Impact of Stressors on Performance of Weaned Calves
An Overview of Our Research on Acute Phase Proteins and Beef Cattle Performance

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Introduction

The science of stress is a complex topic. Indeed, many individuals view, define, and address stress differently. Nonetheless, collectively, we can agree that stress is the result of an external or internal pressure which causes an organism to deviate from homeostasis. Professionals engaged in food animal production systems are certainly aware of the range of stressors impacting their animals; however, the repercussions of these events on nutrition and performance considerations are recently becoming better understood. This article discusses research findings directed toward the acute phase protein reaction and performance of beef calves in response to the stressors related to normal production practices such as weaning, vaccination, castration, and transportation.

In food animals, there are a number of common management practices that result in varying degrees of stress which may result in the activation of an inflammatory reaction. These can be both deliberate and non-deliberate in nature. Some common deliberate production practices, which may induce inflammation, are castration or the inclusion of an adjuvant within a vaccine. Production practices which may induce non-deliberate inflammatory responses include transportation, weaning, and commingling. Typically, deliberate production stressors are associated with some degree of tissue injury, whereas non-deliberate production stressors are linked to a disruption in social order, depression, or anxiety. Despite the source of stress, these inflammatory reactions tend to respond similarly in beef cattle and lead to the initiation of the inflammatory reaction, which is initiated by the production of pro-inflammatory proteins. These proteins, called cytokines (namely interleukin -1 [**IL-1**], interleukin-6 [**IL-6**], and tumor necrosis factor – alpha [**TNF-α**]) are the initial instigators of the acute phase reaction, which orchestrates the subsequent production of acute phase proteins (Baumann and Gauldie, 1994) and ultimately metabolism alterations impacting feed intake, nutrient utilization, and growth (Johnson, 1997; Klasing and Korver, 1997). These cytokines are highly pleiotropic and impact food animal performance both (1) directly, by decreasing circulating concentrations of insulin-like growth factor-1 via a reduction in hepatic cell sensitivity to growth hormone (Broussard et al., 2001) and (2) indirectly, by stimulating the production of plasma proteins, thus diverting energy and nutrients away from body weight gain and toward the production of inflammatory proteins and support of the immune system (Johnson, 1997).

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In terms of total protein mass, the combination of immunoglobulin and the acute phase proteins sum the majority of plasma protein mass observed following an acute phase inflammatory reaction. Our research group has placed a significant effort toward the characterization of the acute phase protein response in beef calves to stressors resulting from normal production practices. In doing so, we have specifically targeted three proteins, including haptoglobin, ceruloplasmin, and fibrinogen. These proteins were selected due to their abundance in the blood of cattle responding to inflammatory signals, their acceptable stability during storage (i.e. freezing, thawing, and re-freezing), and their relatively simple and cost-effective measurement in routine assay systems.

Haptoglobin, Ceruloplasmin, and Fibrinogen

The full functional role of haptoglobin in the body is currently not well understood; however, as an acute phase protein, we understand that haptoglobin restricts the availability of iron by forming complexes with free-hemoglobin in the blood. In this manner, haptoglobin can reduce the availability of iron to bacteria and thus aid in reducing bacterial growth (Eaton et al., 1982). Haptoglobin is virtually undetectable in the blood of unstressed cattle. Concentrations increase rapidly during inflammatory distress (Conner and Eckersall, 1988) making haptoglobin the most widely assayed acute phase protein for cattle. The advantages of measuring haptoglobin include the ability of using qualitative and quantitative approaches when comparing animals (i.e. detectable vs. non-detectable concentrations) and its very rapid response time following stimulation. A distinct disadvantage is the potential for assay interference in blood samples that have varying levels of hemolysis.

Ceruloplasmin is the primary copper-transporter-protein in mammals. Its primary function is to deliver copper to tissues throughout the body during periods of inflammation, thus supporting other enzyme systems, such as the powerful antioxidant, copper/zinc superoxide dismutase (McCord and Fridovich, 1968). It is estimated that nearly 95% of the total copper found in blood is associated with ceruloplasmin (Cousins, 1985). Unlike haptoglobin, ceruloplasmin is detectable in both stressed and unstressed cattle, and increases following an inflammatory stimulus can be 2 to 5-fold of baseline concentrations. Ceruloplasmin is highly stable during storage of serum and plasma samples. A notable disadvantage, however, is its responsiveness to nutritional copper status. Low and high concentrations of ceruloplasmin can sometimes be related to copper intake, and it’s reactiveness as an acute phase protein is strongly associated to nutritional copper status (Arthington et al., 2003; Arthington et al., 1996).

Fibrinogen is an important protein for blood clotting and tissue repair. Similar to ceruloplasmin, fibrinogen concentrations are detectable in both stressed and unstressed animals and are recognized as a marker of inflammation in cattle (Sutton and Hobman, 1975). During an inflammatory reaction, the magnitude of fibrinogen increase can be 5 to 10-fold of baseline. Further, fibrinogen is a very large plasma protein and thus is likely an important contributor to the nutrient and energy pool contributing to the inflammatory reaction. Fibrinogen also has a link to ceruloplasmin, which is not well understood and may have a nutritional consequence. In stressed cattle, fibrinogen concentrations are much greater and ceruloplasmin concentrations less in cattle with low copper status (Arthington et al., 1996).

Acute Phase Protein Reaction and Beef Cattle Performance

Weaning and Transport

During our studies we have identified several instances where calf body weight gain was negatively correlated to blood concentrations of haptoglobin, ceruloplasmin, and/or fibrinogen (Table 1). Although other researchers have linked acute phase protein concentrations to instances of feedlot morbidity and/or disease (Godson et al., 1996; Carter et al., 2002; Berry et al., 2004), our research has focused on response variables collected from overtly healthy cattle being exposed to normal production stressors such as weaning, vaccination, transportation, and castration. Each of these practices will create an inflammatory reaction in cattle, the magnitude of which depends on a variety of factors which are currently not well understood. For example, working cattle through a processing chute 5 times over a 14-d period resulted in a differential acute phase protein response in primiparous vs. multiparous cows (Figure 1; unpublished data). Further, the permanent separation of calves from their dams (weaning) clearly initiates an overt stress response in beef cattle, which results in decreased time spent eating and increased walking and vocalization (Veissier and Le Neindre, 1989; Price et al., 2003). In addition to these behavioral responses, blood concentrations of catecholamines also are increased in calves following weaning (Lefcourt and Elsasser, 1995; Hickey et al., 2003). This stress response may also elicit the onset of the pro-inflammatory response, which appears to be impacted by parity and age of calf at the time of weaning. In one study (unpublished data), we investigated the effects of calf age at weaning and dam parity on the acute phase protein response in both cows and calves. Irrespective of dam parity, calves weaned at an early age (approximately 70 days) had lesser acute phase protein concentrations compared to calves weaned at a normal age (approximately 210 d; Figure 2). These weaning age-related differences in acute phase protein concentrations are important considerations to post-weaning calf performance. In two initial studies, we evaluated the performance of early- vs. normal-weaned calves that were transported for 1,200 km (Study 1; Arthington et al., 2005) and 1,600 km (Study 2; Arthington et al., 2008) prior to feedlot entry. Early-weaned calves were separated from their dams between 70 and 90 d of age, while normal-weaned calves were separated at approximately 300 d of age. Calves were transported on the day of normal weaning. In both studies, early-weaned calves had greater ADG and G:F during the feedlot receiving period ($P \leq 0.03$; Tables 2 and 3). In Study 1 (Table 3), this response continued throughout the growing phase, but treatment groups did not differ in the finishing phase. Nonetheless, overall G:F was greatest ($P = 0.02$) for early- vs. normal-weaned calves when the entire feedlot period was considered.

The calf performance responses revealed in these initial two studies (Tables 2 and 3) were negatively correlated to blood concentrations of acute phase proteins. In both studies, plasma concentrations of ceruloplasmin increased sharply in early- and normal-weaned calves following transport and feedlot entry; however, normal-weaned calves had greater ceruloplasmin peaks and sustained increases compared to early-weaned calves throughout the receiving period (Figures 3 and 4). Haptoglobin peaked sooner than ceruloplasmin and was also greater ($P < 0.05$) in normal- vs. early-weaned calves immediately following transport and feedlot entry (Figure 3). These acute phase protein profiles and associated production responses led us to investigate a potential explanation for the impact of calf weaning age on inflammatory reactions. One possible explanation could be the combined stress of weaning and transport, experienced by the normal- but not early-weaned calves. This could potentially be considered a preconditioning effect and thus impacting the subsequent responses to transport stress and feedlot entry. Although this explanation deserves some merit, it does not fully explain the observed acute phase protein response, because calves weaned at a normal age, but enrolled in a 45-d on-ranch, preconditioning program, fail to exhibit the reduced, post-transport acute phase protein response observed in calves early-weaned at 70 to 90 d of age (Arthington et al., 2008). In a follow up study, we collaborated with Dr. Jeff Carroll (USDA-ARS; Lubbock, TX) who we had recently teamed-up with to profile the bovine pro-inflammatory cytokine response to lipopolysaccharide (**LPS**) challenge (Carroll et al., 2009b). Our model involved evaluating blood concentrations of IL-1, IL-6, and TNF-alpha in serial blood collections following an intravenous infusion of LPS. Early- and normal-weaned calves, similar to those used in the aforementioned studies, were evaluated. In this study (Carroll et al., 2009a), normal-weaned calves experienced much greater increases in concentrations of the pro-inflammatory cytokines compared to early-weaned cohorts (Figure 5). The sampling period (8 h post-LPS challenge) was too short to observe increases in acute phase protein concentrations, nevertheless, the initial signals for the acute phase protein reaction are the pro-inflammatory cytokines evaluated in this study. Thus, we conclude that the observed differences in acute phase protein concentrations and subsequent performance responses of early- vs. normal-weaned, transport-stressed calves is related to initial differences in pro-inflammatory cytokine concentrations at the onset of the inflammatory reaction.

Vaccination

Another common production practice that also initiates inflammation in calves is vaccination. Vaccines are intended to produce immune protection in healthy calves by eliciting a humoral immune response, which stimulates the production of B-lymphocytes and ultimately memory B-cells. These memory cells infer long-term protection by the rapid production of protective antibodies upon future exposure to the disease-causing pathogen. For some vaccines, it is efficacious to include an additive that will stimulate the non-specific branch of the immune system (neutrophils and macrophages), which will directly communicate with the specific-branch of the immune system (lymphocytes) to create a more robust protective immunity (Tizard, 2004). These additives are called adjuvants and when administered along with the vaccine, will cause local tissue inflammation, which has been associated with the production of acute phase proteins (Stokka et al., 1994).

We were interested to learn how the vaccine-induced inflammatory reaction impacted the acute phase protein response in calves and how this response might impact performance. To investigate this, we utilized 23 heifer beef calves randomly assigned to 2 treatments, 1) vaccinated (One Shot, Pfizer Inc.; n = 12), and 2) sterile saline (n = 11). Following vaccination, blood samples were collected for determination of haptoglobin and ceruloplasmin concentrations on d 0, 3, 6, 9, 12, and 15 (Arthington et al., 2010). During this period, individual heifer DMI was measured using an automated feed intake measuring system (GrowSafe; Model 4000E). Initial and final shrunk body weight did not differ ($P > 0.36$) among treatments (Table 4). On d 1, plasma ceruloplasmin and haptoglobin concentrations increased ($P < 0.01$) sharply in vaccinated heifers, but not in saline-injected heifers (Figure 6), and both were greater ($P < 0.05$) in vaccinated vs. saline-injected heifers on d 3 for haptoglobin, and d 3, 6, 9, and 12 for ceruloplasmin, relative to injection. Daily DMI did not differ ($P = 0.66$) among treatments; however, ADG and G:F was greater ($P \leq 0.05$) for control vs. vaccinated heifers (Table 4). These data indicate that in a the short period of time following vaccination, calves administered a *Mannheima haemolytica* vaccine (One Shot) experience an acute phase protein reaction that is associated with reduced ADG and feed efficiency.

Castration

Castration of bull calves is another normal management procedure used in the beef industry. Not surprisingly, the process of castration leads to tissue trauma and the initiation of the inflammatory response. There are several castration methods practiced in the industry ranging from knife-cut surgical castration to “banding-type” processes, which aims at removing the blood supply to the scrotum and eventually death of the associated tissues. There have been several studies investigating the impact of castration on measures of stress and productivity in beef calves. We sought to characterize the effects of multiple methods of post-weaning castration compared to pre-weaning, surgical castration, on the acute phase protein response and measures of feed efficiency (Warnock et al., 2012). In this study, calf ADG during the post-castration period (d 0 to 14) tended ($P = 0.06$) to be affected by treatment. All castrated calves gained less ($P < 0.05$) body weight than calves castrated prior to weaning. However, ADG during the entire experiment (d 0 to 84) was similar ($P = 0.42$) for all treatments, indicating that castrated calves were able to compensate and recover from castration regardless of the castration method used. The acute phase protein response differed among castration method (Figure 7), suggesting that castration methods vary in their influence upon subsequent inflammation and the acute phase protein reaction. The acute phase protein response occurred rapidly regardless of the method, but a delayed inflammatory response was observed when calves were banded (Figure 7). Similar to our previous research results, this study also reported a negative correlation among acute phase protein concentrations and ADG, that is, haptoglobin and ceruloplasmin were both negatively correlated with ADG (-0.60 and -0.55, respectively; $P < 0.0001$).

Acute Phase Proteins: Breed Effects and Reproduction

One interesting note of observation among our studies relates to the impact of "breed" on the correlation of acute phase protein concentrations and performance of stressed calves. This was first recognized by our group during an evaluation of acute phase protein concentrations of calves from a USDA-ARS breeding study in Brooksville, FL. In that study, Brahman, Angus, and Romosinuano breeds were compared (as purebreds and crossbreds) in a weaning and transportation model (Qiu et al., 2007). Weaned and transported calves from Angus sires had the least ceruloplasmin concentrations compared to the other breeds evaluated. Since all calves were exposed to the same stressors (i.e. weaning and transport), it is unlikely that the magnitude of stress was a factor in these results. Instead, we suggest that differences in copper metabolism, as reported by other researchers (Ward et al., 1995), may have contributed to the acute phase protein differences revealed in this study.

We have also investigated the influence of acute phase protein concentrations in lactating beef cows on subsequent attainment of pregnancy. In this study (Cooke et al., 2009), we examined acute phase protein concentrations in cow/calf pairs at the start of the breeding season. Although there were no evident stressors present, ceruloplasmin concentrations were still expected to vary across cows due to nutritional status and basal variations in inflammatory status. These results revealed a significant negative correlation between blood ceruloplasmin concentrations and subsequent likelihood of pregnancy during the breeding season (Figure 8). Although these data implicate a link between acute phase protein concentrations and fertility in beef cows, further research is needed to better clarify this finding.

Summary

The collective results of these studies implicate an important relationship between beef calf performance and the acute phase protein response to normal production stressors. An overreaching goal of these efforts is to seek modifications to beef production practices that help ameliorate the inflammatory response and thus improve calf performance and well-being. A current roadblock relates to our observed breed-associated responses. Since the initial report identifying the lack of correlation between ceruloplasmin and haptoglobin concentrations and performance responses in Angus cattle, we have completed two additional studies with similar results. Given the popularity of the breed in the United States, an inability to adapt these findings to Angus cattle greatly diminishes their value to the beef industry. Further research is needed to better characterize these breed effects and further understand the role of the inflammatory response on measures of fertility in beef cattle.

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Table 1. Effect of acute phase protein concentrations at the time of calf transport on 30-d ADG (simple Pearson correlation coefficients)

Research Publication	**Description**	**Pearson Corr.; R = (Protein[1] x 30 day ADG)**
Arthington et al., 2005 J. Anim. Sci.	40 crossbred steers early-weaned at 90 days of age and transported to feedyard	Cp; -0.59; P < 0.01 Hp; -0.40; P < 0.01
Qiu et al., 2007 J. Anim. Sci.	482 steers; multiple breed comparison study. Calves pre-weaned prior to transport	Cp; -0.31; P < 0.08
Araujo et al., 2010 J. Anim. Sci.	48 Braford steer calves; pre-weaned prior to transport	Cp; -0.26; P < 0.05 Fb; -0.26; P < 0.05

[1] Cp = ceruloplasmin, Hp = haptoglobin, and Fb = fibrinogen

Table 2. Effect of weaning management treatment on steer feedlot performance over a 29-d receiving period [1]

Item	Control	Early-weaned	P	SEM
Body weight, d 0, kg	276	276	0.99	10.4
Body weight, d 1, kg	253	250	0.81	8.6
Body weight, d 29, kg	288	299	0.08	4.1
Shrink, %[2]	8.11	9.25	0.41	0.89
Average daily gain, kg[3]	0.88	1.39	< 0.01	0.08
Average DM intake, % body weight[4]	2.50	2.76	0.06	0.09
Gain to feed[4]	0.12	0.17	< 0.01	0.008

[1] Calf body weight was determined before shipping (d 0), upon arrival (d 1), and at the conclusion of the study (d 29) following a 16 h removal from concentrate and hay. Values are least squares means.

[2] Transport body weight shrink was calculated as the percentage change from the body weight before shipping (d 0) and at arrival (d 1).

[3] Calf ADG during the receiving period was determined by the difference between the final shrunk body weight (d 29) and initial arrival body weight (d 1).

[4] Daily intake of concentrate and hay were determined by subtracting the dry matter (DM) of the daily refusal from the DM of the daily offer of both hay and concentrate. Feed DM was achieved by drying daily samples in a forced-air oven at 55° C for 48 h. Feed efficiency (G:F) calculated by dividing the total DM consumed from d 1 to d 29 into the total body weight gain achieved over this time period.

Table 3. Effects of early- vs. normal weaning age on calf feedlot performance[a]

Period[b]	Early-weaned	Normal-weaned	SEM [c]	*P*
Receiving				
Initial body weight, kg [d]	221	269	10.6	0.03
Average daily gain, kg/d	0.87	0.40	0.10	0.03
DM intake, kg/d	5.65	5.27	0.28	0.36
Gain to feed	0.157	0.081	0.010	0.01
Growing				
Average daily gain, kg/d	1.38	1.18	0.05	0.05
DM intake, kg/d	8.80	8.89	0.35	0.84
Gain to feed	0.159	0.136	0.006	0.06
Finishing				
Final body weight, kg	520	535	17.3	0.57
Average daily gain, kg/d	1.37	1.32	0.12	0.77
DM intake, kg/d	8.70	9.15	0.29	0.33
Gain to feed	0.151	0.141	0.007	0.35
Overall				
Average daily gain, kg/d	1.23	1.25	0.11	0.82
Total body weight gain, kg	295	267	9.3	0.10
Total DM intake, kg	1919	1976	74.9	0.62
Gain to feed	0.155	0.136	0.004	0.02

[a] Early-weaned calves were removed from their dams at 85 d of age. Normal-weaned calves remained with their dams until the day of normal weaning (average age = 300 d).

[b] Receiving diet = d 0 to 28; Growing diet = d 28 to 112; and Finishing diet = d 112 to 215 (slaughter date 1) or d 250 (slaughter date 2). Assignment to slaughter date was achieved by selecting 20 steers with the greatest 12th rib backfat thickness (determined by ultrasonography). Table values are least square means.

[c] Largest SEM of least square means (n = four pens/treatment).

[d] Initial body weight determined on d 3 after feedlot entry once free-choice hay was removed.

Table 4. Effects of vaccination on performance of growing beef heifers

Item	Control[1]	Vaccinated[1]	SEM	*P*
Body weight, kg				
d 0[2]	233	225	9.6	0.55
d 16[2]	251	238	9.8	0.37
Average daily gain, kg	0.52	0.40	0.289	< 0.01
DM intake[3], kg/d	4.20	3.99	0.339	0.66
Gain to feed	0.13	0.10	0.011	0.05

[1] Treatments were, 1) Control (2 mL subcutaneous; sterile saline; n = 11), and 2) *Mannheimia hemolytica* vaccine (2 mL subcutaneous; One Shot; n = 12) administered on d 0.

[2] Individual body weight was determined following a 12 h feed and water withdrawal at the start (d 0) and end (d (16) of the study.

[3] Individual DMI measured using the GrowSafe (Model 4000E) feed intake monitoring system. Heifers were allowed to acclimate for 21 d prior to the start of the study. Values are an average of d 1 to 15.

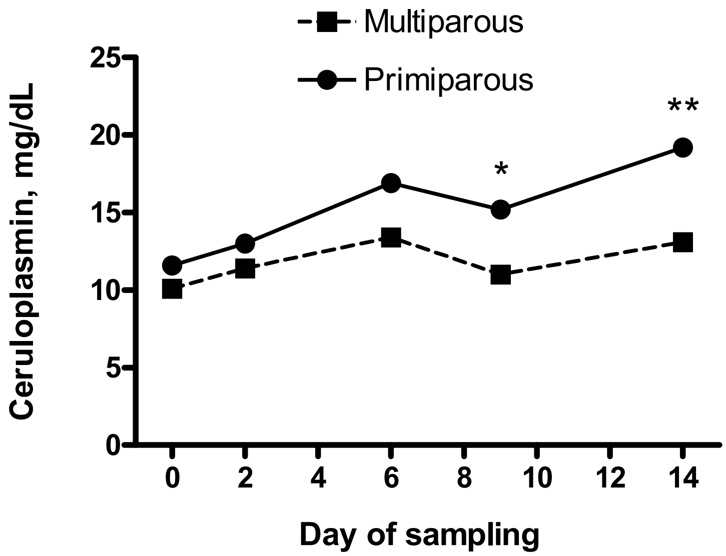


Figure. 1. Effect of parity on plasma concentrations of ceruloplasmin collected from cows moving through a processing chute 5 times over a 14-d period. Mean comparisons within day; * = *P* < 0.05 and ** = *P* < 0.001. Greatest SEM = 2.41.

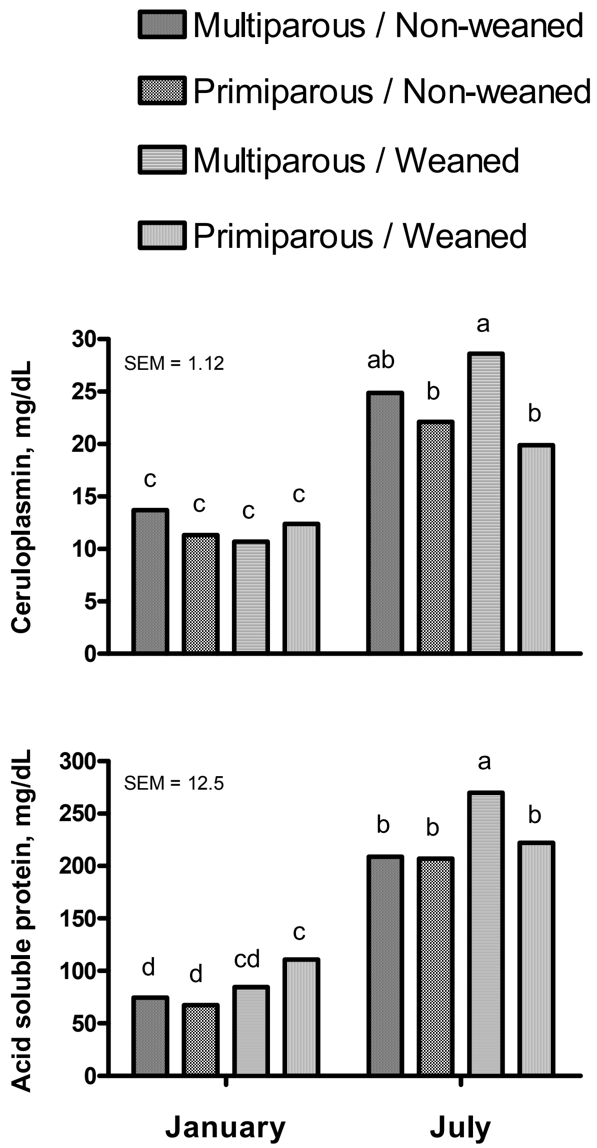


Figure 2. Effects of weaning at an early age (January) vs. a normal age (July) on plasma concentrations of ceruloplasmin and acid soluble protein of calves born to primiparous and multiparous cows. Non-weaned calves served as controls. Within each plot, means with unlike superscripts differ ($P < 0.05$).

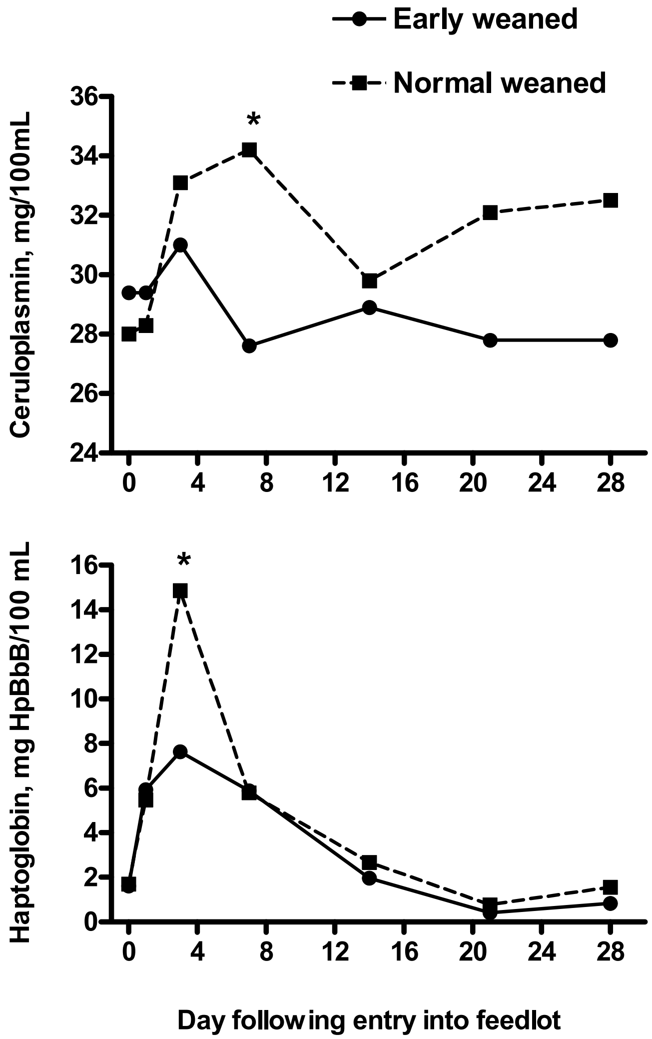


Figure 3. Effect of early calf weaning on plasma ceruloplasmin and haptoglobin concentrations upon entry into a feedyard. Early-weaned calves were removed from their dams at 85 d of age. Normal-weaned calves remained with their dams until the day of normal weaning (average age = 300 d). Upon weaning calves were transported approximately 1200 km directly into a feedyard. Time x treatment; $P < 0.001$. Means differ on d 7 and d 3 for ceruloplasmin and haptoglobin, respectively; *= $P < 0.05$. Pooled SEM = 1.92 and 0.75 for ceruloplasmin and haptoglobin, respectively (n = four pens/treatment). From Arthington et al. (2005).

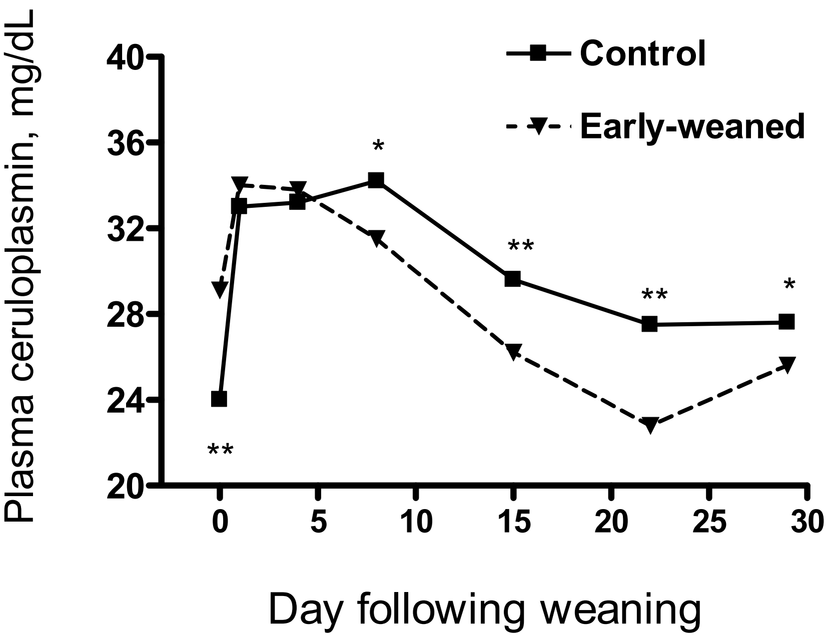


Figure 4. Effect of weaning management on plasma ceruloplasmin concentrations during a 29-d feedlot receiving period. Control animals were weaned at the normal weaning period. Greatest pooled SEM = 1.24. Treatments differ within day; * and ** = *P* < 0.05, and 0.10, respectively. From Arthington et al. (2008).

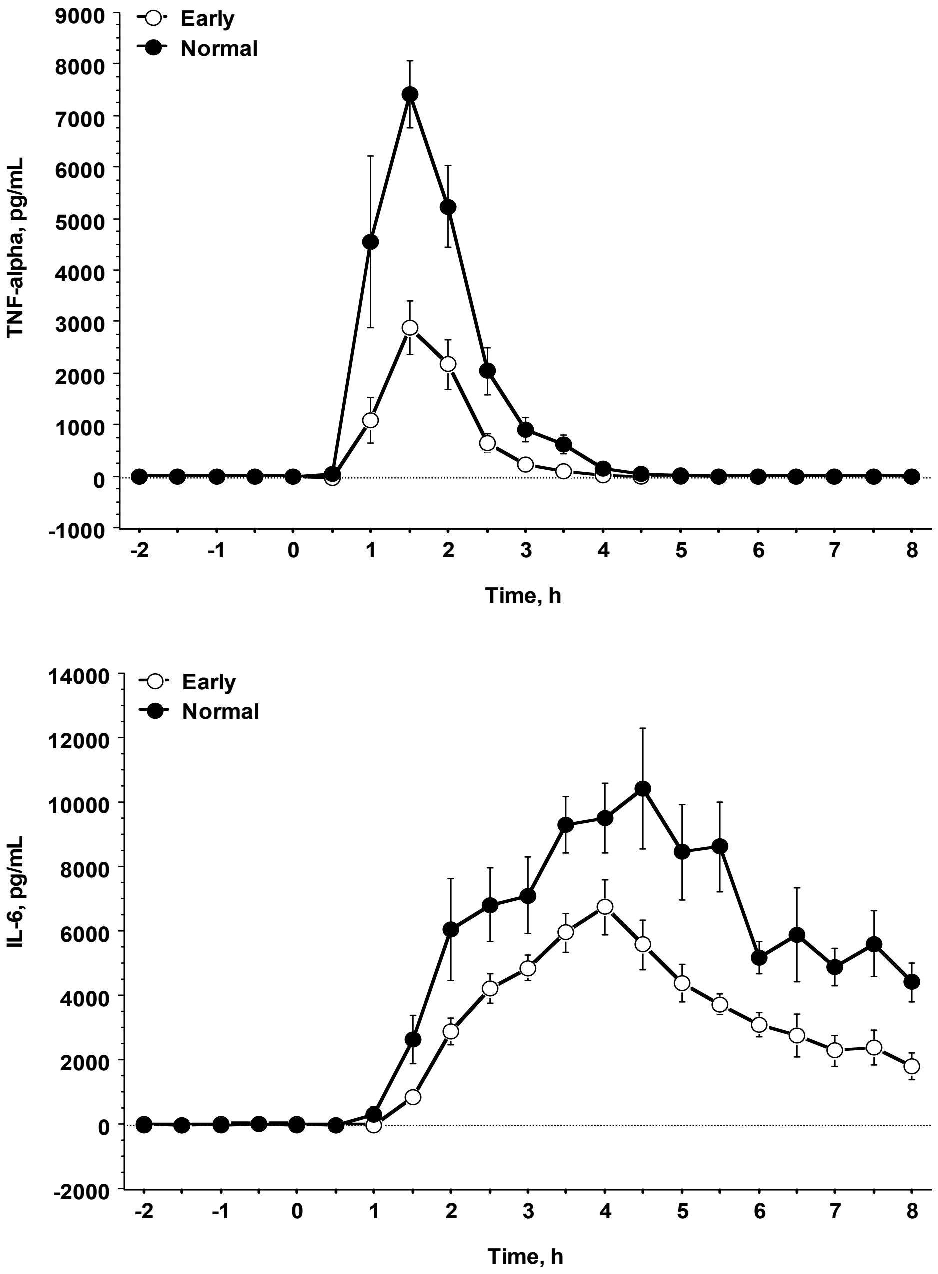


Figure 5. Serum concentrations of TNF and IL-6 in early-weaned (EW) and normal weaned (NW) Brahman × Angus calves following an i.v. bolus dose of lipopolysaccharide (LPS; 1.0 µg/kg BW) via jugular catheter immediately following sample collection at Time 0. Peak serum concentrations of TNF occurred at 1.5 h post-LPS challenge and were approximately 2.6-fold greater ($P < 0.001$) in NW vs. EW calves. Peak serum concentrations of IL-6 occurred at 4.5 h (10455 ± 1888.3 pg/ml) and 4 h (6782 ± 853.5 pg/ml) post-LPS challenge in the NW and EW calves, respectively.

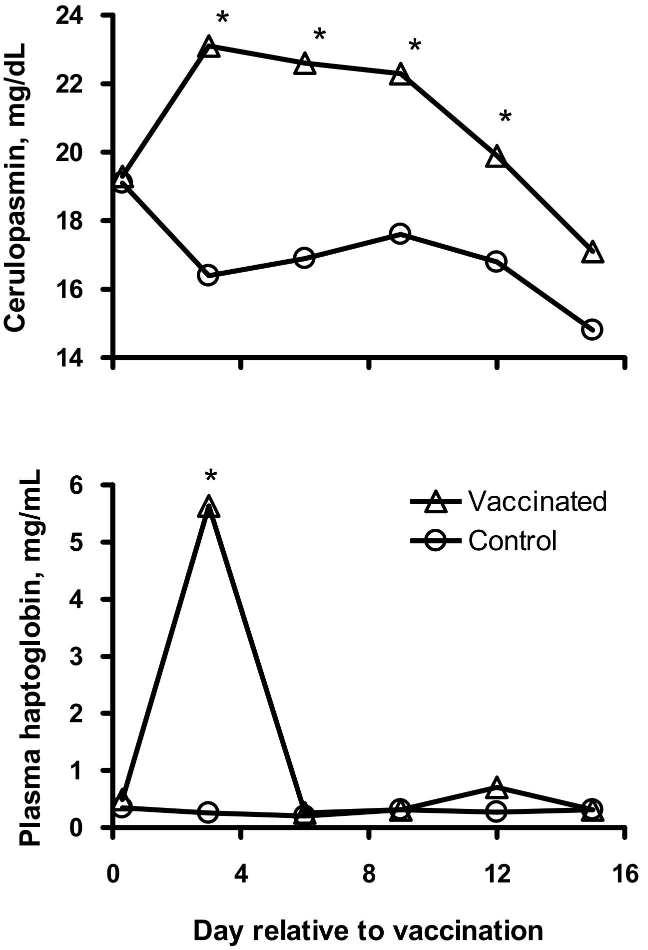
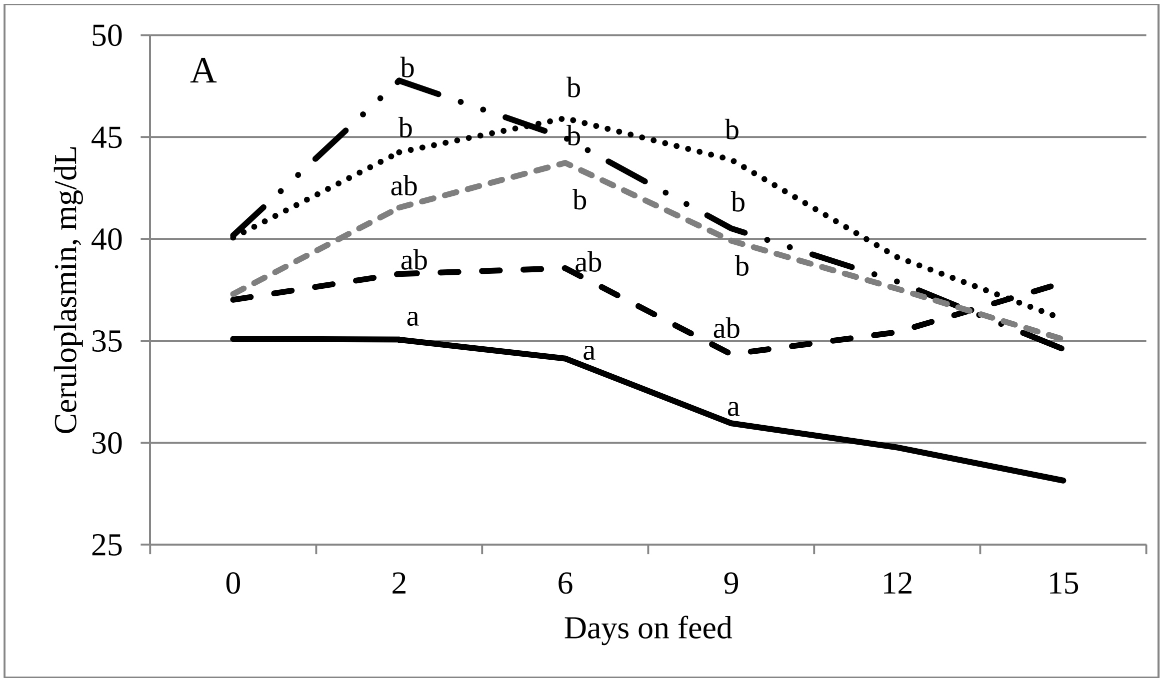


Figure 6. Effect of vaccination (One Shot; Pfizer, Inc.) on plasma ceruloplasmin and haptoglobin concentrations in weaned feedlot heifers over a 15-d sampling period. Treatments within day differ; * = $P < 0.05$.



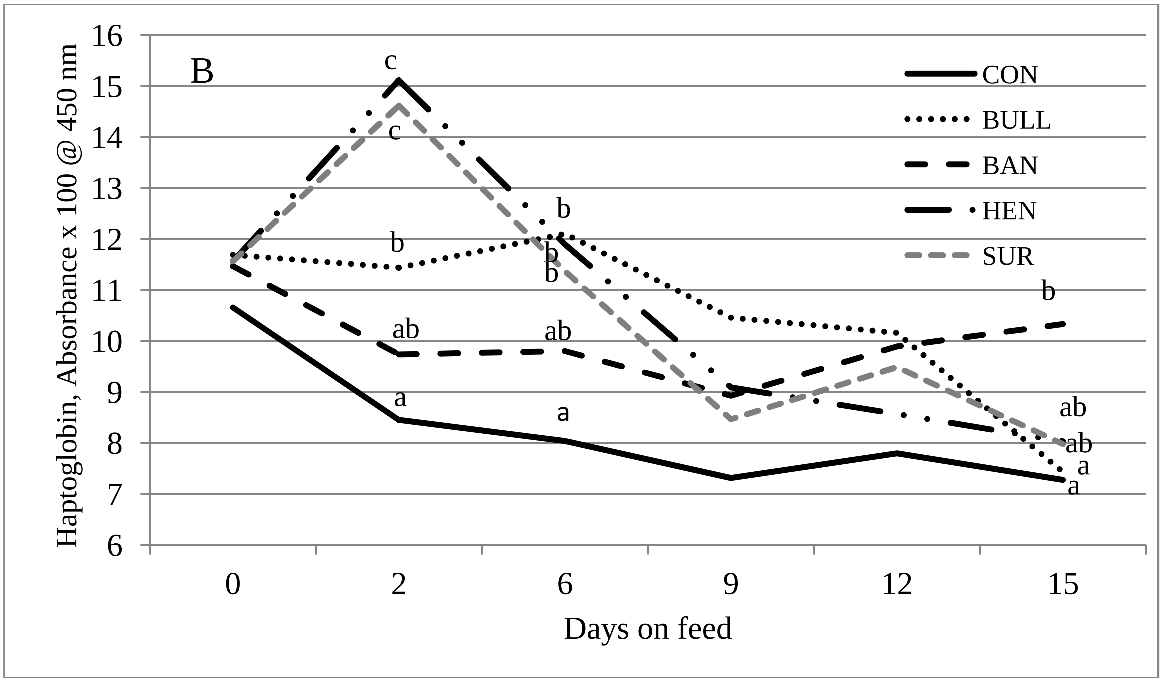


Figure 7. Effect of castration technique and day (*P* < 0.05) on plasma concentration of ceruloplasmin (A) and haptoglobin (B) in beef calves during the post-castration period. CON = steers castrated pre-trial; BULL = intact male calves; BAN = calves banded on d 0; HEN = calves surgically castrated with Henderson castration tool on d 0; SUR = calves surgically castrated with emasculators on d 0. $^{a, b, c}$ Means with different superscripts differ *P* < 0.05.

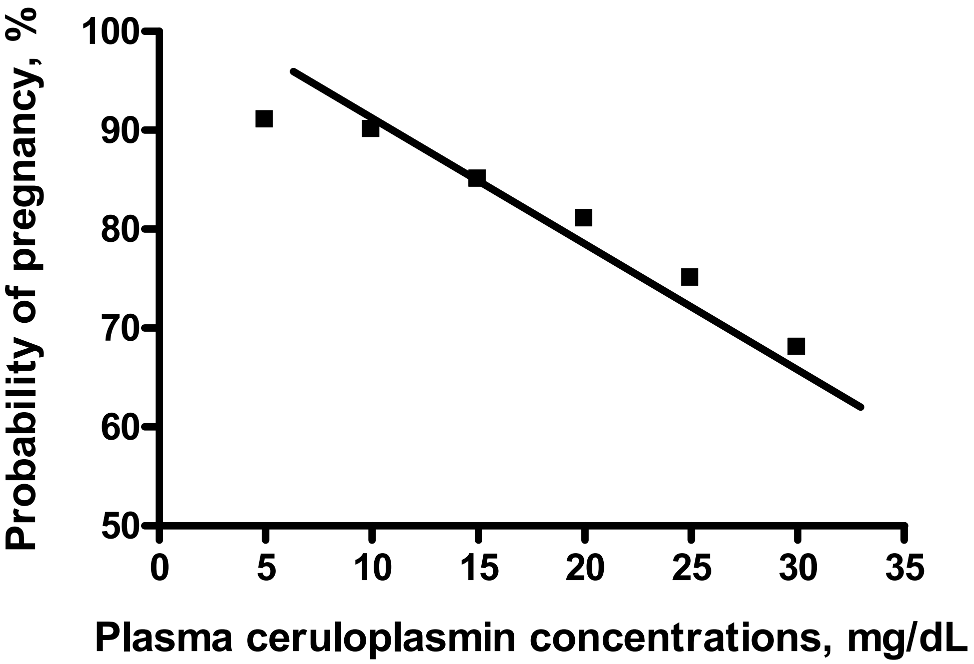


Figure 8. Effects of plasma ceruloplasmin concentrations, assessed at the beginning of the breeding season, on the probability of Brahman-crossbred cows (n = 395) to become pregnant. A linear effect was detected ($P < 0.01$). Adapted from Cooke et al. (2009).

SESSION NOTES