unit increase in log HFC resulted in 0.07 kg/d ($P < 0.\ 10$) increase in postweaning average daily gain (ADG), 19.52 kg increase ($P < 0.10$) in 365-d adjusted yearling weight (YWT), and 0.05 kg/d ($P < 0.02$) increase in birth to yearling ADG. Pasture PM behavior was associated with HFC ($P < 0.05$), with standing cows having fewer flies than those grazing and lying (319 ± 27 vs. 468 ± 52 and 419 ± 38 flies). Exit velocity of cows ($P < 0.0001$) and calves ($P < 0.05$) differed monthly. Results from study clearly demonstrated HFC affected milk production, calf performance, and pasture behavior and temperament measurements.

This dissertation is approved for recommendation
to the Graduate Council

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This has been the longest journey of my life and reaching the end would not have been possible without my families love and support. Words cannot express the overwhelming appreciation and love I have for you all. Our family is and has always been the strong foundation that holds all of us together. When one of us is weak the others are there to hold and support. Your support and love has provided the strength I needed to succeed in this challenge and it will continue to provide the strength I need to succeed in the future.

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DEDICATION

I want to dedicate this dissertation, this degree, and this journey to my husband Travis Mays. You have stood by me, supported me, and held me up. You have provided for me, believed in me, and encouraged me. These past three years have had their challenges and obstacles, but we have overcome them together and created a wonderful family and life in the process. I cannot wait to start the next chapter.

Thank you for being my best friend, husband, and the love of my life.

You ask what I'm doing, and I say, "Loving you".

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INTRODUCTION

The economic impact of horn flies varies in relation to climate, region, pasture characteristics, management techniques, production systems, breed type, and calving periods (Foil and Hogsette, 1994). Economic losses in cattle production due to horn flies has ranged from $730 to $876 million in the United States (Drummond et al., 1987; Kunz et al., 1991). Cattle production losses have not been reported in recent years, but it is reasonable to assume production losses have continued to increase due to increased resistance of horn flies to insecticides.

Beef cattle production traits affected negatively by horn flies include weight gain, weaning weight (WW) and milk production. Loss of weight gain in cattle due to horn flies is correlated to the loss of feeding time and increased energy exerted by the animal to avoid and repel these biting insects (Weimann et al., 1992). The effect of horn flies on weight gain varies, with some studies indicating increased weight gains in cattle treated for horn flies (Brown et al., 1994; DeRouen et al., 2003), while other studies demonstrated no change in weight gain of treated cattle (Hogsette et al., 1991; Sanson et al., 2003). Horn flies have been reported to indirectly impact WW of calves (Cocke et al., 1989; Steelman et al., 1991), with variation also occurring due to genetics and horn fly treatment applications (Gerhardt and Shrode, 1990). Cows treated for horn flies had increased milk production (Block and Lewis, 1986; Minar et al., 1987), while other data reported horn fly treatments did not positively affect milk production (Cheng and Kessler, 1961; Miller et. al., 1973). Therefore, cattle production losses due to horn flies will not and cannot be adequately controlled until effective monitoring and management strategies are established through continued research and better understanding.

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LITERATURE REVIEW

Horn Flies

The horn fly (*Haematobia irritans)* was introduced into the United States from Europe in the late 1800's (Bruce, 1964). Horn flies are currently found throughout Europe, North America, Asia Minor and the Americas, with more recent expansion into South America (Foil and Hogsette, 1994). The common name, horn fly, is used in referral to the clustering of the flies around the base of the horns on cattle. However, in North America horn flies commonly clustered on the backs of cattle (Williams et al., 1985). The backs of cattle provide an ideal location for horn flies to feed and avoid the head and tail of the animal.

Horn flies feed on their host in a head-downward position between 20-40 times per day (Williams et al., 1985; Arther, 1991). Cattle are the primary host, which is the main reason they are described as pasture insects (Williams et al., 1985). Throughout the horn fly lifecycle it remains on the host, with females only leaving to reproduce.

The lifecycle of the horn fly has been reported to range from 1-3 weeks, with adult flies mating at 3-5 days of age (Arther, 1991; Foil and Hogsette, 1994). Horn fly eggs are approximately 1 mm long and are laid within the manure of the cow by the female fly (Williams et al., 1985). The female fly deposits approximately 20 eggs per visit and can lay up to 400 eggs in her lifetime (Arther, 1991). Depending on environmental temperatures, the eggs will hatch within 1-2 days; in 3-10 days larvae will molt twice while feeding and developing within the manure, pupae are then formed and young flies emerge from the pupae stage 6-8 days later (Williams et al., 1985; Arther, 1991). Throughout most of the United States horn flies can enter diapause, or a period of suspended development and growth. They can overwinter as pupae and emerge the following spring as adult horn flies (Foil and Hogsette, 1994). Horn flies are capable of producing 5-10 generations per year (Arther, 1991). The high reproductive rate, short generation intervals, ability to enter diapause, and feeding habits of the horn fly have helped contribute to the negative impact they have on beef cattle production in the United States. In fact, horn flies are considered the most important and economically damaging ectoparasite of pastured cattle (Drummond, 1987).

Economic Impact of Horn Flies. The economic impact of horn flies varies in relation to climate, region, pasture characteristics, management techniques, production systems, breed type, and calving periods (Foil and Hogsette, 1994). One characteristic that remains the same despite these variations is the economic threshold at which horn flies negatively impact cattle production. An economic threshold level of > 200 horn flies per animal has consistently been reported as negatively impacting cattle production (Haufe, 1979; Schreiber et al., 1987).

In the United States cattle production losses due to horn flies have ranged from $730 to $876 million (Drummond et al., 1981; Drummond et al., 1987; Kunz et al., 1991). Production losses have not been reported in recent years, but it is reasonable to assume losses have continued to increase. Lack of data has prevented development of control cost-pest intensity relationships, livestock income-pest-intensity relationships, and producer risk consideration (Steelman, 1976). Cattle production losses due to horn flies will not and cannot be adequately controlled until effective monitoring and management methods are established.

Impact of Horn Flies on Cattle Production Traits. Important aspects of beef cattle production include average daily gain (ADG), total weight gain, weaning weight (WW), and milk production. Loss in feeding time occurs when the animals are interrupted by the annoyance of blood feeding horn flies. Loss of weight gain in cattle corresponds to loss of feeding time and increased energy expenditure (Harvey and Launchbaugh, 1982; Weimann et al., 1992).

The effect of horn flies on ADG and total weight gain varies in the literature. One study compared beef replacement heifers (n = 670) with sire breed types of Angus, Beefmaster, Brahman, Brangus, Charolais, Gelbray, Gelbvieh, Hereford, Red Poll, and Simbrah based on treatment of horn flies for ADG and total weight gain (DeRouen et al., 2003). Horn fly populations of treated heifers were reduced ($P < 0.05$), with horn fly counts of 17.8 flies per side as compared to 111 flies per side of untreated heifers, respectively. Over the fly season (May – September/October) treated heifers had increased (0.414 kg; $P < 0.01$) ADG than untreated heifers (0.370 kg). Overall, total weight gain of treated heifers was 7 kg greater ($P < 0.01$) than untreated heifers (DeRouen et al., 2003). DeRouen et al. (2003) also reported differences in ADG of untreated versus treated pregnant heifers. They showed ADG varied ($P < 0.01$) among heifers resulting in treated heifers having 0.031 kg higher ADG than untreated heifers. Yearling stocker steers and heifers treated for horn flies also gained 0.12 kg per day more than untreated (DeRouen et al., 1995). Other authors have also reported increased gains in cattle with reduced horn flies (Harvey and Brethour, 1979; Haufe, 1982; Kinzer et al., 1984; Brown et al., 1994).

Angus x Brangus cross heifers were evaluated in 3 studies conducted over the fly season, but only 1 study determined horn flies had an impact on total weight gain (Sanson et al., 2003). Horn fly counts were lower ($P < 0.001$) in treated versus untreated heifers in all 3 studies. However, horn fly control had no effect on ADG ($P = 0.604$ and $P = 0.500$) or total weight gain ($P = 0.777$ and $P = 0.500$) in studies 1 and 2. Study 3 showed significant total weight gain ($P < 0.001$) of 10 kg more in treated heifers compared to untreated heifers (Sanson et al., 2003).

Hogsette et al. (1991) also reported reduced horn fly populations did not affect body weight of cows or calves.

Other important production traits of beef cattle include milk production and WW. Campbell (1976) was one of the first to demonstrate increased WW of calves when horn fly control treatments were applied. Hereford cows (n = 1100) were divided into 2 groups, treated and untreated. Weaning weights of steers from the treated group were greater ($P < 0.05$) than steers from the untreated group (175.4 vs. 169.5 kg; Campbell, 1976). Another study reported a linear regression coefficient of 8.1 kg/100 flies ($P = 0.0334$) when weaning weight was regressed on horn fly count in Angus, Charolais, Chianina, Hereford, Polled Hereford, and Red Poll beef cows (Steelman et al., 1991). Other studies have also reported similar results of increased WW or ADG of calves from cows treated for horn flies (Kunz et al., 1984; Cocke et al., 1989).

A seven year study comparing Angus cow/calf pairs from three genetic lines (Control, Select, and Inbred) demonstrated WW of calves varied by year, treatment, and genetics (Gerhardt and Shrode, 1990). Each genetic line was separated into two groups consisting of treated and untreated animals. Analysis determined that in 6 of the 7 years calves demonstrated weight gain differences ($P < 0.05$ and $P < 0.01$) in the Select genetic line when comparing treated versus untreated groups. However, during 4 of these 6 years weight gains were in favor of the untreated groups. When the three genetic lines were combined for analysis no differences between treated versus untreated groups occurred. These results indicate genetic lines used in this study and the use of horn fly treatment versus no treatment resulted in variation of WW for calves (Gerhardt and Shrode, 1990). .

Horn flies and other biting flies are generally thought to negatively impact milk production. Data are limited or nonexistent regarding the effects of horn flies on milk production of beef cows and therefore much of the research has been done using dairy cattle.

Twenty mature Holstein cows were sorted into treated and untreated groups for a 16 week trial during the fly season (Block and Lewis, 1986). Cows were in pasture for 18 hours per day and in the barn 6 hours per day to receive their daily grain allotment and milking. Horn flies accounted for 85.2% of total flies observed and underwent a 99.9% reduction in treated cows. Treated cows produced more ($P < 0.05$) milk during weeks 5, 8, 10, 11, 12 and 13 of the study when compared to untreated cows. Average daily milk production from treated cows was greater ($P < 0.05$) than untreated cows at 1.06 kg d^{-1}. Milk fat and protein percentage in this trial did not differ ($P > 0.05$) between groups (Block and Lewis, 1986). Morgan and Bailie (1980) also reported a higher ($P < 0.01$) in milk yield after fly treatment occurred when using Friesian cows during 2 trials. Mean milk yield/cow/day was reported to rise by 0.8 kg in trial 1 and by 1.0 kg in trial 2 after fly treatment was applied. Riha et al. (1981) and Minar et al. (1987) also reported increased milk yield after fly treatment.

However, milk production data are variable just like the other cattle production traits previously discussed. A three year study conducted on Holstein cows reported fly control did not have an effect on milk production (Cheng and Kessler, 1961). Results indicted horn fly numbers were different ($P < 0.05$) between treated and untreated groups, with untreated animals observed to have > 200 horn flies. As a result of the treatment horn flies were reduced by 99.9%. Despite the significant reduction of horn flies in the treated group milk yield was not different ($P > 0.05$) from the untreated group (Cheng and Kessler, 1961). Other studies indicated similar results, with treatment of flies having no significant effect on milk yield (Neel. 1957 and Miller et. al., 1973).

Horn Fly Variability Factors Among Cattle. Breed type, genetics, management strategies, behavior and/or environment may account for some of the variability reported regarding beef cattle production traits. Breed is often associated with an animal's designation as either horn fly susceptible or resistant (Tugwell et al., 1969; Brown et al., 1994; Steelman et al., 1997). Tugwell et al. (1969) reported that as the percent Brahman increased horn fly numbers decreased ($P < 0.05$). Percent Brahman consisted of 100, 75, 50, 25 and 0 with mean horn fly numbers increasing as the percent Brahman decreased (11 ± 1.4, 47 ± 6.7, 39 ± 4.6, 55 ± 4.5 and 72 ± 7.2, respectively). Steelman et al. (1994) reported similar findings in Brahman x Hereford cows having fewer horn flies during majority of their sampling counts when compared to Angus x Hereford cows. In conclusion, these findings suggest breed may be an important variability factor to consider when estimating horn flies and assigning cattle as either horn fly susceptible or resistant.

Another important variability factor to consider when discussing horn fly infestation on beef cattle is the genetic variation that may be involved. In a multiple year study cows of Angus, Charolais, Chianina, Hereford, Polled Hereford, and Red Poll breeds were used to estimate heritability and repeatability of horn fly density (Brown et al., 1992). Horn fly density for beef cows was reported to have a heritability coefficient of 0.59 and repeatability factor of 0.47 (Brown et al., 1992). Direct heterosis, maternal breed, and direct breed effects for average horn fly density were also reported (Brown et al., 1993). Mackinnon (1990) stated that genetic correlation between parasite resistance and growth of the animal are partly determined by the level of resistance of the genotype or breed.

Cattle Behavior In Response To Horn Flies. An important aspect of dealing with horn flies is animal behavior. To reduce the pain, blood loss, and production losses due to horn flies cattle use a varied arsenal of behaviors to repel or dislodge the biting insects (Mooring et al., 2007). Horn fly avoidance behavioral techniques include tail switching, grouping or bunching, skin twitching, lying, foot stomps and head throwing.

Sixteen Holstein heifers were assigned to be either tail docked or not docked in a study conducted on fly behavior in cattle (Eicher et al., 2001). Results indicated that total flies increased ($P < 0.01$) in docked cows and that fly avoidance behavior reflected this increase. An additional study determined treated steers had greater tail switching ($P < 0.0001$) prior to application of fly treatment (Boland et al., 2008). Steers also showed increased ($P < 0.01$) foot stomping, head throwing, and skin twitching prior to treatment. These data agree with that of Harvey and Launchbaugh (1982) who reported increased tail switching and leg stomping in untreated cattle. These behaviors and data demonstrate that the proportion of blood feeding parasites is negatively associated with the rate of insect-repelling movements (Torr and Mangwire, 2000).

Other research showed animals with larger body mass engage in more ($P < 0.05$) insect-repelling behavior (Mooring et al., 2007). Tail length increases with body mass ($P = 0.0001$), and tail switching and body mass have a positive relationship ($P = 0.02$). Longer tails are reported as more efficient at repelling insects because they cover a broader area of the body surface (Mooring et al., 2007). Evolution may account for providing larger bovid with longer tails due to them attracting more flies and the open pastured environment they usually inhabit (Mooring et al., 2007).

Fly avoidance behaviors observed in pastured animals also include lying and grouping or bunching. Since host-seeking haematophagous insects use visual cues (size and color), airborne chemical cues from the host (carbon dioxide, acetone, and octenol), urine (phenols), feces, skin, and/or body heat it is necessary for animals to exhibit avoidance behaviors (Mooring et al., 2007). The effectiveness of lying down is evident, with reduced exposure of body surface and decreased production of carbon dioxide and sweat being suggested as reasons why laying down decreases the amount of flies attracted to the animal (Espmark et al., 1979).

Grouping or bunching is another type of avoidance behavior associated with biting flies. Cattle have been observed bunching together head first into a tight circle when fly intensity is severe (Hausens and Valiela, 1967; Tesky 1969). When heifers were not treated for flies they were observed bunching more frequently and spent 10 times more time bunched together than treated heifers (Schmidtmann and Valla, 1982). Overall, behavior can be imperative to cattle well-being and successful production.

Horn Fly Management Strategies. Management strategies are of vital importance when controlling horn fly populations. Insecticides have been used to control horn flies since the 1940’s, starting with the use of arsenic and dichlorodiphenyltrichloroethane (DDT; Arther, 1991). In the 1960’s resistance to DDT started to occur and so the number of available compounds to treat horn flies expanded to include other classes of chemicals. A list of insecticides used for horn fly control can be found in Appendix A.

Horn flies have continued to become resistant to the insecticides used to control their populations. However, horn fly control failure is not always a case of resistance, but instead can be caused by inadequate application of insecticides or sudden resurgence of horn fly populations (Quinsberry et al., 1984; Arther, 1991; and Guerrero et al., 1997). Resistance to insecticides containing pyrethroids involves 3 behavioral mechanisms (Arther, 1991). Knock-down resistance (KDR) is the major mechanism, and involves a modification of the binding site so the nerve of the insect is insensitive to the insecticides (Arther, 1991; Guerrero et al., 1997). The second mechanism is metabolic resistance, in which the fly produces enzymes that detoxify the insecticides, and the third mechanism is behavioral. Horn flies are able to detect the presence of insecticides and locate themselves along the ventral aspects of the animal to avoid contact with the chemical (Arther, 1991).

Management of the resistance horn flies have established regarding the chemicals present in insecticides is imperative to the success of these chemicals if they are continued to be used. Immigration of genetically susceptible flies back into a resistant populations, refugia (i.e. providing the insects a refuge to escape from insecticides), and rotation of chemical classes and application methods of insecticides are three things to consider when managing resistance (Arther, 1991). The increased resistance of horn flies to all chemical classes used in insecticides has led to evolving management strategies.

Horn fly control strategies vary and include walk-through traps, insecticide-impregnated ear tags, self-treatment devices such as dust bags and back rubbers, animal activated sprayers, feed additives such as insect growth regulators (IGR) and larvacides, pour on insecticides, and spray on insecticides (Loftin and Corder, 2004). The Regional Research Committee for Livestock Pest Management (NCR-99) has made the following recommendations for horn fly resistance management: 1. only treat when levels exceed 200 flies per animal, 2. separate adults from growing calves, 3. delay early spring treatments, 4. use periodic applications with sprays, dusts, and back rubbers, 5. use IGRs and oral larvacides, 6. late season treatment before diapause, and 7. remove ear tags in the fall. Other horn fly management recommendations include: 1. do not treat for horn flies throughout the season, 2. use slow release devices only during times of peak fly activity, and 3. rotate chemicals present in insecticides annually to decrease resistance (Kunz, 1994). Determining the right management strategies and insecticides to implement can enhance the benefits and the overall production traits of cattle herds. However, the best alternative for resistance to chemical classes used in insecticides is continued research of alternative methods to control horn fly populations and selection for horn fly resistance.

Cattle Production and Performance Traits

Beef Cow Milk Production Traits. Milk production varies depending on age, genetics, breed, nutrition, and period of lactation. Clutter and Nielsen (1987) reported milk production to be 25% higher in mature cows (4 to 5 yr old) when compared with primiparous cows. Frame size did not affect milk yield, with large and small-framed cows on the same nutritional programs having similar milk yield (Holloway and Butts, 1984). Nutritional limitations may exist and prevent the expression of the genetic potential for milk yield, emphasizing the importance of matching sire milk expected progeny differences (EPD) and cow body weight with production environment (Brown et al., 2005).

The effect on milk yield and quality from cows sired by different breeds was demonstrated by Brown and Lalman (2010). Crossbred cows from Brangus dams were sired by Bonsmara, Brangus, Charolais, Gelbvieh, Hereford and Romosinuano bulls and used in the study. Results determined milk yields among Bonsmara, Brangus, Charolais, Gelbvieh and Hereford-sired cows were similar, but Romosinuano sired cows had less ($P < 0.05$) milk yield than all other breed types (Brown and Lalman, 2010). Other studies determined milk yield of Gelbvieh to be greater than Charolais and Hereford (Jenkins and Ferrell , 1992), while Brangus x Angus reciprocal cross cows had greater milk yield than Angus or Brahman cows (Brown et al., 2001).

Milk quality data determined Gelbvieh sired cows had less milk fat than Bonsmara, Charolais, Hereford, and Romosinuano sired cows ($P < 0.05$), but not Brangus sired cows (Brown and Lalman, 2010). Brown et el. (2001) reported the percentage of milk fat for Brahman x Angus reciprocal crosses to be intermediate compared to Angus and Brahman cows. Milk protein percentage of Romosinuano sired cows was greater ($P < 0.05$) than Brangus, Charolais, Gelbvieh, and Hereford sired cows, whereas Bonsmara sired cows had greater milk protein percentage than Charolais and Gelbvieh sired cows ($P < 0.05$; Brown and Lalman, 2010). A different study determined protein percentage was greatest in Brahman x Hereford cows compared to Angus x Hereford, Angus x Charolais, and Brahman x Angus cows (Daley et al., 1987). Brown et al. (2001) also reported percentage of milk protein to be similar among Angus, Brahman, and reciprocal crosses.

Bonsmara and Romosinuano sired cows had similar milk lactose, with both being greater ($P < 0.05$) than Hereford sired cows (Brown and Lalman, 2010). Solids-not-fat (SNF) was similar in Bonsmara and Romosinuano sired cows but greater ($P < 0.05$) than Brangus, Charolais, Gelbvieh, and Hereford sired cows (Brown and Lalman, 2010). Milk urea nitrogen in Brangus sired cows was greater ($P < 0.05$) than Charolais and Gelbvieh sired cows but similar to Bonsmara, Hereford, and Romosinuano sired cows (Brown and Lalman, 2010). Brown and

Lalman (2010) determined somatic cell count (SCC) for Romosinuano sired cows was lesser ($P < 0.05$) than Bonsmara, Charolais, Gelbvieh, and Hereford sired cows, but not Brangus sired cows. The SCC results may indicate Romosinuano influenced cattle may have a greater resistance to mastitis causing organisms than the other breeds used in this study (Brown and Lalman, 2010). Crossbred cows also were reported to possibly have a greater resistance to mastitis causing organisms than purebred cows based on favorable heterosis for SCC (Brown et al., 2001).

Calf Performance Traits. A positive relationship of cow milk yield with calf WW has been demonstrated (Brown and Brown, 2002; Brown et al., 2005). However, conflicting information exists regarding maternal heterosis. Maternal heterosis has been described as having a positive effect on preweaning growth, but the effect may be negative on postweaning ADG (Brown and Dinkel, 1982; Brown et al., 1999). A study using Brangus cows bred to Bonsmara, Brangus, Charolais, Gelbvieh, Hereford, and Romosinuano sires investigated the relationship of milk production with postweaning gain. All calves discussed in this section were assigned to wheat pasture after weaning, with results indicating Charolais sired calves ($P < 0.10$) to be heavier than Bonsmara and Romosinuano sired calves (Wang et al., 2009). Gelbvieh sired calves had greater ($P < 0.10$) postweaning ADG than Bonsmara, Brangus, and Romosinuano sired calves, while Brangus, Bonsmara, and Romosinuano sired calves were exceeded ($P < 0.10$) by Hereford sired calves in post weaning ADG. However, Charolais, Brangus, Bonsmara, and Romosinuano sired calves were all similar in postweaning ADG (Wang et al., 2009).

Other studies reported Charolais sired calves were numerically heavier at weaning than Gelbvieh sired calves or Hereford sired calves (Jenkins and Ferrell 1994), and similar reports were made by Dade et al. (2002) and Oxford et al. (2008) who both described Charolais sired calves as having a 20 kg and 21 kg advantage in WW compared to other breeds used in these studies. A similar 400 day body weight in Hereford, Gelbvieh, and Charolais sired heifers was also reported by Cundiff et al., (2001), while Wheeler et al. (2006) too reported similar 400 day body weight in heifers sired by Hereford, Brangus, and Bonsmara but lesser 400 day body weight in Romosinuano sired heifers.

Sire breed type varied regarding the levels of dam milk yield, with Gelbvieh sired calves benefitting ($P < 0.11$) more from greater milk yield, while Romosinuano sired calves were penalized for greater milk yield (Wang et al., 2009). However, Charolais, Hereford, and Brangus sired calves were nonsignificant ($P > 0.45$) with positive slopes while Bonsmara sired calves were nonsignificant ($P > 0.90$) with negative slopes. Overall, calves on wheat pasture postweaning showed less evidence of the effects of dam milk yield on postweaning ADG than calves on dry lot during postweaning in this study. The results indicate nutritional program postweaning may compensate for preweaning nutrition and/or ADG.

The use of different breed types of cattle may account for the variation that occurs in production traits. Specifically in the southeastern United States *Bos indicus* cattle are utilized in cow-calf production due to their adaptability to hotter climates, parasite resistance and efficiency in utilizing poor-quality forages (Turner, 1980; Brown and Lalman, 2010). The percentage of *Bos indicus,* calf sire, and/or number of crosses used to produce the dams or calves may impact production traits. Due to their adaptability to southern region environments *Bos indicus* cattle may demonstrate different potential regarding production traits. Therefore, breed and environmental impact may be responsible for the variation reported among production and performance traits of beef cows and calves.

Hormones

Cortisol. The glucocorticoid (GC) hormones are active hormones of the adrenocortical axis, with the GC being cortisol in cattle, sheep, and fish (Mormède et al., 2007; Mormède and Terenina, 2012). Cortisol is a cholesterol-derived steroid hormone synthesized in the fascicular zone of the adrenal cortex under the control of the anterior pituitary hormone adrenocorticotropic hormone (ACTH; Mormède et al., 2007; Mormède and Terenina, 2012). Secretion of ACTH and cortisol is pulsatile, with a pulse frequency of about 90 minutes, follows a diurnal cycle, and is influenced by meals, physical activity, and environmental conditions (Mormède et al., 2007).

Cortisol is not soluble in water and is considered lipophilic (Mormède and Terenina, 2012). Circulating cortisol is bound to plasma proteins in the blood, specifically albumin and corticosteroid-binding-globulin (CBG; Gayard, et al., 1996; Breuner and Orchinik 2002). Corticosteriod-binding-globulin is a specialized glycoprotein that binds cortisol with a high affinity and regulates its bioavailability (Gayard, et al., 1996; Breuner and Orchinik 2002). The liposolubility of cortisol and other GC allows for their diffusion in all tissues and cells; allowing them to influence numerous metabolic pathways, the immune system, inflammatory processes, and brain function (Mormède and Terenina, 2012).

Cortisol is often released during exposure to stressful situations or in stressful environments. For example, cattle, sheep, and goats subject to painful procedures such as castrations have been reported to have increased cortisol concentrations (Mellor, 1991; Fischer et al., 1996; and Fisher et al., 1997). Whereas separation from herd mates, mixing with unfamiliar animals, restraint, and transportation are other factors that lead to increased cortisol levels in cattle (Kent and Ewbank, 1983; Boissy and Le Neindre, 1997). Schwinghammer et al. (1986) reported increased blood cortisol concentrations in beef steers exposed to horn flies, while Riley et al. (1994) reported no changes in serum cortisol of yearling heifers exposed to horn flies for 33 days. When 24 Hereford x Angus crossbred beef steers were exposed to varying levels of horn flies (0, 75, 150 and 225 flies) serum cortisol concentrations ($P < 0.11$) decreased in steers exposed to 150 and 225 flies compared to those exposed to 0 and 75 (Presley et al. 1996). These findings suggest the stress response may differ depending on impact and severity perceived by the animal, if the event is acute or chronic, and the animal's synthesis of cortisol.

Cortisol concentrations are reported to differ among species, but evidence is limited regarding the variation in the release rate of cortisol in regards to the severity of the situation or stimulus (Mormède et al., 2007; Mormède and Terenina, 2012). Plasma cortisol levels have been reported to decline after the acute response (Mormède et al., 2007). However, behavioral reaction to the stimulus may still be detected; suggesting continual stress is occurring (Dellmeier et al., 1985; Jensen et al., 1996). The release of cortisol is a slow process, with the cortisol response prolonged until termination of the stressful event (Wegner and Stott, 1972; Veisser and Le Neindre, 1988). The amplitude of the stress response is species dependent, and therefore cortisol is based on the basal concentrations of the species, with cattle basal concentrations very low and often less than 15 nmol/L but can increase up to 60-200 nmol/L (Lay et al., 1992; Boissy and Le Neindre, 1997). Therefore plasma cortisol concentrations are not very informative when trying to detect chronic stress, but perhaps a more adequate measurement could be obtained if blood samples were collected 10 minutes after exposure (Mormède et al., 2007; Mormède and Terenina, 2012). Overall, the stress response, specifically synthesis and release of cortisol, varies among species and an individual animal's response to stressful events and environments.

Prolactin. Prolactin (PRL) is a polypeptide hormone synthesized and secreted from lactotroph or mammotroph cells in the anterior pituitary gland (Freeman et al., 2000). Over 300 separate biological activities have been described to occur due to the hormone PRL (Bole-Feysot et al., 1994). Prolactin has been classified into five categories relating to 1. reproduction (including lactation), 2. osmoregulation, 3. growth promotion, 4. ectodermal structure and 5. synergism with steroids (Nicoll and Bern 1972). The primary category this section will focus on is reproduction, specifically lactation.

Prolactin is an important hormone involved in the control of lactation, and is known to be mammogeneic and lactogenic in both monogastrics and ruminants (Lacasse et al., 2010). The effects of PRL on the mammary gland include growth and development of the mammary gland (mammogenesis), synthesis of milk (lactogenesis), and maintenance of milk secretion (galactopoiesis; Freeman et al., 2000). In cows, milking induced PRL release is correlated with milk production and lactation period, with PRL decreasing during advances in lactation (Koprowski and Tucker, 1973).

In most mammals suppression of PRL strongly inhibits lactation, while milking and suckling stimulates PRL secretion (Flint and Gardner 1994; Lacasse et al., 2010). The effects of increased PRL due to milking and suckling are not related to the milk harvest since PRL can be induced in nonlactating animals by nipple stimulation or suckling (Akers and Lefcourt, 1983). Suckling stimulus is the best-known physiological stimulus affecting PRL secretion and has been characterized as a classical neuronendocrine reflex (Freeman et al., 2000). Although this neuronendocrine reflex is not well understood it is known that the amount of PRL released will decrease as lactation advances (Fuchs et al., 1998; Lacasse et al., 2010), and that suckling is more efficient at inducing the reflex (Lupoli et al., 2001).

Koprowski and Tucker (1973) determined pre-milking serum PRL concentrations differed ($P < 0.01$) over the lactation period in Holstein cows. Serum PRL averaged 33 ± 4 ng/mL at 4 weeks, 68 ± 9 ng/mL at 16 weeks, and then fluctuated between 53 and 64 ng/mL for the remainder of lactation. Milk production was greatest at 8 weeks of lactation (36 kg/day) then gradually declined as lactation advanced, averaging 15 kg/day between the 36th and 44th week of lactation. Concentrations of serum PRL measured immediately after milking were determined to parallel milk production, while average monthly milk production and corresponding average serum PRL concentrations had a correlation coefficient of 0.96 ($P < 0.01$). A decrease in PRL availability from the pituitary as lactation advanced may be responsible for these results (Koprowski and Tucker, 1973). Alternatively, there may also be gradual reduction in the sensitivity of the reflex responsible for PRL release during advanced stages of lactation. Overall, the correlation between milk yield and PRL suggests that serum PRL accounts for only 13 to 4% of differences in milk yield among cows (Koprowski and Tucker, 1973). Therefore, other factor including breed, genetics, and a variety of hormones may be factors in regulating and maintaining milk production in cows.

Another important factor that affects PRL concentrations is ambient temperature. Changes in ambient temperature influence serum PRL concentrations through effects on hypothalamic release-inhibiting factors, which control PRL secretion (Smith et al., 1977). Serum PRL concentrations have been reported to be greater during warmer months as compared to colder months in lactating cows (Koprowski and Tucker, 1973), heifers (Aiken et al., 2007), bulls (Tucker et al., 1974), and calves (Karg and Schams, 1974; Schams and Reinhardt, 1974). Therefore, an inverse relationship between environmental temperatures and serum PRL concentrations has been described (Wettemann and Tucker, 1982). Overall, multiple changes in the animal’s environment, including ambient temperature as well as photoperiod, may influence PRL secretion and inhibition.

To conclude there are other areas of interest regarding PRL, its effects, and what may affect it. These other areas include stress and the immune response. Stress has been shown to dramatically affect PRL secretion. However, due to the synthesis and release of PRL differing based on the nature of the stressor a mechanism has not been described (Freeman et al., 2000). Therefore, one must look at the stressor to determine the cause and effect relationship that may exist with PRL. In the immune response lymphocytes have been reported as sources of PRL, and have been shown to contain dopamine receptors which may be involved in the regulation of lymphocytic PRL production and release (Devins et al., 1992). Prolactin also is a common mediator of the immunoneuroendocrine network where nervous, endocrine, and immune systems communicate with each other (Goffin et al., 1998). Although stress and the immune response were not the main focus of this section they were worth briefly mentioning based on their relevance to the subject matter of this literature review.

Prolactin Genotype

The promoter region of a gene is located upstream of the coding sequence of the gene. A control point for regulated gene transcription is provided by the promoter region of the gene (Bar-Joseph et al., 2003). The promoter region of the PRL gene was identified in cattle (Wolf et al., 1990). Identification of this region was described as the most proximal regulatory region located within the first 300 bases of the 5’ flanking region with the distal enhancer located approximately 1.5 kilobases (kb) from the transcription start site (Nelson et al., 1986; Kim et al., 1988). The prolactin promoter region has been reported to interact with several regulatory factors, including cAMP, thyrotropic releasing hormone, epidermal growth factor (EGF), and estradiol to name a few (Day and Maurer, 1989).

Single nucleotide polymorphisms (SNP) are highly abundant and depending on where they are located different phenotypic results may occur (Syvänen, 2001). In the promoter region of the PRL gene SNP have been identified (Peers et al., 1990). Looper et al. (2010) identified SNP of the promoter region of the PRL gene and their association with profitability traits of beef cattle. The SNP located at position -1,286 (transversion of cytosine (C) to thymine (T)) consisted of homozygous CC, TT, and heterozygous CT genotypes. Homozygous T cows at the C1286T position had reduced ($P < 0.05$) calving rates if grazing tall fescue compared to other genotypes at this location (Looper et al., 2010). Calves from homozygous C cows were less tall at weaning than calves from homozygous T cows (113.5 ± 1.5 vs. 117.0 ± 1.5 cm; $P < 0.10$), with calves from heterozygous CT cows intermediate (115.5 ± 1.5 cm; Looper et al., 2010).

Associations between milk production traits of beef cows and SNP of the PRL promoter region are limited or nonexistent. Milk production traits and associations with SNP of the PRL gene have however been reported in dairy cattle (Brym et al., 2005; Nasrin et al., 2009). Lü et al. (2010) analyzed two SNP in the promoter region of the bovine PRL gene, (-1043A>G and -402A>G). Analysis of their data showed significant associations between the genotypes of the promoter region and milk production traits in Chinese Holsteins (Lü et al., 2010). Milk yield was increased ($P < 0.01$) in cows with homozygous GG genotypes, while fat content was increased ($P < 0.01$) in cows with homozygous AA genotypes. Overall data on the association of specific SNP in the promoter region of the PRL gene in relation to beef cattle production traits are limited and should continue to be investigated.

Temperament

The behavior of cattle has been known to vary from docile to aggressive, with docile being the preferred behavior (Hoppe et al., 2010). Cattle temperament is defined as the response to a situation (Fordyce et al., 1988), with temperament indicated by the response to restraint and handling (Burrow and Corbet, 2000). Therefore, cattle temperament is often assessed and measured during human handling situations, whereas behavior is evaluated without human interaction.

Research has shown that reducing stress during handling will provide advantages to cattle production by increasing productivity and maintaining meat quality (Grandin, 1998). Cattle temperament has been associated with body weight gain, carcass quality, and health (Vetters et al., 2013). Cattle with more excitable temperaments have been documented to have reduced feed efficiency (Petherick et al., 2002), poor meat quality (Voisinet et al., 1997), inhibited milk production (Breuer et al., 2000), and decreased immune function (Fell et al., 1999). While cattle with calm temperaments have been reported to have greater ADG (Burrow, 1997), increased conception rates (Cooke et al., 2011), and reduced incidence of dark cutters (Voisinet et al., 1997).

A cattle's temperament can be measured utilizing various techniques such as pen score (PS), chute score (CS), exit velocity (EV) or flight speed, and exit score. Temperament of beef cattle can also differ based on breed type and sex (Stricklin et al., 1980; Gauly et al., 2001). *Bos indicus* cattle have been described as more temperamental than *Bos taurus* (Voisinet et al., 1997). Selection for temperament among cattle may be beneficial since it has been reported as small to moderately heritable (Burrow and Corbet, 2000; Morris et al., 1994).

Chute score is classified as a restraint technique, while EV is classified as a nonrestraint technique. An issue with CS is the restriction of motion that can occur when the squeeze feature of the side panels and head catch are used on a hydraulic chute. Variation in CS decreases when restricting the range of motion of the animal (Vetters et al., 2013). Another issue with restraint is that some cattle with excitable temperaments may “freeze” under those conditions, and therefore not express their true temperament during the assessment (Burrow and Corbet, 2000). However, EV being a nonrestraint measurement of temperament does not have these issues and has been described as a valuable tool in assessing cattle temperament and a possible indicator of temperament throughout the animal’s lifetime (Curley et al., 2006).

Hoppe et al. (2010) utilized CS when assessing 5 beef cattle breeds; German Angus, Charolais, Hereford, Limousin, and German Simmental. Breed differences varied ($P < 0.001$) for CS, with Charolais and Limousin calves having the largest CS, Hereford calves had the smallest ($P < 0.001$) CS, and German Angus and German Simmental were intermediate. Temperament score differed between male and female calves ($P < 0.01$), with females having a CS of 2.57 ± 0.03 and male calves CS being 1.69 ± 0.03 (Hoppe et al. 2010).

Another study using 66 American Gray Brahman bulls was designed to determine if temperament was repeatable and associated with cortisol (Curley et al., 2006). Temperament measurements consisted of CS, PS, and EV. All three measurements showed positive correlation ($r = 0.35$, $P < 0.05$), with PS ($r = .29$, $P < 0.05$) and EV ($r = 0.26$, $P < 0.05$) also positively correlated with cortisol concentrations on day 0. Day 60 PS and CS were positively correlated ($r = 0.40$, $P < 0.01$), while on day 120 no temperament measurements were correlated. However, on day 120 cortisol concentrations were positively correlated with EV ($r = 0.44$, $P < 0.01$) and PS ($r = 0.25$, $P < 0.05$; Curley et al., 2006).

Human-animal interactions with livestock can contribute to an animal's stress level. The limited habituation to humans usually leads to a negative behavioral response in beef cattle, which can lead to increased work load and risk of injury to the handlers (Le Neindre et al., 2002; Hoppe et al. 2010). An alternative to alleviate negative physiological effects of aggressive temperament from cattle may be acclimation to human handling (Echternkamp, 1984). Acclimation of cattle to human handling has been previously used to prevent elevated concentrations of cortisol in response to human handling (Andrade et al., 2001; Curley et al., 2006), as well as improve temperament measurements as determined by CS, EV, and PS (Cooke et al., 2009ab).

In a study conducted by Cooke et al. (2009a) heifers were acclimated to human handling 3 times per week for 4 weeks prior to the start of the study. Acclimated heifers had reduced ($P < 0.01$) ADG compared to control heifers despite both groups being provided similar pastures and supplements. Cortisol concentrations were also reduced ($P < 0.01$) in acclimated heifers compared to control heifers (37.8 vs. 50.5 ng/mL). Cattle temperament scores consisted of calculating an average score utilizing CS, PS, and EV; however, no treatment effects were detected for temperament scores. Acclimated heifers did however have reduced CS ($P < 0.01$) compared to control heifers (Cooke et al., 2009a). In another study by Cooke et al. (2009b) no treatment affects were detected for temperament measurements of CS ($P = 0.59$), PS ($P = 0.12$), EV ($P = 0.57$), or concentrations of cortisol ($P = 0.88$). Results from these studies indicate acclimation of Brahma cross cows to human interaction varied. Temperament as determined by CS, EV, and PS varied with CS improving in one study but not in the other, cortisol concentrations improved, but ADG was negatively impacted (Cooke et al., 2009ab).

Overall, EV and CS are valid measurements used to assess cattle temperament. Both genetic factors and previous handling experiences contribute to cattle temperaments (Grandin, 1998). The use of low stress handling, acclimation to human handling, and management practices, as well as selecting for cattle based on temperament can all contribute to improved cattle behavior.

Statement of Purpose and Study Objectives

After review of the literature and in conjunction with previous work reported by the University of Arkansas and USDA-ARS Dale Bumpers Small Farms Research Center the purpose of this dissertation was to contribute information to the literature regarding the effects of horn flies on milk production, calf performance, and behavior and temperament of beef cattle. Also, genetic data regarding the use of markers to indentify horn fly resistant cattle is lacking from the literature and therefore served as an additional focus of this research. The information discovered by this research will improve the understanding of the effects of horn flies on beef cow and calf production traits.

The objectives of the study were:

1. To identify beef cows using the PRL promoter gene (C1286T) as a marker for milk production, calf performance traits, and external parasite resistance.
2. To determine the effects of horn flies on milk production traits; including milk yield, somatic cell count, solids-not-fat, milk urea nitrogen, milk fat, milk lactose, and percent protein.

3. To determine the effects of horn flies on calf performance traits; including preweaning ADG, postweaning ADG, 365-d adjusted YWT, and birth to yearling ADG.
4. To determine the effects of horn flies on pasture behavior, CS, EV, and cortisol concentrations.

The study hypothesized that the PRL promoter gene (C1286T) could be used as a marker for selection for improved milk production traits and horn fly resistance in beef cows. Horn flies were also hypothesized to negatively impact calf performance traits and influence pasture behavior and temperament measurements.

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CHAPTER 1

MILK PRODUCTION, CALF PERFOMANCE TRAITS, AND TEMPERAMENT MEASUREMENTS OF BEEF COWS AS AFFECTED BY HORN FLY COUNT AND GENOTYPE

Abstract

Genetic selection for increased milk production, horn fly resistance, and improved calf production traits may be an alternative method to managing beef cattle. The objective of this study was to identify cows using a single nucleotide polymorphism (SNP) in the promoter region of the prolactin (PRL) gene (C1286T). The primary focus was to determine an association with the SNP of the PRL promoter gene and milk production, calf performance, temperament, and horn fly resistance. Cows (n = 53) sired by Bonsmara (BONS; n = 7), Brangus (BRAN; n = 13), Charolais (CHAR; n = 8), Gelbvieh (GELV; n = 5), Hereford (HERF; n = 12), and Romosinuano (ROMO; n = 8) from Brangus dams were genotyped. The cow's Angus-sired offspring were used to assess calf production traits. Cows were maintained on native rangeland pastures throughout the study. Calf birth weight (BW) was determined within 24 hours (h), and calves were weaned at an average of 205 days (d). Estimates of milk yield were collected every 28 d from May to October utilizing a single-cow portable machine beginning approximately 60 d postpartum. Milk weight was adjusted to a 24-h milk yield. A commercial dairy laboratory was responsible for milk quality analysis, including estimates of milk fat, protein, urea nitrogen, somatic cell count (SCC), lactose, and solids-not-fat (SNF). Total horn fly counts were recorded on individual animals while in pasture from 0700 to 0900 h beginning in May and ending in October by the same trained individual throughout the study. Genomic DNA was purified and a specific sequence within the promoter region was amplified using polymerase chain reaction. Amplicons were purified, sequenced, and cows were genotyped. All three genotypes,

homozygous cytosine (CC) and thymine (TT) and heterozygous (CT), were observed in the population. Genotype was not significant in any of the mixed models and therefore means averaged over genotype for milk quality and quantity, calf performance traits, and temperament measurements were determined.

Introduction

Single nucleotide polymorphisms (SNP) are highly abundant and depending on where they are located different phenotypes may result (Syvänen, 2001). Milk production traits and profitability traits in cattle have previously been associated with SNP of the promoter region of the prolactin (PRL) gene. Cows grazing endophyte-infected tall fescue and homozygous thymine (TT) have been reported to have reduced ($P < 0.05$) calving rates compared to other genotypes at this SNP location (Looper et al., 2010). Homozygous cytosine (CC) cows were reported to have shorter calves at weaning than those from TT cows ($P < 0.10$; Looper et al., 2010).

Data regarding associations of milk production traits in beef cows and SNP of the PRL promoter region are limited. However, dairy cows had increased milk yield and increased milk fat with certain genotypes in the promoter region of the PRL gene (Lü et al., 2010). Others have reported milk production traits to be associated with SNP in the coding sequence of the PRL gene but not in the promoter region (Brym et al., 2005; Nasrin et al., 2009).

Data regarding specific PRL genotypes in the promoter region associated with temperament or horn fly resistance have yet to be reported in the literature. The literature does however indicate both temperament and horn fly resistance are genetic traits which are small to moderately heritable (Brown et al., 1992; Burrow and Corbet, 2000; and Morris et al., 1994).

Therefore, due to the lack of genetic data focusing on the promoter region of the PRL gene and its association with milk production, calf performance traits, temperament, and horn fly resistance in beef cattle the objective of this study was to identify cows using a SNP in the promoter region of the PRL gene (C1286T) and associate the genotypes with these traits.

Materials and Methods

Animal Care and Use Committee at the USDA-ARS, Grazinglands Research Laboratory El Reno, OK, and the University of Arkansas and Oklahoma State University's Institutional Animal Care and Use Committee approved the procedures used in this study.

Horn Fly Counts

Total horn fly populations were recorded on individual animals while in pasture from 0700 through 0900 hours (h), every 28 days beginning in May and ending in October. Individual animals were observed by a trained individual throughout the study from a motorized vehicle or on foot, utilizing binoculars for accurate counts if animals were greater than 5 meters (m) away. Horn flies were treated when populations exceeded threshold levels (> 200 flies/animal). This resulted in monthly treatment using Co-Ral (organophosphate; Bayer HealthCare LLC, Animal Health Division, Shawnee Mission, Kansas), which occurred after horn fly counts were recorded. Treatment of horn fly populations beyond threshold levels ensured the animal's health and well being requirements were met and properly maintained according to IUCAC protocols.

Milk Production

Estimates of milk yield and quality were collected from Bonsmara (BONS; n = 7), Brangus (BRAN; n = 13), Charolais (CHAR; n = 8), Gelbvieh (GELV; n = 5), Hereford (HERF; n = 12), and Romosinuano (ROMO; n = 8) sired cows. A single-cow portable machine was utilized to measure milk yield beginning 60 days (d) postpartum. Measurements of milk yield began in late May and ended in late October, and were collected every 28 d during this time period.

Calves were separated from cows at approximately 1900 h the evening prior to milking and held overnight for approximately 14 h, with water provided. No milk-out was conducted prior to separation. Ten minutes prior to milking cows were administered 1.5 mL of acepromazine maleate (10 mg/mL, i.m.) and 1.0 mL of oxytocin (20 USP units/mL). Oxytocin was given immediately before milking to facilitate milk letdown.

Milk was weighed on a digital platform scale and adjusted to a 24 h basis (24-h milk yield) as [(milk weight/14) x 24] (Brown et al., 1996). A commercial dairy laboratory was responsible for milk quality analysis, which included estimates of milk fat, milk protein, milk urea nitrogen, somatic cell count (SCC), milk lactose, and milk solids-not-fat (SNF).

Animal Information

Brangus cows were bred by AI and natural service to randomly selected sires of BONS, BRAN, CHAR, GELV, HERF, and ROMO breeds to produce the cows (n = 53) used in this study. Sires used of each breed type consisted of BONS (n = 4), BRAN (n = 8), CHAR (n = 7), GELV (n = 5), HERF (n = 6), and ROMO (n = 6). Cows utilized for this study were maintained on native rangeland and were born between 2001 through 2006. Cows were bred the spring and summer prior to the study, so as to calve the following spring when the study began.

Calves were sired by Angus bulls using natural service between June and July. Angus bulls were selected based on visual appraisal for soundness and conformation and breeding soundness as determined by a breeding soundness exam. Birth weight (BW) of calves was determined within 24 h of birth and bull calves were castrated by elastration at this time. Calves were weaned in fall at an average age of 205 d when body weight was also determined. Weaning weights (WW) were collected and calves were then maintained on wheat pasture throughout the winter. Yearling weight (YW) was determined at an average age of 365 d.

Body weight of cows was recorded at the same time milking occurred each month. Initial and final body condition score (BCS) of cows also was recorded at this time using a 1-9 scale (1 = to thin to 9 = obese).

Blood Serum Hormone Analysis

Blood samples were collected monthly beginning in May and ending in October via jugular venipuncture using vacutainers (Bectin Dickinson, Franklin Lakes, NJ). Samples were allowed to clot for 24 h at 4˚C and centrifuged at 2,500 x *g* for 25 min (minutes) at 4˚C (Marathon 22KBR, Fisher Scientific, Hermle-Labortechnik, Germany). Serum was then harvested and stored at -20˚C pending analysis.

Serum cortisol (CORT) concentrations were analyzed in duplicate with a solid-phase RIA using components of commercial kits (Siemens Diagnostic, Los Angeles, CA). This assay was performed without prior extraction of individual hormones from serum with the kits utilizing antibody-coated tube technology. Validation of the CORT assay utilizing ruminant serum is described by Kiyma et al. (2004); intra and interassay CV were less than 3% for CORT.

Prolactin (PRL; Spoon and Hallford, 1989) concentrations were also analyzed in duplicate by double-antibody RIA using primary antisera and purified standard and iodination preparations supplied by the National Hormone and Peptide Program (Torrance, CA).

Genotyping

Blood samples were collected via jugular venipuncture using vacutainers (Bectin Dickinson, Franklin Lakes, NJ) containing EDTA. Samples were centrifuged at 2,500 x *g* for 25 min at 4˚C (Marathon 22KBR, Fisher Scientific, Hermle-Labortechnik, Germany) to isolate buffy coat. Buffy coat was harvested and stored at -20˚C until genomic DNA was isolated using a Qiagen extraction kit (Qiagen Inc. Valencia, CA). DNA was diluted to 20 ng/µL prior to sequencing.

Polymerase chain reaction (PCR) primers [forward (5' – AAGTCCCCATAAGCACACTTGG-3') and reverse (5'-CTAACTTTAGGGAGTTCATACTG-3')] were synthesized and supplied by Sigma-Genosys (St. Louis, MO). Primers were used for amplification of a 500-base segment of the bovine PRL promoter region (position -892 to -1,392; Gen-Bank accession numbers AY337763 and AY641989). Genomic DNA template of 100 ng was added to the amplification reaction (50 µL total volume), which contained 2 µL of each primer and 45 µL of platinum PCR Superimx (Invitrogen, Carlsbad, CA). A Peltier thermal cycler (MJ Research, Waltham, MA) was used for PCR. The PCR protocol consisted of an initial 94˚C for 2 min, followed by 35 cycles at 94˚C for 30 sec (second), 55˚C for 1 min and 68˚C for 1 min. The reaction was completed with 68˚C for 10 min and then held at 8˚C. Amplification products were verified by electrophoresis using 2% argarose gels stained with ethidium bromide in 1.0X Tris/Boric Acid/EDTA. Amplicons were purified using the QIAquck PCR purification kit (Qiagen Inc., Valencia, CA). Purified PCR products were sequenced at the University of Arkansas DNA Core Lab using an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA).

Sequences were managed using Bioedit Sequence Alignment Editor (Version 7.0.9.0; http://www.mbio.ncsu.edu/BioEdit/bioedit.html) and compared using the web-based software ClustalW (http://www.ebi.ac.uk/Tools/clustalw2/index.html; European Bioinformatics Institute, Cambridge, UK). A transversion consisting of cytosine (C) to thymine (T) was identified at position -1,286 in the promoter region of the PRL gene. Three genotypes were observed: homozygous cytosine (CC), homozygous thymine (TT) and heterozygous (CT). Assessment of the sequence chromatograms using the ABI Prism ® Sequence Scanner V1.0 (Applied Biosystems, Inc. Foster City, CA) and Bioedit (Hall, 1999) allowed for homozygous and heterozygous allele identification.

Temperament and Behavior

Pasture behavior was recorded twice daily between 0700 through 0900 h and 1300 through 1500 h on the same day horn fly counts were recorded. Cattle were observed and recorded to be grazing, lying or standing.

Chute scores (CS) and exit velocity (EV) measurements were used to determine temperament of cattle. Exit velocity and CS for the current study were obtained monthly for both cows and calves. Chute scores were based on a 1 – 4 scale (1 = calm no movement; 2 = restless shifting; 3 = squirming continuous shaking of the squeeze chute and 4 = rearing, twisting, continuous violent struggle), which was adopted from previously described procedures (Grandin, 1993). Exit velocity was defined as the rate at which the animal exited the squeeze chute and traversed 1.8 m (Curley et al., 2006). Two infrared sensors were used to record EV, (FarmTek Inc., North Wiley, TX) and it was recorded as time [distance(m)/(sec)].

Statistical Analysis

Data were analyzed using PROC MIXED (SAS Institute, Cary, NC). The initial linear model for milk traits, cow temperament measurements, and cow horn fly count included sire breed (fixed), genotype (fixed), sire breed x genotype (fixed), cow nested in sire breed and genotype (random), month (fixed repeated), month x sire breed (fixed), month x genotype (fixed), month x sire breed x genotype (fixed), and a random residual effect. Initial linear models for calf production traits include dam sire breed (fixed), dam genotype (fixed), calf gender (fixed), dam sire breed x dam genotype (fixed), dam sire breed x calf gender (fixed) and calf nested in dam sire breed, genotype, and calf gender (random). Models were reduced when the observed significance levels of F tests were greater than 0.25 according to standard procedures for model reduction. Least squares means and standard errors of milk quality and quantity, calf production traits, and temperament measurements were determined and mean comparisons were done using t statistics where $P < .10$ denoted a difference.

Results

Distribution of genotypes from the promoter region of the PRL gene (C1286T) among the sire breeds is presented in Table 1. Means of milk production and horn fly counts (Table 2.), calf production traits (Table 3.), and temperament measurements (Table 4.) for the three identified genotypes are reported. Statistical analysis of data collected in this study determined genotype did not have an affect ($P > 0.25$) on milk production, calf performance traits, temperament measurements, or horn fly count. The association of genotypes from the promoter region of the PRL gene (C1286T) with milk production, calf production traits, temperament measurements, and horn fly infestation were not significant and will not be reported in subsequent chapters.

Discussion

Prolactin has been described as an important hormone involved in lactation (Lacasse et al., 2010). The promoter region of a gene has been found to provide the control point for regulating gene transcription (Bar-Joseph et al., 2003), and SNP within a gene could account for the phenotypic variations observed (Syvänen, 2001). Therefore, lack of association of the genotypes in the promoter region of the PRL gene (C1286T) in this study with milk production traits, horn fly count and calf performance traits was unexpected.

The results indicate no genotypes (CC, CT, TT) from the promoter region of the PRL gene (C1286T) were associated with milk production or calf performance traits. Other studies have evaluated the association of SNP in the promoter region of the PRL gene with milk production in dairy cattle and found SNP (A1043G) to be associated with milk yield (Lü et al., 2010). Cows' homozygous guanine (GG) had increased ($P < 0.01$) milk yield, while cows' homozygous adenine (AA) had higher ($P < 0.01$) milk fat content (Lü et al., 2010). Other research has found genotypes from the PRL gene, not located in the promoter region, to be associated with milk production traits of dairy cows (Brym et al., 2005; Nasrin et al., 2009). However, research on SNP of the promoter region of the PRL gene in beef cattle and their association with milk production traits is lacking. To my knowledge no literature exists that has evaluated the association of genotypes from the C1286T SNP with milk production traits of beef cattle.

Therefore, in this study perhaps the lack of significance between the selected genotypes (C1286T) and its association with milk production traits indicates the use of this gene for selecting improved milk production in beef cows is not effective. Results also indicate using this gene as a marker for calf performance traits in this study were not beneficial. However, Looper et al. (2010) demonstrated this gene may be useful in selecting for calf height and calving rate in beef cows on specific grazing systems.

Temperament and horn fly resistance have been described as heritable traits (Brown et al., 1992; Burrow and Corbet, 2000; and Morris et al., 1994), but specific genetic markers located in the promoter region of the PRL gene have not been evaluated for these traits. Results from this study indicate genotypes from the promoter region of the PRL gene (C1286T) were not significantly associated with beef cattle temperament measurements or horn fly resistance. In conclusion, selection of cattle using the C1286T gene for milk production, calf performance traits, temperament measurements, and horn fly resistance is not beneficial. However, the use of other SNP located in the PRL promoter region may be useful when selecting for these traits.

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Table 1. Distribution of Genotypes among Sire Breeds.

	Genotype		
Sire Breed	CC	CT	TT
Bonsmara	1	3	3
Brangus	5	8	0
Charolais	3	2	3
Gelbvieh	0	4	1
Hereford	4	4	4
Romosinuano	3	2	3

Table 2. Milk Production Means for Genotypes with Pooled Standard Deviations.

	Genotype			
Variable	CC	CT	TT	SD
Horn Fly Count	351	322	338	303
Milk Yield	7.21	7.56	6.94	2.53
Milk Fat	3.50	3.41	3.26	1.04
Milk Protein	3.30	3.17	3.14	0.45
Milk Lactose	4.86	4.74	4.89	0.49
Milk Urea Nitrogen	11.97	11.00	10.90	4.64
Somatic Cell Count	277.34	173.56	115.57	529.84
Solids-Not-Fat	9.11	8.83	8.96	0.85
Prolactin	35.87	82.29	61.09	79.79

Serum prolactin (ng/mL)
Milk yield (kg/d)
Milk fat, milk protein, milk lactose, and solids-not-fat (%)
Milk urea nitrogen (mg/dL)
Somatic cell count (count x 10)
(P > 0.25), SD = pooled standard deviation

Table 3. Calf Production Trait Means for Genotypes with Pooled Standard Deviations.

	Genotype			
Variable	CC	CT	TT	SD
Birth Weight	20.04	19.36	18.96	9.68
Preweaning ADG	0.41	0.42	0.42	0.05
Weaning Weight	110.55	111.45	111.62	13.29
205-d Weaning Weight	103.93	105.20	104.39	11.48
Yearling Weight	406.29	410.75	392.65	39.61

Birth weight, preweaning average daily gain (ADG), weaning weight, 205-d weaning weight, and yearling weight (kg)

(P > 0.25), SD = pooled standard deviation

Table 4. Temperament Measurement Means of Cows for Genotypes with Pooled Standard Deviations.

	Genotype			
Variable	CC	CT	TT	SD
Cow Exit Velocity	1.96	2.64	4.08	4.48
Cow Chute Score	1.20	1.21	1.09	0.40
Cortisol	18.80	23.59	19.88	10.02

Exit velocity (m/sec)
Cortisol (ng/mL)
($P > 0.25$), SD = pooled standard deviation

CHAPTER II
MILK PRODUCTION TRAITS OF BEEF COWS AS AFFECTED BY HORN FLY COUNT AND SIRE BREED TYPE

Abstract

Horn flies infestations can negatively impact profitability traits of beef cattle. Increased resistance to pesticides has lead to the evaluation of alternative production methods for horn fly control. Cows (n = 53) sired by Bonsmara (BONS; n = 7), Brangus (BRAN; n = 13), Charolais (CHAR; n = 8), Gelbvieh (GELV; n = 5), Hereford (HERF; n = 12), and Romosinuano (ROMO; n = 8) from Brangus dams were used to determine breed differences in horn fly count and the effects of horn fly count on milk yield and quality. Total horn fly counts were recorded on individual animals while in pasture from 0700 to 0900 h beginning in May and ending in October by the same trained individual throughout the study. Estimates of milk yield were collected every 28 d from May to October utilizing a single-cow portable machine beginning approximately 60 d postpartum. Milk weight was adjusted to a 24-h milk yield. A commercial dairy laboratory was responsible for milk quality analysis, including estimates of milk fat, milk protein, milk urea nitrogen, somatic cell count (SCC), milk lactose, and milk solids-not-fat (SNF). Horn fly counts were transformed to natural log horn fly count prior to analysis. Data for milk yield and quality, and horn fly count were analyzed by mixed model least squares using a linear model including sire breed, cow in sire breed group, month, and month x sire breed. Effects of horn fly count on milk yield and quality were estimated by including a linear covariate of log horn fly count and log horn fly count x sire breed. Horn fly counts varied by month ($P <$ 0.0001), with the lowest population recorded in May (99 ± 39 flies) and peaking in August (520 ± 38 flies). Bonsmara and GELV sired cows had greater milk yield compared to HERF sire cows (8.75 ± 0.73 and 8.62 ± 0.86 vs. 6.02 ± 0.57 kg/d; respectively; $P < 0.05$), with CHAR, ROMO and BRAN sired cows intermediate (7.28 ± 0.65, 7.00 ± 0.65, and 7.06 ± 0.56 kg/d; respectively). An effect of sire breed x log horn fly count affected ($P < 0.05$) milk yield. Milk yield was reduced by 0.99 and 0.64 kg/d per unit increase in log horn fly count in GELV and BONS sired cows ($P < 0.05$). The regression coefficient for milk yield on log horn fly count was lesser in GELV sired cows than BRAN, CHAR, HERF, and ROMO sired cows ($P < 0.05$), and lesser in BONS sired cows than BRAN sired cows ($P < 0.05$), where lesser indicates greater reductions in milk yield. An interaction of PRL x sire breed affected ($P < 0.10$) milk yield. For every one unit increase in log horn fly count milk fat decreased by 0.15% ($P < 0.05$), SNF decreased by 0.10% ($P < 0.05$) and milk urea nitrogen decreased by 0.62 mg/dL ($P < 0.02$). Our results indicate horn fly infestation negatively impacts milk yield and quality traits of certain sire breed types. Horn fly numbers also are influenced by sire breed and period of lactation cycle. Therefore, future multi-trait selection for improved, sustainable beef cattle production systems may include selecting for horn fly resistance and milk production traits.

Introduction

Horn flies (*Haematobia irritans*) are often described as pasture insects, with cattle being the primary host (Williams et al., 1985). In North America horn flies are regularly observed clustered on the backs of cattle, which provides an ideal location for horn flies to feed (Williams et al., 1985). Horn flies remain on the host throughout their lifecycle, feeding approximately 20-40 times per day (Arther, 1991).

The cattle response to horn fly stress from feeding has been reported to cause production losses. Production losses have not been reported in recent years, but were last reported to equal $876 million in 1991 (Kunz et al., 1991). With increased resistance to pesticides it is not unreasonable to assume production losses have continued to increase due to the negative effects of horn flies.

Milk production data from cattle affected by horn flies is limited and inconsistent. Milk yield has been reported to increase in cows treated for horn flies (Morgan and Bailie, 1980; Riha et al., 1981; Block and Lewis, 1986; and Minar et al., 1987), while other studies reported no effect on milk yield in cows treated for horn flies (Cheng and Kessler, 1961; Miller et al., 1973). Therefore, the objective of this study was to determine the effects of horn flies on milk production quantity and quality traits of beef cows.

Materials and Methods

The Committee for Animal Welfare at the USDA-ARS, Grazinglands Research Laboratory El Reno, OK and the University of Arkansas Institutional Animal Care and Use Committee approved the procedures used in this study.

Horn Fly Counts

Total horn fly populations were recorded on individual animals while in pasture from 0700 through 0900 hours (h), every 28 days beginning in May and ending in October. Individual animals were observed by a trained individual throughout the study from a motorized vehicle or on foot, utilizing binoculars for accurate counts if animals were greater than 5 meters (m) away. Horn flies were treated when populations exceeded threshold levels (> 200 flies/animal). This resulted in monthly treatment using Co-Ral (organophosphate), which occurred after horn fly counts were recorded. Treatment of horn fly populations beyond threshold levels ensured the animal’s health and well being requirements were met and properly maintained according to IUCAC protocols.

Environmental Temperature

Weather data was collected throughout the study period from the Oklahoma Mesonet Weather Service (El Reno, Oklahoma) located on the research site at the USDA-ARS Grazinglands Research Laboratory approximately 5 miles WNW of El Reno, Oklahoma (Longitude: 35˚ 32’54” N; Latitude: 98˚ 2’11” W). Temperature data was measured every 5 minutes, with the maximum and minimum temperature recorded daily. The average maximum temperature during the study period was 30.7°C, while the average minimum temperature was 19.2°C. Monthly average temperatures (AT), monthly average maximum temperature (MAXT), and monthly average minimum temperature (MINT) were also reported; May (AT: 19.9˚C, MAXT: 27.6˚C, and MINT: 7.3˚C), June (AT: 28.7˚C, MAXT: 32.4˚C, and MINT: 24˚C), July (AT: 31.7˚C, MAXT: 34.1˚C, and MINT: 29.8˚C), August (AT: 30.1˚C, MAXT: 34.9˚C, and MINT: 22.3˚C), September (AT: 20.8˚C, MAXT: 30.7˚C, and MINT: 12.8˚C), and October (AT: 19.2˚C, MAXT: 24.6˚C, and MINT: 14.8˚C).

Milk Production

Estimates of milk yield and quality were collected from Bonsmara (BONS; n = 7), Brangus (BRAN; n = 13), Charolais (CHAR; n = 8), Gelbvieh (GELV; n = 5), Hereford (HERF; n = 12), and Romosinuano (ROMO; n = 8) sired cows. A single-cow portable machine was utilized to measure milk yield beginning 60 days (d) postpartum. Measurement of milk yield began in late May and ended in late October, and occurred every 28 d during this time period.

Calves were separated from cows at approximately 1900 h the evening prior to milking and were held overnight for approximately 14 h, with water provided. No milk-out was conducted prior to separation. Ten minutes prior to milking cows were administered 1.5 mL of acepromazine maleate (10 mg/mL, i.m.) and 1.0 mL of oxytocin (20 USP units/mL). Oxytocin was given immediately before milking to facilitate milk letdown.

Milk was weighed on a digital platform scale and adjusted to a 24 h basis (24-h milk yield) as [(milk weight/14) x 24] (Brown et al., 1996). A commercial dairy laboratory was responsible for milk quality analysis, which included estimates of milk fat, milk protein, milk urea nitrogen, somatic cell count (SCC), milk lactose, and milk solids-not-fat (SNF).

Animal Information

Brangus cows were bred by AI and natural service to randomly selected sires of BONS, BRAN, CHAR, GELV, HERF, and ROMO breeds to produce the cows (n = 53) used in this study. Sires used of each breed type consisted of BONS (n = 4), BRAN (n = 8), CHAR (n = 7), GELV (n = 5), HERF (n = 6), and ROMO (n = 6). Cows utilized for this study were maintained on native rangeland and were born between 2001 through 2006. Cows were bred the spring and summer prior to the study, so as to calve the following spring when the study began.

Calves were sired by Angus bulls using natural service between June and July. Angus bulls were selected based on visual appraisal for soundness and conformation and breeding soundness as determined by a breeding soundness exam. Birth weight (BW) of calves was determined within 24 h of birth and bull calves were castrated by elastration at this time.

Blood Serum Hormone Analysis

Blood samples were collected monthly beginning in May and ending in October via jugular venipuncture using vacutainers (Bectin Dickinson, Franklin Lakes, NJ). Samples were allowed to clot for 24 h at 4˚C and centrifuged at 2,500 x *g* for 25 min (minute) at 4˚C (Marathon 22KBR, Fisher Scientific, Hermle-Labortechnik, Germany). Serum was then harvested and stored at -20˚C pending analysis.

Prolactin (PRL; Spoon and Hallford, 1989) concentrations were analyzed in duplicate by double-antibody RIA using primary antisera and purified standard and iodination preparations supplied by the National Hormone and Peptide Program (Torrance, CA).

Statistical Analysis

Horn fly counts were transformed to natural log horn fly count prior to analysis. Data for milk yield, milk quality, and horn fly count were analyzed by mixed model least squares (SAS Institute, Cary, NC) using a linear model that included sire breed (fixed), cow nested in sire breed (random), month (fixed repeated), and month x sire breed, with calf birth date as a linear covariate. Calf birth date was not a significant covariate and was dropped from the model. Effects of horn fly count on milk yield and quality were estimated by including a linear covariate of log horn fly count (linear) and log horn fly count x sire breed.

The analysis of the regression of PRL on log horn fly count used mixed model least squares, with a linear model of sire breed (fixed), cow nested in sire breed (random), month (fixed repeated), and month x sire breed, log horn fly count (linear) and log horn fly count x sire breed. Prolactin data also was analyzed as the regression of milk yield on PRL with the full linear model including sire breed (fixed), cow nested in sire breed (random), month (fixed repeated), and month x sire breed, PRL (linear), and PRL x sire breed. All models used were reduced in a step-wise procedure by elimination of non significant interactions ($P > 0.25$), and in accordance with appropriate model reduction procedures.

Results

Horn Flies

Horn fly counts of cows varied by month (*P* < 0.0001) with the lowest population recorded in May (99 ± 39 flies) and populations peaking in August (520 ± 38 flies; Figure 1). However, sire breed differences (*P* > 0.25; Figure 2) and a sire breed x month interaction (*P* > 0.10) did not occur in this study.

Milk Yield

Milk yield was affected (*P* < 0.05) by sire breed (Figure 3). Bonsmara and GELV sired cows had increased milk yield when compared to HERF sired cows (8.75 ± 0.73 and 8.62 ± 0.86 versus 6.02 ± 0.57 kg/d; respectively), with CHAR, ROMO and BRAN sired cows intermediate (7.28 ± 0.65, 7.00 ± 0.65, and 7.06 ± 0.56 kg/d; respectively).

An interaction of sire breed and log horn fly count affected (*P* < 0.05) milk yield (Figure 4). Milk yield was reduced by 0.99 and 0.64 kg/d per unit increase in log horn fly count in GELV and BONS sired cows. There was less evidence of horn fly count effects on milk yield in other sire breeds (*P* > 0.25). However, the regression coefficients for other sire breeds were negative, with the exception of BRAN sired cows. The regression coefficient for milk yield on log horn fly count was lesser in GELV sired cows than BRAN, CHAR, HERF, and ROMO sired cows (*P* < 0.01) and lesser in BONS sired cows than BRAN sired cows (*P* < 0.05), where lesser indicates greater reductions in milk yield (Table 1).

A month by log horn fly count interaction also affected (*P* < 0.05) milk yield (Figure 5). Milk yield was reduced by 0.72, 0.68, and 0.71 kg/d per unit increase in log horn fly count in May, June and July. However, the regression coefficients for milk yield in August and September were positive, while the regression coefficient for October was negative.

Milk Quality

Milk lactose and SCC were not affected ($P > 0.25$) by horn flies. However, for every one unit increase in log horn fly count percent milk fat decreased by 0.15% ($P < 0.05$) and percent SNF decreased by 0.10% ($P < 0.05$). Milk urea nitrogen also decreased by 0.62 mg/dL ($P < 0.02$) for every one unit increase in log horn fly count. In HERF sired cows percent milk protein decreased by 0.15% ($P < 0.01$) per unit increase in log horn fly count, with other sire breeds not affected.

Serum Prolactin

The regression of log horn fly count on PRL was not affected ($P > 0.25$) by sire breed. An interaction of PRL and sire breed affected ($P < 0.10$) milk yield (Figure 6). The regression coefficients for all sire breeds except BONS and GELV sired cows were negative, indicating a reduction in milk yield.

Discussion

Variation among breed types and horn fly populations have been reported in the literature, as well as variation among breed types and milk production traits. However, the evaluation of breed type and horn flies and their impact on milk production in beef cows is lacking. The evaluation of this combination and its impact on milk production may be an important contribution to the already existing literature. Monthly horn fly population increase in warmer month(s) compared to cooler months, based on the weather conditions of the geographic location data is collected. Results of this study determined horn fly population followed this trend; increasing in the warmer months and decreasing in cooler months. However, unlike other studies (Tugwell et al., 1969; Brown et al., 1994; and Steelman et al., 1997) our results indicated horn fly populations did not differ between sire breeds. Therefore, the combination of sire breed and the effects of horn flies on milk quality and quantity traits may provide a better understanding of the impact of these variables on beef cow milk production traits.

Milk yield was affected by sire breed in this study, but these results differ compared to that of Brown and Lalman (2010). Romosinuano sired cows had decreased milk yield compared to BONS, BRAN, CHAR, GELV, and HERF sired cows ($P < 0.05$; Brown and Lalman, 2010). However, these same sire breeds were assessed in our study and provided different results, with BONS sired cows having the greatest milk yield compared to other sire breeds. However, the current study is based on one lactation cycle whereas the results of Brown and Lalman (2010) are based on multiple years.

Our study also evaluated the relationship between milk yield and serum PRL concentrations. Milk yield of GELV and BONS sired cows were the only sire breeds which milk yield was not negatively affected by this relationship. Therefore, milk yield may be affected not only by sire breed but also by the synthesis and release of serum PRL, which may vary among individual animals.

The interaction between horn flies and sire breed may also contribute to the differences observed in milk yield in this study. Milk yield was more negatively impacted by horn flies in BONS and GELV sired cows than other sire breeds. Perhaps the greater milk yield observed for these two sire breeds was more negatively impacted by the induced stress caused by horn flies. Hereford sired cows produced the lowest milk yield of all the sire breeds used in this study but were not as negatively impacted by horn flies. Although our results indicate horn flies negatively impact milk yield and this impact is dependent upon sire breed the results from other studies conflict; reporting horn flies may or may not affect milk yield of cows treated or not treated for horn flies (Cheng and Kessler, 1961; Miller et. al., 1973; Morgan and Bailie, 1980; and Block and Lewis, 1986).

Horn flies also negatively impacted milk quality traits; including milk fat, SNF, and urea nitrogen. However, percent milk protein was only affected by horn flies in HERF sired cows. Surprisingly SCC, which is often an indicator of infection or mastitis, was not affected by horn flies. Block and Lewis (1986) reported milk fat and milk protein percentage were not affected ($P > 0.05$) by horn fly treatment or lack of treatment in Holstein cows. Again, there is variation in our results and those of other studies. Explanation of this variation may be other studies utilization of dairy breeds, environmental differences, or geographic location. Overall, our results indicate the effects of horn flies on milk quantity and quality of beef cows used in this study may be dependent upon sire breed.

An additional variable beyond sire breed that should be consider is period of lactation. In this study horn flies had a greater impact on milk production at the beginning of lactation. Initiation of lactation is stressful for the animal, having just given birth and then preparing the body to breed back. The added stress induced by the feeding habits of horn flies in combination with the stress of beginning the lactation cycle may explain the greater reduction in milk yield at the initiation of lactation that we observed. Further research regarding the effects of horn flies on milk production traits of beef cows is needed to confirm and elaborate on the findings of this study. Utilization of different breed types as well as variation in horn fly treatments also would be beneficial in future research.

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Table 1. Regression Coefficients and Standard Errors of Milk Yield on Log Horn Fly Count by Sire Breed.

	Sire Breed					
	BONS	BRAN	CHAR	GELV	HERF	ROMO
Estimate	-0.64^{ab}	0.30^{c}	-0.026^{bc}	-0.99^{a}	-0.17^{bc}	-0.16^{bc}
SE	0.30	0.26	0.22	0.32	.20	0.17

^{a,b,c,d} Values without common superscripts differ ($P < 0.05$).

SE = Standard Errors, BONS = Bonsmara, BRAN = Brangus, CHAR = Charolais, GELV = Gelvbieh, HERF = Hereford, and ROMO = Romosinuano.

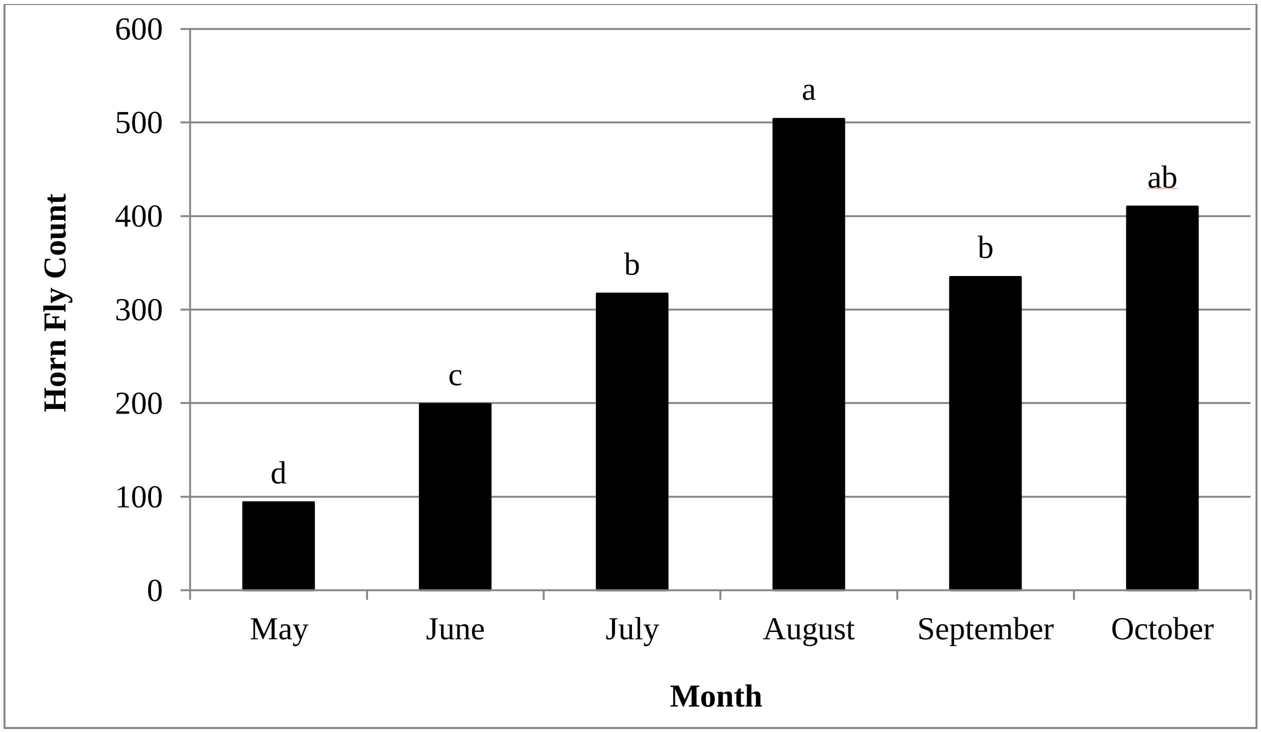

Figure 1. Monthly Horn Fly Count. (*P* < 0.0001; SEM = 37.87). SEM = Standard Error Means. ^{a,b,c,d} Bars without common superscripts differ.

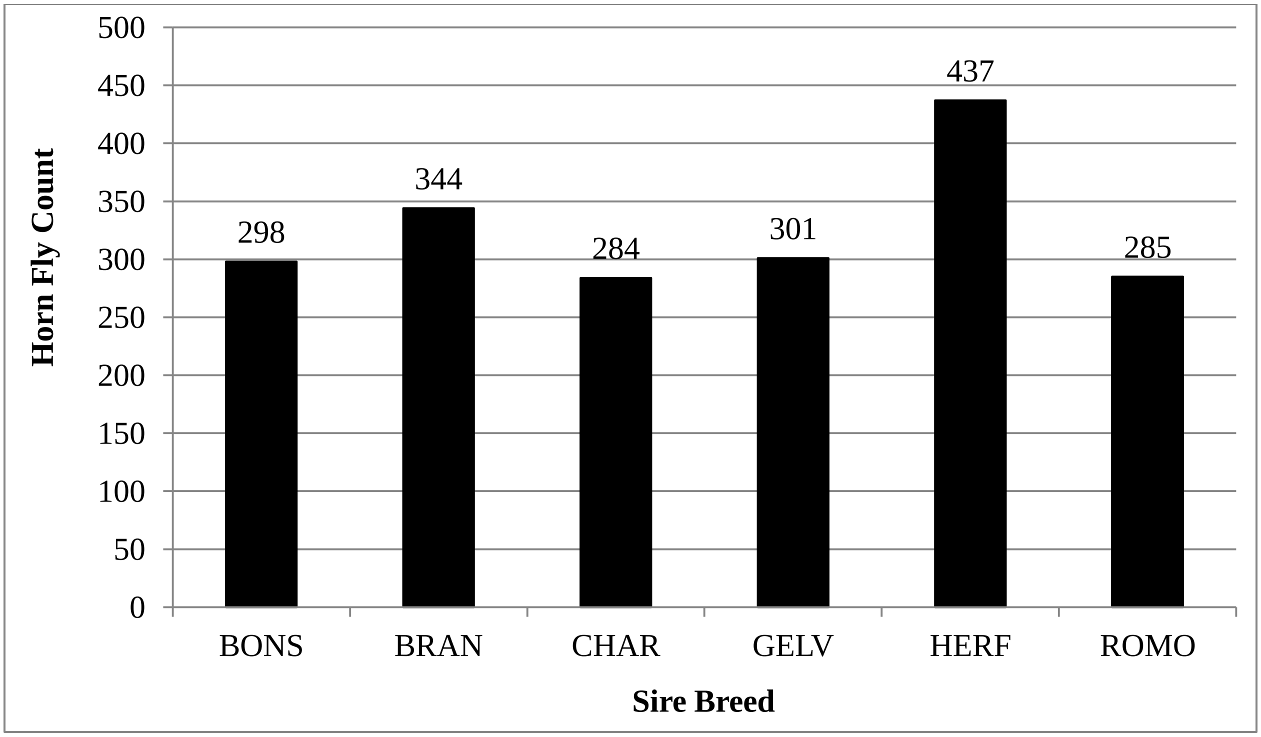

Figure 2. Horn Fly Counts of Sire Breeds. (*P* > 0.25; SEM = 59.42). SEM = Standard Error Means.

BONS = Bonsmara, BRAN = Brangus, CHAR = Charolais, GELV = Gelvbieh, HERF = Hereford, and ROMO = Romosinuano.

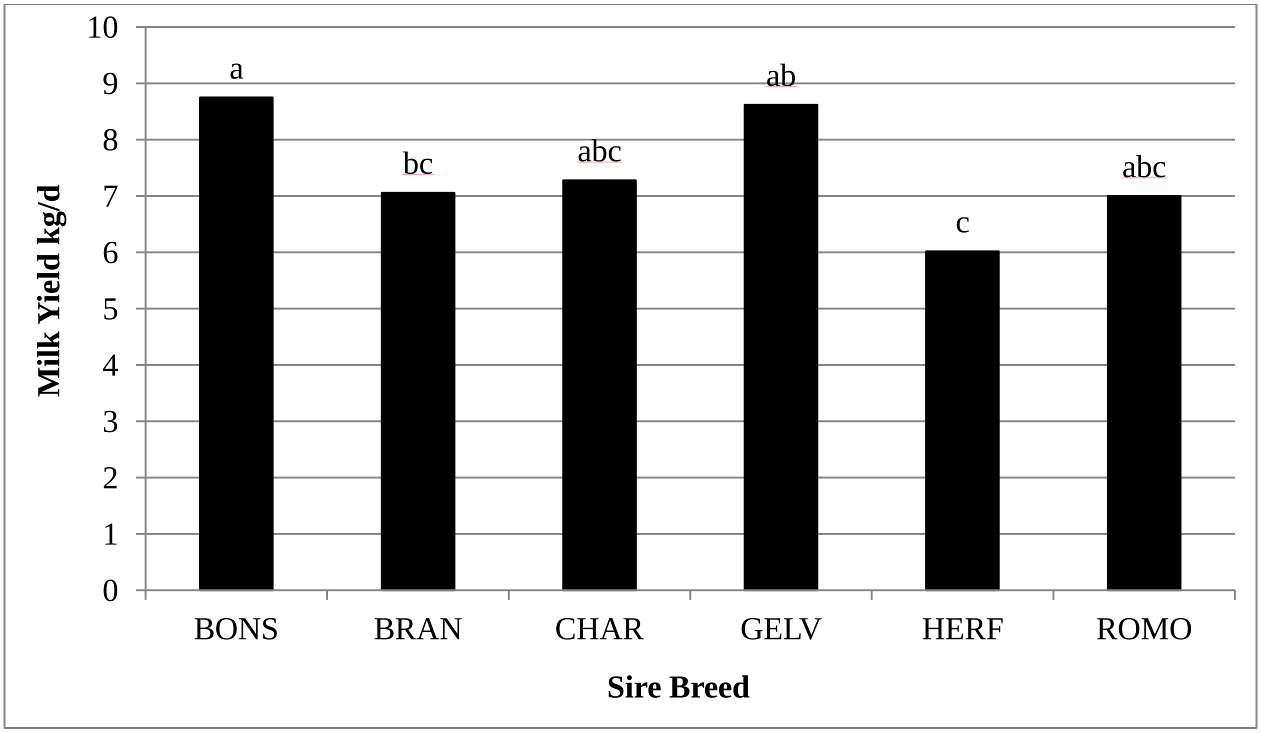

Figure 3. Average Milk Yield of Sire Breeds. (*P* < 0.05; SEM = 0.61). SEM = Standard Error Means. ^{a,b,c} Bars without common superscripts differ.
BONS = Bonsmara, BRAN = Brangus, CHAR = Charolais, GELV = Gelvbieh, HERF = Hereford, and ROMO = Romosinuano.

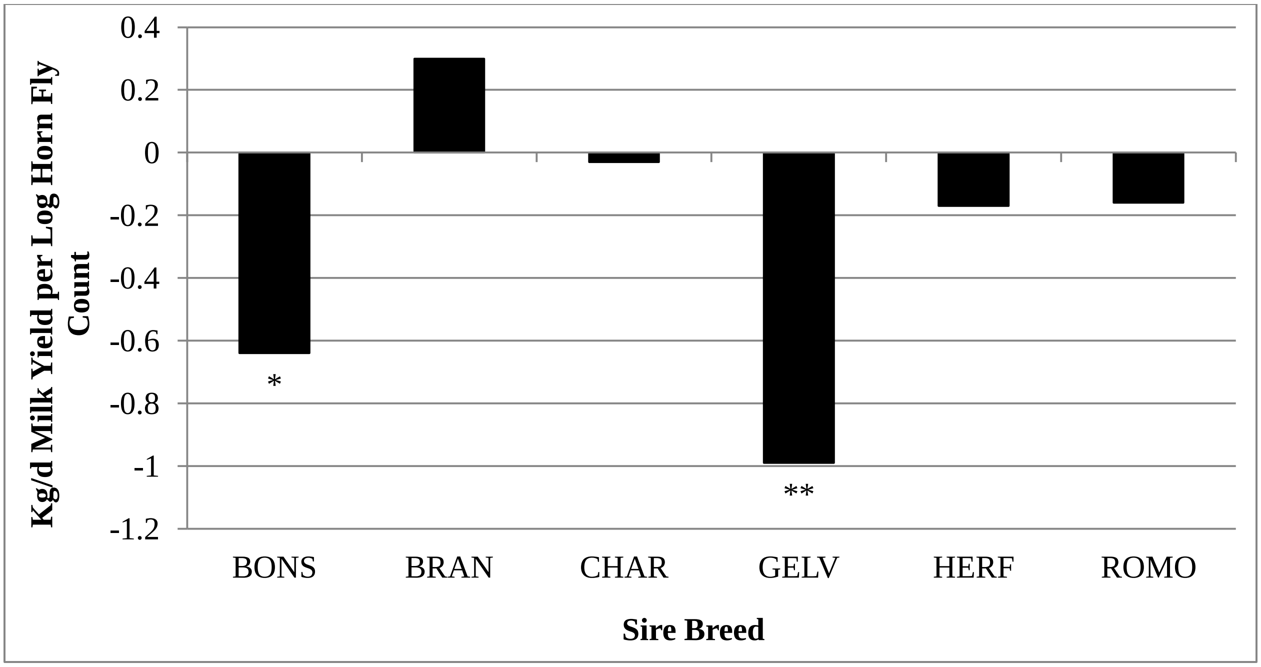

Figure 4. Regression of Milk Yield on Log Horn Fly Count by Sire Breed. ($^{**}P < 0.01$; $^{*}P < 0.05$; SEM = 0.25). SEM = Standard Error Means.
BONS = Bonsmara, BRAN = Brangus, CHAR = Charolais, GELV = Gelvbieh, HERF = Hereford, and ROMO = Romosinuano.

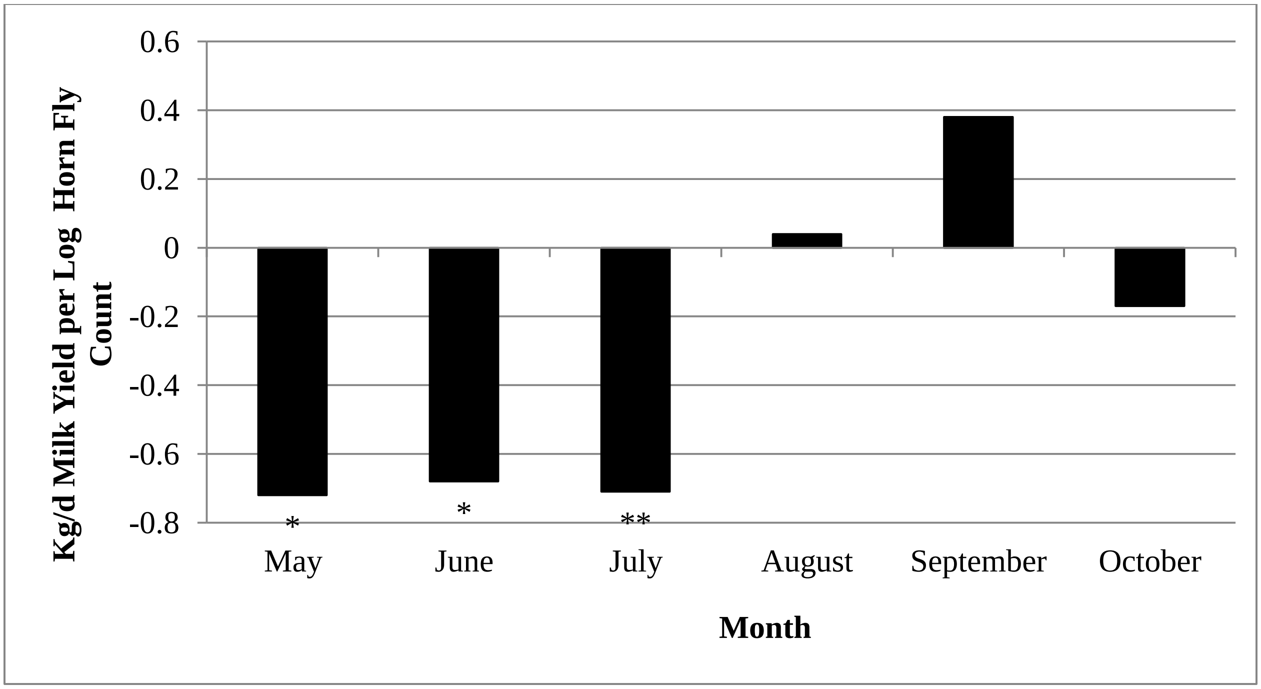

Figure 5. Regression of Milk Yield on Log Horn Fly Count by Month. ($^{**}P < 0.001$; $^{*}P < 0.05$; SEM = 0.32). SEM = Standard Error Means.

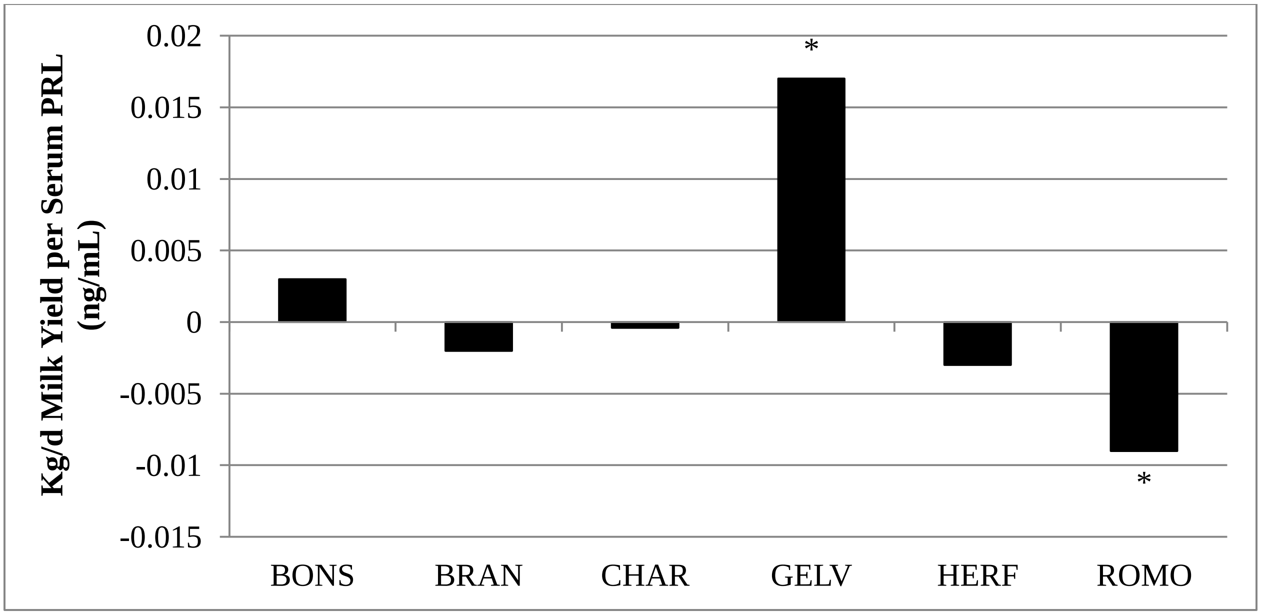

Figure 6. Regression of Milk Yield on Serum Prolactin by Sire Breed. ($^{*}P < 0.05$; SEM = 0.004). SEM = Standard Error Means, PRL = Prolactin concentrations (ng/mL), BONS = Bonsmara, BRAN = Brangus, CHAR = Charolais, GELV = Gelvbieh, HERF = Hereford, and ROMO = Romosinuano.

CHAPTER III
THE EEFCTS OF HORN FLIES AND SIRE BREED OF DAM ON CALF PREWEANING AND POSTWEANING PERFORMANCE TRAITS

Abstract

Weaning weight (WW) and average daily gain (ADG) of beef cattle can be negatively impacted by horn fly infestation. The effects of horn flies on preweaning and postweaning traits of beef calves are less documented. Therefore, the objective of this study was to assess the indirect impact of horn flies on calf performance traits. Angus sired calves (n = 51) from crossbred cows (n = 53) from Brangus dams sired by either Bonsmara (BONS; n = 7), Brangus (BRAN; n = 13), Charolais (CHAR; n = 8), Gelbvieh (GELV; n = 5), Hereford (HERF; n = 12), or Romosinuano (ROMO; n = 8) bulls were evaluated in this study. Total horn fly counts were recorded on individual cows while in pasture from 0700 to 0900 h beginning in May and ending in October by the same trained individual throughout the study. Horn fly counts were transformed to natural log horn fly count prior to analysis. Data for preweaning ADG, postweaning ADG, 365-d adjusted yearling weight (YWT), and birth to yearling ADG were analyzed by mixed model lease squares. The linear models included sire breed, calf gender, and sire breed x calf gender. Effects of horn fly count on these traits were estimated by including a linear covariate of log horn fly count and log horn fly count x sire breed. Preweaning ADG was affected ($P < 0.002$) by sire breed. Romosinuano, BONS, and CHAR calves had greater preweaning ADG (1.00 ± 0.05, 0.99 ± 0.04, 0.99 ± 0.04 kg/d; respectively) compared to BRAN and HERF calves (0.88 ± 0.03 and 0.81 ± 0.03 kg/d), with GELV calves intermediate to ROMO, BONS, CHAR and BRAN (0.98 ± 0.05 kg/day). Preweaning ADG depended on an interaction of cow sire breed and log horn fly count ($P < 0.10$), with results indicating preweaning ADG reduced by 0.19 kg/d per unit increase in log horn fly count in BONS calves ($P < 0.05$). A one unit increase in log horn fly count resulted in

0.07 kg/d ($P < 0.10$) increase in postweaning ADG, 19.52 kg increase ($P < 0.10$) in 365-d adjusted YWT, and 0.05 kg/d ($P < 0.02$) increase in birth to yearling ADG. Calf gender had an effect on postweaning ADG, 365-d adjusted YWT, and birth to yearling ADG, with gain of bull calves greater than heifer calves (0.99 ± 0.02 and 0.91 ± 0.02 kg/d; $P < 0.01$). Horn flies negatively affected preweaning performance of calves from certain cow sire breeds, but horn flies positively affected calf postweaning performance traits. Postweaning management and compensatory gain may explain the results found in this study, but further investigation of the indirect effects of horn flies on calf performance traits is needed.

Introduction

Horn flies (*Haematobia irritans*) negatively impact cattle production traits such as average daily gain (ADG), weaning weight (WW), and milk production (Steelman et al., 1991; Block and Lewis, 1986; and DeRouen et al., 2003). The primary host of the horn fly is pastured cattle, with horn flies consuming approximately 20-40 blood meals per day from their host (Arther, 1991). Weight loss in cattle is attributed to the loss of feeding time and increased energy expenditure spent avoiding and repelling horn flies (Harvey and Launchbaugh, 1982; Weimann et al., 1992). However, the indirect effect of the energy expenditure caused by the avoidance and repelling behavior displayed by the cows may also impact calf performance traits. Although the economic impact of horn flies varies (Drummond et al., 1987; Kunz et al., 1991) production traits are negatively impacted when horn fly levels reach economic threshold (> 200 flies per animal; Haufe, 1979; Schreiber et al., 1987; and Foil and Hogsette, 1994).

However, variability among cattle regarding the impact of horn flies on production traits has been observed and described in the literature. Breed is often associated with an animal’s designation as either horn fly susceptible or horn fly resistant (Tugwell et al., 1969; Brown et al., 1994; and Steelman et al., 1997). Another factor contributing to the variability observed in horn fly populations is increased resistance to insecticides (Quinsberry et al., 1984; Arther, 1991). Cattle production losses due to horn flies cannot and will not be adequately controlled until their effects on all aspects of cattle production are fully understood. Therefore, due to the lack of data existing regarding the effects of horn flies on calf performance traits the objective of this study was to evaluate the indirect effects of horn flies on calf performance traits pre and postweaning.

Materials and Methods

The Committee for Animal Welfare at the USDA-ARS, Grazinglands Research Laboratory El Reno, OK and the University of Arkansas Institutional Animal Care and Use Committee approved the procedures used in this study.

Horn Fly Counts

Total horn fly populations were recorded on individual animals while in pasture from 0700 through 0900 hours (h), every 28 days beginning in May and ending in October. Individual animals were observed by a trained individual throughout the study from a motorized vehicle or on foot, utilizing binoculars for accurate counts if animals were greater than 5 meters (m) away. Horn flies were treated when populations exceeded threshold levels (> 200 flies/animal). This resulted in monthly treatment using Co-Ral (organophosphate), which occurred after horn fly counts were recorded. Treatment of horn fly populations beyond threshold levels ensured the animal’s health and well being requirements were met and properly maintained according to IUCAC protocols.

Animal Information

Brangus cows were bred by AI and natural service to randomly selected sires of Bonsmara (BONS; n=4), Brangus (BRAN; n = 8), Charolais (CHAR; n = 7), Gelbvieh (GELV; n = 5), Hereford (HERF; n = 6), and Romosinuano (ROMO; n = 6) breeds to produce the cows used in this study. Cows (n = 53) utilized consisted of BONS (n = 7), BRAN (n = 13), CHAR (n = 8), GELV (n = 5), HERF (n = 12), and ROMO (n = 8) breed types and were maintained on native rangeland throughout the study. Cows were bred the spring and summer prior to the study so as to calve the following spring when the study began.

Calves were sired by Angus bulls using natural service between June and July. Angus bulls were selected based on visual appraisal for soundness and conformation and breeding soundness as determined by a breeding soundness exam. Birth weight (BW) of calves was determined within 24 h of birth and bull calves were castrated by elastration at this time. Calves were weaned in fall at an average age of 205 d when body weight was also determined. Weaning weights (WW) were collected and calves were then maintained on wheat pasture throughout the winter. Yearling weight (YW) was determined at an average age of 365 d.

Statistical Analysis

Horn fly counts were transformed to natural log horn fly count prior to analysis. Data for preweaning average daily gain (ADG), postweaning ADG, birth to yearling ADG, and 365-d adjusted yearling weight (YWT) were analyzed by mixed model lease squares (SAS Institute, Cary, NC). The linear models included cow sire breed (fixed), calf gender (fixed, sire breed x calf gender (fixed), and a random residual effect. Effects of horn fly count were estimated by including the linear covariate of log horn fly count and log horn fly count x sire breed into the model.

Results

Log horn Fly Counts

Preweaning ADG was not affected by log horn fly count ($P > 0.10$). However, a one unit increase in log horn fly count resulted in 0.07 kg/d ($P < 0.10$) increase in postweaning ADG. A one unit increase in log horn fly count had similar effects on 365-d adjusted YWT, where 19.52 kg increase ($P < 0.10$) occurred for every one unit increase in log horn fly count. Birth to yearling ADG of calves also increased 0.05 kg/d ($P < 0.02$) for every one unit increase in log horn fly count.

Gender

Calf gender had an effect on postweaning ADG, 365-d adjusted YWT, and birth to yearling ADG, with bull calves gaining more than heifer calves (0.99 ± 0.02 and 0.91 ± 0.02 kg/d; $P < 0.01$; Figure 1.). However calf gender did not affect preweaning ADG ($P > 0.25$).

Preweaning ADG

Preweaning ADG was affected ($P < 0.002$) by cow sire breed. Romosinuano, BONS, and CHAR calves had greater preweaning ADG (1.00 ± 0.05, 0.99 ± 0.04, 0.99 ± 0.04 kg/d; respectively) compared with BRAN and HERF calves (0.88 ± 0.03 and 0.81 ± 0.03 kg/d), while GELV calves were intermediate to ROMO, BONS, CHAR, and BRAN calves (0.98 ± 0.05 kg/day; Figure 2.).

Preweaning ADG depended ($P < 0.10$) on an interaction of cow sire breed and log horn fly count, with results indicating preweaning ADG reduced by 0.19 kg/d per one unit increase in log horn fly count in BONS calves ($P < 0.05$), but this same reduction was not observed in other calves (Figure 3.).

Discussion

The effects of horn flies on ADG and total weight gain of cattle has been well documented (Haufe, 1982; Brown et al., 1994; DeRouen et al., 2003; and Sanson et al., 2003). Weaning weight of calves also has been reported to be negatively impacted by horn flies (Campbell, 1976; Kunz et al. 1984; and Cocke et al., 1989). Steelman et al. (1991) reported a linear regression coefficient of 8.1 kg/100 flies ($P = 0.0334$) when weaning weight was regressed on horn fly count.

The results of this study demonstrated that the indirect affect of horn flies negatively impacted preweaning ADG of calves. However, postweaning ADG, 365-d adjusted YWT, and birth to yearling ADG of calves was not negatively affected by horn flies. These production traits instead increased as horn fly populations increased. Gerhardt and Shrode (1990) reported similar results when calves from cows not treated for horn flies had increased weight gains compared to calves from treated cows. This study and ours demonstrate that breed, calf gender, and genetics may play a key role in better understanding the indirect impact of horn flies on calf performance traits. However, postweaning nutrition and management may also be responsible for the results demonstrated in this study.

Despite the negative impact of horn flies on preweaning ADG of the calves in this study all postweaning traits evaluated were positively affected by horn flies. All calves were managed on native rangeland pastures preweaning and wheat pastures postweaning. Preweaning forage has been demonstrated to influence postweaning performance (Brown et al., 2008). In this study perhaps preweaning forage as well as horn fly impact contributed to the negative preweaning gains observed. However, postweaning forage may be a contributing factor to the improved postweaning performance traits reported, and may have enabled the calves to demonstrate compensatory gains. Phillips et al. (2000) determined calves wintered on wheat pasture not only gained faster ($P < 0.01$) but also had heavier ($P < 0.01$) final weights compared to calves wintered on native pastures. Preweaning pasture and horn flies may have contributed to the growth restriction observed in the calves. Therefore, once the growth restrictions were removed growth efficiency improved (Hornick et al., 1998).

A combination of breed type, genetics, and postweaning nutrition may be responsible for the results reported in this study. Horn flies contributed to the decreased preweaning ADG observed but were not responsible for negative affects postweaning. Therefore, perhaps the removal of the calves from the indirect effects of horn flies and their transfer to wheat pastures postweaning were responsible for the compensatory gains observed in this study. Future studies should focus on the effects of horn flies on postweaning traits and different management systems postweaning to evaluate if compensatory gains are in fact occurring.

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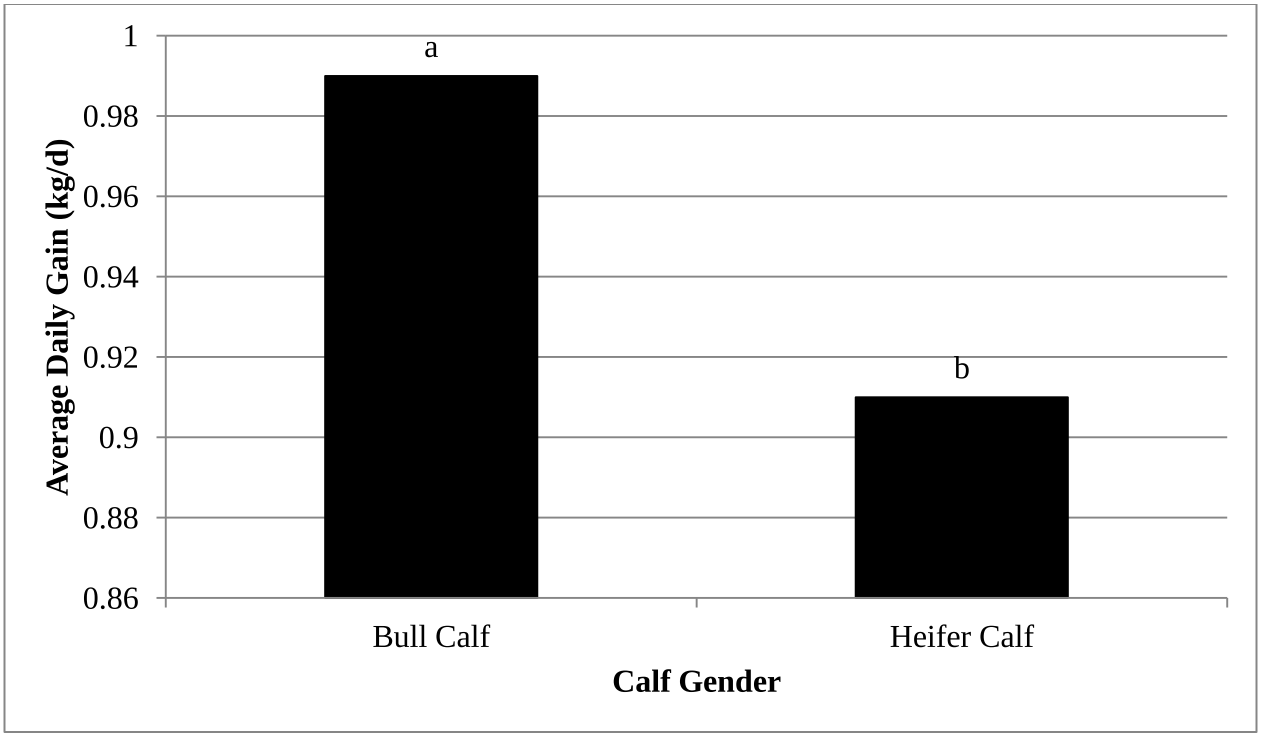

Figure 1. Effects of Horn Flies on Average Daily Gain Calves. ($P < 0.01$; SEM = 0.02). SEM = Standard Error Means. ^{a,b} Bars without common superscripts differ.

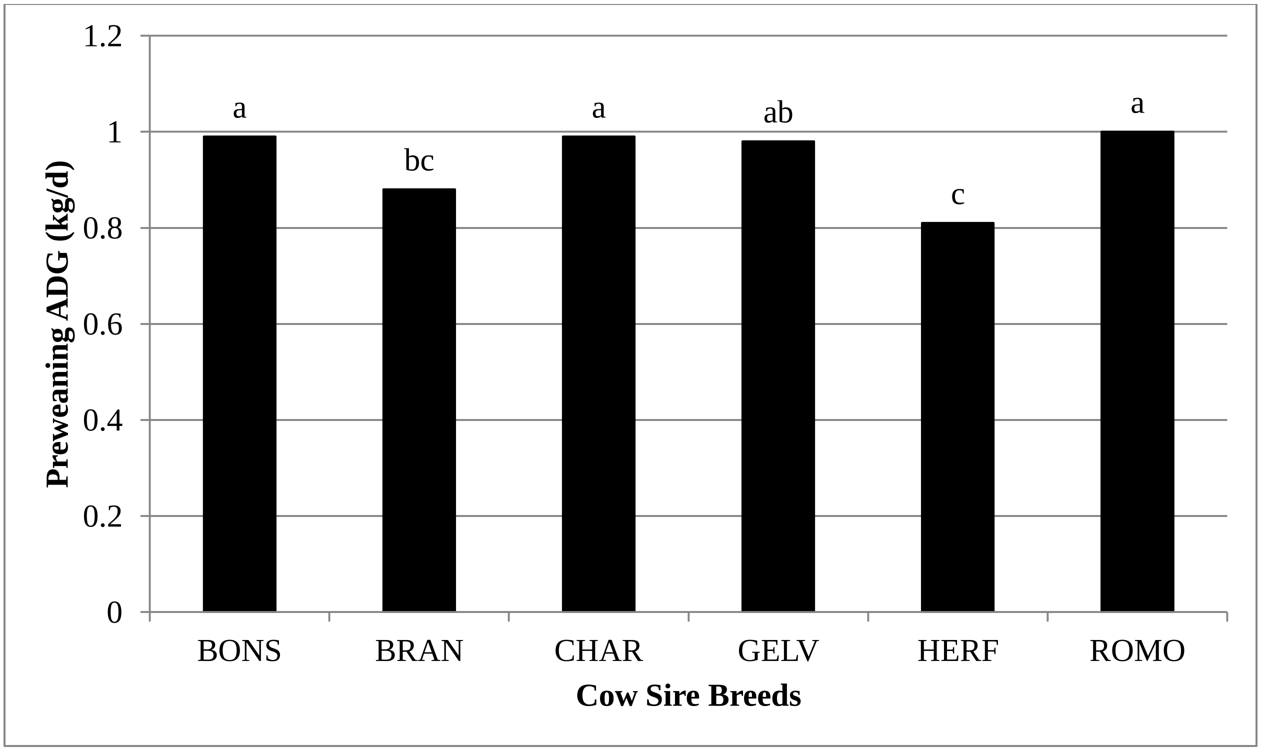

Figure 2. Effects of Sire Breed of Cow on Preweaning Average Daily Gain (ADG) of Calves. (*P* < 0.002; SEM = 0.04). SEM = Standard Error Means. ^{a,b,c,d} Bars without common superscripts differ.

BONS = Bonsmara, BRAN = Brangus, CHAR = Charolais, GELV = Gelvbieh, HERF = Hereford, and ROMO = Romosinuano.

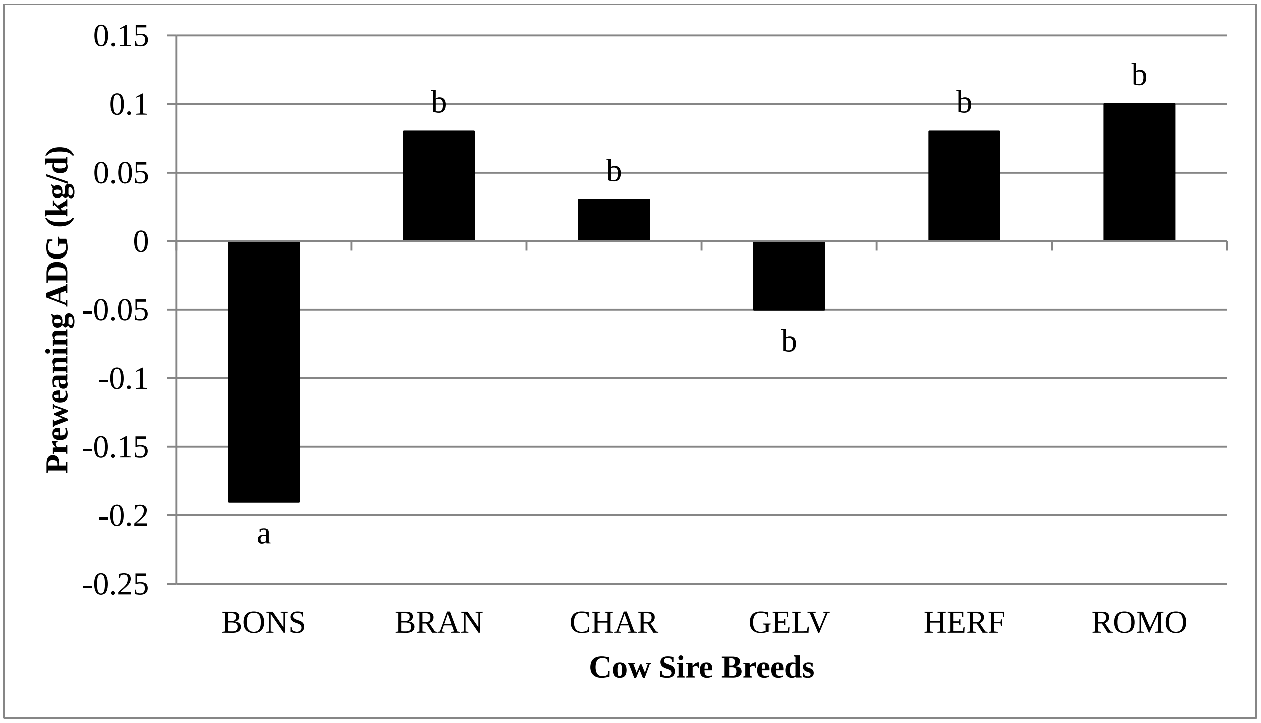

Figure 3. Interaction of Cow Sire Breed and Log Horn Fly Count on Preweaning Average Daily Gain (ADG) of Calves. (*P* < 0.10; SEM = 0.07). SEM = Standard Error Means. ^{a,b} Bars without common superscripts differ.
BONS = Bonsmara, BRAN = Brangus, CHAR = Charolais, GELV = Gelvbieh, HERF = Hereford, and ROMO = Romosinuano.

CHAPTER IV
EFFECT OF HORN FLY INFESTATION ON PASTURE BEHAVIOR AND TEMPERAMENT MEASUREMENTS OF BEEF COW CALF PAIRS

Abstract

Beef cattle profitability traits may be influenced by the effects of horn flies on pasture behavior. Therefore, the objective of this study was to determine if horn flies affected beef cattle pasture behavior and temperament measurements. Crossbred cows (n = 53) from Brangus dams were sired by either Bonsmara (n = 7), Brangus (n = 13), Charolais (n = 8), Gelbvieh (n = 5), Hereford (n = 12), or Romosinuano (n = 8) bulls; and their Angus-sired calves (n = 51) were utilized in this study. Pasture behavior of individual cows was recorded twice a day (AM and PM) along with total horn fly counts (AM only) beginning in May and ending in October. Cattle were observed and behavior was recorded as grazing, lying or standing. Exit velocity (EV) and chute score (CS) was obtained monthly for cows and calves, while serum cortisol (CORT) was determined monthly for cows only. Horn fly counts were transformed to natural log horn fly count prior to analysis. Pasture behavior and horn fly numbers were analyzed by mixed model least squares using a linear model that included sire breed, behavior (AM or PM), and month. The linear model for EV included sire breed, month, and sire breed x month. Effects of horn fly count on serum CORT concentrations were estimated by including a linear covariate of log horn fly count into the linear model of sire breed, month, and sire breed x month. Horn fly counts varied monthly ($P < 0.0001$), with the lowest counts occurring in May (99 ± 39 flies) and the highest count in August (520 ± 38 flies). Pasture behavior in the AM was not associated ($P > 0.25$) with horn fly counts; however PM pasture behavior was ($P < 0.05$). Cows observed grazing and lying had greater horn fly counts than cows observed standing in the afternoon (468 ± 52 and 419 ± 38 versus 319 ± 27 flies; respectively). Exit velocity of both cows ($P < 0.0001$)

and calves ($P < 0.05$) differed by month. Serum CORT concentrations decreased 0.017 ng/mL ($P < 0.01$) for every one unit increase in log horn fly count. Monthly horn fly count was also associated with serum CORT concentrations ($P < 0.0001$). Horn fly count appears to be associated with pasture behavior and temperament measurements of beef cattle in this study. The genetic and physiological mechanisms linking horn fly counts to cattle pasture behavior, temperament measurements, and profitability traits should continue to be the subject of future investigations.

Introduction

The economic impact of horn flies varies in relation to pasture characteristics, management techniques, breed types, and calving periods (Foil and Hogsette, 1994). Horn flies have been reported to feed on their host approximately 20-40 times per day and remain on their host throughout their lifecycle (Williams et al., 1985; Arther, 1991). Economic threshold levels greater than 200 flies per animal have consistently been reported to negatively impact cattle production traits (Haufe, 1979; Schreiber et al., 1987). However, data regarding the impact of horn flies on pasture behavior and temperament of beef cattle is limited.

To reduce pain, blood loss, and production losses caused by horn flies cattle often use a variety of behavioral techniques to repel or dislodge the biting insects (Mooring et al., 2007). Cattle infested with horn flies will use avoidance behaviors, grouping or bunching, and lying to aid in repelling horn flies. When cattle spend more time performing fly avoidance and repelling behaviors and less time grazing production traits can be negatively impacted.

Production traits may also be impacted by the animal’s temperament. A cattle’s temperament can be measured using various techniques such as chute score (CS), exit velocity

(EV) or flight speed, and cortisol (CORT) concentrations. Temperament of beef cattle can differ based on breed type (Stricklin et al., 1980; Gauly et al., 2001), with *Bos indicus* cattle described as more temperamental than *Bos taurus* (Voisinet et al., 1997). Stress during handling and stress caused by horn fly infestation may also contribute to negative production traits and temperament of beef cattle.

The effects of horn flies on cattle pasture behavior and temperament, and therefore indirectly production traits are not known. A better understanding of this relationship led to the objective of this study; evaluation of the effects of horn flies on pasture behavior and temperament of beef cattle.

Materials and Methods

The Committee for Animal Welfare at the USDA-ARS, Grazinglands Research Laboratory El Reno, OK and the University of Arkansas Institutional Animal Care and Use Committee approved the procedures used in this study.

Horn Fly Counts

Total horn fly populations were recorded on individual animals while in pasture from 0700 through 0900 hours (h), every 28 days beginning in May and ending in October. Individual animals were observed by a trained individual throughout the study from a motorized vehicle or on foot, utilizing binoculars for accurate counts if animals were greater than 5 meters (m) away. Horn flies were treated when populations exceeded threshold levels (> 200 flies/animal). This resulted in monthly treatment using Co-Ral (organophosphate), which occurred after horn fly counts were recorded. Treatment of horn fly populations beyond threshold levels ensured the animal’s health and well being requirements were met and properly maintained according to IUCAC protocols.

Environmental Temperature

Weather data was collected throughout the study period from the Oklahoma Mesonet Weather Service (El Reno, Oklahoma) located on the research site at the USDA-ARS Grazinglands Research Laboratory approximately 5 miles WNW of El Reno, Oklahoma (Longitude: 35˚ 32’54” N; Latitude: 98˚ 2’11” W). Temperature data was measured every 5 minutes, with the maximum and minimum temperature recorded daily. The average maximum temperature during the study period was 30.7°C, while the average minimum temperature was 19.2°C. Monthly average temperatures (AT), average maximum temperature (MAXT), and average minimum temperature (MINT) were also reported. May (AT: 19.9˚C, MAXT: 27.6˚C, and MINT: 7.3˚C), June (AT: 28.7˚C, MAXT: 32.4˚C, and MINT: 24˚C), July (AT: 31.7˚C, MAXT: 34.1˚C, and MINT: 29.8˚C), August (AT: 30.1˚C, MAXT: 34.9˚C, and MINT: 22.3˚C), September (AT: 20.8˚C, MAXT: 30.7˚C, and MINT: 12.8˚C), and October (AT: 19.2˚C, MAXT: 24.6˚C, and MINT: 14.8˚C).

Animal Information

Brangus cows were bred by AI and natural service to randomly selected sires of Bonsmara (BONS; n = 4), Brangus (BRAN; n = 8), Charolais (CHAR; n = 7), Gelbvieh (GELV; n = 5), Hereford (HERF; n = 6), and Romosinuano (ROMO; n = 6) breeds to produce the cows used in this study. Cows (n = 53) utilized consisted of BONS (n = 7), BRAN (n = 13), CHAR (n = 8), GELV (n = 5), HERF (n = 12), and ROMO (n = 8) breed types and were maintained on native rangeland throughout the study. Cows were bred the spring and summer prior to the study so as to calve the following spring when the study began.

Calves were sired by Angus bulls using natural service between June and July. Angus bulls were selected based on visual appraisal for soundness and conformation and breeding soundness as determined by a breeding soundness exam. Birth weight (BW) of calves was determined within 24 h of birth and bull calves were castrated by elastration at this time. Calves were weaned in fall at an average age of 205 d when body weight was also determined. Weaning weights (WW) were collected and calves were then maintained on wheat pasture throughout the winter. Yearling weight (YW) was determined at an average age of 365 d.

Blood Serum Hormone Analysis

Blood samples from cows were collected monthly beginning in May and ending in October via jugular venipuncture using vacutainers (Bectin Dickinson, Franklin Lakes, NJ). Samples were allowed to clot for 24 hrs at 4˚C and centrifuged at 2,500 x *g* for 25 min. (minute) at 4˚C (Marathon 22KBR, Fisher Scientific, Hermle-Labortechnik, Germany). Serum was then harvested and stored at -20˚C pending analysis.

Serum cortisol (CORT) concentrations were analyzed in duplicate with a solid-phase RIA using components of commercial kits (Siemens Diagnostic, Los Angeles, CA). This assay was performed without prior extraction of individual hormones from serum with the kits utilizing antibody-coated tube technology. Validation of the CORT assay utilizing ruminant serum is described by Kiyma et al. (2004); intra and interassay CV were less than 3% for CORT.

Temperament and Behavior

Pasture behavior was recorded twice a day between 0700 through 0900 h and 1300 through 1500 h on the same day horn fly counts were recorded. Cattle were observed and pasture behavior was recorded as grazing, lying or standing.

Chute scores (CS) and exit velocity (EV) were measurements used to determine temperament of cattle. Exit velocity and CS for the current study were obtained monthly for both cows and calves. Chute scores were based on a 1 – 4 scale (1 = calm no movement; 2 = restless shifting; 3 = squirming continuous shaking of the squeeze chute and 4 = rearing, twisting, continuous violent struggle), which was adopted from previously described procedures (Grandin, 1993). Exit velocity was defined as the rate at which the animal exited the squeeze chute and traversed 1.8 m (Curley et al., 2006). Two infrared sensors were used to record EV, (FarmTek Inc., North Wiley, TX) and it was recorded as time [distance(m)/(sec)].

Statistical Analysis

Horn fly counts were transformed to natural log horn fly count prior to analysis. Actual fly counts were used to report pasture behavior results due to non-transformed and transformed data results not differing. Pasture behavior and horn fly numbers were analyzed by mixed model least squares (SAS Institute, Cary, NC) using a linear model that included sire breed (fixed), time of day (fixed, either AM or PM), cow nested in sire breed (random), cow x time of day nested in sire breed (random), month (fixed repeated), and appropriate interactions. The linear model for EV excluded time of day effects. Effects of serum CORT concentrations on horn fly count were estimated by including a linear covariate of horn fly count into the linear model. Pearson correlations were calculated to assess the relationship between CS, EV, CORT, and log horn fly count. Temperamental measurements, CS, EV and CORT, were analyzed using log horn fly count in the models.

Results

Horn Flies

Horn fly counts of cows varied by month ($P < 0.0001$), with the lowest population recorded in May (99 ± 39 flies) and populations peaking in August (520 ± 38 flies; Figure 1). However, sire breed differences ($P > 0.25$) and a sire breed x month interaction ($P > 0.10$) did not occur.

Pasture Behavior

Pasture behavior in the AM was not associated ($P > 0.25$) with horn fly counts (Figure 2). However, pasture AM behavior numerically showed grazing cows to have more horn flies than standing or lying cows. Pasture PM behavior was associated ($P < 0.05$) with horn fly counts (Figure 3). Cows observed grazing and lying had greater horn fly counts than cows observed standing in the afternoon (468 ± 52 and 419 ± 38 versus 319 ± 27 flies; respectively).

Exit Velocity

Exit velocity of both cows ($P < 0.0001$) and calves ($P < 0.001$) differed by month. Cow EV was increased in October (1.79 ± 0.2 m/sec) compared to May, June, July and September (1.26 ± 0.2, 1.02 ± 0.2, 1.33 ± 0.2 and 1.40 ± 0.2 m/sec; respectively), with August (1.74 ± 0.2 m/sec) similar to September and October (Figure 4). Calf EV increased in June (0.76 ± 0.1 m/sec) compared to July, August, September and October (0.46 ± 0.1, 0.42 ± 0.1, 0.51 ± 0.1 and 0.41 ± 0.1 m/sec; respectively), while May was intermediate (0.59 ± 0.1 m/sec; Figure 5). Cow EV also was negatively correlated with serum CORT concentrations ($r = -0.12$; $P < 0.05$; Table 1.).

Chute Score

Cow CS was negatively correlated with log horn fly count ($r = -0.09$; $P < 0.10$) but was positively correlated with calf EV ($r = 0.13$; $P < 0.05$) and serum CORT concentrations ($r = 0.24$; $P < 0.01$; Table 1.). Cow CS was not however correlated with calf CS or cow EV. Calf CS was negatively correlated with log horn fly count (r = -0.05; *P* < 0.10) and calf EV (r = -0.15; *P* < 0.05), but was not correlated with cow CS or cow EV (Table 1.).

Serum Cortisol

Serum CORT concentrations decreased 0.017 ng/mL (*P* < 0.01) for every one unit increase in log horn fly count, which agrees with the negative correlation observed (r = -0.20; *P* < 0.01; Table 1.). Serum CORT concentrations differed by month (*P* < 0.0001). Cortisol concentrations were highest in May and September (24.30 ± 1.5 and 23.66 ± 1.5 ng/mL), reduced in August, June, and July (20.42 ± 1.5, 18.51 ± 1.5, and 16.64 ± 1.5 ng/mL; respectively), with October intermediate (22.93 ± 1.5 ng/mL) to May, September, and August (Figure 6.). Sire breed types showed no difference (*P* > 0.25) in serum CORT concentrations with and without log horn fly count as a linear covariate in them model.

Discussion

Host-seeking haematophagous insects, such as horn flies, often use visual cues (size and color), airborne chemical cues from the host (carbon dioxide, acetone, octenol), urine (phenols), feces, skin, and/or body heat to detect a host (Mooring et al., 2007). Therefore, it is necessary for animals to exhibit some form of avoidance behavior such as lying down while in pasture. The effectiveness of lying down has been reported as an ideal pasture behavior due to the reduced exposure of body surface and decreased production of carbon dioxide and sweat, all of which decrease the amount of flies attracted to the animal (Espmark et al., 1979).

Cows displayed no significant change in AM pasture behavior, but numerically cows lying had fewer horn flies than those standing or grazing. However, PM pasture behavior results showed cows standing had significantly reduced horn flies compared to those grazing or lying.

Standing cows have been reported to have warmer core body temperatures than lying cows ($P < 0.01$), with standing occurring more frequently in the afternoon ($P < 0.01$; Allen et al., 2013). These results indicate standing behavior may be associated with time of day and core body temperature of the animal. When considering these factors in relation to our study results perhaps an increase in core body temperature of the cows occurred in the afternoon; influencing PM pasture behavior. Increased core body temperature may be responsible for the reduction of horn fly numbers observed during the afternoon.

Human-animal interaction with pastured beef cattle is often limited due to the added stress put on the animal. However, evaluation of cattle temperaments under restraint or handling conditions can be used to describe the cattle's behavior (Burrow and Corbet, 2000). Exit velocity, a non-restraint technique, and CS, a restraint technique, are two measurements often used to evaluate cattle temperament. Cattle temperament can also differ due to breed and sex (Stricklin et al., 1980; Gauly et al., 2001), with *Bos indicus* cattle described as more temperamental than *Bos taurus* (Voisinet et al., 1997).

Acclimation of cattle to human handling has been used to prevent elevated concentrations of CORT in response to handling (Andrade et al., 200; Curley et al., 2006), as well as improve temperament (Cooke et al., 2009ab). Cow EV was slower, an indicator of a more docile temperament, in earlier months of the study compared to later months. Therefore, acclimation to human handling did not seem to occur in this group of cows, but instead cow EV indicated more agitated/aggressive temperaments as the study progressed. Although speculative, perhaps separation of calf from cow and milk production traits that were evaluated monthly may have contributed to the agitation observed in the animals as the study progressed. Another explanation may be the increase in horn fly numbers observed during later months of the study, and this more negatively influenced the cows' temperament. Calf EV was however increased during earlier months of the study and leveled out as the study progressed. These results indicate the calves became acclimated to human handling as the study progressed.

Cooke et al. (2009a) evaluated cattle temperament using temperament scores that consisted of calculating an average score from CS, pen score (PS) and EV. Results from this study reported human acclimation did not have an effect on heifer temperament scores (Cooke et al., 2009a). Another study conducted by Cooke et al. (2009b) reported no treatment effects on temperament measurements of CS ($P = 0.59$), EV ($P = 0.57$), or concentrations of CORT ($P = 0.88$). These results and ours suggest acclimation of cattle to human interaction does not always positively influence cattle temperament.

Cow CS was positively correlated with serum CORT concentrations and negatively correlated with cow EV. Curley et al. (2006) wanted to determine if temperament was repeatable and associated with CORT concentrations. On day 0 temperament measurements of CS and EV were positively correlated with one another ($r = 0.35$, $P < 0.05$) and EV also was positively correlated with CORT concentration ($r = 0.26$; $P < 0.05$; Curley et al., 2006). Day 120 no temperament measurements were correlated with one another, but CORT concentrations were positively correlated with EV ($r = 0.44$, $P < 0.01$; Curley et al., 2006). Our positive correlation between serum CORT concentrations and cow CS and negative correlation between serum CORT concentrations and cow EV do not agree with the results of Curley et al. (2006). Therefore, it can be concluded that correlation of temperament measurements with serum CORT concentrations vary. The synthesis and release of CORT varies depending on the individual animal's response to stressful events and environmental impact, and therefore may be the variable responsible for the variation in results of these studies..

It is known that CORT is often released during exposure to stressful situations or in stressful environments. For example, cattle, sheep and goats subject to painful procedures such as castrations have been reported to have increased CORT concentrations (Mellor, 1991; Fischer et al., 1996; and Fisher et al., 1997). Separation from herd mates, mixing with unfamiliar animals, restraint, and transportation are other factors that lead to increased CORT concentrations (Kent and Ewbank, 1983; Boissy and Le Neindre, 1997). Horn flies have a negative impact on cattle production traits and behavior, which may be due to the increased stress caused by their feeding habits.

Increased blood CORT concentrations have been reported in beef steers exposed to horn flies (Schwinghammer et al., 1986), while heifers had no change in serum CORT concentrations when exposed to horn flies (Riley et al., 1994). Crossbred beef steers exposed to varying levels of horn flies (0, 75, 150 and 225 flies) had serum CORT concentrations decrease ($P < 0.11$) when exposed to 150 and 225 flies as compared to steers exposed to 0 and 75 flies (Presley et al. 1996). Our research demonstrates serum CORT concentrations were negatively correlated with horn fly infestation, and that serum CORT concentrations decreased per unit increase in log horn fly count. Our results suggest the stress response may differ depending on impact and severity perceived by the animal, if the event is acute or chronic, and the animal's synthesis of CORT.

Cortisol levels are reported to differ among species, but evidence is limited regarding the variation in the release rate of CORT depending upon severity of the situation or stimulus (Mormède and Terenina, 2012). Plasma CORT concentrations have been reported to decline after the acute response, but CORT concentrations are not very informative when trying to detect chronic stress, (Mormède et al., 2007). Monthly serum CORT concentrations differed in our study, with one explanation being the acute stress caused by an EF-5 tornado. The tornado passed through the study location the day before blood samples were collected in May, and perhaps the increased CORT concentrations observed in May were correlated with the stress caused by this event. However, horn fly induced stress would be considered chronic due to lasting the duration of the fly season (May – September/October). However, CORT concentrations are not a good indicator of chronic stress (Mormède et al., 2007). Therefore, results from this study indicate CORT concentrations are not a good biological measurement when evaluating the chronic stress induced by horn flies.

Overall, results from this study evaluated the effects of horn flies on temperament measurements and serum CORT concentrations. Results conclude that temperament measurements vary depending on stress factors beyond that of human handling. Also, serum CORT concentrations may not be a useful indicator of chronic stress induced by horn flies.

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Table 1. Pearson Correlation Coefficients of Temperament Measurements and Log Horn Fly Count.

	Log Horn Fly Count	Cow CS	Calf CS	Cow EV	Calf EV
Cortisol	-0.20^{**}	0.24^{**}	$0.11^{\dagger}$	-0.12^{*}	0.03
Log Horn Fly Count	1.00	$-0.09^{\dagger}$	$-0.05^{\dagger}$	-0.02	-0.01
Cow CS	$-0.09^{\dagger}$	1.00	-0.007	-0.04	0.13^{*}
Calf CS	$-0.05^{\dagger}$	-0.007	1.00	0.04	-0.15^{*}
Cow EV	-0.02	-0.04	0.04	1.00	0.06

($^{**}P < 0.01$; $^{*}P < 0.05$; $^{\dagger}P < 0.10$).

Cortisol (ng/mL)

CS = Chute Score (1 = calm no movement; 2 = restless shifting; 3 = squirming continuous shaking of the squeeze chute and 4 = rearing, twisting, continuous violent struggle).

EV = Exit Velocity (m/sec; rate at which the animal exits the squeeze chute and traverses 1.8 m).

Log Horn Fly Count = log base 10 transformation of actual fly counts.

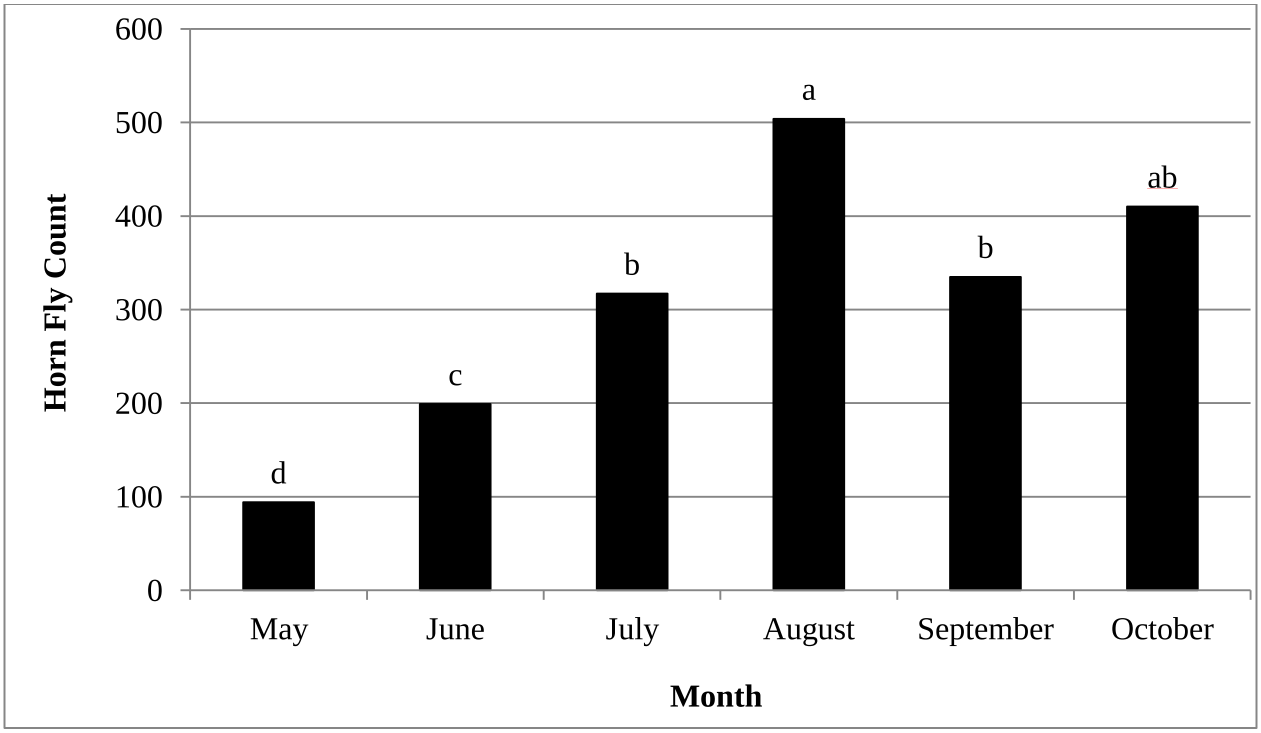

Figure 1. Monthly Horn Fly Count. ($P < 0.0001$; SEM = 37.87).
SEM = Standard Error Means. ^{a,b,c,d} Bars without common superscripts differ.

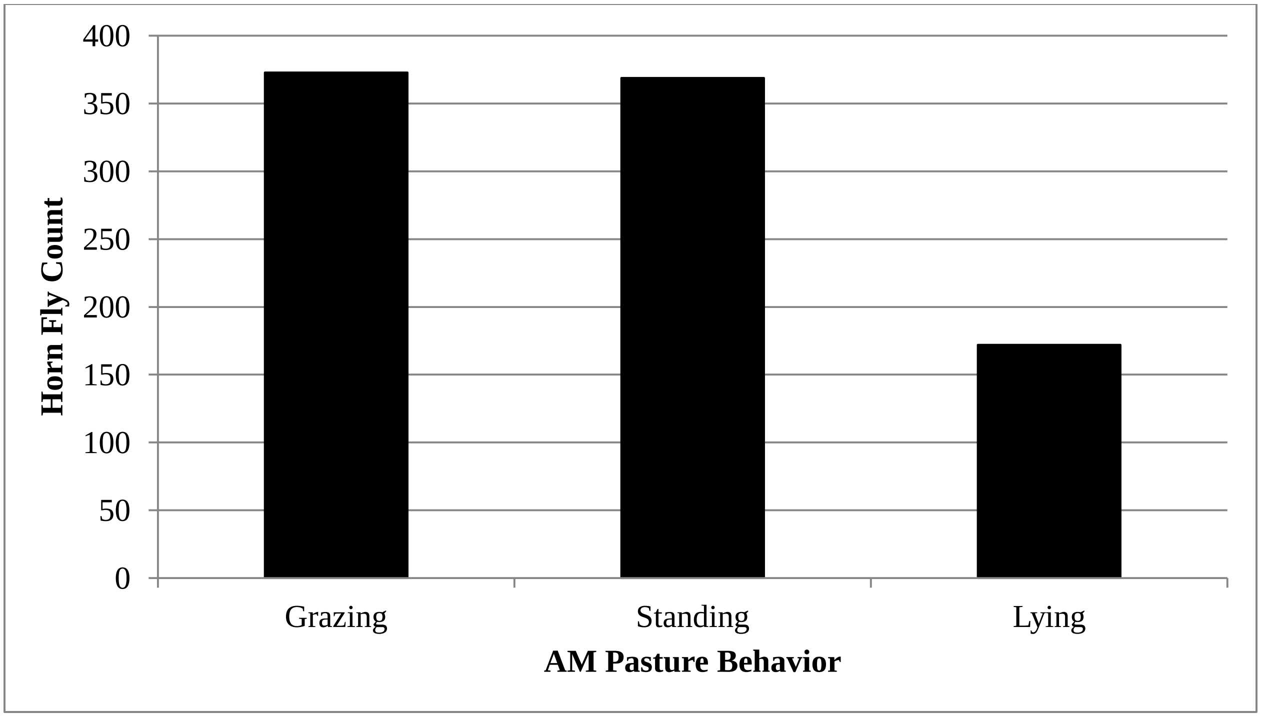

Figure 2. Horn Fly Counts of AM Pasture Behavior. (*P* > 0.25; SEM = 77.84).
SEM = Standard Error Means.

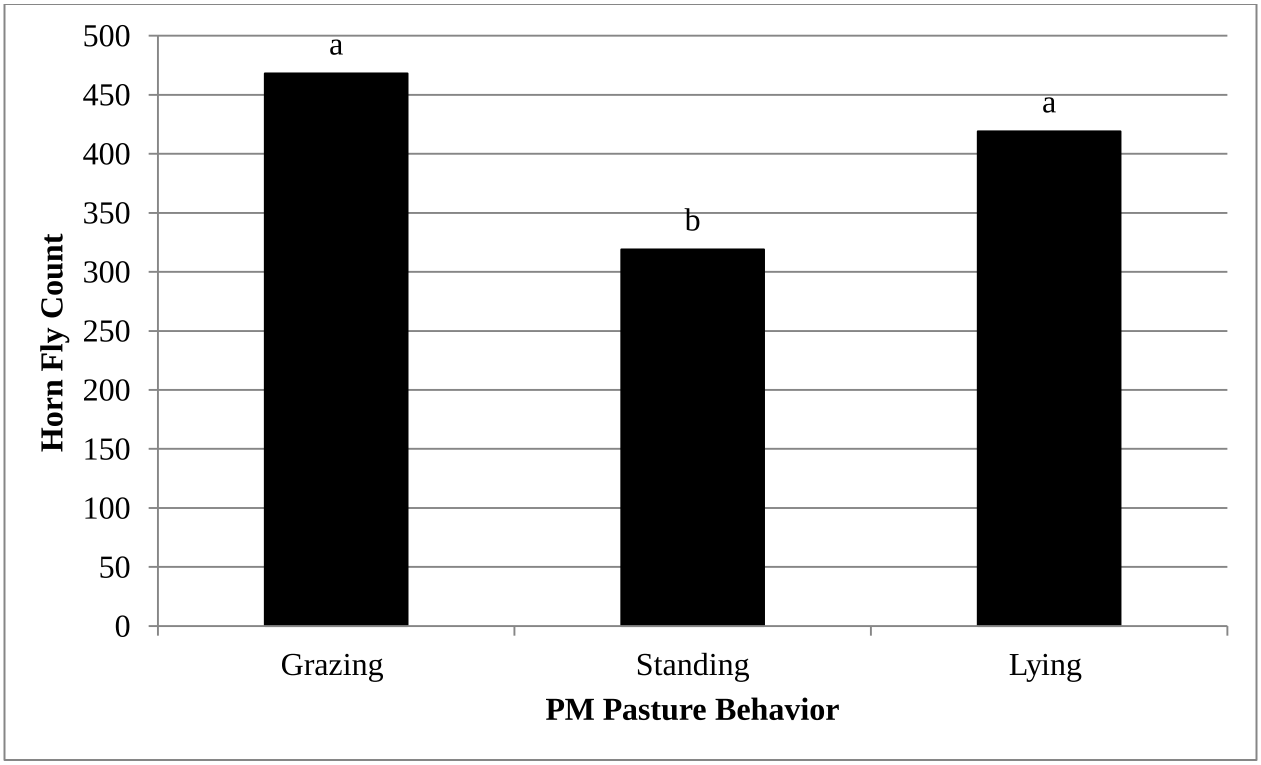

Figure 3. Horn Fly Counts of PM Pasture Behavior. ($P < 0.05$; SEM = 38.85). SEM = Standard Error Means. ^{a,b} Bars without common superscripts differ.

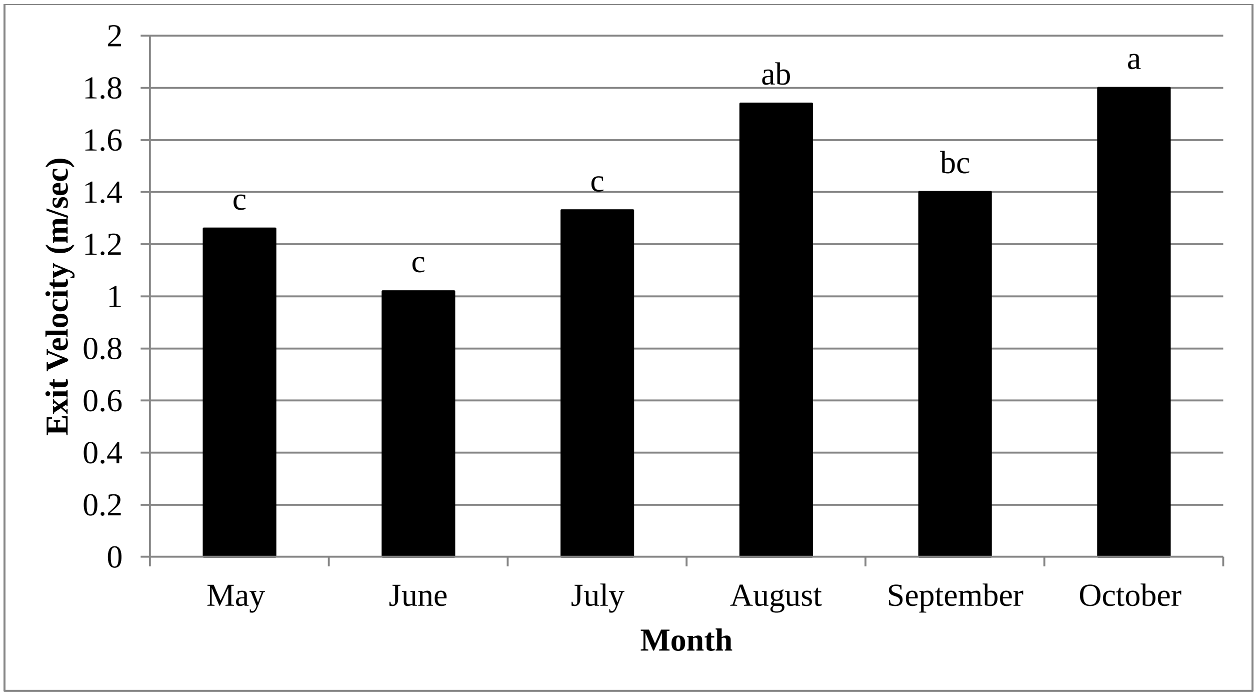

Figure 4. Monthly Exit Velocity of Cows. ($P < 0.001$; SEM = 0.18).
SEM = Standard Error Means. ^{a,b,c,d} Bars without common superscripts differ.

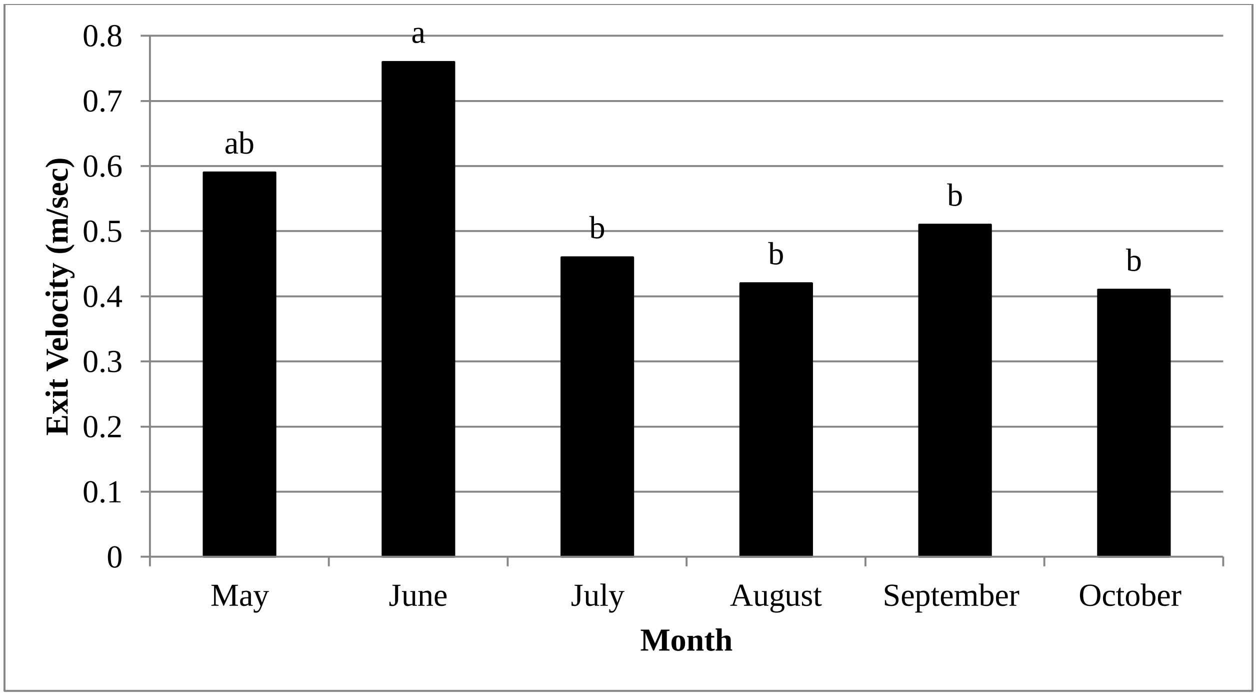

Figure 5. Monthly Exit Velocity of Calves. (*P* < 0.001; SEM = 0.08).
SEM = Standard Error Means. ^{a,b} Bars without common superscripts differ.

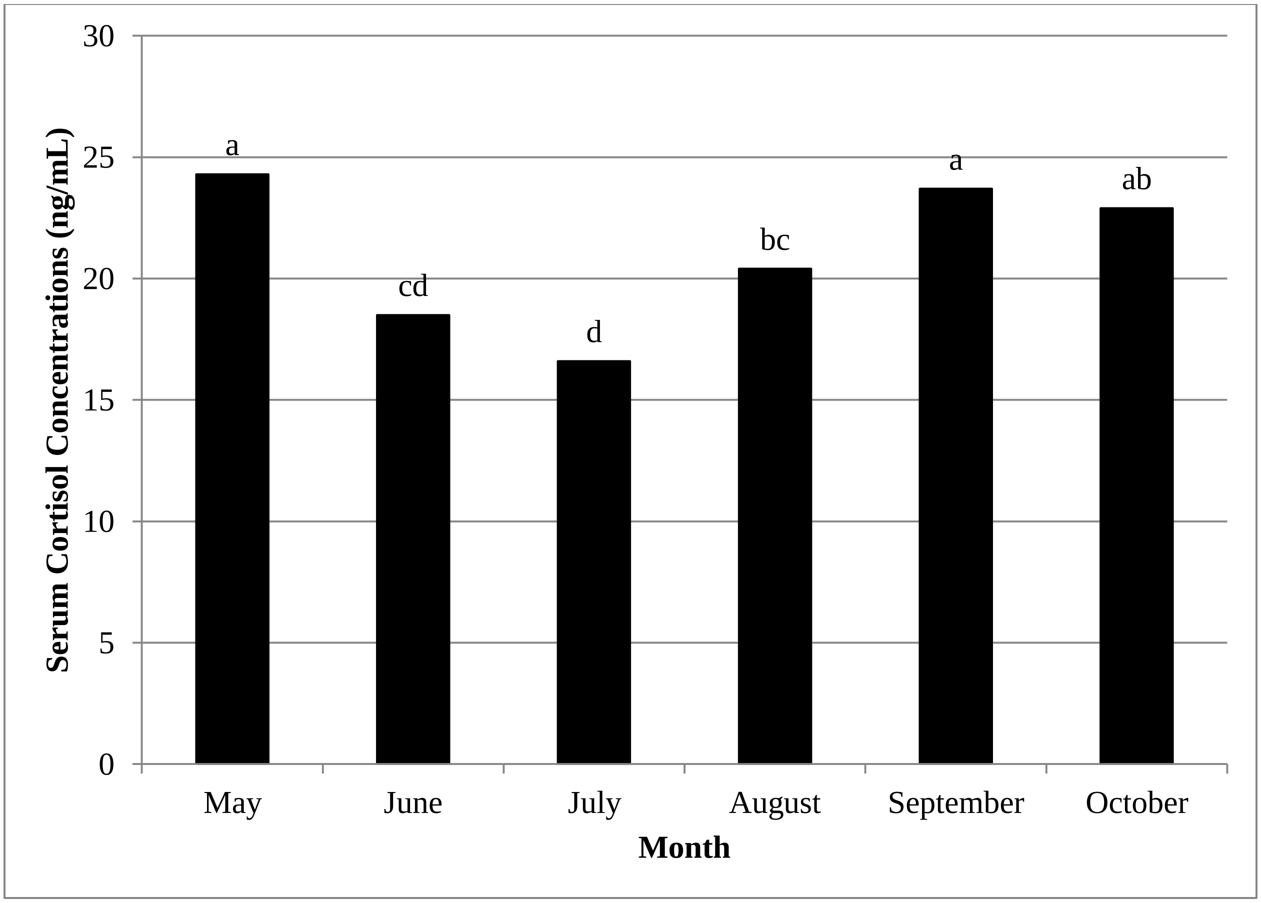

Figure 6. Monthly Serum Cortisol Concentrations of Cows ($P < 0.0001$; SEM = 1.5). SEM = Standard Error Means. ^{a,b,c,d} Bars without common superscripts differ.

CONCLUSION

There was no association between genotypes of the promoter region of the PRL gene (C1286T) with milk production, calf performance traits, temperament measurements, and horn fly resistance in beef cattle used in this study. The use of this SNP as a genetic marker would not be efficient in selecting for these traits in beef cattle based on the results of this study.

Milk yield was affected differently in the six sire breeds (BONS, BRAN, CHAR, GELV, HERF, and ROMO) used in this study. Horn fly numbers impacted milk yield, but the effects were different for each sire breed. Milk yield in all sire breeds was affected negatively except in BRAN sired cows, which had a positive regression coefficient for milk yield. Horn fly numbers also affected milk yield differently during the lactation cycle. Early lactation was negatively impacted by horn flies, while late lactation was not significantly affected by horn flies. These results indicate the impact horn flies have on milk yield is dependent upon sire breed and lactation cycle.

Milk fat, SNF, and urea nitrogen were negatively affected by horn flies. However, milk lactose and SCC were not, while percent protein was only impacted in HERF sired cows. The effects of horn flies on milk quality data in this study appear to not be as dependent upon sire breed or lactation cycle as milk yield, but the results do determine horn flies negatively impact certain milk quality traits.

Sire breed affected preweaning ADG, but so did the interaction of sire breed and log horn fly count. Bonsmara and GELV calves were the only sire breeds with negative regression coefficients for preweaning ADG. However, postweaning traits were not negatively impacted by horn flies. In fact postweaning ADG, 365-d adjusted YWT, and birth to yearling ADG increased per one unit increase in log horn fly count. In conclusion, sire breed and pre and postweaning nutrition may be responsible for the observed results from this study.

Pasture behavior was affected differently in the AM versus the PM by horn flies. Cows observed lying in the AM had numerically fewer horn flies, whereas in the PM cows observed standing had significantly fewer horn flies. Other variables such as cow core body temperature and time of day the observations were collected may have influenced the observed pasture behavior in this study. However, a combination of these factors still resulted in horn fly numbers being associated with specific pasture behaviors that was displayed in this study.

Horn fly numbers, EV, and CORT were all influenced by month. The increase in horn fly numbers observed in August and October may explain the increased EV speeds of cows recorded during these months. Cows did not become acclimated to human handling as the study progressed but instead temperament measurements indicated the cows became more agitated/aggressive. The combination of chronic stress caused by the horn flies as well as the continued stress of separating calf from cow during monthly data collections may have contributed to the temperaments displayed by the animals.

Overall, it can be concluded that horn flies do affect milk production, calf performance traits, pasture behavior, and temperament measurements. However, other factors such as sire breed, lactation cycle, and month may contribute to their effect. The physiological and genetic mechanisms associated with the results observed in this study still need to be determined through future research focusing on horn flies.

Appendix A

Horn Fly Control Insecticides	
Chemical Class	**Common Name**
Organophosphates	Chlorpyrifos Coumaphos Crotoxyphos Diazinon Dichlorvos (DDVP) Fenthion Malathiuon Stirophos Tetrachlorvinphos
Pyrethroids	Cypermethrin Fenvalerate Flucythrinate Permethrin
New Generation Pyrethroids	Cyfluthrin Lamda Cyhalothrin
Macrocyclic Lactone Disaccharide	Ivermectin
Insect Growth Regulant (IGR)	Methoprene
Oral Larvacides	Diflubenzuron Phenothiazine Tetrachlorvinphos