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Effects of pre-weaning feed supplementation and total versus fenceline weaning on the physiology and performance of beef steers

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Forty-eight Angus steers (208 ±15 days of age) housed on pasture with their dams, were divided equally based on initial body weight (BW; 312 ± 28 kg), and assigned randomly to assess whether receiving a palatable, high fiber supplement (YS; 4.5 kg/cow-calf/day) versus no supplement (NS) for 7 days prior to weaning alters the steers' acute stress response following separation from its dam. Steers were weaned (day 0) by fenceline (FS; 12 NS and 12 YS) or total separation (TS; 12 NS and 12 YS). On day 7 the FS group was moved to a pasture lot distant from their dams and adjoining the TS group. All steers were weighed and bled on days -7, 0, 3, 7, 14, and 35 (BW only) and provided access to the supplement on days 0 to 14. By day 0, BW gain increased (P<0.01) and serum interferon-γ (IFN-γ) concentration decreased (P<0.01) in all steers. However, the YS steers exhibited greater (P<0.05) neutrophil to lymphocyte (N:L) ratio and total plasma cortisol (CORT; P=0.09) and ceruloplasmin (CER; P=0.08) concentrations compared with NS steers. From weaning to day 7, NS-TS steers initially experienced a BW loss (P<0.01) followed by a BW gain (P<0.01) when compared with the remaining steers. At 3 day post-weaning, mean hematocrit for YS steers was lower (P < 0.01) than that of NS steers and CORT was greater (P<0.05) for NS-TS vs. YS-TS steers. Red blood cell number, N:L ratio, haptoglobin and IFN-γ concentrations increased (P<0.01) in all steers by day 3 and returned to pre-weaning concentrations thereafter. From days 14 to 35, NS-FS steers gained less (P<0.01) weight compared with YS-FS and all TS steers. These results suggest that providing a high fiber supplement beginning from 7 days prior to weaning may reduce BW loss and temper the steers' acute stress response when weaned using total separation.

Key words: Beef steers, pre-weaning supplementation, stress.

INTRODUCTION

Strategies incorporating a pre-weaning/pre-conditioning program have been investigated and employed with the overall objective of reducing the stress associated with breaking the cow-calf bond. The program may involve providing solid food to calves prior to weaning in an effort to prepare them for receiving at the feedlot (Arthington et al., 2008). Also, a health program is conducted where a range of vaccines are given.

As a consequence, pre-conditioned calves have a lower incidence of morbidity and mortality, gain body weight (BW) faster and reach market weight earlier than those not pre-conditioned (Roeber et al., 2001; Bailey and Stenquist, 1996; Hilton, 2015).

Stress associated with weaning of beef calves has been shown to elicit an acute increase in circulating levels of various blood constituents including cortisol (Lefcourt and Elsasser, 1995; Hickey et al., 2003), the cytokine interferon-γ (Carroll et al., 2009), and the acute phase proteins ceruloplasmin and haptoglobin (Arthington et al., 2003; Qiu et al., 2007) to name a few. Alterations in blood constituents such as these can be attributed to weaning method and degree of separation as well (Hickey et al., 2003; Campistol et al., 2013). The objective of this study was to examine performance and physiological responses in beef steer calves provided there is a palatable high fiber supplement for 7 days prior to weaning, and weaned with or without temporary fenceline contact with their dams. The hypothesis of this experiment was that a 1-week supplementation of the cow-calf pair with the palatable diet would be sufficient time to familiarize the calf to subsequent offering following weaning, thus reducing the acute stress response experienced by the calf following two different methods of separation from its dam.

MATERIALS AND METHODS

Animals and experimental design

All animal procedures were reviewed and approved by the University of Tennessee Animal Care and Use Committee prior to the initiation of this experiment. Forty-eight Angus steer calves (initial BW 312 ± 28 kg; 208 ± 15 days of age (mean ± SD), born and maintained with their dams on an established pasture of mixed orchardgrass (*Dactylis glomerata*) and tall fescue (*Festuca arundinacia*), were used in this study. Steers were initially vaccinated at 138 ± 15 days of age with Cattlemaster® 5 (Pfizer Animal Health, Exton, PA) and Vision® 7 (Intervet Inc., Millsboro, DE), implanted with Ralgro® (Schering-Plough Animal Health Corp., Summit, NJ), and de-wormed with the pour-on Dectomax® (Pfizer Animal Health, Exton, PA), and revaccinated with Cattlemaster® 5 and Vision® 7 at 174 ± 15 days of age.

Steers were equalized by initial BW on day-14 to two groups. Blood samples were collected and additional BW were recorded between 800 and 1000 h, 7 days (day -7) prior to weaning (day 0) and on days 3, 7, 14, and 35 (BW only) post-weaning. Beginning on day -7, steers and their dams (n=24) were offered as a group a high fiber supplement in well-spaced troughs at 4.5 kg/cow-calf pair/day (YS). Ingredient composition of the supplement is presented in Table 1. The remaining steers with their dams served as controls and were not supplemented (NS). All steers were provided the same supplement from weaning to day 14. On day of weaning, 24 steers (12 YS and 12 NS) were randomly selected and moved to a0.81 ha pasture lot adjacent to their dams separated by woven-wire fence with openings too small to accommodate a calf's head (fenceline separation; 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 day 7 following weighing and bleeding, the FS group was transported to a pasture lot adjoining the TS group.

Table 1. Composition and nutrient content of high fiber supplement fed to cow-calf pairs.

Composition	% (As-fed basis)
Cracked corn grain	23.0
Soyhulls (Pelleted)	10.0
Soybean meal	3.3
Cottonseed meal	10.4
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

Blood sample collection and analyses

Blood samples were obtained via jugular venipuncture from each steer while restrained in a squeeze chute with head gate. Each blood sample was collected into two 10.0 ml vacutainer tubes 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, centrifuged, and serum was harvested and stored at -20°C until analyzed for interferon-γ (IFN-γ).

Blood smears were prepared on glass slides and stained with hema-quick stain solution (Hema 3 Stat Pack Cat # 123-869; Fisher Scientific, Sewanee, GA, USA). Smears were examined under oil immersion (100 ×) to differentiate the number of neutrophils and lymphocytes within 100 cells counted, and subsequent calculation of neutrophil to lymphocyte (N:L) ratio. Hematocrit (HCT) values were recorded at each sampling time. Red blood cell (RBC) and white blood cell (WBC) counts were determined (ABC Counter, Grayslake, IL, USA). Plasma total cortisol concentration was analyzed using an RIA procedure (Coat-A-Count, Diagnostic

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Products, Los Angeles, CA, USA) as previously reported in our laboratory (Doherty et al., 2007). Intra- and inter-assay coefficient of variation (CV) 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, respectively.

Plasma haptoglobin concentration was 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. (2007). Intra- and inter-assay CV for haptoglobin were 1.7 and 1.9%, respectively. Plasma ceruloplasmin oxidase activity was analyzed using colorimetric procedures as described by Demetriou et al. (1974). Concentrations were expressed as milligrams per deciliter. Intra- and inter-assay CV for ceruloplasmin were 1.6 and 4.8%, respectively. Serum IFN-γ was assayed per the manufacturer's protocol using a custom-developed multiplex ELISA validated for bovine cytokines (SearchLight, Pierce Biotechnology Inc., Rockford, IL, USA) as reported previously (Carroll et al., 2009) with a detection range of 2.0 to 500 pg/ml. Intra- and inter-assay CV were 11.1 and 8.4%, respectively.

Statistical analysis

Data were analyzed using the MIXED procedure (SAS Institute Inc., Cary, NC, USA) for a completely randomized design with BW at birth and age used as covariates. Calves were initially stratified by BW and then randomly assigned to treatments within strata. The pre-weaning statistical model included supplement with calf as the experimental unit, and repeated measures for data collected on days -7 and 0. The post-weaning model included a supplement by separation factorial with calf as the experimental unit, and repeated measures for data collected on days 0, 3, 7, 14, and 35. Autoregressive correlation was used for repeated measures, and denominator degrees of freedom were adjusted using Kenward-Roger. Least squares means with standard error (SEM) were reported, with Fisher's protected least significant difference mean separation at the 5% significance level. Statistical trends were considered when $0.05 < P < 0.10$. Data were examined for normality (Shapiro-Wilk), extreme observations and equality of variance.

RESULTS AND DISCUSSION

Steer BW and physiological response measurements during the pre- and post-weaning treatment periods are provided in Tables 2 and 3, respectively. The BW gain was not different (P=0.31) between the YS and NS steers over the 7 days prior to separation from their dams (Table 2). Arthington et al. (2008) similarly reported minimal differences in BW gain between creep-fed steer calves provided free-choice access to a concentrate supplement for 45 to 53 days when compared with non-supplemented calves and suggested that this was the result of the limited time of creep feeding. From weaning to day 7, steers not receiving the pre-weaning supplement and total separation (NS-TS) initially experienced a weight loss ($P < 0.01$) followed by a compensatory weight gain ($P < 0.01$) when compared with the remaining steers (Table 3). Price et al. (2003) likewise reported lower weight gain in non-conditioned heifer calves on pasture at 2 and 10 weeks following total separation compared with fenceline separated calves. They also found that preconditioning calves with hay for 10 days prior to weaning in drylots did not improve weight gain following weaning. Weight gain was not different ($P > 0.10$) among steers on days 7 to 14 regardless of dietary supplementation or method of weaning. From days 14 to 35, NS-FS steers gained less ($P < 0.01$) weight compared with YS-FS and all TS steers. Although not statistically different, the mean overall BW gain during post-weaning (days 0 to 35) was numerically greater for YS-TS compared with NS-TS steers.

The hypothesis of this experiment was that a 1-week supplementation of the cow-calf pair with a palatable diet would be sufficient time to familiarize the calf to subsequent offering following weaning, thus reducing the acute stress response experienced by the calf following separation from its dam. We found that 11 of the 12 steers within the NS-TS group experienced acute weight loss over the 3 days following weaning resulting in an overall 5 week post-weaning BW of 11.4 kg less than their supplemented counterpart. Since actual feed consumption was neither measured nor observed in our study due to grazing in the pasture, we can only speculate that 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. Price et al. (2003) reported that calves preconditioned to hay spent a greater percentage of their time eating than non-preconditioned calves (28.9 vs. 21.5%) during the first 3 days after being weaned to drylots.

The HCT of steers did not differ between treatments prior to weaning (Table 2). On day 3, the mean HCT for NS steers was higher ($P < 0.01$) than that for YS steers (38.4 vs. 35.5%; SEM = 0.5; Table 3). All steers had similar but lower ($P < 0.01$) HCT on day 14 compared to day 7 (35.0 vs. 37.9%; SEM = 0.4). No treatment effects were found for RBC count in steers over the sampling times (Tables 2 and 3). The RBC count increased ($P < 0.01$) in all steers from days 0 to 3 (6.9 and 8.0 x 10^6/µl, respectively; SEM = 0.1) and returned to pre-weaning values by day 7. Thus, the elevated HCT for the NS steers does not appear to be a result of polycythemia but may be a sign of dehydration, which has been shown to occur in calves in response to long-distance road transport (Bernardini et al., 2012) and elevated cortisol levels (Parker et al., 2004). Water intake in growing beef cattle was found to be positively correlated with BW gain (Brew et al., 2011), and as noted before, steers in the NS-TS treatment did indeed experience acute weight loss upon weaning. Both weight loss and reduced water intake could be a consequence of this latter treatment group not having previous exposure to the novel diet compounded by the stress of total separation from their dams.

A time × diet interaction was detected for plasma cortisol concentrations, such that cortisol measured on day 0 tended (P=0.09) to be greater in YS than in NS

Table 2. Effects of a fiber supplement on growth performance and physiological responses of pre-weaned beef steers.

Item[1]	Day	Supplement[2]	No Supplement[3]	SEM[4]
Steers, no.	-	24	24	-
Initial BW, kg	-7	319	315	4.2
Total gain, kg	-7 to 0	6.2	7.7	1.0
HCT, %	-7	37.2	37.7	0.7
	0	36.1	37.5	0.6
RBC, × 10^6/µl	-7	7.4	7.7	0.1
	0	7.1	6.7	0.2
WBC, × 10^3/µl	-7	10.1	12.1	0.8
	0	10.4	11.6	0.8
N:L	-7	0.23	0.25	0.03
	0	0.27[a]	0.16[b]	0.03
Cortisol, ng/ml	-7	25.5	21.6	2.0
	0	35.9[c]	26.8[d]	2.9
Haptoglobin, OD × 100	-7	6.0	6.0	0.1
	0	6.0	6.0	0.1
Ceruloplasmin, mg/dl	-7	25.0	24.2	1.0
	0	27.3[c]	22.0[d]	1.8
IFN-γ, pg/dl	-7	10.3	8.8	1.5
	0	6.1	6.1	1.0

[a,b]Within a row, means without common superscripts differ (P<0.05).[c,d]Within a row, means without common superscripts differ (P<0.10). [1]HCT = hematocrit; RBC = red blood cell; WBC = white blood cell; N:L = neutrophil to lymphocyte ratio; OD = optical density; IFN-γ = interferon-γ.[2]Steers provided a fiber supplement beginning 7 days prior to weaning (day 0). [3]Steers not provided a fiber supplement prior to weaning.[4]Standard error of mean.

Table 3. Effects of a fiber supplement on growth performance and physiological responses of post-weaned beef steers.

Item[1]	Day	Supplement[2]		No Supplement[3]		SEM[5]
		Fenceline[4]	Total	Fenceline	Total	
Steers, no.	-	12	12	12	12	-
Initial BW, kg	0	321	322	321	325	7
Gain, kg	0 to 3	0.2[a]	5.8[a]	3.4[a]	-16.0[b]	2.3
	3 to 7	3.0[a]	4.4[a]	5.9[a]	14.1[b]	2.5
	7 to 14	1.0	6.0	7.4	1.5	2.9
	14 to 35	18.0[a]	13.2[a]	5.5[b]	18.9[a]	2.5
Total gain, kg	0 to 35	21.8	29.5	22.9	18.1	3.7

Table 3. Contd.

HCT, %	0	35.9	36.3	37.6	37.4	0.8
	3	35.7[a]	35.3[a]	38.6[b]	38.2[b]	0.8
	7	36.8	37.7	39.5	37.6	0.8
	14	35.1	35.5	34.8	34.8	0.9
RBC, × 10^6/µl	0	7.2	6.9	6.7	6.6	0.3
	3	8.0	7.6	8.3	8.2	0.3
	7	6.8	7.5	7.4	7.1	0.3
	14	7.1	7.0	7.3	7.4	0.2
WBC, × 10^3/µl	0	10.5	10.3	11.7	11.5	1.2
	3	12.9	11.1	12.9	11.6	1.0
	7	13.9	10.4	19.5	13.6	1.9
	14	13.3	12.2	18.4	14.6	3.0
N:L	0	0.30	0.24	0.17	0.14	0.04
	3	0.39	0.32	0.45	0.37	0.06
	7	0.33	0.34	0.32	0.25	0.03
	14	0.24	0.18	0.28	0.40	0.06
Cortisol, ng/ml	0	39.4	32.0	28.9	25.1	3.6
	3	24.2[cd]	22.8[c]	25.2[cd]	34.2[d]	4.2
	7	27.6	21.4	25.2	28.7	4.0
	14	28.2	16.2	16.2	21.7	3.1
Haptoglobin, OD × 100	0	5.8	6.2	5.8	6.1	0.1
	3	6.5	6.2	6.8	6.4	0.3
	7	6.1	6.0	6.0	6.0	0.1
	14	5.6	5.8	6.0	5.7	0.1
Ceruloplasmin, mg/dl	0	28.8	25.9	23.4	20.7	2.7
	3	27.8	25.3	25.8	25.7	1.6
	7	27.0	25.4	25.9	25.8	1.6
	14	24.3	21.5	25.7	21.5	1.7
IFN-γ, pg/dl	0	7.1	5.2	5.4	6.9	1.4
	3	11.3	7.4	10.0	6.9	1.6
	7	6.3	5.1	6.7	5.3	1.3
	14	18.6	11.3	11.9	8.5	2.7

[a,b]Within a row, means without common superscripts differ (P<0.01).[c,d]Within a row, means without common superscripts differ (P<0.05).[1]HCT = hematocrit; RBC = red blood cell; WBC = white blood cell; N:L = neutrophil to lymphocyte ratio; OD = optical density; IFN-γ = interferon-γ.[2]Steers provided a fiber supplement beginning 7 days prior to weaning (day 0). [3]Steers not provided a fiber supplement prior to weaning.[4]Steers within pre-weaning treatment were separated from their dams by fenceline contact on days 0 to 7 followed by relocation to a distant pasture (total), or total alone.[5]Standard error of mean.

steers (Table 2). Feeding activity among the calves and their dams for access to the supplement may have contributed to this elevation in cortisol. Following separation (day 3), the NS-TS steers had greater (P<0.05) plasma cortisol concentration than the YS-TS steers (Table 3). In that the non-supplemented, total separated steers experienced weight loss and possibly dehydration, we contend this increase in cortisol was related to the abrupt separation from their dam and not exposure to the novel feed supplement. Parker et al. (2004) showed that excess cortisol has a suppressive effect on the renin-angiotensin-aldosterone axis in *Bos Indicus* steers, which would interfere with their ability to resist dehydration. Except for the YS-FS treatment group,

the remaining steers exhibited a decline in cortisol concentration from days 7 to 14.

The overall pre-weaning WBC count tended (P=0.06) to be greater in NS compared with YS steers (11.8 vs. 10.3 × 10^3 cells/µl; SEM = 0.6; Table 2). Following weaning, FS steers exhibited overall greater (P < 0.05) WBC count compared with that measured for the TS steers (14.0 vs. 11.7 × 10^3 cells/µl; SEM = 0.6; Table 3). All steers exhibited greater (P < 0.05) WBC counts on days 7 and 14 compared with day 0 (14.0 and 14.5 vs. 11.0 × 10^3 cells/µl, respectively; pooled SEM = 0.9). Regarding changes in differential WBC populations, lymphocyte percentage was greater (P < 0.05) and neutrophil percentage was lower (P < 0.05) on day 0 in NS vs. YS steers (data not shown) resulting in a lower (P < 0.05) N:L ratio for the NS steers (Table 2). From days 0 to 3, all steers experienced a decrease (P < 0.01) in lymphocyte percentage and an increase (P < 0.01) in neutrophil percentage (data not shown) resulting in an increase (P < 0.01) in the N:L ratio from 0.21 to 0.38 (Table 3). This result is consistent with that noted in previous studies as to that the impact weaning stress has on WBC populations in cattle (Hickey et al., 2003; Lynch et al., 2010a,b; Lynch et al., 2012; Campistol et al., 2013). Indeed, the higher neutrophil percentage observed on day 0 in the YS steers is concurrent with the elevated cortisol concentration as noted.

Haptoglobin concentrations did not differ between treatment groups either during the pre- or post-weaning sampling periods (Tables 2 and 3). Overall, haptoglobin concentration measured on day 3 was greater (P < 0.01) than on other days sampled (6.6 vs. 6.0 absorption × 100 at 450 nm; SEM = 0.1). In an earlier study, Lynch et al. (2012) also reported a significant increase in haptoglobin concentration on day 2 following weaning, which was not different between calves having received a concentrate supplement for 26 days prior to weaning compared with non-supplemented calves. On day 0, plasma ceruloplasmin tended (P=0.08) to be greater for YS steers than NS steers (Table 2). As noted, the YS steers sampled on day 0 also had significantly higher cortisol concentrations and higher neutrophil percentage, which resulted in a greater N:L ratio compared with the NS steers. Together, these findings are in agreement with those reported previously indicating a positive relationship between these blood constituents and an acute phase stress response (Cooke and Bohnert, 2011; Campistol et al., 2013; O'Loughlin et al., 2014). Ceruloplasmin concentrations were not different among treatment groups at any time after weaning (Table 3). However, the mean concentrations measured on days 3 and 7 were greater (P < 0.01) than that measured on day 14 (26.1 and 26.0 vs. 23.2 mg/dl; pooled SEM = 0.9). Serum concentrations of IFN-γ did not differ (P = 0.53) as a result of pre- or post-weaning treatment. All steers experienced a decrease (P < 0.01) in IFN-γ from days -7 to 0 (9.5 vs. 6.1 pg/dl; SEM = 0.9; Table 2), which then increased (P < 0.01) by day 3 (8.7 pg/dl; SEM = 0.8; Table 3). The mean IFN-γ concentration returned to pre-weaning concentrations by day 7 but then increased (P<0.01) to their highest concentrations on day 14 (5.8 vs. 12.1 pg/dl; pooled SEM = 0.9).

Collectively, the pre-weaning increase in N:L ratio and plasma cortisol and ceruloplasmin concentrations in the YS steers may indeed be a reflection of increased activity due to competition for the feed supplement. In general, all steers exhibited changes in the growth and physiological indices measured in this study consistent with that associated with acute weaning stress as reported previously (Campistol et al., 2013). However, the results of the present study suggest that providing a high fiber supplement beginning 7 days prior to weaning may reduce body weight loss and temper the steers' acute stress response when weaned using total separation from their dam.

Conflict of interest

The authors have not declared any conflict of interest

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