THESIS

EFFECTS OF TWO-STAGE WEANING WITH NOSE FLAPS APPLIED TO CALVES ON COW PERFORMANCE, CALF PERFORMANCE, CARCASS QUALITY, CALF HUMORAL IMMUNE RESPONSE, AND FERTILITY

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ABSTRACT

EFFECTS OF TWO-STAGE WEANING WITH NOSE FLAPS APPLIED TO CALVES ON COW PERFORMANCE, CALF PERFORMANCE, CARCASS QUALITY, CALF HUMORAL IMMUNE RESPONSE, AND FERTILITY

Two studies were conducted to evaluate the effects of nose flap devices on cow performance, calf performance both pre- and post-separation from the dam, carcass quality, and calf humoral immune response.

The objective of the first study was to determine if QuietWean nose flap (**NF**) devices could be used on calves for a short time, followed by returning calves to normal nursing, to effectively alter dam body condition. This study was conducted at 2 locations using Angus and Angus × Hereford primiparous cows (n = 245). Cow and calf pairs were allocated to 4 treatments in a completely randomized design. Treatments on the calves were: 1) NF for 30 or 31 d while remaining with their dams and removal from dam to the feedlot on d 30 or 31 (**LT-30R**), 2) NF for 4/5 d while remaining with their dams and removal from dam to the feedlot on 30/31 d (**ST-30R**), 3) NF for 4/5 d while remaining with their dams and removal from dam to the feedlot on 60/62 d (**ST-60R**), and 4) no NF while remaining with their dams and removal from dam to the feedlot on 30/31 d (**CON-30R**). Cow BCS and BW were collected on d 0, 60 or 62, and 120 or 122. Cow fat thickness (cm) was measured by ultrasound on d 0 and 60 or 62. Calves were weighed on d 0, and 30 or 31, and 60 or 62. By d 60 or 62, BW from cows in CON-30R was less ($P < 0.05$) than cows in LT-30R or ST-30R. Cows in LT-30R gained more ($P < 0.0001$) from d 0 to 120 or 122 than all other treatment groups, while cows in ST-60R gained less ($P < 0.05$) than all other treatments. Fat thickness as measured by ultrasound did not differ among treatments ($P = 0.18$). While there was no difference in BCS on d 60 or 62 and 120 or 122, cows in ST-60R decreased ($P < 0.001$) in BCS from d 0 to 60 or 62; whereas all other groups increased ($P > 0.05$) in BCS. There was no difference in cow pregnancy rates of the subsequent year among treatments ($P = 0.64$). By d 30 or 31, calves in LT-30R gained less ($P < 0.0001$) than calves from all other treatments. There was no difference ($P < 0.05$) in BW between calves in ST-30R and ST-60R on d 30 or 31; however, both treatments gained less ($P < 0.0001$) than calves in CON-30R. Calves in CON-30R gained more ($P < 0.0001$) than calves in the other treatments. Heifer pregnancy rates in CON-30R tended to be lower than all other treatments ($P = 0.05$). LT-30R heifers were older at calving than ST-30R ($P < 0.05$). Yearling weight (**YWT**), HCW, fat thickness, REA, yield grade (**YG**), and quality grade (**QG**) did not differ among treatments. These results indicate that NF weaning devices can improve performance of cows and heifers without negatively impacting steer carcass quality.

The objective of the second study was to examine the effect of fitting calves with NF devices for 21 d prior to separation from the dam on cow BCS, calf performance both pre- and post-separation from the dam, and humoral immune response to vaccination compared to traditional weaning. This study was conducted using primiparous and multiparous Angus and Hereford cows (n = 113) and their respective Angus, Hereford, and Angus × Hereford calves (161 ± 22.7 d, 179.4 ± 3.92 kg). Cow/calf pairs were allocated to one of 2 treatments in a completely randomized design: 1) NF for 21 d prior to separation from the dam (**NF**), or 2) no NF for 21 d prior to separation from the dam (**CON**). Cow BCS was measured on d -21 and 56 to determine cow performance. Calf separation from the dam occurred on d 0. Calf performance was determined for 21-d before separation from the dam and during the feedlot period (d 1 post-separation from the dam through d 195). Modified-live vaccinations were administered on d -21 and 1. Calves were weighed on d -21, 1, 7, 14, 21, 28, and 195 and jugular blood samples were collected on d -21, 1, 14, and 28. Cow BCS and change in BCS were similar across treatments ($P > 0.05$). There was no difference ($P > 0.05$) in calf BW on d 1, 7 or 28 between treatments; however, CON calves tended to have greater BW on d 14 ($P = 0.09$), 21 ($P = 0.07$), and 195 ($P = 0.07$). Control calves had greater ($P < 0.05$) ADG from d -21 to 1 compared to NF calves. However, ADG from d -21 to 195 was similar between treatments ($P > 0.05$). There was a tendency for CON calves to have greater ($P = 0.08$) DMI from d 22 to 28 with no difference ($P > 0.05$) between treatments at remaining times or during the 28-d post-separation period. Feed intake, efficiency and morbidity were similar across treatments ($P > 0.05$). Serum neutralization tests for bovine viral diarrhea virus type 1 (**BVDV-1**) and bovine herpesvirus type 1 (**BHV-1**) were used to measure humoral response to vaccination. Serum antibody titers to BVDV-1 tended to be greater ($P = 0.08$) for CON calves on d 0 and were greater ($P < 0.05$) by d 28. By d 28, more ($P < 0.05$) CON calves reached seroconversion than NF calves, with 82.1% of CON calves and 66.7% of NF calves reaching seroconversion. Serum antibody titers for BHV-1 were greater ($P < 0.05$) on d 0 and 28 for CON calves. Seroconversion to BHV-1 was greatest ($P < 0.05$) on d 14 for both treatments but did not differ ($P > 0.05$), with 82.5% of NF calves and 85.5% of CON calves reaching seroconversion. There was no difference ($P > 0.05$) in antibody titers to BHV-1 on d 14. An ovalbumin (**OVA**) challenge was conducted with a subset (n = 57) of calves to evaluate the humoral immune response to foreign protein during the initial post-separation period. There were no differences in OVA specific immunoglobulin G (**IgG**) among treatments ($P > 0.05$). These results indicate that NF devices did not influence calf performance, feed intake, feed efficiency, or morbidity during the initial post-separation period. Serum titers and seroconversion to BVDV-1 and BHV-1 were decreased in NF calves, however primary immune response to ovalbumin was not affected. More research is needed to determine long-term effects of NF weaning devices on calf performance and immune response, however they appear to be an adequate alternative to traditional weaning as it does not negatively effect calf performance or health status.

Key Words: beef calves, feedlot performance, immune response, nose flaps, weaning

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CHAPTER I

REVIEW OF LITERATURE

Consumer Concerns and Animal Welfare

In today's animal production industry, consumer concerns about how and where livestock animals are raised, as well as animal welfare, are of increasing importance but vary from person to person (De Jonge and van Trijp, 2013). Many common husbandry practices used in the animal production industry are not fully understood by consumers, and instead leave a sense of confusion. De Jonge and van Trijp (2013) asked consumers to answer questions regarding broiler production systems and evaluated them in regards to their perception of "animal friendliness," or how humane the practices were. Two hundred nine students at a Dutch University from a variety of majors participated in this study. Participants were seated at a computer and began a computer program that used a conjoint analysis approach to determine their values regarding different systems in broiler production. Using a paired comparison, participants had to rate one system over the other as to which was more animal friendly, and by how much: -10 as profile A is more friendly, and +10 as profile B is more friendly. Results found that while some practices such as outdoor access and stocking density had a large impact on their perception of animal friendliness, the degree as to which how much a certain production system impacted their perception of animal friendliness greatly differed. Many participants had positive outlooks towards organic systems, as they perceived them to be more humane than conventional systems, therefore were more likely to consider organic more animal friendly. Husbandry practices that were important for consumers who had never been on a farm were different than those who had farm experience. In addition, consumers who had farm experience valued different husbandry practices than those who lived on a farm, therefore it may be difficult to recognize which welfare concerns are of most importance.

Consumers around the world are concerned about animal welfare (Mitchell, 2001) and it is important to keep these concerns at the forefront of livestock industry priorities for both foreign and domestic consumers. While consumers may not fully understand the meaning of labels placed on food packages, there are some marketing strategies that they believe increase the quality of the product. Harper and Makatouni (2002) examined consumer perception of organic foods and farm animal welfare by conducting 4 focus groups with 6 to 8 parents each. In order to participate in the study, consumers had to have an understanding of the meaning of “organic,” have children 4 to 11 years old, be the primary purchaser of food in their family, and be a part of the lower-middle through upper-middle socio-economic class. The first 2 focus groups were conducted solely with consumers who purchased organic food products, while the last 2 focus groups were made solely of consumers who did not buy organic food. Participants were questioned as a group about concerns they had regarding food safety, the source of their food, changes they have made because of food concerns, and what their perception of organic food was. All participants understood the correct definition of organic to be food grown without the use of pesticides, fertilizers, or genetically modified organisms, and animals raised without the use of growth hormones or antibiotics (USDA, 2015). However, many consumers believed that organic eggs indicated that chickens were raised free-range, or vice-versa; which they believed was more humane to animals and resulted in higher quality than non-organic eggs. While this is not a correct definition of the term “organic,” it does signify that consumers are concerned in today’s market about how animals that provide products they purchase were raised and treated.

The National Beef Quality Audit (**NBQA**) conducted in 2011 identified the top quality concerns affecting the beef industry and how different sectors describe meat quality. Among the top quality concerns, appearing in the first audit to date, are “how/where the food was raised” and “food safety,” (Igo et al., 2013). Consumers that have become increasingly separated from agriculture are beginning to become more interested in food production systems and with animal welfare concerns. According to the 2011 NBQA, a primary carcass defect in the beef industry was bruising (McKeith, 2012). Twenty-three percent of carcasses evaluated exhibited bruising, and the number of bruises classified as major, critical, and extreme increased from 2.0% in the 2005 NBQA to 3.1% in the 2011 NBQA. This increase in the intensity of bruising in beef cattle indicates that issues regarding animal welfare prior to harvest need to be resolved and have led to expansion of low-stress cattle handling methods and techniques.

Stress at Weaning

Weaning is a particularly stressful event in a young beef animal’s life. Traditional weaning methods include sudden separation of calves from their dams followed by transportation to another location where they experience nutritional, social, and environmental stress in one event. Calves that are abruptly weaned exhibit increased behaviors associated with stress such as vocalizing and walking (Haley et al., 2005; Lucas et al., 2007; Lambertz et al., 2014) as well as increased plasma cortisol and noradrenaline concentrations, indicators of stress (Hickey et al., 2003).

Stress has the capability to cause immunosuppression and increased disease susceptibility. Blecha et al. (1984) reported decreased lymphocyte blastogenic responses of the immune system in calves experiencing transportation stress. Lymphocytes increase in response to infection and help the body produce an immune response necessary to respond to a vaccination or infection (Murphy et al., 2011). If a calf experiences increased stress accompanying common husbandry practices at the time of vaccination, the ability to develop immunity to disease may be limited and may leave the animal vulnerable to disease once entering into the feedlot.

Hickey et al. (2003) sought to investigate the effects of abrupt weaning on physiological indicators of stress and calf immune response. The study used 36 male and 36 heifer Limousin and Charolais cross calves, blocked by sex, weight, and breed of dam, and separated into an abruptly weaned group or the un-weaned, control group. Blood samples were taken at -168, 24, 48, and 168 h after calves were abruptly weaned from their dams. Abruptly weaned calves were found to have increased plasma cortisol and noradrenaline concentrations, both indicators of stress. Additionally, calves that were abruptly weaned experienced decreased leukocyte concentrations, cells that protect the body against disease, and increased neutrophil:lymphocyte ratio, an indicator of subclinical inflammation. This indicates that not only does weaning increase markers associated with stress, but also has the ability to suppress the immune response.

Transportation alone, not coupled with weaning, can be a stressful event for a young calf. Stanger et al. (2005) studied the effects of 72 h of transport on the immune system of 10 *Bos indicus* steers aged 15 to 18 mo of age. Blood samples were collected 2 d prior to transportation at -48 h, immediately after transportation at 72 h, and 6 d later at 216 h. Total leukocyte and eosinophil count were suppressed immediately following transportation. Lymphocyte proliferation was also suppressed, but tended to be elevated compared to baseline after 6 d. This immunosuppression indicated that calves in this study could have been vulnerable to infection for up to 6 d after transportation.

To further examine the direct effects of abrupt weaning, Lynch et al. (2010) examined 16 male calves from Limousin × Holstein-Friesian and Simmental × Holstein-Friesian breeding. On d 0, calves were placed into 1 of 2 housing treatments: 1) abrupt weaning, or 2) non-weaned (control), where calves were placed into housing with their dams. Blood samples were collected on d -7, 0, 2, 7, and 14. By d 2, abruptly weaned calves exhibited increased neutrophils and leukocytes, and diminished cytotoxic T (**CD8+**) lymphocytes and phagocytic neutrophils. This multifaceted effect on the immune response of calves represented a sizable suppression of immune response due to abrupt weaning. This stress, coupled with transportation stress, may have a more dramatic effect on calf health.

Mitigating stress experienced during common husbandry practices can prevent immunosuppression and decrease morbidity in calves. Meloxicam is a nonsteroidal anti-inflammatory drug that reduces the cytokines responsible for promoting pain and inflammation. When used properly, this drug can be used to mitigate stress associated with many stressful husbandry practices. Coetzee et al. (2012) demonstrated the beneficial effects of mitigating castration stress through the use of meloxicam. Two hundred fifty-eight British crossbred bulls aged 8 to 10 mo were used in this study. Animals were either received as steers (n = 113) or bulls surgically castrated upon arrival (n = 145). Both groups were given either a placebo per os (**PO**), or a dose of meloxicam at 1 mg/kg BW PO. Animals were then grouped in pens by treatment. Pen feed intake, morbidity, mortality, and steer weights were collected to monitor feedlot performance post-receiving. While castrated steers exhibited decreased ADG and G:F, treatment with meloxicam decreased first pull rate by pen and incidence of bovine respiratory disease. The use of meloxicam to mitigate castration stress therefore shows promise to maintain the health of receiving bulls.

The use of meloxicam has also been shown to negate the negative effects of dehorning. Heinrich et al. (2009) used 60 Holstein heifers 6 to 12 weeks of age to observe how Meloxicam can mitigate postsurgical stress associated with cautery dehorning. Meloxicam calves (n = 30) received an IM injection of meloxicam at a rate of 0.5 mg/kg BW. As a measure of stress, heart and respiratory rates were monitored after dehorning and blood samples were taken at 0.0, 0.5, 1.0, 1.5, 2.0, 4.0, 6.0, and 24.0 h post surgery. While there were no differences in respiratory rate or serum cortisol concentration, control calves had greater heart rates than calves that received meloxicam. Both groups exhibited elevated serum cortisol concentration that remained elevated 24 h post surgery, however meloxicam calves had less serum cortisol concentration from 0 to 6 h. In addition, by 1.5 h when lidocaine generally loses effectiveness, only control calves exhibited an increase in cortisol. These data indicated that meloxicam was indeed effective in mitigating stress associated with pain experienced by dehorning.

Alternative Weaning Methods

As medications such as meloxicam can only be used for pain mitigation and cannot be used for weaning stress, there are many alternative management strategies available to producers to decrease stress at weaning and prepare calves for separation from the dam and the post-weaning period.

Preconditioning can be an important part of insuring calf health when a calf enters future sectors of the beef industry. Roeber et al. (2001) examined different preweaning management strategies on feedlot performance and carcass quality in feedlot steers. Steers were purchased from the value-added calf programs including Certified Preconditioned for Health (n = 95) and Kentucky Cattlemen's Association Gold Tag (n = 90), or were purchased from local auction markets (n = 88) and transported to a Colorado feedyard. Average daily gain at reimplant, overall ADG, and BW were collected to evaluate feedlot performance. Morbidity rates were recorded by number of hospital visits. Preconditioned calves had lower morbidity rates than calves purchased from auction markets, with percentage of calves requiring at least one hospital visit being 34.7 and 77.3% respectively. Mortality rates were also less for preconditioned calves, with 1.1% mortality rate for preconditioned calves and 11.4% mortality rate for auction market calves. Preconditioned calves exhibited fewer treatments for morbidity, thus having the potential to decrease health costs and increase overall profitability per animal.

In order to determine the effect of commingling calves from varying backgrounds, Step et al. (2008) observed steer calves during a 42-d receiving period from 4 sources: 1) traditionally weaned calves from a single ranch, 2) calves weaned without a vaccination on a ranch 45 d before shipping, 3) calves weaned on a ranch and given a modified-live vaccine (**MLV**) 45 d before shipping, and 4) calves purchased from auction markets. Calves that were weaned 45 d before shipping were found to have greater DMI than calves that were traditionally weaned and were less likely to be treated for morbidity than market calves or traditionally weaned calves. Calves weaned for 45 d prior to shipping exhibited 0.0% mortality, whereas market calves exhibited 3.1% mortality and were pulled earlier for their first and second treatments. Subsequently, health costs were only $8.30 and $8.93 for calves that were weaned without a vaccination 45 d prior to shipping and calves weaned with a MLV 45 d prior to shipping, respectively, compared to $13.54 for market calves and $13.24 for traditionally weaned calves.

Data from this study support that weaning calves prior to transportation to the feedlot where they will be commingled with calves from various backgrounds can improve health and DMI in calves during the receiving period.

Early weaning calves for a longer period of time before transportation may serve as another low-stress weaning strategy available to producers, as it may be able to spread out the different types of stress calves experience at the time of weaning over a prolonged amount of time. Arthington et al. (2005) sought to investigate possible differences in stress tolerance between early and normally weaned calves and observed performance and acute-phase protein concentrations of these calves post-transportation and during the receiving period. This study used 40 Brahman x English steer calves that were approximately 89 d of age for early weaning and 300 d of age at normal weaning. Early-weaned calves were placed on pasture with supplemental feed for approximately 200 d until normal weaning. At this time, all calves were transported 1,200 km to the research facility. Blood was collected by jugular venipuncture at weaning, immediately post-transportation, and 1, 3, 7, 14, 21, and 28 d post-transportation to determine stress during weaning, transportation, and receiving. While early-weaned calves weighed less than normally weaned calves by the time of normal weaning and by d 28 of the receiving period, they experienced greater G:F during the receiving and growing periods with no differences during the finishing period in ADG, DMI, or G:F. Ceruloplasmin, an indicator of stress, continued to increase until d 7 after transportation for normal weaned calves and was greater in normal weaned calves. Haptoglobin, another indicator of stress, peaked at 4 d post transportation for both treatments and was greater for normal weaned calves. As the calves in this study experienced the nutritional stress of weaning separately from the stress of transportation, it may account for the differences in stress observed in this study. Early-weaned calves experience enhanced feed efficiency during the feeding period and less stress during the receiving period, indicating that this system may serve as a beneficial alternative protocol for weaning calves.

In the National Animal Health Monitoring System 2008 Beef Cow report, research found that only half of beef producers held calves for preconditioning (USDA, 2008). Of all operations, only 16.6% held calves for 1 to 31 d, 13.8% held calves for 32 to 61 d, and 10.1% held calves for 123 d or more. Even more alarming was that 60.6 % of operations, making up 30.9% of the population of sold beef calves, did not vaccinate calves pre-weaning. This number increased as herd size decreased, as 73.7% of operations with 1 to 49 hd did not vaccinate their calves, 36.9% of 50 to 99 hd operations, 28.3% of 100 to 199 hd operations, and 18.0% of 200 or more hd operations. This leaves much room for improvement in the beef industry to better prepare calves for the post-weaning period.

There are several alternative methods to traditional weaning that can decrease calf stress at weaning, while being easily applicable to producers. One alternative system is fenceline weaning. This alternative allows the calf to experience nutritional stress of weaning separately from transportation stress, while allowing the calf to stay in proximity of their dam during the process. Price et al. (2003) sought to observe the differences in weaning stress exhibited by calves weaned through fenceline (**FEN**) weaning, separation from the dam to pasture, separation from the dam to drylot and preconditioned to hay, separation from the dam to drylot and not preconditioned to hay, and control calves that were not weaned and remained with their dams. Time spent eating, walking, and lying down was monitored to determine stress. Calf BW was collected weekly for 10 weeks prior to weaning to monitor performance. While there was no difference in time spent eating between FEN and control calves, FEN calves spent more time eating and vocalized less than calves separated from their dams to pasture and calves separated from their dams to drylot without preconditioning. Fenceline calves gained 95% more weight postweaning than normally weaned calves during the first 2 weeks post-separation from their dams, and gained more weight during the entire post-separation period. This study concluded that fenceline weaning could be a beneficial alternative to traditional weaning to decrease stress observed by traditionally weaned calves and increase postweaning gain.

Weaning through the use of a nose flap (**NF**) device serves as a unique opportunity to wean calves while allowing the calf to stay with the dam, which may serve as a less stressful method. These NF are fitted to calves for a short period of time, such as 4 to 7 d, to inhibit the calf from nursing while allowing the calf to stay in physical contact with the dam. Boland et al. (2008) compared FEN, NF, and traditional weaning methods and their effects on behavior, blood metabolites, and calf performance. The study used 108 Angus-cross calves that were approximately 220 ± 18 d of age in Experiment 1, and 54 Angus-cross steers that were approximately 228 ± 13 d of age in Experiment 2. On d -7, FEN calves were placed in paddocks adjacent to their dams and NF calves received a NF. On d 0, all calves were removed from their dams. Before removal from dams and transport, NF calves had reduced time eating and ADG than FEN or traditionally weaned calves. However after transport, FEN and NF weaned calves spent more time eating and less time walking than traditionally weaned calves. Overall, FEN calves had a greater ADG than NF calves in this study. However, research by Enríquez et al. (2009) observed conflicting results. The authors' study had similar objectives to observe behavior and performance using FEN, NF, and traditional weaning methods. Forty-eight Hereford and Hereford × Angus calves averaging 180 ± 1.3 d of age were used. On d -17, FEN calves were placed on the opposite side of the fence from their dams and NF calves were fitted with NF. On d 0, all calves were separated from their dams. Behavior was monitored by time spent vocalizing, playing, walking, ruminating, suckling attempts, fenceline pacing, grazing, seeking, lying, drinking water, and distance to the fenceline. Body weight and ADG were measured to monitor calf performance. On d -17 and over the course of the observation period, FEN calves vocalized more than NF calves. Both NF and FEN calves vocalized less at weaning than traditionally weaned calves. During the entire observation period from d -17 to d 5, NF calves spent less time vocalizing, walking, and pacing the fenceline than FEN calves. Overall, traditionally weaned calves exhibited greater ADG than NF or FEN calves, and FEN calves had increased ADG than NF calves. However, NF calves had increased ADG than FEN calves from d 7 to 21, so it may have been necessary to monitor postweaning ADG for a longer period of time to observe long-term effects of weaning method. Over the entire course of the pre- and post-weaning period observed in this study, NF weaning was able to decrease behaviors associated with weaning stress compared to FEN weaning.

Another study by Lambertz et al. (2014) observed differences in behavior and performance in calves weaned with NF compared to traditional weaning. Using 192 German Angus and Simmental cows and their respective German Angus, Simmental, and German Angus × Simmental calves, pairs were placed into either traditional weaning (n = 103) or NF weaning (n = 89). Nose flap calves were fitted with NF for 7-d prior to separation. Behavior of a subset of calves (n = 20) was monitored for 3 d prior to weaning and 4 d post-weaning. Cows with traditionally weaned calves vocalized 5 times more frequently than cows with NF weaned calves. Nose flap calves spent less time walking than traditionally weaned calves during the first two days of separation. An earlier study by Haley et al. (2005) found similar results. Four trials were conducted to measure the effectiveness of NF in minimizing behavior associated with stress and to monitor calf performance. In Trial 1, calves were fitted with NF for either 14 d (n = 58) or 3 d (n = 58) prior to separation, or were traditionally weaned (n = 74). Traditionally weaned calves were found to vocalize 20 times more often in the first three days than NF calves and spent more time walking, less time laying down, and less time eating than NF calves. Average daily gain was suppressed in NF calves compared to traditionally weaned calves, and was increasingly suppressed in calves that wore NF for 14 d. Contrarily, NF calves had increased ADG during the initial week post-separation. In Trials 2 (n = 100) and 3 (n = 52), calves were fitted with NF for 5 d (n = 50, 26) prior to separation or were traditionally weaned (n = 50, 26). The combined trials show similar results, with NF calves exhibiting greater ADG over traditionally weaned calves during the first week post-separation. In Trial 4, calves were fitted with NF for 4 d (n = 25) prior to separation or were traditionally weaned (n = 25) and observed for walking behavior. Results found that throughout the 4 d before separation to the 4 d after separation, NF calves walked approximately 2.7 km/d less than traditionally weaned calves. Similar to data found by Boland et al. (2008), NF calves over the 4 trials exhibited suppressed weight gain compared to traditional weaning before separation from the dam, however exhibited greater post-weaning ADG than traditionally weaned calves. This study supports previous research that the use NF weaning devices can minimize behaviors associated with stress experienced by traditionally weaned calves. Depending on the protocol, this method will not hinder calf performance.

Disease on Carcass Quality and Performance

Mitigating stress that occurs during husbandry practices is not only a welfare concern, but may also help increase animal performance. Not only does increased incidence of disease and illness disrupt an animal's well being, it is also detrimental to the beef industry. Waggoner et al. (2007) observed the records of 813 steers during the finishing phase from 2000 to 2003 to examine the effects of morbidity on feedlot performance. Carcass traits of interest included HCW, subcutaneous fat thickness, LM area, marbling score, QG, and YG. Of the 23.9% of steers that were treated, 78.5% were treated once and 21.5% were treated twice or more. Steers that were untreated exhibited increased ADG and decreased days on feed than treated steers, however carcass quality was similar from treated and untreated steers. Treatment cost was $0.00, $28.43, and $62.63 for untreated steers, steers treated once, and steers treated twice, respectively. Between increased health costs and a tendency for carcass price to decrease with increasing morbidity rate, overall gross income per animal was less for treated steers. Untreated steers had a gross income of $856.36, compared to $827.84 for steers treated once and only $683.69 for steers treated twice or more. This data concludes that morbidity has a negative effect on feedlot performance and overall profitability.

Research by Reinhardt et al. (2012) supported that the more times an animal is treated for disease, the larger the decrease of performance and carcass quality. The study observed Angus steers (n = 17,919) over the course of a decade at a single feedlot to further evaluate the effects of feedlot health on performance and carcass traits. Morbidity rate was recorded as either not treated, treated once, treated twice, or treated twice or more times. Performance and carcass characteristics of interest included ADG, final BW, HCW, QG, and YG. Initial BW at receiving was important to morbidity, as there was a negative relationship between the two variables. Average daily gain, final BW, HCW, QG, and YG were increasingly negatively affected as the number of treatments a steer received increased. The more times an animal was treated for a disease, the greater the negative effect on overall performance and carcass merit. Similar results were observed by research completed by Schneider et al. (2009) where increased incidence of disease greatly diminished carcass quality. The study observed 5,976 cattle at 10 different feedlots from 2003 – 2006. Health was monitored specifically for bovine respiratory disease (**BRD**) and recorded as animals not receiving treatment, and those receiving treatment once, twice, or three or more times. The incidence of BRD in this study was dramatically decreased compared to the previous study, as 8.7% of cattle exhibited symptoms of respiratory disease. Of those cattle treated, 53% were treated once, 34% treated twice, and 13% treated three or more times. Overall ADG and final BW were less for treated cattle, and tended to be less for HCW. Untreated steers exhibited greater subcutaneous fat cover and marbling score than treated steers. Steers grading Choice or better were 71% of untreated cattle, 57% for cattle treated once, 55% for cattle treated twice, and 52% for cattle treated three times or more. This dramatic decrease in performance and carcass quality, paired with increase health costs, translated to greater decreases in overall carcass value. Cattle treated once, twice, and three or more times had a decline in carcass value of $23.23, $30.15, and $54.01 respectively. This remains an enormous loss to the beef industry, and may be able to be alleviated through better preparation of the steer's immune system before entering into the feedlot.

Fenceline weaning has been shown to have a decrease in morbidity rate, with traditionally weaned calves experiencing twice treatment rate for disease (Boyles et al., 2007). Boyles et al. (2007) observed 280 steer calves during feedlot receiving that were weaned in three different weaning methods: 1) traditional weaning where calves are weaned at shipping, 2) weaned 30 d prior to shipping and placed in a drylot, and 3) calves that were FEN weaned 30 d prior to shipping. While there were no differences in ADG during the 30 d receiving period between FEN and traditionally weaned calves, FEN calves had greater DMI. In addition, FEN calves had less morbidity rates at 15% compared to 28% of traditionally weaned calves and 38% of calves that were placed in the drylot 30 d prior to shipping. The superior calf health due to alternative weaning method may have the potential to improve carcass quality that is decreased due to morbidity.

Steers that are treated for disease experience decreased ADG and more days on feed than healthy steers (Waggoner et al., 2007), as well as decreased HCW and marbling scores (Schneider et al., 2009). This leads to increased cost of production and lower gross income, with a tendency for treated steers to have lower price carcasses (Waggoner et al., 2007). Carcasses from steers treated up to three times show a reduction in value of up to $54.01 compared to carcasses from healthy steers, thus decreasing overall profitability (Schneider et al., 2009). Seventy-five percent of treated steers are treated within the first 55-d of the receiving period (Schneider et al., 2009). This indicates that the receiving period is a crucial time period. Ensuring that calves entering into the feedlot have robust immune systems to remain healthy may increase production of high quality carcasses with minimal health costs. Research by Macartney et al. (2003) observed 12,313 conventional and market calves for health performance in the first 28 d in the feedlot from 1999 – 2000. Twenty-one percent of the calves observed were treated for BRD. Calves that were vaccinated were 0.68 times as likely to be treated for disease compared to market calves. This data indicate that vaccinating calves serves as a key role in increasing calf health during the receiving period, however there is still more work that can be done to maximize calf health through ensuring vaccine effectiveness.

Stress and Response to Vaccination

Minimizing stress associated with weaning may improve the effectiveness of vaccines and decreasing incidence of morbidity. Two-stage weaning strategies may be able to serve as a low-stress weaning method by allowing the calf to begin to break the social bond with the dam before physical separation from the dam, therefore decreasing behaviors commonly associated with weaning stress (Haley et al., 2005; Lambertz et al., 2014). Traditional weaning increases plasma cortisol and neutrophil:lymphocyte ratio (Hickey et al., 2003) as well as diminished CD8+ lymphocytes and phagocytic neutrophils (Lynch et al., 2010), which indicate that weaning stress may inhibit vaccine effectiveness.

Many calf vaccination protocols may include a vaccine administered at weaning and subsequent transportation. As it is not recommended for vaccinations to be given during a stressful event, this may disrupt the effectiveness of the vaccine. Richeson et al. (2008) sought to investigate the effects of delaying the MLV vaccine until 14 d post transportation on infectious bovine rhinotracheitis (**IBR**) serum titers. The study used 528 crossbred bull and steer calves that were randomly allocated to one of two MLV vaccination protocols: 1) MLV vaccination upon arrival (**AMLV**), or 2) delayed (14 d) MLV vaccination (**DMLV**). Body weight was recorded on d 0, 14, 28, and 42 and blood samples were taken on d 14, 28, and 42. DMLV Calves weighed greater from d 0 to 14 and during the entire receiving period from d 0 to 42. DMLV calves also had a greater seroconversion rate for IBR on d 42, with over 40% DMLV seroconverting and just over 20% AMLV calves. However, morbidity rates were extremely high for this study and did not differ among treatment. Seventy-five percent of AMLV calves were treated once for BRD, and 25.1% were retreated. Sixty-three percent of DMLV were treated once, and 30.8% needed to be retreated. These morbidity rates for BRD are much higher than seen in previous studies (Reinhardt et al., 2012; Waggoner et al., 2007) as 63.5 – 72.5% of calves needed treatment for BRD, which may account for some of the concentration seen in serum titers.

Further research from the previous study evaluated the effects of delaying clostridial (**CLOS**) or modified live vaccination for respiratory (**RESP**) 14 d after arrival in the feedlot on calf performance and serum antibodies for bovine viral diarrhea virus (Richeson et al., 2009). This study used crossbred bull (n = 207) and steer (n = 57) calves randomly allocated to one of 4 treatments: 1) CLOS and RESP on-arrival (ACAR), 2) CLOS on-arrival, delayed RESP (ACDR), 3) delayed RESP, on-arrival CLOS (DCAR), or 4) delayed CLOS, delayed RESP (DCDR). On d 0, 14, 28, 42, and 56, BW was measured to monitor calf performance and blood samples were collected to measure serum antibody concentration and immune response. Calf performance did not differ across treatments. Calves that received RESP on arrival experienced much greater titers for BVDV on d 14 and 28 than calves that received a delayed RESP, but did not differ by d 42. Delaying either CLOS or RESP vaccinations did not affect morbidity, however again, the calves in the current study experienced much higher morbidity rates than seen previously (Reinhardt et al., 2012; Waggoner et al., 2007) as 60.7 – 75.3% of calves needed treatment for clostridial and BRD, which may account for the elevated serum titer concentrations.

Several calf vaccinations protocols implemented at weaning include an initial vaccine to be given several weeks prior to weaning with a booster given at weaning. However, as stated earlier, it is not recommended to give a vaccination at a high-stress event. Therefore, Downey et al. (2013) investigated weaning calves either at the initial vaccination (n = 508) or the booster vaccination (n = 496) in the presence of maternal antibodies. This study used purebred American

Angus calves that averaged 139 d of age when weaned at the initial vaccine and 128 d when weaned at the booster vaccine. Serum titer concentrations for BVDV – type 2 were measured every 21 d to monitor vaccine effectiveness. Calves weaned at the initial vaccination exhibited greater overall response to vaccine. As data indicated an influence of maternal antibodies to vaccine effectiveness, this increase in titers may have been suppressed. However, as calves weaned at the booster vaccine experienced stress associated with weaning, it is to be expected that they would have suppressed antibody response to the booster vaccine. A research study by Tait et al. (2013) evaluated the effect of weaning time of the calves in the previous study on subsequent yearling performance and carcass quality. Yearling weights were measured along with fat thickness and REA as measured by ultrasound to monitor performance. Carcass traits of interest included HCW, fat thickness, REA, KPH, marbling score, YG, WBSF, and meat pH. There was a positive association found between calves that had high antibody concentrations to having increased yearling weight. Similarly, calves with greater overall antibody response exhibited increased ADG as high-responding calves weaned at the initial vaccination gained weight more quickly than low or nonresponder calves. There was a positive relationship between high antibody concentrations with a more favorable increase in meat pH. Additionally, high-responding calves that were weaned at the initial vaccination exhibited a favorable, decrease in WBSF. No other performance or carcass traits were effected by treatment, however the data exhibited in this study support that providing calves with a robust immune system prior to entering into the feedlot is an important step in ensuring increased performance and carcass quality.

Cow Performance

While low-stress weaning methods are important for calf health and performance, cow performance is a key component of a productive operation. Weaning calves decreases the energy requirement of cows by taking away the energy requirements for lactation. Cows approaching a moderate BCS of 3 from a 1 to 5 scale exhibit increased pregnancy rates of 100% ± 6 compared to cows less than BCS 3 exhibiting decreasing body condition of 69% ± 10 (Houghton et al., 1990). Therefore, it is important to allow cows to increase in BCS to increase pregnancy rates. Early weaning calves reduces postpartum interval (**PPI**) by 24.3 d and increases first service conception rate by 21.7% (Houghton et al., 1990). Alternative weaning methods may serve as a unique opportunity to wean calves in a less stressful approach next to their dams while allowing cows less than a moderate BCS to gain condition for rebreeding. It is important to allow adequate time for cows to reach proper condition in order to conceive another pregnancy. Cow BCS increases and pregnancy rates improve as calf age at weaning decreases (Myers et al., 1999). Cows with early weaning calves exhibit decreased annual costs, and increased BCS and weight when compared to cows with normally- or late-weaned calves (Story et al., 2000). However, it can be useful to continue the use of milk from the dam as a beneficial supplement to growing calves. Therefore, it is important to equally consider both cow and calf gains to optimize their performance in the weaning process.

Conclusion

Calves weaned at the booster vaccination showed suppressed final antibody levels, which may be attributed to weaning stress. If weaning stress is minimized through a low-stress weaning protocol, this may be able to increase vaccine effectiveness and better prepare calves for feedlot entry. Nose flap weaning devices offer a unique opportunity to implement this type of vaccination and weaning protocol. Using a system including an initial vaccine given several weeks prior to weaning, nose flaps could be implemented at the same time as the initial vaccine without additional labor or processing. Calves would then receive the booster vaccination at weaning and transportation as usual. This protocol may be a unique opportunity to increase vaccine effectiveness without negatively impacting morbidity rates during the feedlot-receiving period. Additionally, as nose flaps essentially prevent calves from nursing, this may serve as an opportunity to allow cows to increase in condition before going into winter to increase rebreeding rates during the following season.

As forage quality and quantity varies by region, there are inconsistencies in the effect of nose flaps and other two-stage weaning strategies on calf performance. There is currently a lack of data evaluating weaning strategy as it effects long-term calf performance such as heifer pregnancy rate and steer carcass quality, as well as subsequent year cow pregnancy rates. Additionally, there is a little research evaluating the effect of weaning strategy on calf humoral immune response to vaccination. Research is needed to investigate the use of nose flaps weaning devices and how it affects calf performance, cow performance, calf humoral immune response, and carcass quality.

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CHAPTER II

EFFECTS OF QUIETWEAN NOSE FLAP DEVICES APPLIED TO CALVES ON COW AND CALF PERFORMANCE, CARCASS TRAITS, AND HEIFER FERTILITY

Summary: The objective of this study was to determine if QuietWean nose flap (**NF**) devices could be used on calves for a short time, followed by returning calves to normal nursing, to effectively alter dam body condition. This study was conducted at 2 locations using Angus and Angus × Hereford primiparous cows (n = 245). Cow and calf pairs were allocated to 4 treatments in a completely randomized design. Treatments on the calves were: 1) NF for 30 or 31 d while remaining with their dams and removal from dam to the feedlot on d 30 or 31 (**LT-30R**), 2) NF for 4/5 d while remaining with their dams and removal from dam to the feedlot on 30/31 d (**ST-30R**), 3) NF for 4/5 d while remaining with their dams and removal from dam to the feedlot on 60/62 d (**ST-60R**), and 4) no NF while remaining with their dams and removal from dam to the feedlot on 30/31 d (**CON-30R**). Cow BCS and BW were collected on d 0, 60 or 62, and 120 or 122. Cow fat thickness (cm) was measured by ultrasound on d 0 and 60 or 62. Calves were weighed on d 0, and 30 or 31, and 60 or 62. By d 60 or 62, BW from cows in CON-30R was less ($P < 0.05$) than cows in LT-30R or ST-30R. Cows in LT-30R gained more ($P < 0.0001$) from d 0 to 120 or 122 than all other treatment groups, while cows in ST-60R gained less ($P < 0.05$) than all other treatments. Fat thickness as measured by ultrasound did not differ among treatments ($P = 0.18$). While there was no difference in BCS on d 60 or 62 and 120 or 122, cows in ST-60R decreased ($P < 0.001$) in BCS from d 0 to 60 or 62; whereas all other groups increased ($P > 0.05$) in BCS. There was no difference in cow pregnancy rates of the subsequent year among treatments ($P = 0.64$). By d 30 or 31, calves in LT-30R gained less ($P < 0.0001$) than calves from all other treatments. There was no difference ($P < 0.05$) in BW between calves in ST-30R and ST-60R on d 30 or 31; however, both treatments gained less ($P < 0.0001$) than calves in CON-30R. Calves in CON-30R gained more ($P < 0.0001$) than calves in the other treatments. Heifer pregnancy rates in CON-30R tended to be lower than all other treatments ($P = 0.05$). LT-30R heifers were older at calving than ST-30R ($P < 0.05$). Yearling weight (**YWT**), HCW, fat thickness, REA, yield grade (**YG**), and quality grade (**QG**) did not differ among treatments. These results indicate that NF weaning devices can improve performance of cows and heifers without negatively impacting steer carcass quality.

Key words: beef calves, BCS, fat thickness, nose flap, two-stage weaning

INTRODUCTION

Typical weaning methods generally include sudden separation of calves from their dams. Removing calves from cows decreases the energy requirements of the cows and allows them to begin improving in body condition. As pregnancy rates are greater in cows maintaining moderate BCS or rising towards moderate (Houghton et al., 1990), it is important to allow adequate time for cows to reach proper condition in order to conceive another pregnancy. Cow BCS increases and corresponding year pregnancy rates improve as calf age at weaning decreases (Myers et al., 1999). However, it can be useful to continue the use of milk from the dam as a beneficial supplement to growing calves. Therefore, it is important to equally consider both cow and calf gains to optimize their performance in the weaning process. Several weaning methods have been studied to observe their effects on cow and calf performance after weaning (Price et al., 2003, Enriquez et al., 2009). One method has been the use of nose flap (**NF**) weaning devices. A NF inhibits the nursing of calves while continuing to allow them to consume feed and water.

The objectives of this study were to: 1) examine the effect of NF devices on cow body condition while allowing calves to remain on pasture with their dams, and 2) to examine the effect of NF on heifer performance and steer carcass traits.

MATERIALS AND METHODS

This experiment was conducted following Colorado State University Animal Care and Use Committee approval at the Eastern Colorado Research Center.

Experimental Design and Treatments

The experiment was a completely randomized design. Two pasture locations were used for this study, each containing herds consisting of 153 (location 1) and 92 (location 2) primiparous Angus and Angus x Hereford cows (n = 245), 113 steer, 123 heifer, and 9 bull calves. Cows were diagnosed for pregnancy and calving records were evaluated before the study. Non-pregnant cows and cows with incomplete records were removed from the study. Remaining cow/calf pairs were then randomly assigned by breed, calf age, and calf gender to 1 of 4 treatments at each location: 1) NF for 30- or 31-d (long-term; **LT**) while remaining with dam followed by removal (**R)** from dam to a feedlot on d 30 or 31 (**LT-30R,** n = 62), 2) NF for 4- or 5-d (short-term; **ST**) while remaining with dam followed by R from dam to a feedlot on d 30 or 31 (**ST-30R,** n = 61),

3) NF for 4- or 5- d while remaining with dam followed by R from dam to a feedlot on d 60 or 62 (**ST-60R**, n = 61), and 4) no NF while remaining with dam followed by R from dam to a feedlot on d 30 or 31 (**CON-30R,** n = 61). As seen in Figure 1, on d 0 calves in LT-30R, ST-30R, and ST-60R treatment groups were fitted with QuietWean NF (JDA Livestock Innovations Ltd, Saskatchewan, Canada) and returned to their dams. On d 4 or 5, depending on location, pairs were again gathered and NF were removed from the ST-30R and ST-60R groups. On d 30 or 31, cows and calves were gathered and NF from LT-30R group were removed. Calves from the LT-30R, ST-30R, and CON-30R were then separated from dams and placed in a feedlot. The ST-60R group remained on pasture with their dams until d 60 or 62. Body condition score as described by Wagner et al. (1988) and BW of cows were collected on d 0, 60 or 62, and 120 or 122. Cow fat thickness (cm) was measured by ultrasound on d 0 and 60 or 62. Calves were weighed on d 0, and 30 or 31, and 60 or 62.

Postweaning Animal Management

After all calves were removed from their dams and placed in the feedlot, calves were placed on a growing diet for 30 d (Table 2.1). Heifers (n = 123) were placed on pasture after calves were separated for 150 d until artificial insemination using a 5-d estrous synchronization protocol with controlled internal drug release (**CIDR**; EAZI-BREED CIDR, 1.38 g of progesterone, Zoetis, Florham Park, NJ) and given an injection of 100 µg of GnRH (Factrel, Gonadorelin, Zoetis, Florham Park, NJ) i.m. After 5 d, CIDRs were removed and heifers were given an injection of 25 mg PGF2α (Lutalyse, Dinoprost tromethamine, Zoetis). Artificial insemination took place 72 h after CIDR removal and heifers were given an injection of GnRH.

Bulls were placed with heifers 10 d after AI. Heifers were checked for pregnancy 85 d after AI to determine approximate age of the fetus, pregnancy rate to AI, and overall season-long pregnancy rate. Replacement heifers were selected (n = 21) and those not chosen as replacements were sold as pregnant heifers. Age at calving was collected for replacement heifers the following year.

After the 30 d growing diet, steers (n = 113) were placed on cornstalks for 135 d. Yearling weights (**YWT**) were collected, and steers were placed on pasture for an additional 150 d before being brought to the feedlot to be finished. One steer died while on pasture, and 15 steers were sold. Remaining steers (n = 97) were placed in the feedlot and fed a finishing diet (Table 2.1). Steers were fed for 120 d before being harvested. Hot carcass weights were collected at harvest, and the following carcass characteristics were measured 24 h postmortem: 1) yield grade (**YG**), 2) quality grade (**QG**), 3) subcutaneous fat thickness over the 12th rib, and 4) ribeye area (**REA**).

Statistical Analysis

Data for cow BCS, BW, fat thickness, and calf BW were analyzed as a completely random design using the PROC Mixed procedure of SAS (v. 9.2; SAS Inst. Inc., Cary, NC) to produce a mixed model accounting for breed, sex, and sire, with cow or calf as the experimental unit. Heifer and cow pregnancy data were analyzed using the PROC Glimmix procedure of SAS to produce a binomial model with heifer or cow as the experimental unit. There was a location × treatment interaction ($P = 0.02$) for calf BW, so data for calf BW were analyzed by location. There were no location × treatment interactions ($P > 0.05$) for BCS, BW, fat thickness, heifer pregnancy, or steer carcass quality variables, so data were pooled across locations. Means were separated using the LSMEANS procedure of SAS using least significant differences when $P <$ 0.05.

RESULTS AND DISCUSSION

Cow Performance

On d 60 or 62, CON-30R cows weighed less ($P < 0.05$) than LT-30R and ST-30R cows (Table 2.2). Cows from the LT-30R treatment gained more from d 0 to 60 or 62 ($P < 0.05$) and 0 to 120 or 122 ($P < 0.0001$) than all other treatment groups. There was no difference ($P = 0.85$) in gain between cows from the ST-30R and CON-30R from d 0 to 120 or 122. Cows from the ST-60R gained less ($P < 0.05$) from d 0 to 120 or 122 than all other treatment groups. It is important to note that there was no difference in BW between ST-60R and CON-30R cows on d 60 or 62, as the former treatment included nursing calves for an additional 30 d than the latter.

There was no difference ($P = 0.51$) in cow fat thickness as measured by ultrasound among treatment groups on d 60 or 62 (Table 2.3). As seen in Table 2.4, there was no difference across treatments in cow BCS at the beginning of the study ($P = 0.39$). By d 60 or 62, cows from ST-60R had a lower ($P < 0.01$) BCS than all other treatments. There was no difference ($P < 0.05$) between cows from ST-30R and CON-30R by d 120 or 122, however BCS was greater ($P >$ 0.05) for ST-30R cows than ST-60R cows by this time. Cows from ST-60R experienced less (P < 0.05) BCS gain than all other treatments from d 0 to 60 or 62. While there were no differences ($P > 0.05$) in cow BCS from d 60 or 62 to 120 or 122, total change in BCS gain was lower ($P <$ 0.05) for cows from ST-60R than all other treatments. It is important to note that fat thickness as measured by ultrasound is an objective method of measuring condition, whereas BCS is a subjective method. However, there are benefits of using BCS as a measure of condition as it is measured based on the appearance of the fat cover of multiple different parts of the animal (Eversole et al., 2009), whereas ultrasound fat thickness is only measured at one location over the 12th rib.

Dams were observed for pregnancy post breeding season the following year (Table 2.5). There was no difference ($P > 0.05$) in AI pregnancy rates amongst treatment groups. Similarly, there was no difference ($P > 0.05$) in season-long pregnancy rate among treatments. It is important to note that the percentage of cows observed pregnant by AI is on the lower edge of average, as average pregnancy rate for the AI protocol described previously is 43.6-58.8% (Hall et al., 2009).

These results support previous findings that have found weaning calves earlier resulted in improved cow body condition (Myers et al., 1999; Story et al., 2000). Results of the current study, however, do not support research by Pruitt et al. (2000) who observed that in a calving season beginning in mid-March, cows that weaned calves in mid-September had higher pregnancy rates in the subsequent year than cows that weaned calves in mid-October. As CON-30R cows in the current study were already at a high pregnancy rate of 98.8 ± 2.4%, there was little room for dramatic improvement.

Calf Performance

There was a location × treatment interaction ($P \leq 0.02$) for calf BW. This was likely due to a difference in the forage quality and availability to calves on pasture; however, forage quality was not monitored in the current study. As seen in Table 2.6, the average BW of calves at location 2 began lower ($P < 0.05$) than location 1. Due to this, data were reported by location.

There was no difference in calf BW on d 0 or 30 at location 1. Calves in CON-30R gained more ($P < 0.0001$) than all other treatment groups from d 0 to 30. There was no difference ($P > 0.05$) in gain between calves in ST-30R or ST-60R, and LT-30R calves gained less ($P < 0.01$) than calves in all other treatments from d 0 to 30. From d 30 to 60, only ST-60R calves remained in the pasture with their dams. By d 60, ST-60R calves weighed less ($P < 0.05$) than all other treatments and gained less ($P < 0.05$) BW from d 0 to 60. Over the entire period from d 0 to 60, LT-30R calves gained less ($P < 0.05$) than CON-30R calves, however there was no difference ($P > 0.05$) in gain between ST-30R and CON-30R calves.

At location 2, CON-30R calves weighed and gained more ($P < 0.05$) than LT-30R calves by d 31. These results were expected, as the NF prevented nursing from those calves for the entire 31-d period. Once removed from their dams and moved to the feedlot on d 31, there was no difference ($P > 0.05$) in calf BW among LT-30R, ST-30R, or CON-30R calves. For both locations, calf BW on d 60 or 62 and change in calf BW from d 0 to 60 or 62 was lower ($P < 0.05$) in calves from ST-60R than all other treatments. At this time, the other calves had already entered the feedlot and received a more energy dense diet. These findings support those reported by Haley et al. (2005), in which the use of NF weaning devices for 14 d lowered calf ADG during implementation of the treatment, but ADG was not suppressed once calves were removed from their dams.

Heifer Pregnancy

As seen in Table 2.7, overall pregnancy rates from CON-30R heifers were less ($P < 0.05$) than ST-30R heifers, and tended to be less than LT-30R ($P = 0.07$) and ST-60R ($P = 0.08$) heifers. LT-30R heifers were older ($P < 0.05$) at calving than ST-30R and ST-60R heifers, and tended to calve later in the calving season than ST-60R heifers ($P = 0.09$). However, there was no difference ($P > 0.05$) in age at calving or calving date within the calving season between LT-30R and CON-30R heifers. The findings in the current study support previous findings (Lusby et al., 1981) that early-weaned heifers have higher pregnancy rates than normally weaned calves. In the current study, however, NF calves were allowed to remain on pasture with their dams throughout the weaning process as opposed to traditional weaning. This indicates that two-stage weaning protocols may improve heifer pregnancy rates.

Steer Carcass Quality

Carcass data can be found in Table 2.8. There was no difference ($P > 0.05$) in YWT among treatments. In addition, there were no differences ($P > 0.05$) for any carcass traits measured in this study among treatments. This supports previous findings where no differences in carcass quality were found among early- and normally weaned calves (Myers et al., 1999; Fluharty et al., 2000), indicating that NF are not detrimental to carcass quality.

IMPLICATIONS

Through implementation of nose flaps for a 4 or 5 d period and removal of calves after 30 d, cow and calf performance data indicate that a similar protocol could maintain calf ADG and improve condition of cows to optimize calf and cow performance. Increased calf weights in location 1 indicate that this group may have been overgrazed, and calf weights were not suppressed after nose flaps were implemented as seen in location 2. This indicates that in periods where forage is plentiful, nose flaps may be a useful tool to wean calves alongside their dams without inhibiting calf growth. In addition, heifer pregnancy and steer carcass data indicate that two-stage weaning protocols using nose flaps may be beneficial to reproductive development in heifers and may benefit producers, while maintaining value in the carcasses from steers from the same group. Additional research is needed to further examine the use of nose flaps as a weaning protocol and the effect on cow and calf performance.

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Treatment	d 0	4 or 5	30 or 31	60 or 62
LT-30R	NF placed	-	NF removed Calves removed from dams	-
ST-30R	NF placed	NF removed	Calves removed from dams	-
ST-60R	NF placed	NF removed	-	Calves removed from dams
CON-30R	-		Calves removed from dams	-

Figure 2.1. Schedule of nose flap weaning treatments applied to calves[1]

[1] ST = short-term, LT = long-term, NF = nose flap, R = removal from dam.

Table 2.1. Composition of growing diet fed to newly weaned steer and heifer calves for 30 d post-removal from the dam and finishing diet fed for 120 d to yearling steers (DM basis)

	Diet	
Item	Growing	Finishing
Ingredients, %		
Dry flaked barley	24.8	29.9
Dry flaked corn	15.9	30.1
Ground wheat straw	26.1	0.0
Dried distillers grains	30.7	30.9
Liquid supplement[1]	2.5	2.5
Sorghum silage	0.0	6.6
Diet composition		
CP, %	15.4	17.3
DM, %	86.4	68.6
NEm, Mcal/kg	1.71	1.98
NEg, Mcal/kg	0.99	1.33

[1] Liquid supplement = molasses-based containing monensin, vitamins, and minerals.

Table 2.2. Least squares means for the effect of two-stage weaning with nose flaps applied to calves at two locations on cow BW[1]

	Treatment[2]					
Item	LT-30R	ST-30R	ST-60R	CON-30R	SEM	*P* - Value
BW, kg						
d 0	497.5	510.5	505.9	501.1	5.7	0.40
d 60/62	516.6^{a}	517.7^{a}	502.3^{ab}	498.1^{b}	5.7	0.03
d 120/122	535.5	535.0	523.2	525.8	5.7	0.24
BW gain or loss, kg						
Δ d 0 to 60 or 62	19.3^{a}	7.1^{b}	0.4^{bc}	-3.2^{c}	3.8	<0.001
Δ d 60 or 62 to d 120 or 122	18.8^{ab}	18.6^{ab}	16.5^{a}	27.7^{b}	3.4	0.09
Δ d 0 to 120 or 122	38.3^{a}	25.9^{b}	16.9^{c}	25.2^{b}	2.7	<0.0001

[1] There was no location × treatment interaction ($P > 0.05$).

[2] LT-30R = nose flaps (NF) for 30 or 31 d while remaining with dam, calves removed (**R**) from dam on d 30 or 31; ST-30R = NF for 4 or 5 d while remaining with dam, calves removed from dam on d 30 or 31; ST-60R = NF for 4 or 5 d while remaining with dam, calves removed from dam on d 60 or 62; CON-30R = no NF while remaining with dam, removed from dam on d 30 or 31.

[a-c] Means within a row without common superscripts differ ($P < 0.05$).

Table 2.3. Least squares means for the effect of two-stage weaning with nose flaps applied to calves at 2 locations on cow subcutaneous fat thickness measured by ultrasound on d 0 and 60 or 62[1]

	Treatment[2]					
Item	LT-30R	ST-30R	ST-60R	CON-30R	SEM	*P* - Value
Fat thickness, cm						
d 0	1.30	1.12	1.19	1.32	0.076	0.18
d 60 or 62	0.84	0.89	0.76	0.91	0.076	0.51
Fat gain or loss, cm						
Δ d 0 to 60 or 62	-0.48	-0.23	-0.46	-0.43	0.102	0.34

[1] There was no location × treatment interaction ($P > 0.05$).

[2] LT-30R = nose flaps (NF) for 30 or 31 d while remaining with dam, calves removed (**R)** from dam on d 30 or 31; ST-30R = NF for 4 or 5 d while remaining with dam, calves removed from dam on d 30 or 31; ST-60R = NF for 4 or 5 d while remaining with dam, calves removed from dam on d 60 or 62; CON-30R = no NF while remaining with dam, removed from dam on d 30 or 31.

Table 2.4. Least squares means for the effect of two-stage weaning with nose flaps applied to calves at 2 locations on cow BCS[1]

	Treatment[2]					
Item	LT-30R	ST-30R	ST-60R	CON-30R	SEM	*P* - Value
BCS[3]						
d 0	5.43	5.55	5.48	5.48	0.07	0.39
d 60 or 62	5.59^{a}	5.65^{a}	5.42^{b}	5.62^{a}	0.08	<0.01
d 120 or 122	5.51^{ab}	5.57^{a}	5.40^{b}	5.52^{ab}	0.08	0.10
BCS gain or loss						
Δ d 0 to 60 or 62	0.16^{a}	0.10^{a}	-0.07^{b}	0.14^{a}	0.04	<0.001
Δ d 60 or 62 to d 120 or 122	-0.07	-0.09	-0.03	-0.10	0.05	0.77
Δ d 0 to 120 or 122	0.09^{a}	-0.002^{a}	-0.09^{b}	0.04^{a}	0.05	0.08

[1] There was no location × treatment interaction ($P > 0.05$).

[2] LT-30R = nose flaps (NF) for 30 or 31 d while remaining with dam, calves removed (**R)** from dam on d 30 or 31; ST-30R = NF for 4 or 5 d while remaining with dam, calves removed from dam on d 30 or 31; ST-60R = NF for 4 or 5 d while remaining with dam, calves removed from dam on d 60 or 62; CON-30R = no NF while remaining with dam, removed from dam on d 30 or 31.

[3] BCS scale was 1 = thin, 9 = obese (Wagner et al., 1988).

[a,b] Means within a row without common superscripts differ ($P < 0.05$).

Table 2.5. Least squares means for the effect of two-stage weaning with nose flaps applied to calves at 2 locations on dam pregnancy[1]

	Treatment[2]					
Item	LT-30R	ST-30R	ST-60R	CON-30R	SEM	*P* - Value
Pregnancy rate to AI, %	43.3	46.1	44.8	42.8	10.1	0.98
Overall season-long pregnancy rate, %	95.6	97.6	97.8	98.8	2.4	0.64

[1] There was no location × treatment interaction ($P > 0.05$).

[2] LT-30R = nose flaps (NF) for 30 or 31 d while remaining with dam, calves removed (**R**) from dam on d 30 or 31; ST-30R = NF for 4 or 5 d while remaining with dam, calves removed from dam on d 30 or 31; ST-60R = NF for 4 or 5 d while remaining with dam, calves removed from dam on d 60 or 62; CON-30R = no NF while remaining with dam, removed from dam on d 30 or 31.

Table 2.6. Least squares means for the effect of two-stage weaning with nose flaps applied to calves at 2 locations on calf BW by location[1]

	Treatment[2]					
Item	LT-30R	ST-30R	ST-60R	CON-30R	SEM	*P* - Value
Location 1:						
BW, kg						
d 0	227.1	221.7	222.9	218.5	4.1	0.54
d 30	247.1	246.2	247.4	250.1	4.2	0.92
d 60	281.4^{a}	279.8^{a}	259.0^{b}	281.3^{a}	4.2	0.0002
BW gain or loss, kg						
Δ d 0 to 30	20.1^{a}	24.5^{b}	24.0^{b}	31.6^{c}	1.3	<0.0001
Δ d 0 to 60	54.1^{a}	58.1^{ac}	36.1^{b}	62.7^{c}	2.2	<0.0001
Location 2:						
BW, kg						
d 0	196.2	202.1	201.7	201.1	5.2	0.84
d 31	212.7^{a}	227.2^{ab}	226.2^{ab}	228.0^{b}	5.3	0.13
d 62	242.2^{a}	262.7^{a}	221.3^{b}	263.1^{a}	3.4	<0.0001
BW gain or loss, kg						
Δ d 0 to 31	16.1^{a}	24.3^{b}	24.5^{b}	26.9^{b}	1.8	<0.001
Δ d 0 to 62	50.3^{a}	59.9^{b}	41.2^{c}	62.0^{b}	4.9	<0.0001

[1] Because of a location × treatment interaction (*P* = 0.02), calf BW data are reported by location.

[2] LT-30R = nose flaps (NF) for 30 or 31 d while remaining with dam, calves removed (**R**) from dam on d 30 or 31; ST-30R = NF for 4 or 5 d while remaining with dam, calves removed from dam on d 30 or 31; ST-60R = NF for 4 or 5 d while remaining with dam, calves removed from dam on d 60 or 62; CON-30R = no NF while remaining with dam, removed from dam on d 30 or 31.

[a-c] Means within a row without common superscripts differ (*P* < 0.05).

Table 2.7. Least squares means for the effect of two-stage weaning with nose flaps applied to calves at 2 locations on pregnancy of 21 nulliparous heifers[1]

	Treatment[1]					
Item	LT-30R	ST-30R	ST-60R	CON-30R	SEM	*P* - Value
Pregnant to AI, %	45.5	43.7	57.2	23.8	16.9	0.13
Overall season-long pregnancy rate, %	92.2^{ab}	96.1^{a}	90.1^{ab}	70.5^{b}	6.3	0.05
Age at calving, d	753.7^{a}	717.3^{b}	719.3^{b}	739.3^{ab}	7.9	0.02
Calving date, d[3]	34.3	14.3	14.0	24.3	6.2	0.07

[1] There was no location × treatment interaction ($P > 0.05$).

[2] LT-30R = nose flaps (**NF**) for 30 or 31 d while remaining with dam, calves removed (**R**) from dam on d 30 or 31; ST-30R = NF for 4 or 5 d while remaining with dam, calves removed from dam on d 30 or 31; ST-60R = NF for 4 or 5 d while remaining with dam, calves removed from dam on d 60 or 62; CON-30R = no NF while remaining with dam, removed from dam on d 30 or 31.

[3] Day of calving season.

[a,b] Means within a row without common superscripts differ ($P < 0.05$).

Table 2.8. Least squares means for the effect of two-stage weaning with nose flaps applied to calves at 2 locations on steer carcass traits[1]

	Treatment[2]					
Item	LT-30R	ST-30R	ST-60R	CON-30R	SEM	*P* - Value
YWT, kg[3]	441.4	454.4	448.9	445.8	5.9	0.48
HCW, kg	433.8	439.7	429.5	429.8	6.0	0.60
Fat thickness, cm	1.75	1.65	1.75	1.78	0.076	0.56
REA, cm^2	83.5	84.2	83.0	83.5	1.4	0.95
YG[4]	4.1	4.0	4.1	4.1	0.1	0.91
Quality Grade:						
Prime, %	0.0	11.5	4.8	20.0	4.73	0.54
Upper 2/3 choice, %	68.0	57.8	57.1	48.0	9.96	0.57
Low choice, %	32.0	30.8	38.1	32.0	9.57	0.95

[1] There was no location × treatment interaction ($P > 0.05$).

[2] LT-30R = nose flaps (NF) for 30 or 31 d while remaining with dam, calves removed (**R)** from dam on d 30 or 31; ST-30R = NF for 4 or 5 d while remaining with dam, calves removed from dam on d 30 or 31; ST-60R = NF for 4 or 5 d while remaining with dam, calves removed from dam on d 60 or 62; CON-30R = no NF while remaining with dam, removed from dam on d 30 or 31.

[3] YWT = yearling weight.

[4] YG = yield grade.

CHAPTER III

EFFECTS OF TWO-STAGE WEANING WITH NOSE FLAP DEVICES APPLIED TO CALVES ON COW BODY CONDITION, CALF PERFORMANCE, AND CALF HUMORAL IMMUNE RESPONSE

Summary: The objective of this study was to examine the effect of fitting calves with NF devices for 21 d prior to separation from the dam on cow BCS, calf performance both pre- and post-separation from the dam, and humoral immune response to vaccination compared to traditional weaning. This study was conducted using primiparous and multiparous Angus and Hereford cows (n = 113) and their respective Angus, Hereford, and Angus × Hereford calves (161 ± 22.7 d, 179.4 ± 3.92 kg). Cow/calf pairs were allocated to one of 2 treatments in a completely randomized design: 1) NF for 21 d prior to separation from the dam (**NF**), or 2) no NF for 21 d prior to separation from the dam (**CON**). Cow BCS was measured on d -21 and 56 to determine cow performance. Calf separation from the dam occurred on d 0. Calf performance was determined for 21-d before separation from the dam and during the feedlot period (d 1 post-separation from the dam through d 195). Modified-live vaccinations were administered on d -21 and 1. Calves were weighed on d -21, 1, 7, 14, 21, 28, and 195 and jugular blood samples were collected on d -21, 1, 14, and 28. Cow BCS and change in BCS were similar across treatments ($P > 0.05$). There was no difference ($P > 0.05$) in calf BW on d 1, 7 or 28 between treatments; however, CON calves tended to have greater BW on d 14 ($P = 0.09$), 21 ($P = 0.07$), and 195 ($P = 0.07$). Control calves had greater ($P < 0.05$) ADG from d -21 to 1 compared to NF calves. However, ADG from d -21 to 195 was similar between treatments ($P > 0.05$). There was a tendency for CON calves to have greater ($P = 0.08$) DMI from d 22 to 28 with no difference ($P > 0.05$) between treatments at remaining times or during the 28-d post-separation period. Feed intake, efficiency and morbidity were similar across treatments ($P > 0.05$). Serum neutralization tests for bovine viral diarrhea virus type 1 (**BVDV-1**) and bovine herpesvirus type 1 (**BHV-1**) were used to measure humoral response to vaccination. Serum antibody titers to BVDV-1 tended to be greater ($P = 0.08$) for CON calves on d 0 and were greater ($P < 0.05$) by d 28. By d 28, more ($P < 0.05$) CON calves reached seroconversion than NF calves, with 82.1% of CON calves and 66.7% of NF calves reaching seroconversion. Serum antibody titers for BHV-1 were greater ($P < 0.05$) on d 0 and 28 for CON calves. Seroconversion to BHV-1 was greatest ($P < 0.05$) on d 14 for both treatments but did not differ ($P > 0.05$), with 82.5% of NF calves and 85.5% of CON calves reaching seroconversion. There was no difference ($P > 0.05$) in antibody titers to BHV-1 on d 14. An ovalbumin (**OVA**) challenge was conducted with a subset ($n = 57$) of calves to evaluate the humoral immune response to foreign protein during the initial post-separation from the dam period. There were no differences in OVA specific immunoglobulin G (**IgG**) among treatments ($P > 0.05$). These results indicate that NF devices did not influence calf performance, feed intake, feed efficiency, or morbidity during the initial post-separation from the dam period. Serum titers and seroconversion to BVDV-1 and BHV-1 were decreased in NF calves, however primary immune response to ovalbumin was not affected. More research is needed to determine long-term effects of NF weaning devices on calf performance and immune response, however they appear to be an adequate alternative to traditional weaning as it does not negatively effect calf performance or health status.

Key Words: beef calves, feedlot performance, immune response, nose flaps, weaning

INTRODUCTION

Blecha et al. (1984) reported decreased lymphocyte blastogenic responses of the immune system in calves experiencing transportation stress, indicating that stress has the ability to cause immunosuppression and increased disease susceptibility. Lymphocytes increase in response to infection and help the body produce an immune response necessary to respond to a vaccination or infection (Murphy et al., 2011). Abrupt weaning has also been observed to reduce immune function through decreased lymphocytes and neutrophils (Lynch et al., 2010). Not only does increased incidence of disease associated with stress disrupt an animal's well being, it is also detrimental to the beef industry as feedlot steers treated for disease have reduced performance and carcass merit (Schneider et al., 2009).

Minimizing stress associated with weaning may improve the effectiveness of vaccines. Two-stage weaning strategies may be able to serve as a low-stress weaning method by allowing the calf to begin to break the social bond with the dam before physical separation from the dam, therefore decreasing behaviors commonly associated with weaning stress (Haley et al., 2005; Lambertz et al., 2014). Fenceline weaning has been shown to decrease morbidity rates to 15% compared to 28% for traditionally weaned calves (Boyles et al., 2007). Proactive calf vaccination protocols include a vaccine administered a few weeks before weaning and a booster vaccine administered at weaning. This may serve as an opportunity to initiate a two-stage weaning protocol without processing calves an additional time.

Our hypothesis was that NF devices used for 21 d prior to weaning are a low-stress alternative to traditional weaning that would improve vaccine effectiveness without negatively impacting performance post-separation from the dam. The objective of this study was to evaluate the effects of nose flap (**NF**) weaning devices on cow performance, calf performance, both pre- and post-separation from the dam, and calf humoral immune response.

MATERIALS AND METHODS

This experiment was conducted following approval by the Colorado State University Animal Care and Use Committee.

One hundred and thirteen Angus, Hereford, and Angus × Hereford calves and primiparous and multiparous purebred Angus and Hereford dams were utilized in this experiment. On d -98 at 63 ± 22.7 d of age, all calves received a clostridial antigen vaccination (Ultrabac 7, Zoetis, Florham Park, NJ) and a modified-live intranasal vaccine containing bovine respiratory syncytial virus, infections bovine rhinotracheitis virus, and parainfluenza-3 virus (Inforce 3, Zoetis). On d -48 at 103 ± 22.7 d of age, all calves received another modified-live intranasal vaccine containing bovine respiratory syncytial virus, infections bovine rhinotracheitis virus, and parainfluenza-3 virus (Inforce 3, Zoetis). Cow BCS as described by Wagner et al. (1988) was collected on d -21 and 56 (Table 3.1). Calves were vaccinated with a modified live vaccine containing bovine rhinotracheitis, bovine viral diarrhea virus, parainfluenza 3 and bovine respiratory syncytial virus (Bovi-Shield GOLD 5, Zoetis) on d -21 and 1. Vaccination storage and handling protocols consistent with Beef Quality Assurance Guidelines (BQA, 2010) were followed, as vaccines stayed within an insulated container when not in use and small amounts were mixed at a time. Calves were separated from their dams on d 0 and weighed on d -21, 1, 7, 14, 21, 28, and 195. A subset (n = 57) of calves was challenged with an ovalbumin (**OVA**) injection on d 1. Blood was collected from all calves via jugular venipuncture on d -21, 1, 14, and 28 into individually marked vacuum tubes (Vacutainer, BD, Franklin Lakes, NJ). Samples were transported to the laboratory on ice and allowed to coagulate overnight before processing.

Experimental Design and Treatments

The experiment was a completely randomized design. Body weight at approximately 114 ± 22.7 d of age, breed, sex, and age of the calves were evenly distributed amongst treatments and cow/calf pairs were allotted to 1 of 2 treatments: 1) NF for 21 d prior to separation from the dam (**NF**), or 2) no NF for 21 d prior to separation from the dam (**CON**). On d -21, calves in the NF group were fitted with QuietWean NF (JDA Livestock Innovations Ltd, Saskatchewan, Canada) and returned to their dams. Average age of calves on d -21 was 161 ± 22.7 d of age.

On d 0, cow/calf pairs were gathered and calves were separated from their dams. All calves were then transported approximately 3.5 h (257 km) to Colorado State University's feedlot research facility. After an overnight rest period, calves were weighed, bled, and administered a booster vaccine (Bovi-Shield GOLD 5, Zoetis) on d 1. To measure feed intake and efficiency, a subset (n = 75) of calves was assigned to 1 of 8 pens by sex and treatment with 4 pens per treatment. Calves excessively greater or less than the mean BW were commingled by sex and treatment in a group pen, and data from these calves were included in calf performance and morbidity, but not included for feed intake or efficiency. Another subset (n = 57) of calves received an OVA challenge to examine humoral immune response to a foreign protein immediately post-separation from the dam.

Calves received a feedlot starter ration upon entering the feedlot (Table 3.2). During the first week, supplemental grass hay was included in the ration. Orts were collected weekly, weighed, and subtracted from the amount fed to calculate DMI. After the 28-d feeding period, heifer calves were commingled together and bull calves were commingled together.

Trained feedlot staff monitored calves daily for signs of morbidity. Treatment records were collected and analyzed for calves treated either once or 2 or more times for respiratory illness, digestive conditions, lameness, or other ailments.

Serum Neutralization

After blood samples were allowed to coagulate overnight in a refrigerator at 5°C, they were centrifuged at room temperature (25°C) at 1,000 x *g* for 10 min to separate serum. Serum was removed, placed into a sterile tube labeled with animal identification and d, and stored at -5°C until processing.

Serum neutralization tests against bovine viral diarrhea virus (**BVDV-1**; CSU NVSL 140BVD9701) and bovine herpesvirus (**BHV-1**; CSU Cooper Strain) were completed to analyze serum titers. Tests were conducted at the Colorado State University Veterinary Diagnostic laboratory. Serum was heat inactivated at 56°C for 30 min. Minimal essential medium was added to 96-well sterile culture plates, followed by positive control anti-serum and serum sample. Each sample was completed singularly within a single plate in two-fold dilutions. Virus diluted to 100 50% tissue culture infective dose was added to each well and allowed to incubate for 1 hr in a 37°C CO_2 cell culture incubator. After incubation, 150 µl of cell suspension was added to each well and again allowed to incubate in a 37°C CO_2 cell culture incubator. Following 3 d incubation, cells were observed for cytopathic effect (**CPE**). Titers were reported as log base 2 transformation of the reciprocal of the average greatest dilution that observed no CPE. A 0 antibody concentration was reported for serum that showed CPE at 1:4 dilution for IBR and 1:8 dilution for BVDV. Serum titers that showed a four-fold increase in serum titer concentration were considered seroconverted.

Ovalbumin Challenge

In order to observe the primary immune response at weaning and transportation, a subset (n = 57) of calves was injected with ovalbumin to elicit a primary response to a secondary antigen on d 1. Two mL of a solution containing 160 mg ovalbumin (**OVA**; Sigma A5503, Sigma Aldrich, St. Louis, MO), 60 mL Freund's Incomplete Adjuvant (Sigma F-5506, Sigma-Aldrich), and 60 mL of phosphate buffered saline were injected SC and 1 mL IM providing a total dose of 4,000 µg of ovalbumin per animal (Dorton et al., 2003). Serum was removed from blood samples in the procedure listed previously.

Analyses of serum antibody titers specific to OVA were conducted using the ELISA procedure described by Engvall and Perlmann (1972). Ninety six-well sterile culture plates were coated with OVA and incubated at 4°C for at least 18 h. Ovalbumin was aspirated from each well, and washed with a solution composed of Tris-Buffered Saline and Tween 20 a total of 3 times. Two hundred µl of TBST was added to each well and incubated at 21°C for a minimum of 1 h. Plates were aspirated, washed once, and filled with 100 µl of serum diluted with TBST. After 1 h incubation at 21°C, wells were aspirated and washed 5 times. One hundred µl of OVA specific IgG were added to each well and incubated at 21°C for 1 h. After incubation, wells were aspirated and washed an additional 5 times. To detect antibody, 100 µl of enzyme substrate was added to each well. To stop the reaction, 100 µl of H_2SO_4 was added to each well. Plates were read at 450 nm using a microtiter plate reader to observe serum OVA immunoglobulin G (**IgG**) concentrations. Titers were reported as log base 10 transformation of the reciprocal of the average greatest dilution.

Statistical Analysis

Data were analyzed as a completely randomized design using a mixed model using the mixed procedure of SAS (v. 9.2; SAS Inst. Inc., Cary, NC) with cow or calf as the experimental unit for cow BCS, calf BW, calf ADG, and serum titer concentration for BVDV-1, BHV-1, and OVA. Pen was used as the experimental unit for feed intake and efficiency. Initial cow BCS and serum antibody titer for BVDV-1 tended ($P = 0.09$) to differ, therefore they were each included as a covariate in each respective model. No effects ($P > 0.05$) were discovered for breed, age, gender, or sire for either BVDV-1 or BHV-1 titers. Binomial data (e.g. morbidity) were analyzed using the PROC Glimmix procedure of SAS to produce a binomial model with calf as the experimental unit for calf morbidity. No effects ($P > 0.05$) were discovered for age, sex, breed, or pen.

RESULTS

Cow Performance

Cow BCS data are included in Table 3.3. There was a tendency ($P = 0.09$) for a difference in BCS among cows when treatments were initiated; therefore initial BCS was included in the model as a covariate. There was no difference ($P > 0.05$) in cow BCS across treatments post-separation at pregnancy diagnosis on d 56. Overall change in BCS was also calculated. Cows from both treatments increased numerically in BCS post-separation; however, there was no difference in BCS change between NF and CON treatments ($P > 0.05$).

Calf Performance

As seen in Table 3.4, there was no difference ($P > 0.05$) in calf BW between NF and CON treatments at the beginning of the study, and there was no ($P > 0.05$) treatment × day interaction. There was no difference ($P > 0.05$) in BW between treatments on d 1 or 7 ($P > 0.05$). There was a tendency for CON calves to weigh more on d 14 ($P = 0.09$) and 21 ($P = 0.07$), but there was no difference in BW by d 28. On d 195, there was a tendency ($P = 0.07$) for CON calves to weigh more than NF calves.

All calves gained weight during the study ($P < 0.001$). Calves from the CON group had greater ADG ($P < 0.05$) than NF calves from d -21 to 1 when NF were present in the pre-weaning period. During the post-separation period, there was no difference in ADG ($P > 0.05$) across treatments. During the entire period pre- to post-separation from the dam, there was no difference in ADG ($P > 0.05$) between NF and CON calves. In addition, there was no difference ($P > 0.05$) in ADG from NF administration on d -21 to yearling weights on d 195.

Calf Feed Efficiency

Dry matter intake and G:F were monitored and analyzed during the 28-d post-separation from the dam period to determine feed intake and efficiency and can be found in Table 3.5.

There was no ($P > 0.35$) treatment × week interaction for DMI. During the post-separation period, DMI increased for both treatments ($P < 0.001$) during the 28-d post-separation period. There was no difference ($P > 0.05$) in DMI between NF and CON calves from d 1 to 21. There was a tendency ($P = 0.08$) for CON calves to have a greater DMI than NF calves late in the feeding period from d 22 to 28; however, there was no difference ($P > 0.05$) in overall DMI during the entire post-separation period.

There was no ($P > 0.35$) treatment × week interaction for G:F ($P = 0.23$). During the first week post-separation from d 1 to 7, both treatments had greater G:F ($P < 0.001$) than later observations. There was no difference ($P > 0.05$) in G:F between NF and CON calves during the weekly measurements. Over the duration of the post-separation period, there was no difference ($P > 0.05$) in G:F between NF and CON calves.

Morbidity and Humoral Immune Response

Morbidity rates can be found in Table 3.6. There was no difference ($P > 0.05$) between NF and CON calves treated for illness either once or 2 or more times during the 28-d post-separation period. To further analyze morbidity rates in calves treated once, cause for treatment was evaluated. In some instances, there was more than one cause for treatment. There were no differences ($P = 0.45$) between percentage of calves treated for respiratory illness, digestive conditions, or lameness. One hundred percent of NF calves treated once were treated for respiratory disease, and 33.3% of NF calves treated once were treated for digestive conditions. Eighty percent of CON calves treated once were treated for respiratory disease, and 20% were treated for lameness. Percentage of calves treated twice or more was 3.5% for NF calves and 5.4% for CON calves. No mortalities were observed for either treatment group.

Serum Titer Concentration

Serum samples were collected to measure humoral antibody titer response to vaccination. Serum antibody titer concentrations can be found in Table 3.7. Initial serum antibody titer concentrations for BVDV-1 were greater ($P = 0.006$) for NF calves, therefore initial antibody titer was included as a covariate in the model. A treatment × time interaction was observed for serum antibody concentration for BVDV-1 and BHV-1 ($P < 0.0001$). There was a tendency for NF calves to have higher serum antibody concentration for BVD-1 at d 0, however there was no difference among treatment by d 14. Control calves had greater ($P = 0.04$) serum antibody concentration for BVDV-1 on d 28. There was no difference ($P > 0.05$) in percentage of calves that had reached seroconversion by d 0 or 14, however by d 28, when antibody concentration peaked, more ($P < 0.05$) CON calves had seroconverted.

There were no differences ($P > 0.05$) in serum antibody concentration for BHV-1 on d -21. On d 0, CON calves had greater ($P < 0.05$) serum antibody concentration for BHV-1 than NF calves and more ($P < 0.05$) CON calves had seroconverted. However, there was no difference ($P > 0.05$) in serum antibody concentration or seroconversion when antibody titers peaked on d 14. By d 28, serum antibody concentration was greater ($P < 0.05$) for CON calves and more ($P < 0.05$) CON calves had seroconverted.

Ovalbumin Titer Concentration

No effects (*P* > 0.05) of breed, age, gender, or sire were found for OVA IgG antibody titers. As seen in Table 3.5, there was a difference (*P* < 0.0001) observed for serum antibodies to OVA by time, but no differences among treatments (*P* = 0.97). Serum antibody concentration was greater (*P* < 0.05) on d 14 than d 0, with no difference (*P* > 0.05) between d 14 and 28 for either treatment. Antibody concentration increased (*P* < 0.05) from 0 to 3.7 for both treatments by d 14, and to 3.77 and 3.75 by d 28 for NF and CON calves, respectively.

DISCUSSION

During the study, cow performance did not differ across treatments. While cow BCS was not monitored on d 0, these findings contradict previous studies where BCS improved when calves were early weaned (Myers et al., 1999, Story et al., 2000). In cows at 4.2 to 4.3 BCS, Myers et al. (1999) observed an increase in cow BCS when calves were weaned at 90 and 152 d, compared to 215 d. Cows in the present study were at a moderate BCS of 5.7 ± 0.10 at the initiation of the study, which may have left a narrow window for improvement. Price et al. (2000) observed an increase in BCS when comparing cows at an average of 5.4 to 5.5 when calves were weaned at 150 compared to 210 d, and another increase when compared to weaning at 270 d. In the present study, NF calves were prevented from suckling for only 21 d, compared to 60 d or more in other studies, which may have been too short of a time to cause a difference in cow BCS.

The decreased calf ADG for NF calves from d -21 to 1 supports previous studies (Haley et al., 2005, Burke et al., 2009). Burke et al. (2009) observed similar findings, as NF calves gained less weight compared to traditionally and fenceline weaned calves during 7 d pre-weaning; however, ADG was not hindered during the 7 d post-separation period. Haley et al. (2005) also observed suppressed ADG compared to control calves when NF were used for both 3 and 14 d; however, ADG was greater for NF calves during the 7 d post-separation when compared to traditionally weaned calves. As NF calves in the current study were effectively removed from milk intake 21 d before CON calves and transitioned from their dam's milk and range forage to only range forage, these results were to be expected. However, the tendency for NF calves to weigh less than CON calves in the current study contradicts a previous study that found no long-term effects of alternative weaning methods (Lambertz et al., 2014). This may be due to the fact that in the current study, NF calves were removed from milk 21 d earlier than CON calves. In the future, it may be beneficial to separate and wean CON calves from their dams at the time NF are applied to specifically evaluate the low-stress capability of NF.

Dry matter intake did not differ between treatments. This is somewhat contradictory to research conducted by Haley et al. (2005), who observed that NF calves spent more time eating during the first few days post-separation than CON calves. Similarly, Price et al. (2003) observed fence-line weaned calves spent more time eating during the first 7 d post-separation than CON calves. Both studies observed CON calves spending more time walking than calves subjected to a low-stress weaning alternative. While behavioral effects were not measured in the current study, weaning method did not have an effect on DMI or G:F.

Possible explanations for the lack of difference in morbidity rate could be due to the fact that calves used in the present study were not "high-risk" calves and experienced a low morbidity rate. Across the country, 16.2% of feedlot cattle in feedlots greater than 1,000 animals are treated for respiratory disease (USDA, 2013). Previous research has observed morbidity rates for traditionally weaned calves during the first 28 d after being placed in the feedlot as 28% (Boyles et al., 2007) and may reach as high as 60.7 to 75.3% (Richeson et al., 2009). Cows and calves used in this study were on a proactive vaccination program discussed previously, and therefore morbidity rates may not differ among treatments as greatly as calves that were considered high-risk. In addition, calves were penned according to sex and treatment with their companions rather than commingled with unfamiliar calves that may occur in a conventional feedlot setting.

Research investigating the interaction between vaccine effectiveness and weaning stress has been limited. Recent research investigating delaying vaccination until after weaning has helped to shed light on possible explanations for results seen in serum antibody titers in the current study. Implementing a NF protocol at the time of the pre-weaning (d -21) vaccine reduced antibody titer concentration and decreased the amount of calves seroconverting to BVDV-1 and BHV-1 up to 28 d after separation from the dam. Tait et al. (2013) investigated various weaning times with a two-vaccine vaccination protocol with the booster vaccine given an average of 21.1 d after the initial vaccine. The authors reported that calves weaned at the initial vaccine had a greater antibody titer response to BVDV-2 and increased ADG than calves weaned at the time the booster vaccine was given, indicating that providing the booster vaccination after stress associated with traditional weaning increases antibody titer. As antibody titer and percentage of calves that seroconverted to BVDV-1 and BHV-1 were suppressed in NF calves in the current study, this may indicate that stress experienced with suppression of nursing may have hindered vaccine effectiveness that was still present 28 d post separation from the dam. Pollock et al. (1991) suggested that cell-mediated immunity may be a better indicator of immunological status than humoral immunity for calves older than 5 mo of age. As cell-mediated immunity was not measured in the current study, more research needs to be conducted to fully understand the interactions between vaccination and weaning.

Contradictory to the antibody titer concentrations to BVDV-1 and BHV-1, there were no differences in antibody titers to OVA amongst treatments. As OVA is a foreign antigen, the immune system is able to create a primary response that mimics that of foreign antigens the calf may encounter once entering the feedlot. This indicates that the primary immune response was not affected as all calves initiated a robust response in reaction to the OVA; therefore, NF did not hinder the primary immune response.

IMPLICATIONS

Observations seen by administration of nose flaps for a 21-d period prior to weaning indicate that there was no impact on calf performance, feed efficiency, or calf morbidity during pre- or post-separation from the dam periods. As calves weaned through the use of nose flaps were effectively weaned 21 d prior to control calves, separating control calves from the dam at the same time nose flaps were applied may have provided another model to compare growth parameters in calves. A longer post-separation feeding period may be needed in future studies to monitor long-term effects of nose flap devices on feedlot performance and feed efficiency. Serum antibody titers and seroconversion to bovine viral diarrhea virus and bovine herpesvirus were decreased when nose flaps were applied at the pre-weaning vaccination, however primary immune response to ovalbumin given immediately after calves were removed from their dams and transported to the feedlot was not different among treatments. Additional research is needed to fully understand the interactions between weaning, transportation, and vaccine effectiveness.

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Table 3.1 Timeline of actions applied to cow/calf pairs

Item	Timeline (d)								
	-21	0	1	7	14	21	28	56	195
Cow BCS	X							X	
Application of NF[1] to NF treatment	X								
Modified-live vaccine	X		X						
Ovalbumin challenge			X						
Separation from dam and transportation to feedlot		X							
Blood sample	X		X		X		X		
Calf BW	X		X	X	X	X	X		X

[1] NF = nose flap

Table 3.2 Ingredient and composition (DM basis) of receiving ration consumed by calves during the 28-d period after separation from the dam

Item	Diet
Ingredient, %	
Ground alfalfa	6.8
Wheat straw	23.4
Corn silage	11.7
Cracked corn	22.9
Dry distillers grains	30.8
Limestone	2.1
Salt	0.3
Molasses-based CP, vitamin, and mineral supplement	2.0
Diet composition:	
CP, %	15.2
DM, %	72.6
NEm, Mcal/kg	1.47
NEg, Mcal/kg	0.92

Table 3.3 Least squares means for the effect of nose flap (**NF**) administration in calves for 21 d prior to weaning on cow BCS[1]

	Treatment			
Item	NF[2]	CON[3]	SEM	*P* - Value
Cow BCS				
d -21	5.72	5.59	0.10	0.44
d 56	5.68	5.66	0.08	0.82
Change in cow BCS	0.04	0.06	0.12	0.92

[1] BCS scale was 1 = thin, 9 = obese (Wagner et al., 1988).

[2] Nose flaps were administered to calves for 21 d while remaining with dams prior to all calves being removed from dams on d 0 and transported to a feedyard and evaluated for 28 d.

[3] CON = control group; did not receive NF for 21 d while remaining with dams.

Table 3.4 Least squares means for the effect of nose flap (**NF**) administration in calves for 21 d prior to weaning on calf BW and gain

	Treatment			
Item	NF[1]	CON[2]	SEM	*P* - Value
BW, kg[3]				
d -21	177.5	181.4	6.9	0.61
d 1	190.2	200.5	6.9	0.18
d 7	206.7	218.9	6.9	0.12
d 14	211.6	224.8	6.9	0.09
d 21	229.3	243.2	6.9	0.07
d 28	231.1	243.0	6.9	0.12
d 195	402.1	416.1	6.9	0.07
ADG, kg/d[4]				
d -21 to 1	0.6	0.9	0.1	0.03
d 1 to 28	1.5	1.5	0.1	0.67
d -21 to 28	1.1	1.3	0.1	0.23
d -21 to d 195	1.1	1.1	0.1	0.73

[1] Nose flaps were administered to calves for 21 d while remaining with dams prior to all calves being removed from dams on d 0 and transported to a feedyard and evaluated for 28 d.

[2] CON = control group; did not receive NF for 21 d while remaining with dams.

[3] Day effect ($P < 0.0001$), treatment × day interaction ($P = 0.56$).

[4] Day effect ($P < 0.0001$), treatment × day interaction ($P = 0.91$).

Table 3.5 Least squares means for the effect of nose flap (**NF**) administration in calves for 21 d prior to weaning on calf feed intake and efficiency during the 28-d period after separation from the dam

	Treatment			
Item	NF[1]	CON[2]	SEM	*P* - Value
DMI, kg/d[3]				
d 1 to 7	4.8	4.8	0.4	0.99
d 8 to 14	6.1	6.4	0.4	0.61
d 15 to 21	6.6	7.1	0.4	0.34
d 22 to 28	6.6	7.7	0.4	0.08
d 1 to 28	6.2	6.3	0.4	0.87
G:F[4]				
d 1 to 7	0.53	0.50	0.02	0.30
d 8 to 14	0.10	0.11	0.02	0.78
d 15 to 21	0.24	0.22	0.02	0.44
d 22 to 28	0.13	0.10	0.02	0.42
d 1 to 28	0.24	0.21	0.01	0.27

[1] Nose flaps were administered to calves for 21 d while remaining with dams prior to all calves being removed from dams on d 0 and transported to a feedyard and evaluated for 28 d.

[2] CON = control group; did not receive NF for 21 d while remaining with dams.

[3] Week effect ($P < 0.0001$), treatment × week interaction ($P = 0.21$).

[4] Week effect ($P < 0.0001$), treatment × day interaction ($P = 0.81$).

Table 3.6 Least squares means for the effect of nose flap (**NF**) administration in calves for 21 d prior to weaning on calf health status during the 28-d period after separation from the dam

	Treatment			
Item	NF[1]	CON[2]	SEM	*P* - Value
Morbidity				
Treated once, %[3]	5.3	8.9	3.4	0.45
Respiratory, %	100.0	80.0	9.0	0.98
Digestive, %	33.3	0.0	13.6	0.98
Lameness, %	0.0	20.0	9.0	0.98
Other, %	0.0	0.0	-	-
Treated twice or more, %	3.5	5.4	2.7	0.64
Mortality, %	0.0	0.0	-	-

[1] Nose flaps were administered to calves for 21 d while remaining with dams prior to all calves being removed from dams on d 0 and transported to a feedyard and evaluated for 28 d.

[2] CON = control group; did not receive NF for 21 d while remaining with dams.

[2] Reason for treatment was distinguished for animals treated once. Some calves had symptoms associated with more than 1 condition.

Table 3.7. Least squares means for the effect of nose flap (**NF**) administration in calves for 21 d prior to weaning on serum titer concentration for bovine viral diarrhea virus (**BVDV**), bovine herpesvirus (**BHV**), and ovalbumin (**OVA**)

	Treatment			
Item	NF[1]	CON[2]	SEM	*P* - Value
Serum neutralization titer				
BVDV type 1[3], $\log_2$[4]				
d -21	2.32	1.14	0.30	<0.01
d 0	2.85	2.05	0.31	0.08
d 14	4.32	4.87	0.31	0.23
d 28	4.35	5.73	0.31	<0.01
BHV type 1[5], $\log_2$[6]				
d -21	0.93	0.55	0.13	0.25
d 0	2.34	3.36	0.29	<0.01
d 14	4.67	5.15	0.25	0.15
d 28	3.61	4.29	0.24	0.04
OVA[7] IgG titer, $\log_{10}$[8]				
d 0	0.0	0.0	-	-
d 14	3.70	3.70	0.07	0.92
d 28	3.77	3.75	0.06	0.76
Seroconversion to BVDV type 1, %				
d 0	1.8	3.6	2.1	0.57
d 14	17.5	30.9	5.6	0.11
d 28	28.1	51.8	6.3	0.01
Seroconversion to BHV type 1, %				
d 0	32.1	62.5	6.4	0.002
d 14	82.5	85.5	4.9	0.67
d 28	66.7	82.1	5.7	0.07

[1] Nose flaps were administered to calves for 21 d while remaining with dams prior to all calves being removed from dams on d 0 and transported to a feedyard and evaluated for 28 d.

[2] CON = control group; did not receive NF for 21 d while remaining with dams.

[3] BVDV = Bovine viral diarrhea virus.

[4] Day effect ($P < 0.0001$), treatment × d interaction ($P < 0.001$).

[5] BHV = Bovine herpesvirus.

[6] Day effect ($P < 0.0001$), treatment × d interaction ($P = 0.002$).

[7] OVA = ovalbumin.

[8] Day effect ($P < 0.0001$), treatment × d interaction ($P = 0.97$).

APPENDIX I

SAS Code for Chapter II

Code for analyzing cow or calf BW, and change in BW:

```
PROC MIXED; CLASS TRT TAG DAY;
MODEL cwt=TRT|DAY /ddfm=KR;
random TAG(TRT);
repeated DAY/subject=TAG(trt) type=ar(1) r rcorr;
LSMEANS TRT|DAY ;
SLICE TRT*DAY /SLICEBY=DAY PDIFF;
run;
PROC MIXED; CLASS TRT TAG DAY;
MODEL wt0to120=TRT|DAY/ddfm=KR;
random TAG(TRT);
LSMEANS TRT|DAY /pdiff;
SLICE TRT*DAY /SLICEBY=DAY ;
run;
```

Code for analyzing cow fat thickness and change in fat thickness:

```
PROC MIXED; CLASS TRT TAG DAY;
MODEL FAT=TRT|DAY /ddfm=KR;
random TAG(TRT);
```

```
repeated DAY/subject=TAG(trt) type=ar(1) r rcorr;
LSMEANS TRT|DAY ;
SLICE TRT*DAY /SLICEBY=DAY PDIFF;
run;
PROC MIXED; CLASS TRT TAG DAY;
MODEL FAT0TO60=TRT|DAY/ddfm=KR;
random TAG(TRT);
LSMEANS TRT|DAY /pdiff;
SLICE TRT*DAY /SLICEBY=DAY ;
run;
```

Code for analyzing cow BCS and change in BCS:

```
PROC MIXED; CLASS TRT TAG DAY;
MODEL BCS=TRT|DAY /ddfm=KR;
random TAG(TRT);
repeated DAY/subject=TAG(trt) type=ar(1) r rcorr;
LSMEANS TRT|DAY ;
SLICE TRT*DAY /SLICEBY=DAY PDIFF;
run;
PROC MIXED; CLASS TRT TAG DAY;
MODEL BCS0TO120=TRT|DAY/ddfm=KR;
random TAG(TRT);
LSMEANS TRT|DAY /pdiff;
```

```
  SLICE TRT*DAY /SLICEBY=DAY ;
  run;
```

Code for analyzing cow and heifer pregnancy rate:

```
PROC GLIMMIX;
CLASS TRT AITECH SEMENTECH BULL PREGSTATUS LOCATION; WHERE
PREGSTATUS=1;
MODEL PREGSTATUS(REF=FIRST)= TRT LOCATION/DIST=BINARY SOLUTION;
RANDOM AITECH SEMENTECH BULL;
lsmeans TRT /pdiff ilink;
RUN;
```

Code for analyzing nulliparous heifer age at calving or calving date:

```
PROC MIXED; CLASS TRT CALFID LOCATION  ;
MODEL CALVDAY=TRT  /ddfm=KR;
LSMEANS TRT /PDIFF adjust=tukey;
RUN;
```

Code for analyzing YWT, HCW, fat thickness, REA, and YG:

```
PROC MIXED;
CLASS TRT YG LOCATION;
MODEL YWT = TRT/ddfm=BW;
LSMEANS TRT /PDIFF ADJ=TUKEY;
```

```
RUN;
```

Code for analyzing percent of carcasses grading low choice, upper 2/3 choice, or low prime:

```
PROC GLIMMIX;

CLASS TRT LOWCH UPPERCH PRIME LOCATION;

MODEL PRIME(REF=FIRST)= TRT /DIST=BINARY SOLUTION;

lsmeans TRT /pdiff ilink;

RUN;
```

SAS Code for Chapter III

Code for analyzing cow BCS or change in cow BCS

```
PROC MIXED; CLASS TRT DAY COWID; WHERE DAY>0;

MODEL BCS =trt BCS_21 /ddfm=BW;

repeated day/subject=cowid(trt) type=ar(1) r rcorr;

LSMEANS TRT /PDIFF ADJ=TUKEY;

RUN;
```

Code for analyzing calf BW:

```
PROC MIXED; CLASS TRT DAY SEX BREED PEN CALFID;

MODEL WEIGHT=TRT|DAY /ddfm=KR;

repeated DAY/subject =CALFID(TRT) type=ar(1) r rcorr;

LSMEANS TRT|DAY /PDIFF ;

SLICE TRT*DAY /SLICEBY=DAY PDIFF;

RUN;
```

Code for analyzing calf ADG, DMI, and G:F:

```
PROC MIXED;

CLASS TRT WEEK SEX PEN;

MODEL ADGWEEK=TRT|WEEK  /ddfm=KR;

repeated WEEK/subject=PEN(TRT) type=ar(1) r rcorr;

LSMEANS WEEK /PDIFF ADJUST=TUKEY;
```

```
RUN;
```

Code for analyzing calf morbidity:

```
PROC GLIMMIX;
CLASS SEX TRT TREAT1X RESPIR DIGEST LAME;
MODEL TREAT1X(REF=FIRST)= TRT /DIST=BINARY SOLUTION;
LSMEANS TRT  /PDIFF ADJUST=TUKEY;
RUN;
```

Code for analyzing treated once for morbidity by respiratory, digestive, or lameness:

```
PROC GLIMMIX;
CLASS SEX TRT TREAT1X RESPIR DIGEST LAME OTHER TREAT1X; WHERE
TREAT1X=1;
MODEL DIGEST(REF=FIRST)= TRT /DIST=BINARY SOLUTION;
LSMEANS TRT  /PDIFF ILINK
RUN;
%MACRO MORBIDITY(Y);
PROC GLIMMIX;
CLASS SEX TRT TREAT1X RESPIR DIGEST LAME OTHER TREAT2X; WHERE
TREAT2X=1;
MODEL &Y.(REF=FIRST)= TRT /DIST=BINARY SOLUTION;
LSMEANS TRT  /PDIFF ILINK;
%MEND;
```

```
     %MORBIDITY(RESPIR); %MORBIDITY(DIGEST); %MORBIDITY(LAME);
     %MORBIDITY(OTHER);
     RUN;
```

Code for analyzing BVDV, BHV, or OVA serum titer concentration:

```
     PROC MIXED; CLASS TRT DAY CALFID ;
     MODEL LOG2 = TRT|DAY  /ddfm=KR RESIDUAL;
     repeated DAY/subject=CALFID(trt) type=ar(1) r rcorr;
     LSMEANS TRT|DAY ;
     SLICE TRT*DAY /SLICEBY=DAY PDIFF;
     RUN;
```

Code for analyzing BVDV serum titer concentration with covariate:

```
     PROC MIXED; CLASS TRT  DAY CALFID  ;
     MODEL LOG2 = TRT|DAY Day_21 /ddfm=KR RESIDUAL;
     repeated DAY/subject=CALFID(trt) type=ar(1) r rcorr;
     LSMEANS TRT|DAY ;
     SLICE TRT*DAY /SLICEBY=DAY PDIFF;
     RUN;
```

Code for analyzing seroconversion to BVD or BHV:

```
PROC GLIMMIX;
CLASS TRT CALFID;
```

```
MODEL d28(REF=FIRST)= TRT /DIST=BINARY SOLUTION;

lsmeans trt /pdiff ilink;

RUN;
```