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The Effects of Weaning Strategy on the Physiology and Performance of Beef Calves

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To the Graduate Council:

I am submitting herewith a thesis written by Cristina Campistol entitled "The Effects of Weaning Strategy on the Physiology and Performance of Beef Calves." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

Henry G. Kattesh, Major Professor

We have read this thesis and recommend its acceptance:

John C. Waller, Emmit Rawls, Gina M. Pighetti

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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THE EFFECTS OF WEANING STRATEGY ON THE PHYSIOLOGY AND PERFORMANCE OF BEEF CALVES

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Cristina Campistol
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ABSTRACT

Two experiments examined growth performance and physiological measures of stress in pre- and postweaned Angus steers (313 ± 24.5 kg; n = 48/Exp.), where steers were fitted with (YD) or without (ND) an anti-suckling device (Exp. 1), or provided (YS) or not provided (NS) a supplement (Exp. 2) for 7 d and weaned (d 7) by fenceline (FS) or total separation (TS). Steers in Exp. 1 were weighed and bled on d 0, 3, 7, 10, 14, 21, and 42, and in Exp. 2, on d 0, 7, 10, 14, and 21 and provided a supplement on d 7-21. In Exp. 1, weight gain was not different ($P = 0.74$) between ND and YD steers during preweaning. The YD-FS steers lost weight ($P = 0.01$) by d 10 compared with YD-TS steers. Hematocrit (Hct) increased ($P = 0.04$) in YD but not ND steers on d 3. Neutrophil:lymphocyte (N:L) ratio increased ($P < 0.01$) in all steers by d 7. Cortisol values in YD-FS steers were higher ($P < 0.05$) compared with YD-TS steers on d 10 and 21. The ND-FS steers had higher ($P = 0.04$) interferon-gamma (IFN) concentrations on d 10 compared with all YD steers. Haptoglobin (HAP) values increased ($P < 0.01$) in all steers by d 3. The FS steers had higher ($P < 0.01$) ceruloplasmin (CER) values by d 10 than TS steers regardless of preweaning treatment. Ovalbumin-specific IgG increased ($P < 0.01$) in all steers 10 d following its administration. In Exp. 2, NS-TS steers lost weight ($P < 0.01$) between d 7 and 10 compared with the remaining steers. The YS steers had higher cortisol, N:L ratio and CER on d 7 compared with NS steers. Moreover, NS steers

had higher ($P < 0.01$) Hct on d 10 than YS steers. Based upon physiological and growth performance data, it may be concluded that use of an anti-suckling device prior to weaning does not improve the animals' well-being and, providing a high fiber supplement beginning 7 d prior to weaning may temper the animals' stress response due to weaning when total separation is employed.

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LITERATURE REVIEW

Weaning of beef cattle involves the separation of a cow-calf pair. Beef calves are naturally weaned between 7 and 14 mo of age, during which time the cow progressively rejects the suckling of the calf, while the calf starts ingesting solid food (Price, 2008). However, beef producers tend to wean their calves at around 7 mo so that cows can return to estrous and rebreed on an annual cycle (Price, 2008). Weaning time will differ mainly between breed and geographic location (NAHMS-USDA, 2008). Calves located in the West and Southern Plain regions of the United States are weaned at 8 mo of age. In the North Central, Northern Plains and Southeast regions, calves are weaned at 7 mo of age (ERS-USDA, 2001). There are additional factors that producers consider when deciding at what age to wean their calves. These include: ending of a grazing lease or permit, forage availability, physical condition of the cow, market price, cash flow, and tradition (NAHMS-USDA, 2008).

Weaning Strategies

Weaning is stressful for both the calf and the cow. This stress is mainly caused by the loss of social contact between the cow-calf pair and the denial of suckling (Price, 2008). It is known that these two weaning components affect the calf's response in different

ways (Haley et al., 2005). The stress produced by weaning may appear in forms of increased movement, decreased food consumption, and increased vocalization by the calf (Price, 2008). It is known that cow and calf vocalize so that they can identify their voices and be reunited (Von Keyserlingk, 2007). Upon weaning, calves are mixed with other calves, which can lead to aggressive behaviors between individuals (Weary et al., 2007).

The traditional method of weaning beef cattle is performed by separating calves from their dams without applying any preconditioning treatment prior to weaning. This method is also referred to as abrupt separation. At weaning, calves can be total separated to a distant pasture or farm, or fenceline separated. As an alternative to the traditional method, weaning strategies incorporating a pre-conditioning program have been investigated and employed with the overall objective of reducing the stress associated with breaking the cow-calf bond. The goals of a pre-conditioning program are to prepare calves to be more efficient and also lower the risk of possible health problems in the future. The market demand of pre-conditioned calves has increased during the past few years (Bailey and Stenquist, 1996).

Preconditioning involves giving solid food to calves to advance rumen development so that they can be weaned earlier. Also, a health program is conducted where calves are vaccinated with infectious bovine rhinotracheitis (IBR), parainfluenza-3 (PI3), bovine viral diarrhea virus (BVDV) and bovine respiratory syncytial virus (BRSV). As a consequence, they will gain weight faster and reach market weight by 14-15 mo compared to an average range of 16-18 mo (Bailey and Stenquist, 1996). In addition,

early weaning was found to be beneficial in terms of calves being more stress tolerant during transportation (Arthington et al., 2005), and more social with other calves (Veissier et al., 1989). A 45 d pre-conditioning program (feeding, vaccinations and labor) is estimated to cost approximately $49/head (Donnell et al., 2008). When the market price of calves' increases, the cost of a pre-conditioning program will be negligible, since the sale price will be high enough to cover those expenses. In addition, feedlots are more receptive to buying pre-conditioned calves to reduce death losses (Bailey and Stenquist, 1996).

A two-stage method of weaning beef cattle was first investigated and reported by Haley et al. (2001). This method involves the prevention of nursing by the calf (stage 1) for a brief period before separation of the cow and calf (stage 2). Fitting the calf with a nose-flap device for 4-14 d before separation prevents the calf from nursing, but does not interfere with the calf from grazing, eating, or drinking (Grandin, 2008). Calves weaned using the two-stage method followed by fenceline separation have generated a higher net return (once feed and health programs were covered) at 58 d following weaning ($79.63), than did calves weaned by the traditional method ($45.97) as a result of higher ADG (Rawls, 2009).

Studies directed at the advantages of weaning beef calves in two stages compared with the traditional method, relative to the animal's well-being, have been primarily directed at measures of behavior and growth performance, with limited studies regarding measures of physiological change. Haley et al. (2005) conducted two trials documenting behavioral

changes of calves weaned using the two-stage method. In the first trial, calves of mixed breed and sex (n = 190, 187 ± 13 d of age at weaning) were fitted with an anti-suckling device 3 or 14 d prior to weaning, or kept as a control group (still nursing). Behavioral observations began 24 h following weaning and continued for two additional days. Calves fitted with the device for 3 d stayed closer to their mothers compared with control calves and calves fitted with the device for 14 d Two-stage weaned calves, following total separation from their dams, vocalized and walked less and spent more time laying down and eating than did control calves. Frequency of vocalization did not differ between calves prevented from nursing for 3 or 14 d. However, calves fitted with the anti-suckling device for 14 d walked and laid down more than calves fitted with the device for 3 d. In the second trial, calves fitted with the anti-suckling device for 4 d prior to weaning walked more (steps/d) than the control group. However, upon total separation from their dams, control calves walked more than calves that had been fitted with the device for 14 d. Overall, walking frequency from 4 d preweaning to 4 d postweaning was greater for the control calves (Haley et al., 2005).

Upon weaning, calves can be fenceline or totally separated. Fenceline separation involves the relocation of calves to an adjoining pasture for a brief period (~ 7 d), which prevents them from nursing, but allows them some social contact (visual, vocal, tactile) with their dams. In contrast, total separation practically eliminates all forms of social interaction between a cow and their offspring. Price et al. (2003) examined the effects of fenceline vs. total separation weaning strategies in beef calves (Angus x Hereford; 203-

228 d of age at weaning), and found that fenceline separated calves spent more time eating, less time walking and more time lying down than total separated calves during the first 7 d postweaning. The differences between treatments were greater within 3 d after weaning. In addition, fenceline separated calves were successfully weaned 5 or 6 d following the separation from their dams (Price, 2008).

Growth Performance

Cattle producers are concerned with raising their animals to a certain weight with the best meat quality possible to obtain the maximum economical benefit from each carcass. Stress is one of the most critical factors that can influence the growth performance of calves. The stress of weaning can lead to a decrease in feed intake and therefore a decrease in ADG. In addition, cattle that lose weight are more susceptible to health problems, which can increase the health cost if they become ill.

In the previously described study by Haley et al. (2005), ADG was lower (~0.4 kg/d) for calves during the time they were fitted with the anti-suckling device compared with calves without the device (~0.85 kg/d). In addition, during that same period of time, calves fitted with the anti-suckling device for 3 d prior to weaning (all calves were total separated in the study), exhibited higher ADG than calves fitted with the device for 14 d before weaning. During the first week following weaning, two-stage weaned calves gained ($P < 0.001$) more weight than did control calves. Within the two-stage weaned group, the calves that had been prevented from nursing for 3 d prior to weaning gained

more weight compared with calves prevented from nursing for 14 d.

Over the 44 d postweaning period, the two-stage weaned calves had a similar weight gain as the control calves (nursed until weaning), and the group that had been prevented from nursing for 14 d gained less weight than the group prevented from nursing for 3 d (Haley et al., 2005). The authors concluded from these results that during preweaning, calves that had been fitted with the anti-suckling device gained less weight than did calves without device. In addition, two-stage weaned calves gained more weight than control calves during postweaning.

In two additional trials, calves (181-189 d of age) were prevented from nursing for 5 d prior to weaning and were compared with the control group that was allowed to nurse their dams until weaning (Haley et al., 2005). Results of the first trial indicated no difference in ADG between calves of the two treatments during preweaning. However, in the second trial, calves that were prevented from nursing for 5 d prior to weaning exhibited a lower ADG than the control group during that same period of time. During the entire postweaning period (d 0 to 28) of the second trial, two-stage weaned calves had a greater ADG than calves that were still nursing. From these results the researchers concluded that there is a lack of evidence to confirm that two-stage weaned calves gain more weight than control calves during postweaning, since ADG data was not consistent between trials. In addition, ADG was not measured in all of the trials performed in the study.

When comparing fenceline vs. total separation, fenceline separated calves gained more weight than total separated calves during the two weeks following weaning. Moreover, total separated calves remained lighter than fenceline separated calves even 10 wk postweaning (Price et al., 2003). All calves in this study were weaned abruptly, and treatments were performed for only 1 wk following weaning. Subsequently, all calves were relocated together on pasture and were divided into two groups (n = 50).

Several approaches have been reported using preconditioning either by providing a dietary supplement to the lactating cow or to the calf following weaning. There are limited studies investigating the effects of diet supplementation directly to the calves themselves prior to weaning.

The effects of providing a pre-conditioning diet to the lactating cow on growth performance of the nursing calf have been found to differ depending on the composition of the supplement. Lake et al. (2006) reported no difference in ADG for calves nursing their dams fed Foxtail millet hay and a low fat supplement or fat supplement alone versus a supplement composed of high-linoleate safflower seed until d 40 of lactation. Providing a protein supplement to lactating cows every 3rd d during lactation was reported to increase weight gain of the calves (Short et al., 1996).

Schoonmaker et al. (2004) reported that overall ADG was greater in steers weaned at 119 d of age and fed a 50% high moisture corn and 30% corn silage during the 140 d following weaning compared to steers weaned at 204 d of age and provided a 70% corn silage diet for 54 d following weaning. These results suggest that providing a supplement

with less percentage of corn silage when weaning calves at an early age may be more beneficial than providing a supplement with a higher percentage of corn silage to calves that are weaned at the traditional age of weaning. It was also noted that the group that had been fed the high fiber diet and weaned at 119 d had greater fat depth at 260 d, lower percentage of longissimus muscle fat, and reduced shear force. In contrast, calves weaned at 204 d of age had heavier carcasses and larger longissimus muscle area.

Supplementing calves following weaning has not always proven to be beneficial regarding growth performance. Calves supplemented with Se and vitamin E for 28 d following weaning improved growth performance from d 0-42 postweaning (Swecker et al., 2008). In addition, the time of the day when giving the supplement has been reported to have different effects on ADG. Calves provided with pelleted corn gluten feed at 0.5% of BW each morning (0700 h) during the first 60 d following weaning, had a higher ADG than calves provided the supplement each day at noon (Scaglia et al., 2009). From these results the authors suggested that eating behavior was influenced by the time of the day the supplement was given and consequently had an effect on ADG.

Providing a highly palatable feed to the calf for a brief period prior to weaning may make the transition from milk to solid food less difficult (Bailey et al., 1996). Blanco et al. (2008) performed a study in Spain with 28 Brown Swiss cow-calf pairs where calves were weaned at 90 or 150 d of age, with or without a dietary starter concentrate mix provided prior to weaning. Of the calves that were weaned at 150 d of age, those receiving the supplement exhibited greater ADG compared to the non-supplemented

calves. No differences were found for weight gain between the early weaned calves as a result of supplementation. From d 150 to slaughter, ADG for all treatments was lowest among the early weaned and supplemented group of calves. Regarding carcass quality, early weaned calves had a greater dressing percentage (warm carcass/shrunk live wt) and fatness score than the traditionally-weaned calves, regardless of whether they were fed supplement. The non-supplemented calves exhibited the poorest carcass quality.

Physiological measures of stress

There are mainly two types of stress response: non-threatening and threatening (Moberg, 1999). The latter involves a risk for the animal's well-being and is also defined as distress. Stress, as defined by Moberg (1999), is the biological response(s) elicited when an individual perceives a threat to its homeostasis. The stress response begins with the recognition of a stimulus, which the animals' central nervous system (CNS) needs to identify as a possible threat to its homeostasis. If the stimulus is recognized as a threat, a biological response will result related to behavior and also to the nervous, neuroendocrine, and immune systems.

The first and most frequent biological response to a stress is directed through changes in behavior, where for instance, if the animal is in the sun and its body temperature is rising in a way that could threaten its homeostasis, the animal will relocate to a shaded area. The autonomic and endocrine systems can respond as well by redirecting more blood to a certain part of the animal's body, or synthesizing and releasing certain

hormones that will help the biological machinery return to homeostasis. However, the autonomic nervous system (ANS) stress response is short lasting, and for this reason has not been studied to as great extent as the endocrine response with regard to its long-term effects on the well-being of the animal. The pituitary is regulated, in part, by the hypothalamus and will enhance the connection between the CNS and the endocrine system. Therefore, the neuroendocrine system appears central to the understanding of stress on the animal's overall well-being (Moberg, 1999). The difficulty of studying the stress response exists mainly because the responses produced can vary depending on both the animal and the stressor. Therefore, different animals may not secrete the same hormones when they are under stress, and for this reason, the measurement of stress should not be limited to neuroendocrine changes alone.

An animal is subjected to many stimuli during its life. However, not all the stimuli can be considered as stressors, only those that result in the animal entering a prepathological state. The prepathological state, as proposed by Moberg (1999), occurs when the animal has been stressed and becomes vulnerable to developing a pathology. A pathology may be any condition that may impair or threaten to impair the organisms' ability to perform its normal functions, develop or reproduce. If the animal is in a prepathological state for a long time and becomes vulnerable to developing a pathology, it will enter the pathological state. Therefore, the likelihood of the animal developing a pathology is positively correlated with the length of time that the animal is in the prepathological state. The pathological state does not necessarily mean that the animal

will suffer a disease; in most cases it can be described as a loss of weight, changes in behavior, or irregularities in reproduction and development.

Cortisol. The measure of this glucocorticoid hormone, produced by the adrenal cortex, is most commonly used when assessing an animal's stress response. When an animal encounters a significant stressor, the hypothalamus releases corticotropin-releasing hormone (CRH) which is transported via the hypothalamic-pituitary portal circulation to the anterior pituitary gland for the production and release of adrenocorticotropic hormone (ACTH). This trophic hormone is responsible for the synthesis and release of glucocorticoids (i.e. cortisol). Elevated blood levels of cortisol can produce a negative feedback effect inhibiting further synthesis and release of CRH and ACTH. Cortisol is a hormone that can increase in a matter of minutes (Moberg et al., 2000).

From a summary of several studies looking at the effects of handling cattle on cortisol concentrations, it was suggested that values higher that 70 ng/mL in cows or steers might indicate inappropriate handling or use of poor equipment (Grandin, 1997). Baseline cortisol concentrations in beef cattle typically range from 0.5 to 9 ng/mL (Tennessen et al., 1984; Mitchell et al., 1988). Lefcourt et al. (1995) reported finding an increase in cortisol values in Angus x Hereford cows and their calves (4 to 6 mo of age) before and after temporary separation. Two blood samples were taken following separation, the first one was taken 4 min after separation and the second one was taken 45 min after separation. In addition, two more blood samples were taken the following day, immediately before and 4 min after calves were reunited with their dams. Control calves

and dams were not separated from each other during the experiment. The results showed that cortisol values did not differ in any of the groups from immediately prior to or 4 min after separation. However, cortisol values increased in separated dams, control dams and control calves 45 min following separation, but did not differ in the separated calves compared to values before separation. The results regarding cortisol values before and after calves were reunited with their dams showed that values before and 4 min after being reunited increased in separated dams, whereas cortisol values of the other groups did not differ. The researchers concluded that the increases in cortisol might have been more likely due to restraint while collecting blood samples, than due to weaning. Regarding gender, cortisol was shown to be higher in heifers than in steers, independent of the treatment applied, which was consistent in different transport and commingling conditions of newly weaned calves (Arthington et al., 2003).

Acute Phase Proteins. Ceruloplasmin is an acute phase protein (APP) produced by the liver that carries Cu in the blood as its major function but also has an influence in the metabolism of Fe. Ceruloplasmin has been related to weaning stress, increasing in concentration in stressed steers and tending to decrease in concentration in unstressed steers (Ward et al., 1999). In addition, ceruloplasmin concentrations were found to increase 14 d after the animals experienced stress (Ward et al., 1999). Cooke et al., (2009), studying the effects of acclimatizing beef heifers to handling, found no correlation between ceruloplasmin and cortisol concentrations. Moreover, cortisol values decreased in acclimated heifers compared to control heifers, and ceruloplasmin values

were not different between acclimated and non-acclimated heifers. Arthington et al. (2003) studied the effects of transportation and commingling of newly weaned Brahman-crossbred calves on APP. Calves were either transported for 3 h or not transported. Blood samples were taken at weaning and during the day following transportation. The researchers found that ceruloplasmin levels increased in calves during transportation compared to nontransported calves. After studying several APP, the researchers concluded that ceruloplasmin and fibrinogen were the acute phase proteins most affected by transportation stress in calves.

Haptoglobin, another APP, binds free plasma hemoglobin which allows enzymes to have access to hemoglobin and also prevents excretion of Fe by the kidneys (Kushner et. al., 1993). A positive relationship between haptoglobin and total WBC has been shown using haptoglobin deficient mice (Huntoon et al., 2008). A decrease in lymphocytes was observed in the haptoglobin deficient mice, which exhibited a decrease in lymphoid organ size; primarily the spleen. Arthington et al. (2003) in the study described previously, reported that in contrast to ceruloplasmin, haptoglobin appeared to be higher in steers that were not transported (Arthington et al., 2003).

Previous studies have lookeding at the effects of weaning on APP. Regarding APP as a whole, Qui et al (2007) reported that in response to weaning, APP increased in beef calves over time. Arthington et al., (2005) performed an experiment studying the differences in APP response measured in Brahman/English calves weaned at 3 versus 10 mo of age. The results showed that calves that had been weaned at 10 mo of age had

higher ceruloplasmin levels at d 3 and 7 postweaningcompared to that measured in early weaned calves. Haptoglobin concentrations increased in calves weaned at 3 and 10 mo. In addition, the calves weaned at 10 mo of age had higher concentrations of haptoglobin 3 d following weaning compared to the early weaned calves.

WBC, N:L ratio, and IFN-γ. The immune system is developed so that the animal can cope with possible threats to its health. It has been demonstrated that when animals are stressed, their immune system will not respond adequately because some components like neutrophil function (Salak et al., 1993) will be impaired, and therefore they will be more susceptible to diseases (Salak-Johnson et al., 2006).

The immune system responses can be classified into innate and adaptive immunity. Innate immunity is defined as the first line of defense; it is not highly specific and is considered a fast or acute response (Carroll et al., 2008). This type of immunity relies upon phagocytes, inflammatory releasing cells and natural killer cells (Carroll et al., 2008).

The second type of immunity is adaptive immunity, which differs from innate immunity mainly because it is specific for antigens and is enhanced over time. It is initiated by macrophages and dendritic cells, which expose the antigens to lymphocytes (Salak-Johnson et al., 2006). As noted earlier, weaning involves the separation of a cow-calf pair. The calf receives passive transfer of colostral immunoglobulins from the cow within the first day following birth. Upon weaning, the active immune status of the calf must be functioning in order for the animal to cope with novel pathogens. The stress

of weaning can compromise their immune system, since immune functions do not perform as well under stress conditions, and therefore the immune system becomes more vulnerable (Price, 2008).

The WBC's form part of the immune system. The normal range for WBC concentration in adult cattle is 4000-12000/µL (Malmo, 1993). There are two subclasses of WBC in blood; granulocytes (neutrophils, eosinophils, and basophils) and agranulocytes (lymphocytes and monocytes). An increased WBC count may indicate an infection, whereas a decreased count may indicate weakness from a long illness (Frandson, 2003). Neutrophils and lymphocytes are the predominant WBC in the circulation, and in cattle, lymphocytes are generally greater in number than neutrophils.

When total neutrophil numbers are increased, it is usually a sign of a bacterial infection or some form of extreme stress. Thus, performing a WBC differential count and calculating the neutrophil-to-lymphocyte (N:L) ratio can be useful in assessing the animals' health status and well-being (Frandson, 2003).

In a study performed by Anderson et al. (1999), dexamethasone (a synthetic glucocorticoid that simulates the effects of adrenal glucocorticoids) was used to reproduce the physiological stress response in cattle. They found that the N:L ratio and WBC count increased in steers 1 d following the administration of dexamethasone compared to the control steers which received a sterile saline solution. The researchers concluded that hematological parameters are sensitive to the effects of dexamethasone, however additional studies should be performed to prove that these measures are good

indicators of stress regarding the cattle's well-being.

Interferon gamma (IFN-γ) is a pro-inflammatory cytokine that is produced by T-lymphocytes, natural killer T cells, B cells, natural killer T cells and antigen-presenting cells (APC) (Shroder et al., 2004). The production of IFN-γ is positively regulated by other cytokines that are synthesized by APC, mainly interleukin (IL) 12 and 18 (Shroder et al., 2004). In the study by Anderson et al. (1999), IFN-γ did not appear to be affected by dexamethasone treatment, since values of control calves did not differ between values of treated calves. Moreover, the release of IFN-γ has been found to be related to the age of the calves. Carroll et al. (2009) administered endotoxin (LPS) to challenge the immune system of calves weaned at different ages. The results showed that calves weaned at 80 d of age had greater concentrations of IFN-γ than calves weaned at 250 d of age. The authors concluded from this study that the immune system of younger calves may be more efficient when it comes to recognizing foreign endotoxins.

Circulating level of IgG in response to ovalbumin (OVA) has been used in several studies to investigate the humoral immune response in animals subjected to various stressors. Lake et al., (2006) reported an experiment where OVA was administered to calves nursing cows fed different diets. The dams were provided either a low-fat control or a supplement based on cracked high-linoleate safflower seed during the first 40 d of lactation. The calves were injected with OVA (15 mg s.c.) at 21 and 35 d of age, and blood was collected weekly from d 14 to 42. The calves whose dams were fed the linoleate supplement experienced a decrease in total antibody production in response to

OVA throughout the study. The researchers concluded that the antibody production of the suckling calves may have been inhibited due to a decrease in lymphocyte development) resulting from the lipid supplementation to the dams.

Other studies regarding IgG in response to OVA have been performed using older calves. Lefevre et al, (2009) measured antibody titers, memory B cells and plasma cells in four, 4-8 mo old calves immunized twice with OVA 49 d apart. The researchers found an increase in OVA specific IgG titers from d 6 to 15 post-injection, which plateaued by d 49. A secondary response was observed at 3-4 d following the second injection, and reached greater levels than the primary response. The response of the plasma cells was very different from the response of the antibody titers. By d 6-7, a sharp increase in plasma cells was observed in the primary response, whereas in the secondary response a sharp peak was also shown, but this one occurred by d 3-4. Memory B cells showed an increase by d 15-20 in the primary response. During the secondary response, a decrease in memory B cells was observed immediately after d 0, followed by an increase by d 5-6. The researchers suggested that a decrease of memory B cells might have resulted from the cells becoming sequestered in the lymphoid organs.

Ward et al. (1993) examined the effects of dietary Cu supplementation ($CuSO_4$ or CuLys) on the immune response following injection of OVA. The researchers found that levels of IgG increased 7 d after injection and a linear response was shown from d 7-21. However, this increase was not related to any of the dietary treatments. Another study performed by Ward et al. (1999) looked at the effects of dietary and/or injected Cu on

immune response in Angus steer calves weaned at 7 mo of age. Twenty-two of 42 calves on study were injected with Cu 28 d before weaning, weaned and then transported to the feedlot. The day following weaning (d 0), calves were separated in pens (2 calves/pen) by treatment. The steers that had been injected with Cu were provided a supplement with 7.5 mg of Cu, as $CuSO_4$/ kg of DM, and the steers that had not been injected with Cu were provided a basal diet with no Cu supplement. In addition, one steer from each pen was injected intradermally with 150µg of phytohemagglutinin (PHA), and on d 133 of the growing phase, all steers were injected with OVA. The results showed that the antibody titers to OVA were higher in steers that had been Cu-supplemented. The author suggested that the immune function (humoral response) may also be altered by Cu-deficiency, however, this effect was only apparent after an extended period of time.

Ahola et al. (2005) also reported a study analyzing the effects of lifetime mineral supplementation (Cu, Zn, and Mn) on antibody response to OVA administered to calves during the finishing phase. The study showed that the increase of antibody in response to OVA was not related to mineral supplementation of the calves. The authors concluded that lifetime supplementation has an effect on health and performance only when there is a mineral deficiency.

RBC and Hematocrit. The number of RBC's within the circulation is influenced mainly by its rate of production, destruction, and loss due to circulation. The life-span of RBC's in adult cattle is approximately 160 d. In addition, the concentration of RBC's in adult cattle ranges from 5.0 to 10.0 x 10^6/µL (Malmo, 1993). An increase in RBC

concentration from 7 x10^6/µL to 9.9 x 10^6/µL was reported in Holstein dairy calves bled frequently until 84 d of age. In dairy/beef crossed calves sampled during the first 83 d of life, elevated concentration of RBC's were noted in blood samples having correspondingly high WBC concentrations (Knowles et al., 2000).

Hematocrit (Hct) refers to the percentage of a blood sample that is occupied by RBC's. An abnormally high Hct may indicate that the animal is producing too many RBC's or is dehydrated. Conversely, a low Hct could indicate that the animal is anemic (Frandson et al., 2003). Knowles et al., (2000), in the same study noted above, found a range of Hct values to be between 30-50%, where higher Hct values corresponded with a higher WBC count. Mohri et al. (2007) reported finding age-related changes in Hct measured in cattle, with values decreasing from 27 to 24% between birth and 4 wk of age and then increasing to 31 % by 12 wk of age.

From the studies previously described it is clear there is a lack of experiments performed related to both pre- and post weaning strategies and the measurement of physiological variables. For this reason, our hypothesis for the present study is that a combination of weaning strategies may reduce stress at the time of weaning, based on growth performance and select physiological measures. Two experiments were performed to document the effects of two different weaning strategies on the physiology and growth performance of beef calves. In the first experiment, steers were weaned using the two-stage vs. abrupt method with or without temporary fenceline contact with their dams. In the second experiment, steers were provided a high fiber supplement for 7 d

prior to weaning and weaned with or without temporary fenceline contact with their dams.

CHAPTER II

MATERIALS AND METHODS

All animal procedures were reviewed and approved by the University of Tennessee Animal Care and Use Committee (IACUC Protocol No. 1776), which were in accordance with the applicable portions of the Animal Welfare Act and the "Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching" by FASS. The studies were conducted at the Grassland Unit of the Plateau Research and Educational Center (PREC) in Crossville, TN. Some of the males were castrated when initially vaccinated (18 in Exp. 1 and 10 in Exp. 2), the rest of the males were castrated at birth.

Experiment 1

Forty-eight Angus and Angus X Gelbvieh steers (BW 314 ± 20.5 kg; age 231 ± 15.4 d), born and maintained with their dams on mixed orchardgrass/tall fescue pastures, were used in Fall, 2008. At 147 ± 15.4 d of age, steers were vaccinated with Cattlemaster® 5 (Pfizer Animal Health, Exton, PA), Vision® 7 (Intervet Inc., Millsboro, DE), implanted with Ralgro® (Schering-Plough Animal Health Corp., Summit, NJ), and de-wormed with Dectomax® (Pfizer Animal Health, Exton, PA) pour-on, and revaccinated with Cattlemaster® 5 and Vision® 7 at 175 ± 15.4 d of age. Steers were provided Purina® (St.Louis, MO) Preconditioning/ Receiving Chow® 3130 (6.8-9.1 kg/steer/d) for the first

10 d postweaning, and Co-Op 14% hi-energy with Rumensin® 80 (Elanco, Greenfield, IN) diluted at 40 g/T and given at 2.7 kg/steer/d with hay fed *ad libitum* thereafter. No antibiotics were included within the diets.

One week before beginning the study (d -7), all steers were weighed and randomly allotted in similar number and BW to one of two preweaning treatments as described below. Blood samples, and additional weights, were collected on d 0 (d of weaning), 3, 7, 10, 14, 21, and 42 between 0800 and 1000 h. On d 0, the humoral immune response was evaluated by injecting steers in the neck region with 3 mL (2 mL subcutaneous, 1 mL intradermal) of a solution containing 4 mg of ovalbumin (OVA; A5503, Sigma, St. Louis, MO) in 1.5 mL of Freund's Incomplete Adjuvant (FIA, Sigma F5506) and 1.5 mL of sterile phosphate buffered saline (PBS). Steers (n = 24) were fitted with a nose-flap weaning device (**YD**; C18349N Nasco; Fort Atkinson, WI; http://www.enasco. com/product/ C18349N) to prevent calves nursing from their dams. The remaining steers (n = 24) served as controls (**ND**). The animals were then rejoined with their dams on pasture. On d 7, at the time of weighing and bleeding, the YD steers had their nose device removed and all animals were then separated from their dams. Twenty-four steers (12 YD and 12 ND) were moved to a 0.81 ha pasture lot adjacent to their dams separated by woven-wire fence with openings too small to accommodate a calf's head (fenceline contact; **FS**). The remaining steers were separated from their dams and transported to a distant pasture lot such that the vocalizations of either group could not be heard by the other (total separation; **TS**). On d 14 following weighing and bleeding, the FS group was

transported to a pasture lot adjoining the TS group. Flow charts of the experimental design, and experimental procedures performed, are provided in Figures 1 and 2, respectively.

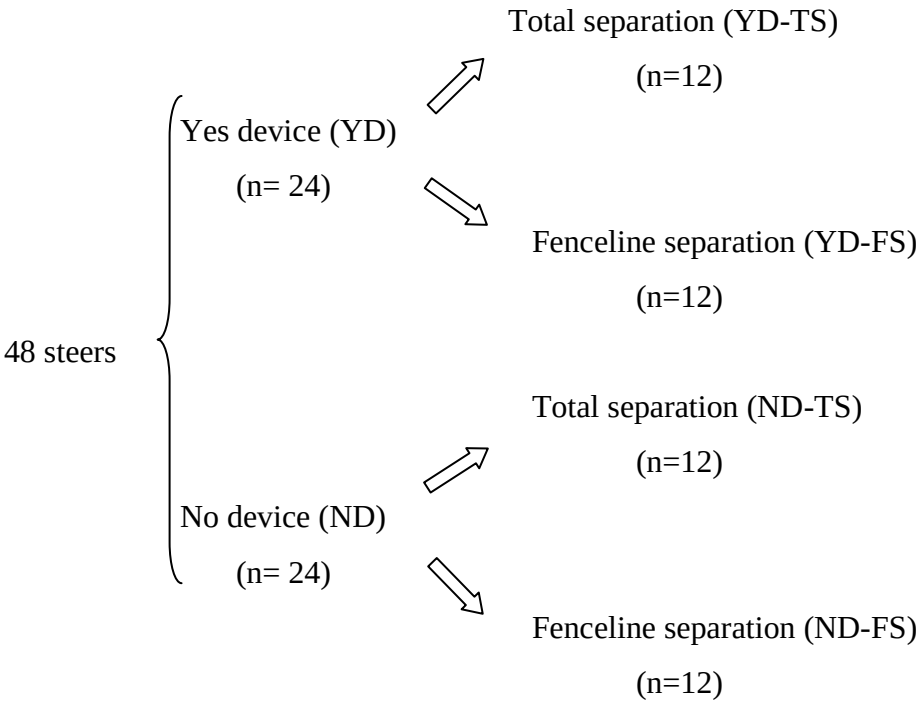

Figure 1. Design of Exp. 1.

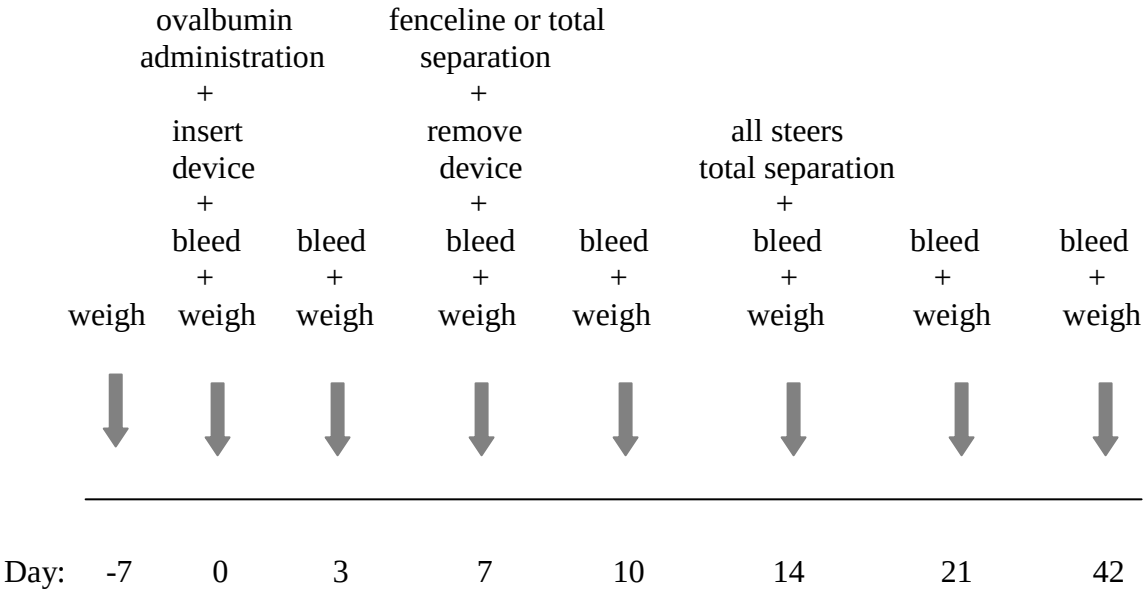

Figure 2. Procedures performed at each time period of Exp. 1.

Experiment 2

Forty-eight Angus steers (BW 312 ± 27.9 kg; age 208 ± 15.16 d), born and maintained with their dams on mixed orchardgrass/ tall fescue pastures, were used in Fall, 2009. Steers were initially vaccinated at 138 ± 15 d of age, and revaccinated at 174 ± 15 d of age, as described in Exp. 1.

One week before beginning the study (d -7), all steers were weighed and randomly allotted in similar number and BW to one of two preweaning treatments. Beginning one week prior to weaning (d 0), steers and their dams (n = 24) were offered a highly palatable high fiber supplement in well-spaced troughs at 4.5 kg/cow-calf pair/d (**YS**). Ingredient composition of the diet is presented in Table 1. The remaining steers with their dams served as controls and were not supplemented (**NS**; n = 24). All steers were provided the same supplement from weaning to d 21. No antibiotics were included within the supplement. On d 7, twelve steers from each treatment were moved to a 0.81 ha pasture lot adjacent to their dams separated by woven-wire fence with openings too small to accommodate a calf's head (fenceline contact; **FS**). The remaining steers were separated from their dams and transported to a distant pasture lot such that the vocalizations of either group could not be heard by the other (total separation; **TS**). On d 14, the FS group was transported to a pasture lot adjoining the TS group. Flow charts of the experimental design, and experimental procedures performed, are provided in Figures 3 and 4, respectively.

Table 1. Composition and nutrient content of supplement fed to calves in Exp. 2.

Composition	% (As-fed basis)
Cracked corn grain	23.0
Soyhulls (Pelleted)	10.0
Soybean meal	3.3
Cottonseed meal	10.5
Cane Molasses	5.0
TM salt	0.3
Citrus Pulp (Pelleted)	20.0
Cottonseed hulls	28.0
Nutrient content	% (Dry Matter basis)
TDN	71.79
CP	12.66
Ca	0.59
P	0.28
K	1.17
CF	22.67
Salt	0.28

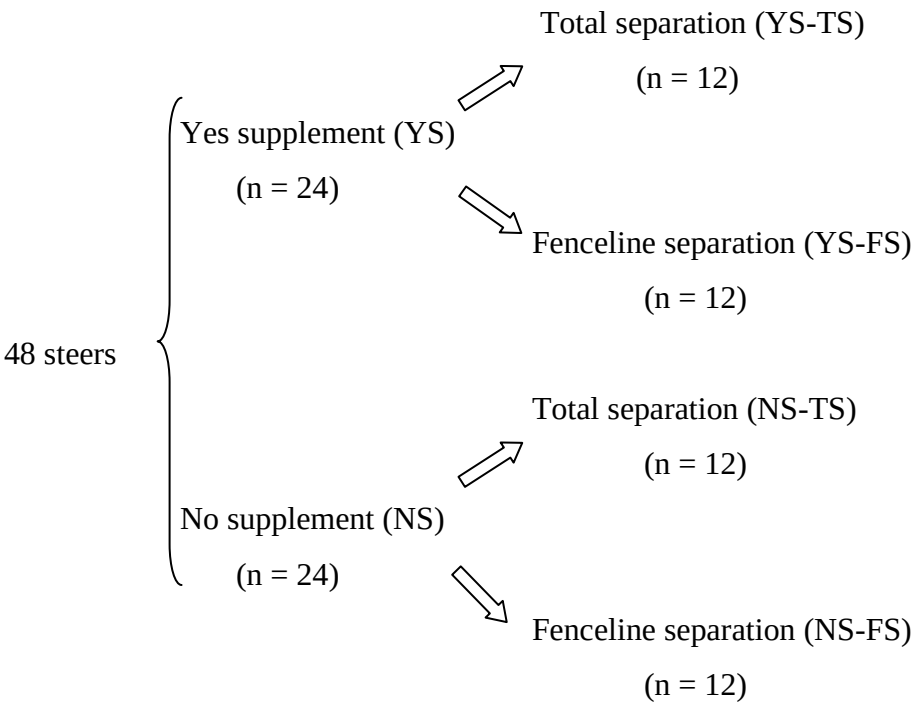

Figure 3. Design of Exp. 2.

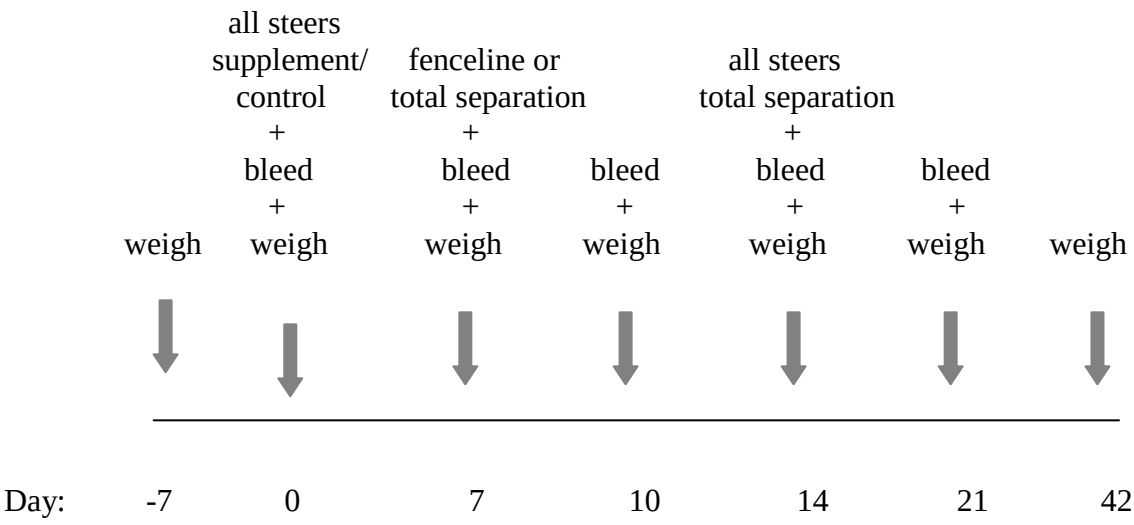

Figure 4. Procedures performed at each time period of Exp. 2.

Sample Collection and Analyses

Blood samples were obtained from each steer while restrained in a squeeze chute with head gate. Each blood sample was collected into two 10.0 mL vacutainers with and without lithium heparin (Cat. No. 02-689-7, 02-683-60; Fisher Scientific, Sewanee, GA). Blood smears were made from heparinized whole blood for hematological analyses. Plasma was collected from heparinized blood following centrifugation at 2,000 × *g* for 20 min at 4°C, aliquoted into three 1.8 mL cryogenic vials, and stored at -20°C until analyzed for total cortisol, haptoglobin and ceruloplasmin. The non-heparinized blood was refrigerated overnight at 4°C, serum was harvested and stored at -20°C until analyzed for IgG to ovalbumin (Exp. 1 only), and interferon-gamma (IFN-γ).

Hematological parameters. Blood smears were prepared on glass slides and stained with hema-quick stain solutions (Hema 3 Stat Pack Cat # 123-869; Fisher Scientific, Sewanee, GA). Smears were examined under oil immersion objective (100 ×) to differentiate the number of neutrophils and lymphocytes within 100 cells counted, and subsequent calculation of neutrophils-to-lymphocyte (N:L) ratio. Hematocrit values were recorded at each sampling time. Red and white blood cell number/μL of whole blood were determined in Exp. 2 using a Hyperion Dual Ratio Diluter Mallinckrodt count machine and shaker.

Cortisol. Plasma total cortisol concentrations were analyzed using an RIA procedure

(Coat-A-Count, Diagnostic Products, Los Angeles, CA) as previously reported in our laboratory (Doherty et al., 2007). Cortisol concentration was expressed as nanograms per milliliter.

Intra- and interassay CV for Exp. 1 were 10.8 and 4.6% for low (43.3 ng/mL), 6.7 and 7.6% for medium (45.8 ng/mL), and 6.8 and 8.8% for high (91.9 ng/mL) cortisol standards. Intra- and interassay CV for Exp. 2 were 5.7 and 14.7% for low (14.0 ng/mL) and 11.8 and 9.0% for high (54.1 ng/mL) cortisol standards.

Acute Phase Proteins. Plasma haptoglobin concentrations were determined in duplicate samples by measuring haptoglobin/hemoglobin complexing by the estimation of differences in peroxidase activity and read in units of absorption × 100 at 450 nanometer (Makimura and Suzuki, 1982) following assay quality controls as described by Qiu et al. (2008). Intra- and interassay CV for haptoglobin were 2.8% and 11.5%, respectively for Exp. 1, and 1.7 and 1.9%, respectively for Exp. 2.

Plasma ceruloplasmin oxidase activity was analyzed using a colorimetric procedure as reported by Qiu et al. (2007). Concentrations were expressed as milligrams per deciliter. Intra- and interassay CV for ceruloplasmin were 2.6 and 6.8%, respectively for Exp. 1, and 1.6 and 4.8 %, respectively for Exp. 2.

Ovalbumin-specific IgG. Ovalbumin-specific IgG antibody titers in response to inoculation with ovalbumin (see above for Exp. 1) were detected by ELISA (Engvall et

al., 1972). The optical density (OD) values were read at 450 nm using an ELISA reader, and expressed as a ratio of a positive control sample present on each plate.

IFN-γ. Serum was analyzed using a custom-developed ELISA validated for bovine IFN-γ (SearchLight, Pierce Biotechnology Inc., Rockford, IL) with a detection range of 2.0 to 500 pg/mL. Intra- and interassay CV were 11.1 and 8.4%, respectively.

Statistical Analysis

Data were analyzed using the MIXED procedure of SAS (2003, SAS Institute Inc., Cary, NC) for a completely randomized block design (CRD) with covariate on birth weight and age. The treatment design was repeated measures performed on 7 (Exp. 1) and 6 (Exp. 2) time periods, with factorial in the whole plot. Calves were blocked by initial weight and randomly assigned to treatments. Fixed effects of the Exp. 1 were the following: device, separation, device * separation, time, device * time, separation * time, device * separation * time, birth weight and age. Classification variables were: steer, device, separation, time and bleed. Random variables used were: steer (device * separation), time * steer (device * separation). The statistical model of Exp. 1 was as follows:

$$Y = \mu + D + S + D * S + A\ (D*S) + T + T * D + T * S + T * D * S + T * A\ (D*S) + A + BW + e,$$

where: Y = dependent variable

μ = population mean

D = device

S = separation

T = time

A = age

BW = birth weight

e = standard error

In Exp. 1, three different approaches (models) were made based on the general model, since treatments were performed at different time periods. The first model included all treatments and time periods. The second model included d 0, 3, 7 and device. The third model included d 7, 10, 14, 21, 42 and separation as a treatment in addition to device.

Fixed effects of Exp. 2 were the following: supplement, separation, supplement * separation, time, supplement * time, separation * time, supplement * separation * time, birth weight and age. Classification variables were: steer, supplement, separation, time and bleed. Random variables used were: steer (supplement * separation), time * steer (supplement * separation). The statistical model of Exp. 2 was as follows:

$$Y = \mu + Su + S + Su * S + A\ (Su * S) + T + T * Su + T * S + T * Su * S + T * A\ (Su * S) + A + BW + e,$$

where: Y = dependent variable

μ = population mean

Su = supplement

S = separation

T = time

A = age

BW = birth weight

E = standard error

In both experiments, data were represented as LSM ($P < 0.05$) with SE and the degrees of freedom were calculated with the Kenward-Roger method. Repeated measures correlation was found using an autoregressive procedure and correlations between dependent variables were performed using Pearson correlation coefficients. Diagnostics were checked for normality (Shapiro-Wilk), extreme observations and equality of variance.

CHAPTER III

RESULTS

Experiment 1 (Growth Performance)

Growth performance of steers, represented by weight gain between time points, is presented in Table 2. Weight gain was not different ($P = 0.74$) between control steers (ND) and steers fitted with the anti-suckling device (YD) over the 7 d before separation from their dams. Steers fitted with the anti-suckling device and fenceline separated (YD-FS) lost weight compared with device fitted and total separated (YD-TS) steers during the first 3 d following weaning (-1.5 vs. 6.2 ± 2.0 kg; $P < 0.01$). From d 10 to 14, weight gain was greater ($P < 0.05$) in ND steers (4.7 ± 1.6 kg) compared with YD steers (1.5 ± 1.6 kg). On d 14 to 21, regardless of preweaning treatment, the FS steers gained more weight ($P < 0.01$) than did the TS steers (10.6 vs. 1.9 ± 1.5 kg). From d 21 to 42, YD-FS steers gained less ($P = 0.02$) than YD-TS steers (17.3 vs. 29.0 ± 3.5 kg). However, overall weight gain during the postweaning period (d 7 to 42), was greater ($P < 0.01$) for YD-TS steers compared with YD-FS steers (40.2 vs. 28.2 ± 3.6 kg).

Experiment 1 (Physiological Measures)

Hematocrit measures of steers did not differ between treatments within each sampling period prior to or following weaning. However, within treatment, YD steers experienced

Table 2. Weight gain (kg) of steers fitted with or without an anti-suckling device prior to weaning and weaned by fenceline (FS) or total (TS) separation[1].

Treatment	Initial wt (d 0)	d 0-3	d 3-7	**d 0-7**	Initial wt (d 7)	d 7-10	d 10-14	d 14-21	d 21-42	**d 7-42**
Device (n = 24)	317.8[a]	6.9[a]	4.5[a]	11.4[a]						
FS (n = 12)					335.0[a]	-1.5[b]	1.4[a]	11.0[a]	17.3[b]	28.2[b]
TS (n = 12)					323.4[a]	6.2[a]	1.6[a]	3.6[b]	29.0[a]	40.2[a]
No device (n = 24)	316.0[a]	8.2[a]	2.7[a]	10.9[a]						
FS (n = 12)					330.0[a]	1.5[ab]	4.7[b]	10.3[a]	21.3[ab]	38.4[ab]
TS (n = 12)					323.9[a]	5.1[a]	4.8[b]	0.2[b]	26.7[ab]	36.7[ab]
pooled SE:	± 4.1	± 1.6	± 1.1	± 1.9	± 5.9	± 2.0	± 1.6	± 1.5	± 3.5	± 3.6

[1] Values are means ± SE of steers between each weighing according to pre- (d 0 to 7) and post- (d 7 to 42) weaning treatment. Steers were fitted with an anti-suckling device (device, n = 24) or no device (control, n = 24) on d 0-7. Following weaning (d 7), steers within pre-weaning treatment were separated from their dams by fenceline (FS, n = 24) or total (TS, n = 24) weaning method.

[a, b] Within a column, means without a common superscript letter differ ($P < 0.05$).

an increase ($P = 0.04$) in hematocrit within the first 3 d following placement of the device (Figure 5). Following weaning, all steers experienced a decrease ($P = 0.03$) in hematocrit from d 10 to 14 (Figure 6). No differences were found between FS or TS steers for hematocrit values measured from d 7 to 42.

Lymphocyte percentage decreased ($P < 0.01$) in all steers from d 3 to 7 (72.4 vs. 66.6 ± 1.76 %; Table 3). There was a trend ($P = 0.07$) observed in lymphocyte percentage between device treatments on d 7, where ND steers had a higher lymphocyte percentage than did YD steers (70.0 vs. 63.2 ± 2.5%). Following weaning, no differences were found between treatments. However, lymphocyte percentage tended ($P = 0.07$) to increase over time (Table 4). Neutrophil percentage increased ($P < 0.05$) in all steers from d 0 to 7 (25.56 vs. 31.12 ± 1.73%; Table 3). During postweaning, values for all steers increased ($P < 0.05$) from d 10 to 21 (33.95 vs. 27.0 ± 2.0%; Table 4). The resultant N:L ratio increased ($P < 0.01$) from d 3 to 7 in all steers. Following weaning, the N:L ratio decreased ($P < 0.05$) in all steers from d 10 to 14. Within YD steers, YD-FS had a higher ($P < 0.05$) N:L ratio than did the YD-TS steers measured on d 21 (0.4 vs. 0.3 ± 0.04; Table 5).

Cortisol concentrations measured from d 0 and 7 were not different ($P = 0.56$) between steers with or without the anti-suckling device (Table 6). Cortisol concentration decreased ($P < 0.01$) in all steers regardless of treatment between d 0 (37.9 ± 6.7 ng/mL) and d 3 (30.6 ± 6.7 ng/mL). After weaning, YD-FS steers had higher ($P < 0.05$) cortisol

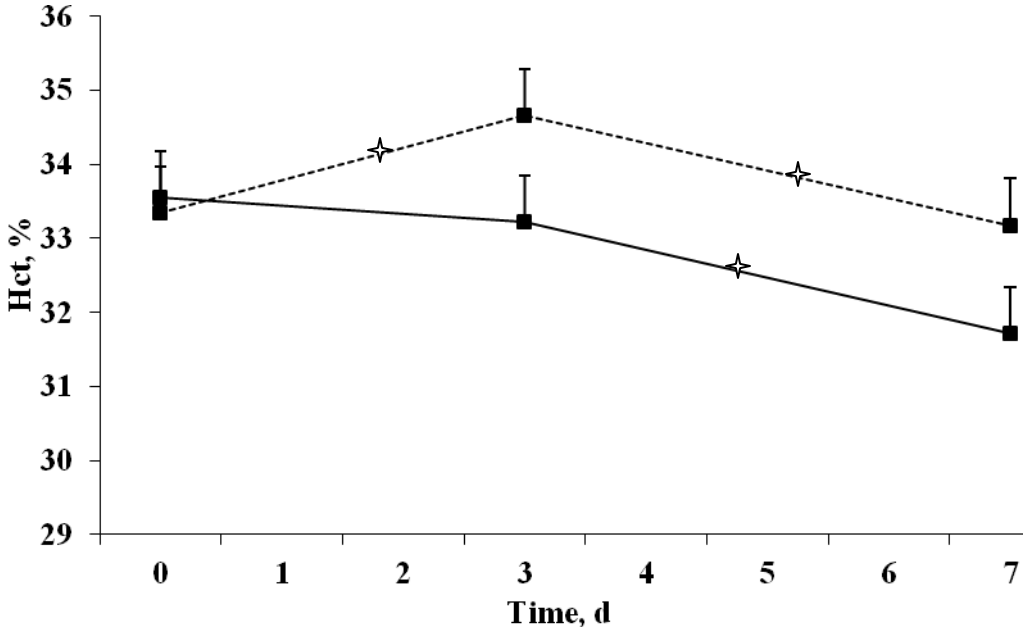

Figure 5. Hematocrit values of steers during 7 d with (---■---) or without (——■——) an anti-suckling device prior to weaning (n = 24 per treatment).
✧ Mean values between successive sampling days within a given treatment differed ($P < 0.05$).

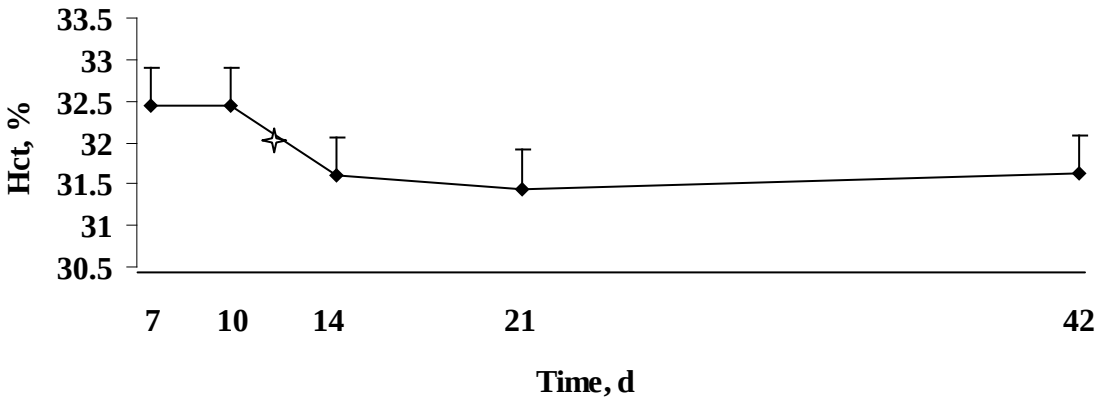

Figure 6. Hematocrit values for all steers measured between d 7 to 42 postweaning. ✧ Mean values between successive sampling days within a given treatment differed ($P < 0.05$).

Table 3. Percentage of neutrophils and lymphocytes measured in all steers prior to weaning (d 0 to 7).

Day	Lymphocytes	Neutrophils
0	72.9[a]	25.6[b]
3	72.4[a]	27.1[ab]
7	66.6[b]	31.1[a]
pooled SE	± 1.8	± 1.7

[a, b, c] Within a column, means lacking a common superscript letter differ ($P < 0.05$).

Table 4. Percentage of neutrophils and lymphocytes measured in all steers following weaning (d 7 to 42).

Day	Lymphocytes	Neutrophils
7	66.6[ab]	31.1[ab]
10	63.5[b]	33.9[a]
14	68.0[ab]	28.9[abc]
21	70.0[a]	27.0[bc]
42	70.4[a]	25.4[c]
pooled SE	± 2.0	± 2.0

[a, b, c] Within a column, means lacking a common superscript letter differ ($P < 0.05$).

Table 5. N:L ratio of steers fitted with or without an anti-suckling device prior to weaning and weaned by fenceline (FS) or total (TS) separation[1].

Treatment	d 0	d 3	d 7	d 10	d 14	d 21	d 42
Device	0.3[a]	0.4[a]	0.6[a]				
FS (n = 12)				0.8[a]	0.5[ab]	0.4[a]	0.5[a]
TS (n = 12)				0.6[a]	0.6[a]	0.3[b]	0.4[a]
No device	0.4[a]	0.4[a]	0.5[a]				
FS (n = 12)				0.5[a]	0.3[c]	0.4[ab]	0.4[a]
TS (n = 12)				0.4[a]	0.4[bc]	0.3[ab]	0.3[a]
pooled SE	± 0.05	± 0.05	± 0.08	± 0.1[a]	± 0.05	± 0.04	± 0.08

[1] Values are means ± SE of steers on each day according to pre- (d 0 to 7) and post- (d 7 to 42) weaning treatment. Steers were fitted with an anti-suckling device (device, n = 24) or no device (control, n = 24) on d 0 to 7. Following weaning (d 7), steers within pre-weaning treatment were separated from their dams by fenceline (FS, n = 24) or total (TS, n = 24) weaning method.

[a, b] Within a column, means lacking a common superscript letter differ ($P < 0.05$).

Table 6. Plasma cortisol concentrations (ng/mL) of steers fitted with or without an anti-suckling device prior to weaning and weaned by fenceline (FS) or total (TS) separation[1].

Treatment	d 0	d 3	d 7	d 10	d 14	d 21	d 42
Device	38.6[a]	30.0[a]	36.2[a]				
FS (n = 12)				36.0[a]	37.01[a]	34.5[a]	41.2[a]
TS (n = 12)				22.1[b]	26.2[a]	21.2[b]	29.3[ab]
No device	37.3[a]	31.2[a]	31.4[a]				
FS (n = 12)				32.5[ab]	24.7[a]	26.7[ab]	24.7[b]
TS (n = 12)				33.3[ab]	31.2[a]	25.5[ab]	25.6[b]
pooled SE	± 3.4	± 3.4	± 3.2	± 4.5	± 4.2	± 3.9	± 4.7

[1] Values are means ± SE of steers on each day according to pre- (d 0 to 7) and post- (d 7 to 42) weaning treatment. Steers were fitted with an anti-suckling device (device, n = 24) or no device (control, n = 24) on d 0 to 7. Following weaning (d 7), steers within preweaning treatment were separated from their dams by fenceline (FS, n = 24) or total (TS, n = 24) weaning method.

[a, b] Within a column, means lacking a common superscript letter differ ($P < 0.05$).

values compared with YD-TS steers on d 10 (36.0 vs. 22.1 ± 4.5 ng/mL) and d 21 (34.5 vs. 21.2 ± 3.9 ng/mL; Table 6).

No differences were found in plasma haptoglobin values measured in YD and ND steers between d 0 and 7 (Figure 7). However, values increased ($P < 0.01$) in all steers by d 3, and decreased ($P < 0.01$) by d 7. Following weaning, haptoglobin values in all steers decreased ($P < 0.0$) from d 7 to 10 (0.07 vs. 0.06 ± 0.003 Abs at 450nm x 100), and were not different following d 10 (Figure 8).

Preweaning treatment had no affect on plasma ceruloplasmin concentrations measured at any time prior to or following weaning (data not shown). Ceruloplasmin concentrations increased ($P < 0.01$) in all steers, regardless of device treatment, from d 0 to 7 (25.08 vs. 31.03 ± 0.86 mg/dL). Postweaning treatment differences were seen on d 10, where values for FS steers were greater ($P = 0.03$) than that measured for TS steers (Figure 9). Ceruloplasmin concentrations tended ($P = 0.07$) to remain higher on d 14 for the FS steers, but were similar between steers from d 21 to 42 (Figure 9).

Steers fitted with an anti-suckling device prior to weaning had similar IFN- γ concentrations than steers without device (data not shown). On d 10, the ND-FS steers had higher ($P = 0.04$) IFN- γ concentrations compared with all YD steers (Figure 10).

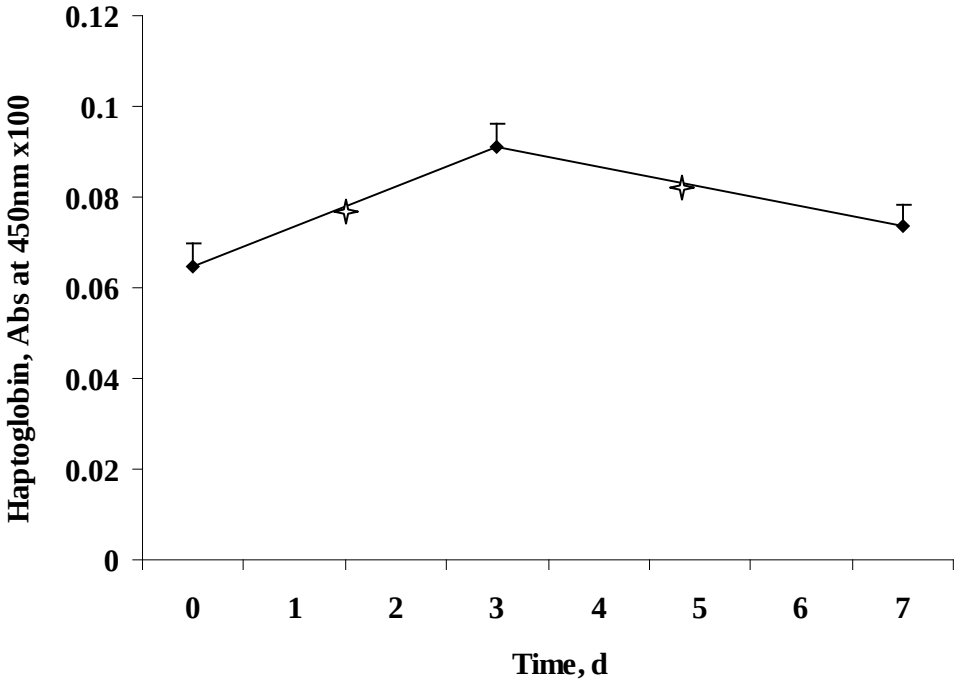

Figure 7. Plasma haptoglobin values of all steers measured preweaning (d 7 = d of weaning) in Absorbance at 450 nm x 100.
✧ Mean values between successive sampling days within a given treatment differed ($P <$ 0.05).

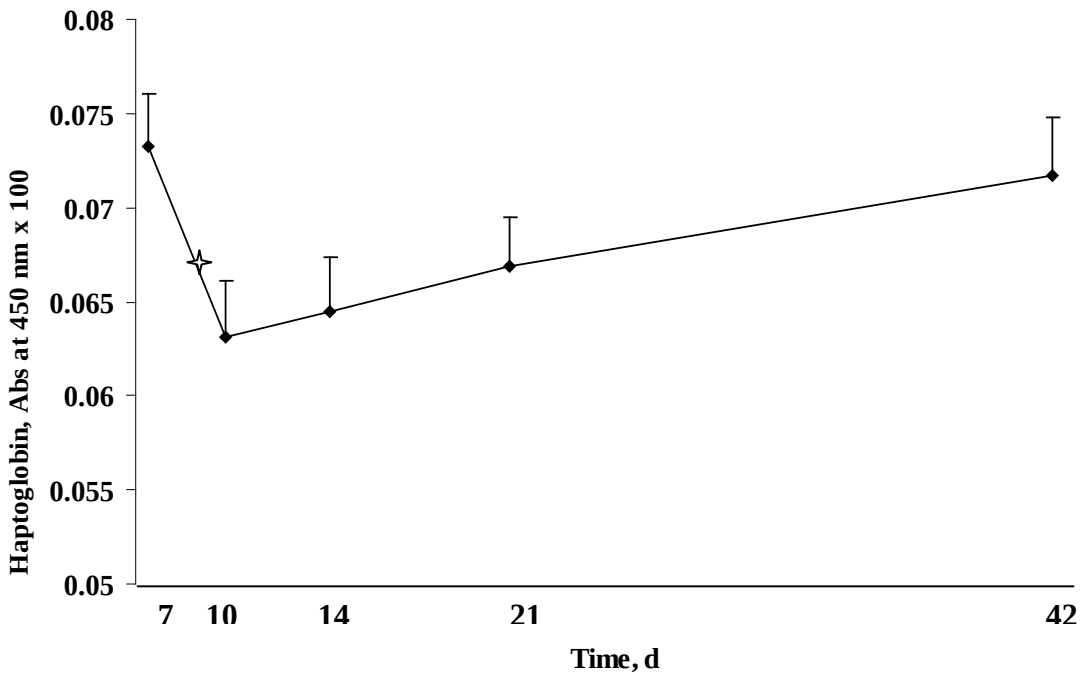

Figure 8. Plasma haptoglobin values of all steers measured postweaning (d 7 = d of weaning) in Absorbance at 450nm x 100.
✧ Mean values between successive sampling days within a given treatment differed ($P <$ 0.05).

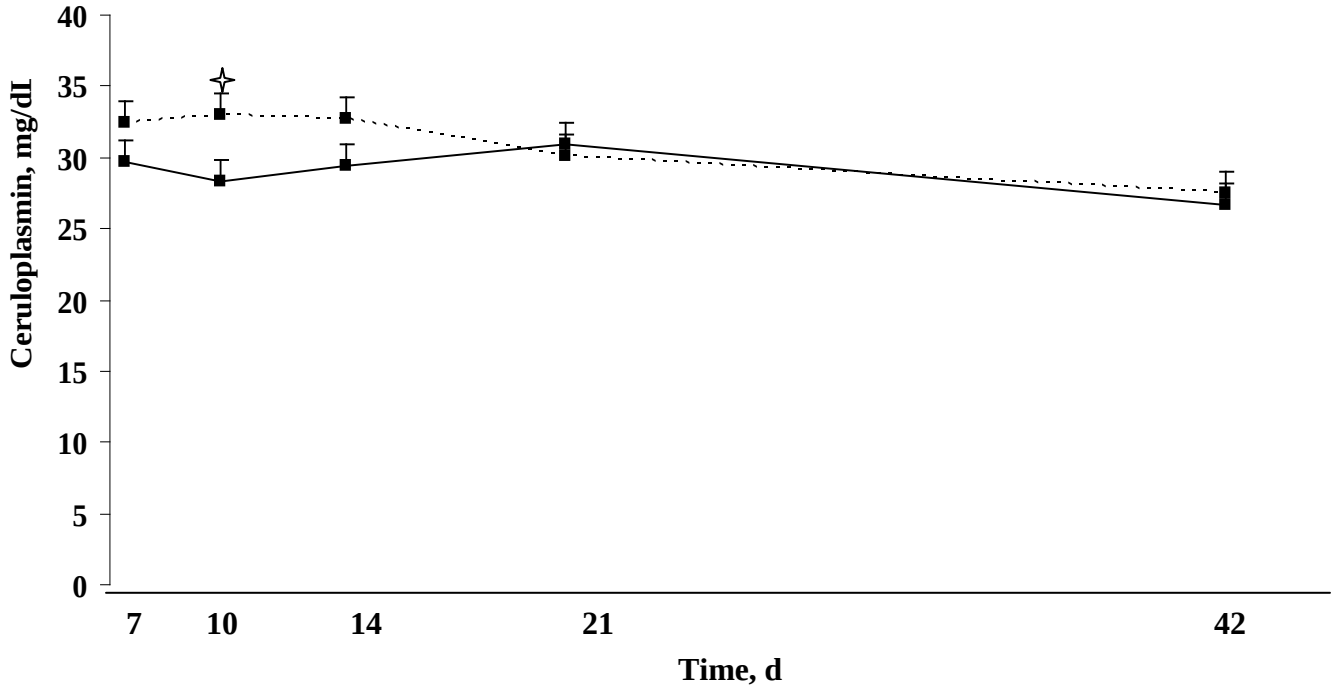

Figure 9. Plasma ceruloplasmin concentrations of steers weaned by fenceline (--■--) or total (—■—) separation.measured postweaning (d 7 = d of weaning).
✧ d 10: FS different from TS ($P < 0.05$).

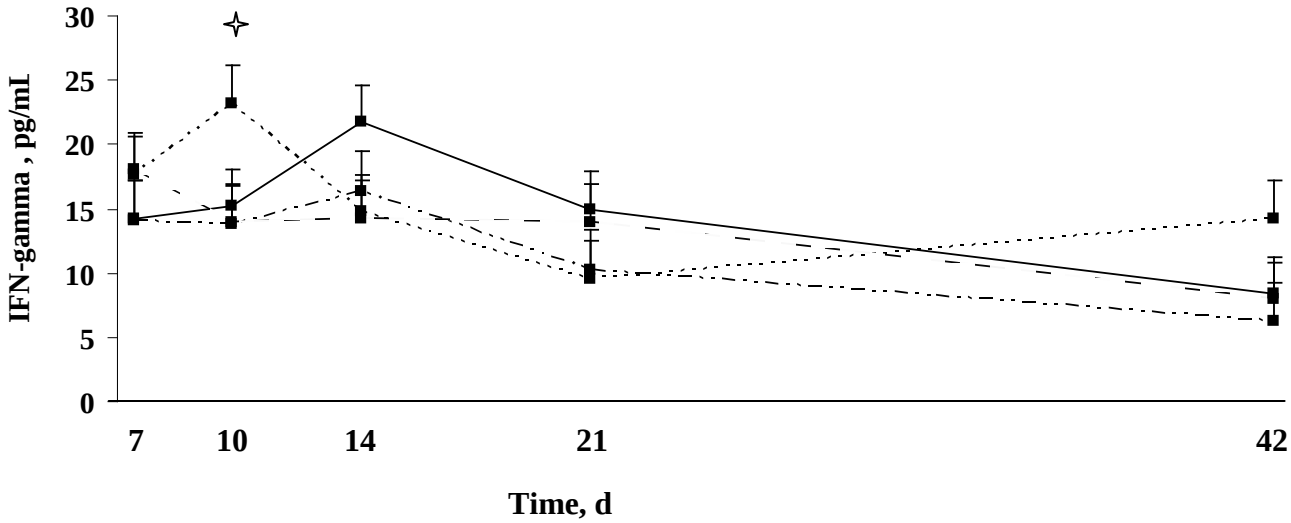

Figure 10. Serum IFN- γ concentrations of steers by device and weaning treatment following weaning (d 7 = d of weaning)[1].

[1]On d 7 steers that had been fitted an anti-suckling device (YD, n = 24), were assigned either fenceline (YD-FS – · – · ■– · – ·, n = 12) or total separation (YD-TS ····■···, n = 12). Steers without an anti-suckling device (ND, n =24), were assigned fenceline (ND-FS - - -■- - -, n = 12) or total separation (ND-TS ——■——, n = 12).

✧ d 10: ND-FS different from YD-FS and YD-TS ($P < 0.05$).

No differences in IgG antibody titer to ovalbumin were found between steers due to pre- or post-weaning treatment (data not shown). The antibody titer to ovalbumin increased ($P < 0.01$) in all steers within 10 d following ovalbumin administration (Figure 11).

Average body weight was positively associated with IgG ($P = 0.01$; r = 0.37), hematocrit was positively related with RBC ($P < 0.01$; r = 0.51), cortisol ($P < 0.01$; r = 0.22), and haptoglobin ($P < 0.05$; r = 0.16). In addition, RBC was positively associated with cortisol ($P < 0.01$; r = 0.22) and negatively related with IgG ($P = 0.01$; r = 0.26).

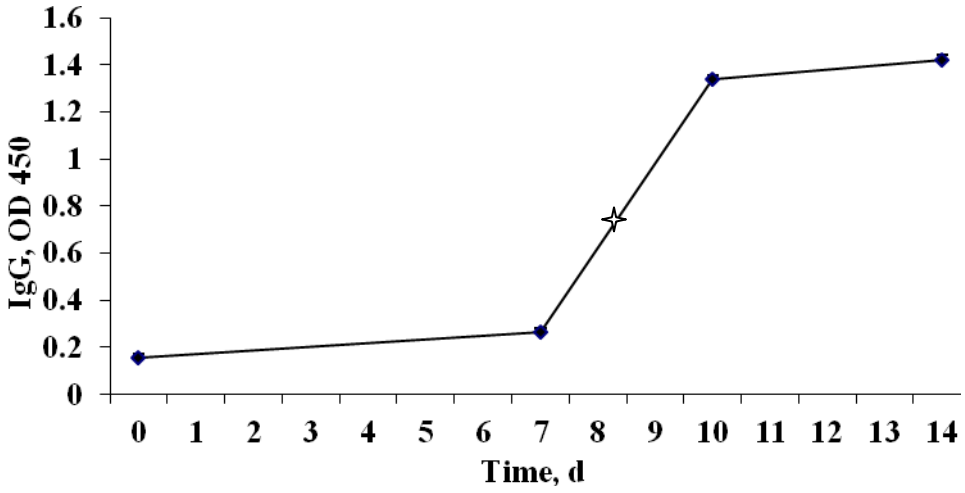

Figure 11. Serum IgG antibody specific for ovalbumin following ovalbumin administration on d 0.
✧ Mean values between successive sampling days within a given treatment differed ($P < 0.05$).

Experiment 2 (Growth Performance)

Growth performance of steers, represented by weight gain between time points, is presented in Table 7. Weight gain was not different ($P = 0.31$) between control steers (NS) and steers provided with a high fiber supplement (YS) over the 7 d before separation from their dams. Steers not provided with a high fiber supplement and total separated (NS-TS) lost weight during the first 3 d following weaning compared with the other steers. Conversely, from d 10 to 14, NS-TS steers gained ($P < 0.01$) more weight than did the remaining steers. On d 14 to 21, NS-FS steers experienced less ($P < 0.01$) weight gain compared with the NS-TS, YS-FS, and YS-TS steers (4.3 vs. 19.5, 18.6, and 13.0 kg, respectively). From d 21-42, NS-FS steers gained ($P < 0.01$) more weight than did the NS-TS and YS-FS steers (Table 7). Overall weight gain during postweaning was greater ($P < 0.01$) for YS-TS (29.5 ± 3.7 kg) compared with NS-TS (18.1 ± 3.7 kg) steers.

Experiment 2 (Physiological Measures)

Hematocrit values of steers did not differ between treatments prior to weaning. However, hematocrit tended ($P = 0.09$) to be higher for NS compared with YS steers on d 7. On d 10, NS steers had higher ($P < 0.01$) hematocrit values than YS steers (38.4 vs 35.4 ± 0.8%; Table 8). On d 14, all steers had similar values ($P = 0.14$), and by d 21 hematocrit values of all steers decreased ($P < 0.01$).

Table 7. Weight gain (kg) of steers provided or not provided with a high fiber supplement prior to weaning and weaned by fenceline (FS) or total (TS) separation[1].

Treatment	Initial wt (d 0)	d 0-7	d 7-10	d 10-14	d 14-21	d 21-42	d 7-42
Supplement (n=24)	318.7[a]	6.2[a]					
FS (n = 12)			0.3[a]	2.8[b]	18.6[a]	0.03[b]	21.8[ab]
TS (n = 12)			5.8[a]	4.4[b]	13.0[a]	6.2[ab]	29.5[a]
No supplement (n=24)	315.2[a]	7.7[a]					
FS (n = 12)			3.3[a]	6.1[b]	4.3[b]	9.0[a]	22.9[ab]
TS (n = 12)			-16.0[b]	14.0[a]	19.5[a]	0.5[b]	18.1[b]
pooled SE:	± 4.2	± 1.0	± 2.5	± 2.4	± 2.3	± 2.7	± 3.7

[1] Values are means ± SE of steers between each weighing according to pre- (d 0 to 7) and post- (d 7 to 42) weaning treatment. Steers were fed (supplement, n = 24) or not fed (control, n = 24) a supplement on d 0-7. Following weaning (d 7), steers within the pre-weaning treatment were separated from their dams by fenceline (FS, n = 24) or total (TS, n = 24) weaning method.

[a, b] Within a column, means lacking a common superscript letter differ ($P < 0.05$)

Table 8. Hematocrit (%) of steers provided or not provided with a high fiber supplement prior to weaning and weaned by fenceline (FS) or total (TS) separation[1].

Treatment	d 0	d 7	d 10	d 14	d 21
Supplement (n=24)	37.10[a]	36.1[a]			
FS (n = 12)			35.56[b]	36.79[a]	35.24[a]
TS (n = 12)			35.32[b]	37.71[a]	35.43[a]
No supplement (n=24)	37.74[a]	37.50[a]			
FS (n = 12)			38.73[a]	39.41[a]	34.50[a]
TS (n = 12)			38.13[a]	37.67[a]	34.95[a]
pooled SE	± 0.7	± 0.6	± 0.8	± 0.9	± 0.8

[1] Values are means ± SE of steers according to pre- (d 0 to 7) and post- (d 7 to 42) weaning treatment. Steers were fed (supplement, n = 24) or not fed (control, n = 24) a high fiber supplement on d 0 to 7. Following weaning (d 7), steers within pre-weaning treatment were separated from their dams by fenceline (FS, n = 24) or total (TS, n = 24) weaning method.

[a, b] Within a column, means lacking a common superscript letter differ ($P < 0.05$)

Lymphocyte percentage decreased ($P < 0.01$) from 81.1 to 72.7%, and neutrophil percentages increased ($P < 0.01$) from 15.9 to 25.9% in all steers from d 7 to 10 (Table 9). On d 7, NS steers had higher lymphocyte (84.9 vs. 77.4 ± 1.8; $P < 0.01$) and lower neutrophil percentage than YS steers (12.5 vs. 19.4 ± 1.7%; $P < 0.01$; Table 10). On the day of weaning, N: L ratio for NS steers was lower ($P < 0.01$) than that of YS steers (0.15 vs. 0.27 ± 0.03; Table 11). Following weaning, N: L ratio increased in all steers ($P < 0.01$) from d 7 to 10, and decreased ($P < 0.01$) from d 10 to 14. Within the total separated steers, NS-TS had a higher ($P = 0.02$) N: L ratio on d 21 than YS-TS steers (0.39 vs. 0.18 ± 0.06; Table 11).

Overall, WBC concentrations were found higher (13.6 vs. 11.4 ± 0.6 x 10^3 mL; $P < 0.01$) in NS steers compared with the YS steers. In addition, FS steers had overall higher (13.4 vs. 11.6 ± 0.6 x 10^3 mL; $P < 0.05$) values than TS steers. No differences were found in WBC concentrations between treatments during pre- and postweaning. From d 7 to 14, WBC concentrations increased (10.0 vs. 14.0 ± 0.8 x 10^3 mL; $P < 0.01$; Figure 12).

No differences ($P = 0.53$) in RBC concentrations were found between treatments. All steers exhibited decreased (7.5 vs 6.8 ± 0.1 x 10^6/mL; $P < 0.01$) RBC concentrations from d 0 to 7. Following weaning, values of all steers increased (6.8 vs. 8.0 ± 0.1 x 10^6/mL; $P < 0.01$) from d 7 to 10 (Figure 13).

Table 9. Percent distribution of neutrophils and lymphocytes measured in all steers prior (d 0-7) and following weaning (d 7-21).

Day	Lymphocytes	Neutrophils
0	80.7[a]	17.9[bc]
7	81.1[a]	15.9[c]
10	72.7[c]	25.9[a]
14	75.8b[c]	22.7[a]
21	77.1[b]	19.2[b]
pooled SE	±1.3	± 1.2

[a, b, c] Within a column, means lacking a common superscript letter differ ($P < 0.05$)

Table 10. Percentage of neutrophils and lymphocytes measured in steers provided (YS) or not provided (NS) a high fiber supplement prior to weaning.

Day	Treatment	Lymphocytes	Neutrophils
0	NS	80.6^{ab}	18.1^{de}
7	NS	84.9^{a}	12.5^{f}
10	NS	71.6^{d}	26.8^{a}
14	NS	77.1^{bc}	21.2b^{cd}
21	NS	73.8^{cd}	22.6^{abc}
0	YS	80.8^{ab}	17.8^{cde}
7	YS	77.4^{bc}	19.4^{cde}
10	YS	73.8^{cd}	24.9^{ab}
14	YS	74.5^{cd}	24.2^{ab}
21	YS	80.4^{ab}	15.8^{ef}
pooled SE		± 1.83	± 1.74

$^{a-f}$ Within a column, means lacking a common superscript letter differ ($P < 0.05$)

Table 11. N:L ratio of steers provided or not provided with a high fiber supplement prior to weaning and weaned by fenceline (FS) or total (TS) separation[1].

Treatment	d 0	d 7	d 10	d 14	d 21
Supplement (n=24)	0.25[a]	0.27[a]			
FS (n = 12)			0.41[a]	0.33[a]	0.23[ab]
TS (n = 12)			0.32[a]	0.34[a]	0.18[b]
No supplement (n=24)	0.23[a]	0.15[b]			
FS (n = 12)			0.42[a]	0.32[a]	0.30[ab]
TS (n = 12)			0.39[a]	0.25[a]	0.39[a]
pooled SE	± 0.03	± 0.03	± 0.06	± 0.03	± 0.06

[1] Values are means ± SE of steers according to pre- (d 0 to 7) and post- (d 7 to 42) weaning treatment. Steers were fed (supplement, n = 24) or not fed (control, n = 24) a high fiber supplement on d 0 to 7. Following weaning (d 7), steers within pre-weaning treatment were separated from their dams by fenceline (FS, n=24) or total (TS, n = 24) weaning method.

[a, b] Within a column, means lacking a common superscript letter differ ($P < 0.05$)

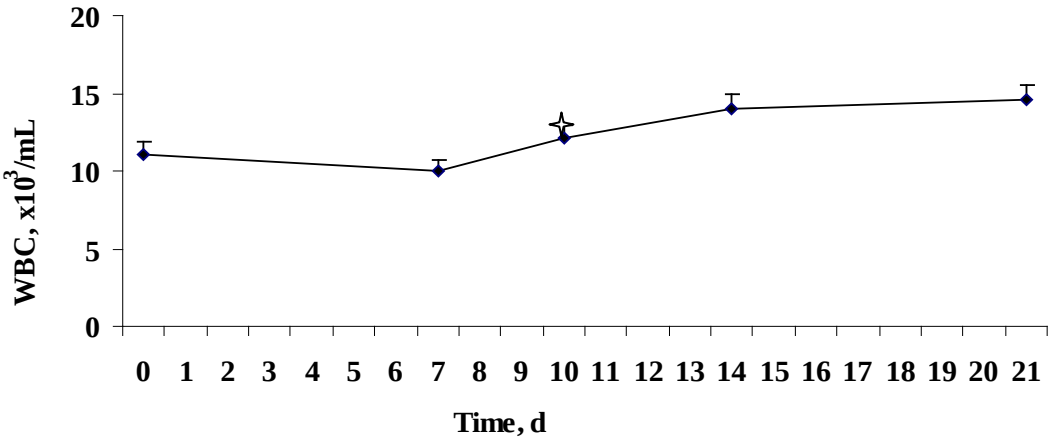

Figure 12. WBC concentrations of all steers pre- (d 0 to 7) and postweaning (d 7 to 21). ✧ Means on d 7 and 14 differ ($P < 0.05$)

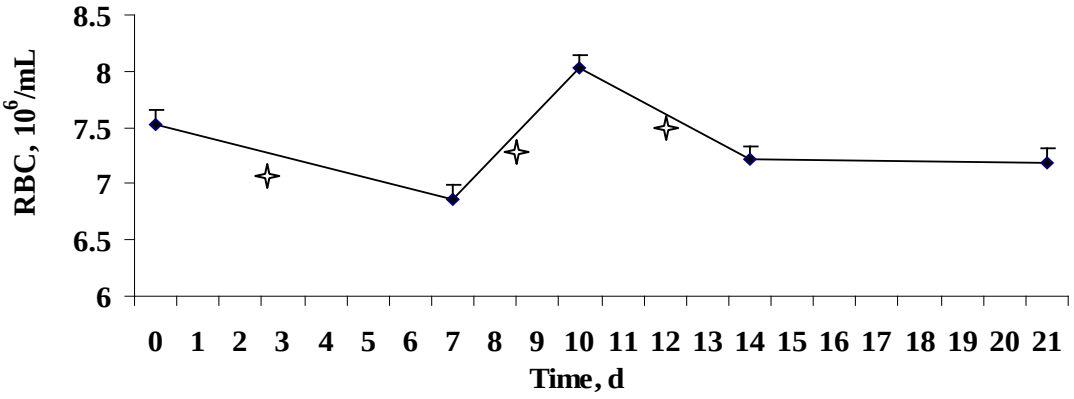

Figure 13. RBC concentrations of all steers pre- (d 0 to 7) and postweaning (d 7 to 21). ✧Mean values between successive sampling days within a given treatment differed ($P <$ 0.05).

On d 7, YS steers had higher ($P < 0.01$) cortisol concentrations than NS steers (35.7 vs. 27 ± 2.6 ng/mL; Figure 14). Cortisol increased in all steers from d 0 to 7 and decreased from d 14 to 21 ($P < 0.01$), regardless of pre- and postweaning treatment.

Haptoglobin values did not differ between treatments either during the preweaning (d 0 to 7), or postweaning (d 7 to 21) periods. All steers experienced an increase ($P < 0.01$) in haptoglobin values 3 d following weaning (d 10) and a decrease ($P < 0.01$) by d 14 (Figure 15).

On d 7 ceruloplasmin concentrations were higher ($P = 0.02$) in YS steers compared with NS steers (Table 12). During the postweaning period, no differences were found between treatments on d 10 and 14. By d 21, NS-TS steers had lower ($P = 0.04$) ceruloplasmin values than NS-FS steers (Table 12).

IFN- γ concentrations did not differ between treatments during the preweaning (d 0 to 7), or postweaning (d 7 to 21) periods. All steers decreased ($P < 0.01$) IFN- γ concentrations during preweaning (d 0 to 7). Following weaning, all steers showed an increase in IFN- γ concentrations 3 d after weaning (Figure 16).

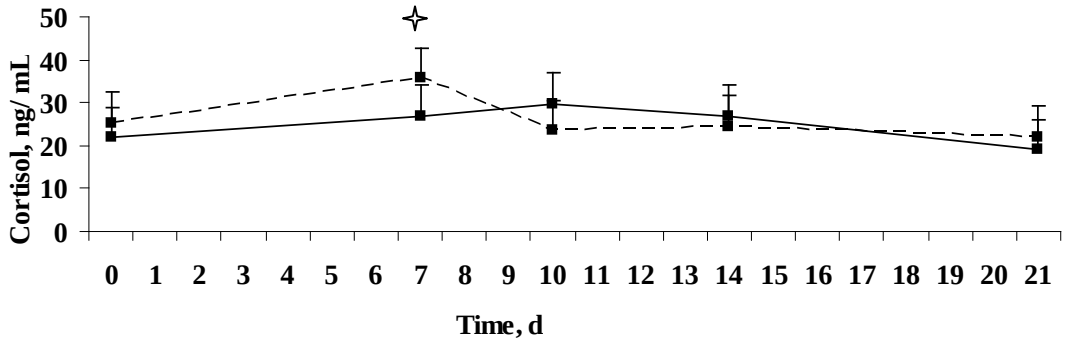

Figure 14. Plasma cortisol concentrations of steers pre- (d 0 to 7) and postweaning (d 7 to 21) provided (YS - -■- -, n = 24) or not provided (NS —■—, n = 24) with a high fiber supplement prior to weaning[1].

✧d 7: NS different from YS ($P < 0$ .05).

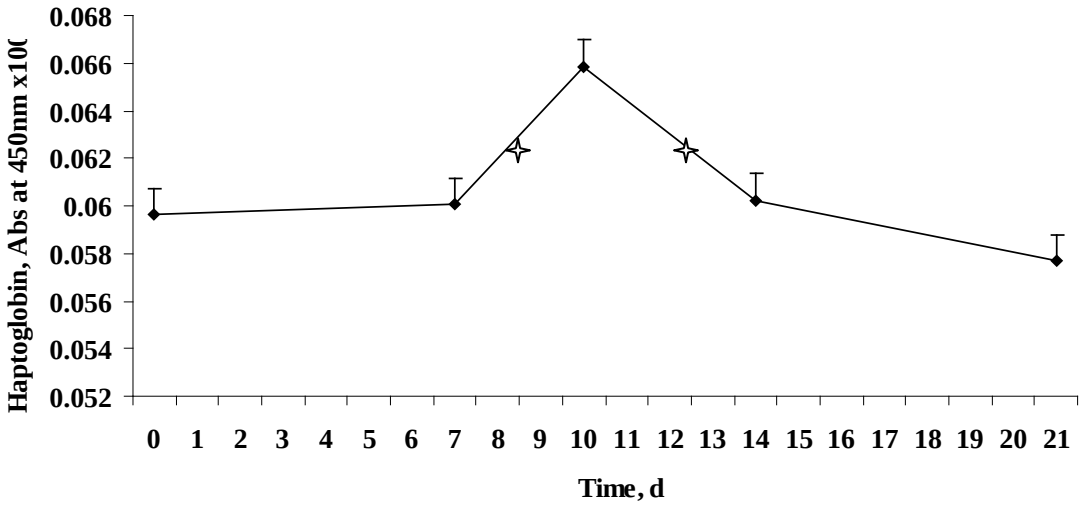

Figure 15. Plasma haptoglobin values of all steers during pre- (d 0 to 7) and postweaning (d 7 to 21) in Absorbance at 450nm x 100.
✧ Means on d 7 and 14 differ ($P < 0.05$)

Table 12. Plasma ceruloplasmin concentrations (mg/dL) of steers provided or not provided with a high fiber supplement prior to weaning and weaned by fenceline (FS) or total (TS) separation[1].

Treatment	d 0	d 7	d 10	d 14	d 21
Supplement (n=24)	25.39[a]	27.85[a]			
FS (n = 12)			28.45[a]	27.64[a]	24.09[ab]
TS (n = 12)			25.56[a]	25.56[a]	22.77[ab]
No supplement (n=24)	24.70[a]	23.56[b]			
FS (n = 12)			26.16[a]	26.27[a]	26.75[a]
TS (n = 12)			26.24[a]	26.47[a]	21.76[b]
pooled SE	± 1.00	± 1.2	± 1.6	± 1.6	± 1.5

[1] Values are means ± SE of steers according to pre- (d 0 to 7) and post- (d 7 to 42) weaning treatment. Steers were fed (supplement, n = 24) or not fed (control, n = 24) a high fiber supplement on d 0 to 7. Following weaning (d 7), steers within preweaning treatment were separated from their dams by fenceline (FS, n = 24) or total (TS, n = 24) weaning method.

[a, b] Within a column, means lacking a common superscript letter differ ($P < 0.05$)

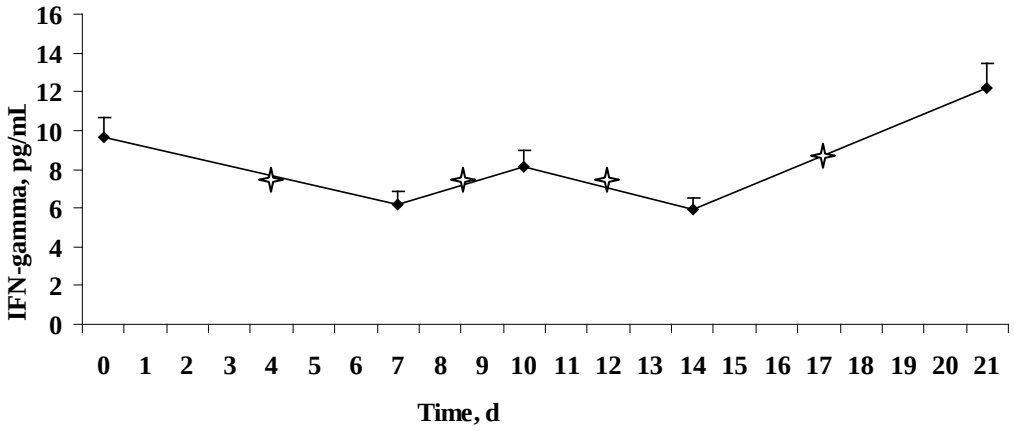

Figure 16. Serum IFN- γ concentrations of all steers pre- (d 0 to 7) and postweaning (d 7 to 21).
✧ Mean values between successive sampling days within a given treatment differed ($P < 0.05$).

Cortisol concentrations were found to be negatively correlated with average body weight ($P < 0.05$; r = 0.13) and positively correlated with haptoglobin concentrations ($P < 0.05$; r = 0.13). In addition, RBC was observed to be positively correlated with hematocrit ($P < 0.01$; r = 0.45), N:L ratio ($P < 0.01$; r = 0.18), neutrophil percentage ($P < 0.01$; r = 0.19), haptoglobin ($P < 0.01$; r = 0.19), and negatively correlated with lymphocyte percentage ($P < 0.05$; r = 0.17). A positive relationship was found between haptoglobin and ceruloplasmin ($P < 0.01$; r = 0.23).

CHAPTER IV

DISCUSSION

Preweaning Growth Performance

Experiment 1. Results from the present study (Exp. 1) showed that steer calves fitted with an anti-suckling device experienced similar growth performance compared with control calves during the period in which the device was employed.

It was shown previously that calves (187 ± 13 d of age) fitted with an anti-suckling device for 3 or 14 d prior to weaning had lower ADG than did calves still nursing (Haley et al., 2005). In addition, calves fitted with the device for 3 d exhibited higher ADG than calves fitted with the device for 14 d. These researchers reported that calves with the device stayed closer to their dams than did calves that were still nursing. Haley et al. (2005) performed a second (189 ± 10 d of age; n = 100; mixed sexes), and third trial (181 ± 13.7 d of age; n= 52; heifers) where calves were prevented from nursing for 5 d prior to weaning. The ADG during preweaning in the second trial did not differ between calves with or without the device. However, in the third trial, calves fitted with the device were found to have lower ADG compared to the calves that were allowed to nurse their dams. The authors proposed that the conflicting results between the three trials could be the result of a higher quality pasture that calves in the second trial had available to them during the preweaning period. All of the calves in the present two experiments, regardless

of pre- or postweaning treatment, were maintained on pastures of similar quality. Thus, the lack of improvement in growth performance of calves fitted with an anti-suckling device for 7 d as noted in the present study reaffirms that reported by Haley et al. (2005) and coworkers in their last trial.

An alternative explanation for the improved ADG that Haley et al. (2005) and coworkers observed for the calves in the second trial may be associated with the age of the calves when the device was applied. Their calves were weaned at 186 ± 12.2 d of age (average age for all trials), which was approximately 2 mo younger than those in the present study (Exp. 1). Beef calves are naturally weaned by their dams as early as 7 mo of age, depending on the breed and forage availability (Price, 2008). In the present study, the average age of the calves at weaning was 245 ± 15.4 d (Exp. 1). It is possible that at the time the calves where fitted with the anti-suckling device, they had been self-weaned making the device ineffective in discouraging nursing.

Experiment 2. Dietary supplementation of calves for 7 d prior to weaning did not increase average daily gain (ADG) at weaning compared to that measured in the non-supplemented calves. However, no gain was expected to be observed during preweaning due to the low amount of supplement given (~4.5 kg/cow-calf pair).

Loy et al. (2002) reported a study where nursing calves were provided with different supplements. Groups of calves were provided three different supplements over a 3 mo period: 100% soybean hulls at 385 g/d; 68% soybean hulls and 32% soybean meal (SBM) at 375g/d; 80% xylose-treated SBM, 16% feather meal and 4% blood meal at 375g/d. In

addition, there was a control group which was not supplemented. The researchers found that overall, calves provided with a supplement exhibited a faster rate of gain than did the nonsupplemented calves. However, calves from this study were provided supplement for a longer period of time than calves in the present study (Exp. 2), having supplement for only 1 wk prior to weaning.

Blanco et al. (2008) reported that calves not provided with a supplement and still nursing had a lower ADG than calves that were still nursing and supplemented. The supplement, consisting of 34% ground corn, 28% ground barley, 17.9 SBM, 8% bran and 12% milk replacer, was provided *ad libitum* from birth to weaning. However, calves in this study were weaned at 150 d of age (5 mo) in contrast to our study which were 233.5 ± 15.5 d of age (7.8 mo) (average of Exp.1 and Exp. 2) when weaned. . Differences in weight gain have been previously reported between early and normal weaned calves, where early weaned calves (89 d of age) were lighter at 300 d of age compared to calves that were still nursing (Arthington, et al., 2005).

Postweaning Growth Performance

Experiment 1. In the present study, steers that had been fitted with an anti-suckling device and were fenceline separated experienced a loss of weight compared to steers from the other treatments. Differences between preweaning treatments were found during the second week following weaning, where fenceline separated steers experienced a compensatory gain and therefore had greater weight gains compared to total separated

steers regardless of preweaning treatment. In addition, there was a device by separation interaction observed during the third week following weaning. Within steers fitted with an anti-suckling device, those that were total separated had a higher weight gain than fenceline separated steers. Overall weight gains during the postweaning period were greater for steers fitted with an anti-suckling device and total separated than steers fitted with an anti-suckling device and fenceline separated.

As reported by Haley et al. (2005), two-stage weaned calves gained more weight than control calves during the week following weaning. Within two-stage weaned calves, the group that had been prevented from nursing for 3 d prior to weaning gained more weight compared with calves prevented from nursing for 14 d during the same period. In the second and third trials performed by Haley et al. (2005), the combined data from the first week following weaning indicated that two-stage weaned calves gained more weight than the control calves (abruptly weaned). In our study, weight gain measured during the first week following weaning was similar between total separated steers regardless of whether they were fitted with the anti-suckling device or not. However, within the fenceline separated group, steers that had been fitted with the device lost weight when compared with those without the device. This may suggest that the use of the device was not advantageous for calves that were fenceline separated, and that the use of the anti-suckling device should only be applied on calves that are to be subsequently weaned by total separation. In addition, we also found that within the group of steers not fitted with the anti-suckling device, weight gain measured during the week following

weaning was similar regardless of whether they were fenceline or total separated. This is in discordance with what Price et al. (2003) observed, where fenceline weaned calves gained more weight following weaning than total separated calves.

Regarding the entire postweaning period, we observed that within the group fitted with the anti-suckling device, total separated calves had a greater overall weight gain than fenceline separated calves. This might suggest that calves that were fitted with an anti-suckling device were more responsive to weaning than calves not fitted with the device, since calves without the device did not have a different weight gain between postweaning treatments when looking at the whole postweaning period. This is in contrast with what Haley et al. (2005) found during postweaning (d 0-44), where two-stage weaned calves had a similar weight gain than control calves that were not weaned, and within two-stage weaned calves, the group that had been prevented from nursing for 14 d gained less weight than the group prevented from nursing for 3 d. The present study did not have a control group (not weaned) to compare it with, as a difference from the previous study reported. However, the present study was studying the effects of different weaning strategies that producers could perform, and not weaning the calves at that time would not be a preferable option to choose from. In addition, the length of days that calves had the device fitted would be an intermediate value between the 3 and 14 d that Haley et al. (2005) performed in the reported study, which is consistent with the fact that the longer the device was fitted, the more sensitive calves were to lose weight. Moreover, Price et al. (2003) showed that fenceline separated calves had a higher weight than total separated

calves at 10 wk following weaning. In the present study, calves were weighed for last time at 5 wk following weaning, therefore, we do not know if fenceline separated calves compensated their weight during the following weeks. However, given our results, the most remarkable differences were seen during the weeks following weaning.

Experiment 2. In the present study, nonsupplemented steers weaned by total separation lost weight during the 3 d following weaning compared to the other groups of steers. The weight loss for 11 of the 12 steers within this group was between 9 and 23 kg. By not having prior exposure to the supplement, the steers may have been reluctant to consume it upon weaning since this was their first exposure to the supplement.

The loss of weight that steers experienced during the 3 d following weaning is critical due to the fact that depending on the producer, steers could have been transported to the feedlot at that time and given that loss of weight, they would have been more susceptible to developing diseases.

Price et al. (2003) found that non- preconditioned (following weaning) and total separated calves did not differ in the percentage of time walking. In addition, they measured the percentage of time calves spent eating following weaning and noted that total separated calves relocated to pasture spent approximately 23.7% of the time eating.;Calves that were total separated and preconditioned to hay and moved to a drylot spent 28.9%, and calves that were total separated and not preconditioned to hay and moved to drylot spent 21.5 % of their time eating during the 3 d postweaning.In our study, nonsupplemented and fenceline separated steers exhibited a lower weight

gain following total separation (d 14-21) compared to the other treatment groups. Therefore, it would appear that providing a high fiber supplement for a minimum of 7 d prior to weaning, would be beneficial for growth performance of calves when weaned using temporary fenceline separation.

Steers that were supplemented during preweaning and were total separated, had a higher total weight gain than non-supplemented steers and total separated. The cost of the supplement provided during the 7 d prior to weaning was $0.175/kg. Since each cow-calf pair consumed 4.5kg/day, that would mean that during the preweaning period, they consumed 31.5kg, which would cost $5.51. The difference in wt gain between supplemented and non-supplemented steers was 11.4kg (Table 7), and given a steer sale price of $84.41/cwt (www.tnbeefcattleinitiative.org), that would represent a benefit of $21.20. Moreover, discounting the cost of the supplement ($5.51) would give us a gross return of $15.69 for each calf (does not include labor or equipment cost). Therefore, providing a supplement during preweaning to steers, is cost efficient when total separation is performed.

Price et al. (2003) reported that fenceline separated and not supplemented calves had higher weight gains than total separated calves (regardless of the diet). In addition, fenceline separated calves (Angus x Hereford; heifer; 205 d of age) gained 95% more weight than total separated calves during the 2 wk following weaning. Similar results were observed in the present study during wk 1 and 2 following weaning, where greater weight gains were found in calves that were fenceline separated compared to total

separated calves, regardless of the preweaning treatment.

Physiological measures

The present study (Exp. 1) found that steers with the anti-suckling device experienced an increase in Hct values by d 3 following placement of the device compared with steers not fitted with the device. The anti-suckling device only prevents calves from nursing, and does not appear to interfere with eating or drinking (Haley et al., 2005). Since weight gains among the steers were similar over the 7 d prior to weaning, regardless of treatment, this would suggest that the device did not interfere with the calves' ability to consume pasture. A low Hct has been associated with dehydration (Wright et al., 2000), and this could be associated with the reduced fluid intake from the elimination of nursing and/or consumption of water. However, 3 d after the device was fitted, calves experienced a decrease in Hct which may imply that once the steers became accustomed to the device, they began to take in more water as a substitute for absence of milk. Steers from Exp. 2 that were supplemented and nonsupplemented had similar Hct values during preweaning. However, once steers were weaned, the group that had not been supplemented showed a higher Hct at 3 d following weaning compared to supplemented steers. In addition, an increase in RBC was found in all steers following weaning, regardless the preweaning treatment. Calves weaned at (150 d) have been reported to have higher RBC and Hct baseline values than calves weaned at an earlier age (90 d) (Blanco et al., 2009). Following weaning, steers not familiar with the supplement may

have experienced immediate competition from the steers that were accustomed to receiving the supplement. A high hematocrit has been reported in dairy cows during the first two mo of lactation and decreases during the third and fourth mo, and is not correlated with body weight (Lane and Campbell, 1969).

The increase in haptoglobin, ceruloplasmin and N:L ratio exhibited by all steers during preweaning in Exp. 1, could have been the result of handling associated with the corralling and bleeding of the steers, since changes were noted in all of the animals regardless of treatment. Haptoglobin has been described as a regulating protein of the immune cell response, where mice that were haptoglobin deficient exhibited an impaired immune response (Huntoon et al., 2008). However, haptoglobin and ceruloplasmin have been previously found not to differ between calves acclimated to handling vs. not acclimated to handling (Cooke et al., 2009). This would contradict the results found in the present study, where an increase of these acute phase proteins were observed in response to pre- and post-weaning treatments by all steers which would indicate that the increase found might have been due to handling than to treatment. The study performed by Cooke et al. (2009) was studying the physiological and performance response of processing Braford and Brahman x Angus heifers through the handling facilities 3 times per week during the 30 d following weaning and no weaning treatments were applied.

Cortisol values in Exp. 1 did not differ between treatments during preweaning. However, during postweaning, the calves fitted with the anti-suckling device and fenceline separated had greater cortisol values 3 d following weaning compared with total

separated steers and fenceline separated. The removal of physical contact might have increased the activity of the calf trying to go back to the other side of the fence, despite the removal of the nursing bond between cow and calf.

Steers in Exp. 2 given a supplement prior to weaning, had higher cortisol values before being weaned than nonsupplemented steers. We hypothesize this might be due to competition between calves for the supplement. In addition, no differences between treatments were observed in cortisol values between calves during postweaning. Another explanation for the previous results reported from Exp. 2 could be that sincegcortisol can increase or decrease in several minutes, levels may have varied while collecting blood samples, since studies have reported an increase in cortisol concentrations in cattle caused by handling during the collection of blood samples (Hopster et al., 1999) and branding (Lay et al., 1992). Moreover, it was suggested by Hopster et al. (1999) that blood samples should be collected within 1 min after the animal is restrained, in order to obtain meaningful cortisol concentrations regarding the treatments applied instead of the handling. Care was taken to obtain the blood samples within 1-2 min following restraint of the animal, however, some steers were more fractious than others and blood samples were collected within a range of 30 sec to 2.5 min. In addition, calves did not follow the same order every time they were bled.

In that cortisol, N:L ratio, and ceruloplasmin values during preweaning were greater in supplemented compared to nonsupplemented calves The availability of the high fiber

supplement may have lead to an increase in activity and competition between calves, which could explain the increase in these physiological parameters.

Steers in Exp. 1 that were fenceline separated and not fitted with the anti-suckling device, exhibited an increase in IFN-γ as measured 3 d postweaning. Results of a recent study found the cytokine IFN-γ was associated with the inflammation caused by the bacterial challenge produced by administration of LPS (Carroll et al., 2009), and has been previously described as inhibiting the synthesis of haptoglobin (Yoshioka et al., 2002). In addition, Carroll et al. (2009) reported that IFN-γ is related with age of the calves. Higher concentrations of IFN-γ were found in early weaned calves (80 d of age) compared to traditionally weaned calves (250 d of age). No previous studies have been reported regarding pre- and postweaning strategies, only with early and normal weaning. However, in the present study, IFN-γ increased in fenceline separated calves that had not been fitted previously with the device.

In Exp. 2, an increase in WBC and IFN-γ was found in all steers following weaning, regardless of pre- and postweaning treatment. Hickey et al. (2003) reported that weaning had no affect on total WBC concentration of calves, while Blanco et al. (2009) observed an increase in total WBC more pronounced in early weaned calves than traditionally weaned calves. In addition, WBC has been shown to decrease in steers following transportation, and IFN-γ values were observed not to decrease following transport (Stanger et al., 2005).

In Exp.1, lymphocyte percentage decreased and neutrophil percentage increased in all

steers 1wk following the insertion of device and administration of ovalbumin. There was a trend observed in lymphocyte percentage between treatments on d 7, where steers not fitted with the device had a higher lymphocyte percentage than did steers fitted with the device. Following weaning, no differences were found between treatments,. even though lymphocyte percentage tended to increase over time. Lymphocyte and neutrophil number has been reported not to be different between steers before and after transportation (Stanger et al., 2005). However, lymphocyte function was observed to be suppressed following transportation (Stanger et al., 2005). In Experiment 2, lymphocyte percentage decreased and neutrophil percentage increased in all steers during the 3 d following weaning, which is in agreement with that reported earlier by Hickey et al.(2003) where the lymphocyte proportion decreased following weaning. Moreover, in the present study (Exp. 1), steers that had not been provided with a supplement had higher lymphocyte and lower neutrophil percentage than steers provided with a supplement 1 wk following weaning.

Generation of IgG-specific antibodies to ovalbumin, measured in the first study to assess immune function, was evident by 10 d following administration of ovalbumin, and was not related to device or degree of separation. Lefevre, et al., (2008) reported a similar response time, to our study, where antibodies to ovalbumin were significantly elevated 6 to 15 d after OVA administration in 4 to 8 mo-old cattle. Treatment differences in IgG following the administration of ovalbumin, have only been found in studies related to the effect of supplementation given to calves. Ward and Spears (1999) found an increase in

antibodies in calves that had been Cu-supplemented, while Ahola et al., (2005) reported no affect on antibody response to ovalbumin in calves that had been supplemented.

CHAPTER V

IMPLICATIONS

Strategies to decrease stress during weaning have been developed over the years. A common practice that has been used at preweaning during this last decade is the two stage weaning, where an anti-suckling device is fitted on the nose of the calf for a period of days prior to weaning. This device involves the prevention of nursing by the calf, but does not interfere with the calf from grazing, eating or drinking. Some studies have reported to use this method successfully. However, most of the studies conducted using the anti-suckling device have been based on behavioral and growth performance measures. The present study did not show that the two-stage weaning method was beneficial for steers based upon growth performance and specific physiologica of stress. Although, age of the calves may have been an important factor in our results.. Further studies should be done, using younger calves at the time of weaning. In addition, the practice of providing a high fiber supplement prior to weaning was successful in the case of total separated calves.

In both experiments, the postweaning treatment was the same; either fenceline or total separation. The fact that postweaning treatments results were found to be different between the two experiments could imply that the effect of the preweaning treatments was overshadowed or overlapped by the effect of the postweaning treatments. nother

possible explanation could be due to the effect of year, since the steers used in each experiment were exposed to different environmental conditions of that specific year, even though both experiments were performed at the same location.

Moreover, it would be beneficial to conduct some behavioral measures in addition to growth performance and physiological measures in order to make the studies more comparable.

Finally, a tracking of the steers until postslaughter would be benefitial in order o study the effects of pre- and postweaning methods on carcass and meat quality, since few or no studies performed regarding pre- and postweaning methods have included a follow up until slaughter, and most of the studies that have looked at the effects of weaning on meat quality have only compared the effects of early and traditionally weaned calves.

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In 2008, these interests led her to the United States, where she enrolled as a graduate student in the Department of Animal Science at the University of Tennessee, Knoxville. The work for her Master of Science degree was focused primarily on the Health and Well-Being of Beef Cattle. She has retained her dual citizenship (Spanish-US) and hopes to continue to live and work in the United States indefinitely.