

Effects of timing of anabolic implant insertion on growth and immunity of recently weaned beef steers¹

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ABSTRACT: We evaluated the effects of timing of estrogenic implant insertion, relative to weaning, on growth performance and measurements of innate and humoral immunity of beef calves. On d -14, Angus × Simmental crossbred steers ($n = 48$; BW = 217 ± 5 kg; age = 191 ± 3 d) were stratified by BW, age, and cow parity and randomly assigned to receive no implant (NOIP) or 36 mg of zeranol on d -14, 0, or 14, relative to weaning (IP-14, IP0, and IP+14, respectively; 12 steers/treatment). From d -14 to 0, cow-calf pairs remained on a single, tall-fescue pasture with no access to concentrate supplementation. Steers were weaned on d 0, stratified by treatment and BW, and then allocated into 1 of 16 drylot pens to receive daily free-choice access to a corn silage-based diet during the preconditioning phase (d 0 to 56). Steers were vaccinated against infectious bovine rhinotracheitis (IBRV), bovine viral diarrhea virus (BVDV), and *Clostridium* on d -27 and 0. From d 56 to 252 (postpreconditioning phase), steers remained in their respective feedlot pens and were provided free-choice access to corn silage-based growing (d 56 to 167) and finishing total mixed rations (d 168 to 252). Body weight on d 0 did not differ among treatments ($P \geq 0.29$) but was greater for

IP-14 and IP0 than NOIP and IP+14 steers on d 14, 42, and 56 ($P \leq 0.05$). Treatment effects were not detected for G:F and DMI from d 0 to 56 ($P \geq 0.34$), but ADG from d -14 to 56 was greater for IP-14 compared to NOIP ($P \leq 0.05$) and intermediate for IP0 and IP+14 steers. Plasma IGF-1 concentrations were greater for IP-14 than NOIP ($P \leq 0.05$) and intermediate for IP0 and IP+14 steers on d -7, 0, 14, and 21. Plasma concentrations of cortisol and haptoglobin and serum titers against BVDV types 1a and 2 did not differ among treatments from d 0 to 56 ($P \geq 0.37$). However, serum IBRV titers were greater for IP+14 than NOIP, IP-14, and IP0 steers ($P \leq 0.02$). On d 252, BW was greater for IP-14 and IP0 than NOIP steers ($P \leq 0.05$) and intermediate for IP+14 steers, but ADG and G:F from d 57 to 252 and carcass characteristics at slaughter did not differ among treatments ($P \geq 0.16$). Thus, the 36-mg zeranol implant did not elicit an inflammatory response or affect the overall vaccine response of steers (except for IBRV titers). However, growth of steers during a 56-d preconditioning period was enhanced by administering 36-mg zeranol implant 14 d before weaning, without affecting subsequent postpreconditioning growth and carcass characteristics at slaughter.

Key words: acute-phase response, beef steers, immune, implant, preconditioning, vaccination

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doi:10.2527/jas2016-0470

INTRODUCTION

Numerous anabolic implants have been used in the United States to reduce feed costs and improve gain and feed efficiency of beef cattle (Duckett and Pratt, 2014). However, the interaction between anabolic implant efficacy, physiological stress, and vaccine response has not been widely explored. In fact, we are unaware of studies evaluating the effects of anabolic implanting on the acute-phase response (APR), which typically occurs

¹Partial financial support for this research was provided by Zoetis Animal Health. Appreciation is expressed to Dean Askew and Gregory Shaeffer (North Carolina State University, Raleigh) for their assistance during this study.

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Received March 14, 2016.

Accepted May 5, 2016.

in recently weaned beef calves for 14 d after an immunological challenge, such as vaccination, weaning, and feedlot entry (Cooke et al., 2011; Arthington et al., 2013; Artioli et al., 2015; Moriel et al., 2015). During APR, cortisol is released and has been shown to induce immune suppression effects (Salak-Johnson and McGlone, 2007), weaken the innate immune response (Dai and McMurray, 1998), and impair antibody production (Salak-Johnson and McGlone, 2007). In contrast, anabolic implants increase the synthesis of local and systemic IGF-1 (Dayton and White, 2008), promoting muscle synthesis, which could alleviate the negative effects of APR on growth performance (O'Connor et al., 2008).

We hypothesized that a single estrogenic implant insertion to recently weaned beef steers would exacerbate the existing APR, leading to suppressed growth and vaccine response against pathogens associated with bovine respiratory disease (BRD), which could be prevented by shifting the timing of implant insertion relative to weaning. Therefore, our objectives were to evaluate the effects of timing of estrogenic implant insertion, relative to weaning, on growth performance and measurements of innate and humoral immunity of beef calves.

MATERIALS AND METHODS

All procedures for the experiment conducted at the Butner Beef Cattle Field Laboratory (Butner, NC) were approved by the Institutional Animal Care and Use Committee of North Carolina State University (15-096-A).

Animals, Diets, and Sample Collection

Prewaning (d -14 to 0) and Preconditioning (d 0 to 56) Phases. On d -14, Angus × Simmental crossbred steers ($n = 48$; 217 ± 5 kg of BW; 191 ± 3 d of age) were stratified by BW, age, and cow parity and randomly assigned to receive no estrogenic implant (NOIP; $n = 12$ steers) or a 36-mg zeranol implant (Ralgro, Merck Animal Health, Summit, NJ) on d -14, 0, or 14, relative to weaning (IP-14, IP0, and IP+14, respectively; $n = 12$ steers/treatment). The 14-d interval difference on implant insertion among treatments was selected because plasma concentrations of haptoglobin and cortisol (indicators of APR) are typically greatest between 3 and 7 d and return to baseline concentrations within 10 to 14 d after weaning, vaccination, and feedlot entry (Arthington et al., 2013; Moriel and Arthington, 2013; Artioli et al., 2015; Moriel et al., 2015). From d -14 to 0, all cow-calf pairs remained on a single 20-ha tall-fescue pasture (*Lolium arundinaceum*; 21% CP and 62% TDN; DM basis) with free-choice access to water and no access to concentrate supplementation. All steers were weaned on d 0, strati-

Table 1. Nutritional composition of total mixed ration (TMR) provided to steers from d 0 to 56 (preconditioning), 56 to 167 (growing), and 168 to 252 (finishing phase)¹

Item	Preconditioning TMR (DM basis)	Growing TMR (DM basis)	Finishing TMR (DM basis)
Corn silage, %	71.4	77.8	16.7
Ground corn, %	7.8	7.6	69.7
Soybean meal, %	19.6	23.4	11.8
Limestone, %	0.91	0.89	1.48
Inorganic trace mineral salt, ² %	0.28	0.27	0.28
Rumensin 90, ³ %	0.011	0.011	0.018
Vitamin ADE, ⁴ %	0.034	0.033	0.037
DM, %	35.7	40.5	71.5
CP, %	18.0	16.0	15.0
TDN, ⁵ %	73.0	75.0	85.0
NDF, %	28.6	39.3	16.8
ADF, %	16.1	24.2	8.2
NE _m , ⁶ Mcal/kg	1.74	2.06	2.17
NE _g , ⁶ Mcal/kg	1.12	1.31	1.48
Ca, %	0.46	0.64	0.62
K, %	0.92	1.56	1.32
Mg, %	0.22	0.25	0.16
P, %	0.28	0.26	0.28
S, %	0.15	0.33	0.23

¹Samples of preconditioning TMR were pooled by week, whereas samples of growing and finishing TMR were pooled by month. All samples were sent in duplicate to a commercial laboratory for wet chemistry analysis (Dairy One Laboratory, Ithaca, NY).

²Cargill Animal Nutrition, Minnetonka, MN (56.3% Cl, 32.9% Na, 0.91% S, 72 mg/kg Co, 5,000 mg/kg Cu, 104 mg/kg I, 2,500 mg/kg Mn, 104 mg/kg Se, and 10,000 mg/kg Zn).

³Rumensin 90 (Elanco, Greenfield, IN; 200 g monensin/kg of product).

⁴Provimi North America Inc. (Brookville, OH; 2,043,000 IU/kg vitamin A, 450,000 IU/kg vitamin D₃, and 900 IU vitamin E).

⁵Calculated as described by Weiss et al. (1992).

⁶Calculated using equations of NRC (2000).

fied by treatment and BW, and immediately allocated into 1 of 16 concrete, slatted floor pens (11 m²/pen; 3 steers/pen) in a fully covered, drylot facility. Steers were provided daily free-choice access to a preconditioning corn silage total mixed ration (TMR) at 0800 h from d 0 to 56 (Table 1). Accumulated diet refused (as fed) was measured once weekly. Diet DM refused and offered was obtained by drying weekly samples of diets refused and offered in a forced-air oven at 56°C for 48 h. Daily DMI was determined by subtracting the weekly DM refused from the weekly DM offered and then dividing by 7 d. Samples of diet offered and refused were collected weekly, pooled by week, and sent in duplicate for chemical composition using wet chemistry procedures at a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY). Samples were analyzed for concentrations of CP (method 984.13; AOAC, 2006), ADF (method 973.18 modified for use in an Ankom 200 fiber analyzer;

Ankom Technology Corp., Fairport, NY; AOAC, 2006), and NDF (Van Soest et al., 1991; modified for use in an Ankom 200 fiber analyzer; Ankom Technology Corp.). Concentrations of TDN were calculated as proposed by Weiss et al. (1992), whereas NE_m and NE_g were calculated using equations from NRC (2000).

All steers were treated with doramectin for internal and external parasites on d -27 (5 mL subcutaneous; Dectomax injectable, Zoetis Animal Health, Kalamazoo, MI) and received 2 mL subcutaneous of Bovi Shield Gold FP5 VL5 and Ultrabac 7 (Zoetis Animal Health) on d -27 and 0. Shrunken BW of steers was obtained on d -14 and 56 after 12 h of feed and water withdrawal. Additional BW of steers was obtained on 2 consecutive days immediately before feeding at 14-d intervals from d 0 to 42. Shrunken BW was not obtained from d 0 to 42 to avoid disturbing feeding behavior and avoid an unnecessary shrink-induced physiological stress that could interfere with plasma measurements and vaccine response (Marques et al., 2012).

Blood samples were collected via jugular venipuncture in tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ) containing sodium heparin (158 United States Pharmacopeia units) for plasma harvest on d -14, -13, -10, -7, 0, 1, 3, 7, 14, 15, 17, 21, and 28 to determine the plasma concentrations of IGF-1, cortisol, and haptoglobin. In addition, blood samples from jugular vein were collected into tubes containing no additives (Vacutainer, Becton Dickinson) for serum harvest on d -14 and 56 to determine serum titers against infectious bovine rhinotracheitis virus (IBRV) and bovine viral diarrhea virus (BVDV) types 1a and 2. Blood samples were immediately placed on ice following collection and then centrifuged at $1,200 \times g$ for 25 min at 4°C. Plasma and serum samples were stored frozen at -20°C until later analysis.

Postpreconditioning phase (d 56 to 252). Steers remained in the same drylot facility and pen distribution during the postpreconditioning phase, which was divided into growing (d 56 to 167) and finishing periods (d 168 to 252). The growing and finishing TMR are described in Table 1 and were provided ad libitum at 0800 h from d 56 to 167 and 168 to 252, respectively. Accumulated diet refused (as fed) was measured once weekly. Diet DM offered was determined by drying diet samples in a forced-air oven at 55°C for 48 h. Samples of growing and finishing diets were pooled by month and sent in duplicate for chemical composition using wet chemistry procedures at a commercial laboratory (Dairy One Forage Laboratory). Steer BW was determined before feeding at 0800 h every 28 d from d 56 to 252.

On d 139, all steers were administered 200 mg trenbolone acetate and 40 mg estradiol 17 β (Revalor XS; Merck Animal Health, Summit, NJ). All steers were slaughtered on d 253 at the USDA-inspected

Cargill Meats Solutions Corporation (Wyalusing, PA). Longissimus muscle area, back fat thickness, KPH percentage, marbling scores, carcass quality, and yield grade were determined 48 h after slaughter by a qualified personnel from the Meats Laboratory at Penn State University (University Park, PA).

Laboratory Analyses

Plasma concentrations of haptoglobin were assessed in duplicate using a biochemical assay measuring haptoglobin-hemoglobin complex by the estimation of differences in peroxidase activity (Cooke and Arthington, 2013). Inter- and intra-assay CV of haptoglobin assays were 5.3% and 2.2%, respectively. Plasma cortisol concentrations were analyzed in duplicate samples using a single chemiluminescent enzyme immunoassay (Immulite 1000; Siemens Medical Solutions Diagnostics, Los Angeles, CA). Intra-assay CV for cortisol assay was 1.9%. Plasma IGF-1 concentrations were analyzed using commercial enzyme-linked immunosorbent assay kits (SG100; R&D Systems Inc., Minneapolis, MN) previously validated for bovine samples (Moriel et al., 2012). Inter- and intra-assay CV for IGF-1 assay were 2.6% and 3.0%, respectively.

Serum antibody titers against IBRV and BVDV-1a and -2 were determined by the Oklahoma Animal Disease and Diagnostic Laboratory using a virus neutralization test (Rosenbaum et al., 1970). Serum titers were reported as the \log_2 of the greatest dilution of serum that provided complete protection of the cells (lowest and greatest tested dilution = 1:4 and 1:256, respectively; Richeson et al., 2008; Artioli et al., 2015; Moriel et al., 2015). For the seroconversion analysis, samples with serum neutralization values of <4 were considered negative and assigned a value of 0, whereas samples with serum neutralization values ≥ 4 were considered positive and assigned a value of 1. Then the assigned values (0 or 1) were used to calculate the positive seroconversion (percentage of steers with positive serum neutralization) to IBRV and BVDV-1a and -2.

Statistical Analyses

Except for seroconversion and carcass characteristics, all data were analyzed as a completely randomized design using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC, version 9.3) with Satterthwaite approximation to determine the denominator degrees of freedom for the test of fixed effects. Pen was considered the experimental unit for analysis of DMI and G:F (pre- and postpreconditioning), which were tested for fixed effects of treatment using pen(treatment) as the random variable. Steer was considered the experi-

Table 2. Body weight of steers during preconditioning (d 0 to 56), growing (d 56 to 167), and finishing (d 168 to 252) phases¹

BW, kg	Treatment				SEM	P-value, treatment × day
	NOIP	IP-14	IP0	IP+14		
Preconditioning phase (d 0 to 56) ²						
d 0	223 ^a	225 ^a	222 ^a	222 ^a	2.7	0.03
d 14	244 ^a	252 ^b	251 ^b	246 ^a		
d 28	264 ^a	274 ^b	269 ^{a,b}	263 ^a		
d 42	281 ^a	295 ^b	291 ^b	283 ^a		
d 56	301 ^a	315 ^b	312 ^b	304 ^a		
Growing and finishing phases (d 56 to 252) ³						
d 84	334 ^a	352 ^a	350 ^a	342 ^a	12.0	0.04
d 112	376 ^a	394 ^a	392 ^a	386 ^a		
d 140	413 ^a	432 ^a	429 ^a	424 ^a		
d 168	461 ^a	485 ^a	481 ^a	473 ^a		
d 196	504 ^a	543 ^b	528 ^{a,b}	518 ^a		
d 224	537 ^a	578 ^b	568 ^b	550 ^a		
d 252	590 ^a	632 ^b	614 ^b	606 ^{a,b}		

^{a,b}Within day, means without a common superscript differ ($P \leq 0.05$).

¹Steers received no estrogenic implant (NOIP) or 36 mg of zeranol on d -14, 0, or 14, relative to weaning (IP-14, IP0, and IP+14, respectively; $n = 12$ steers/treatment).

²Shrunk BW was obtained on d -14 and 56 after 12 h of feed and water withdrawal. Additional BW was obtained on 2 consecutive days before feeding at 14-d intervals from d 0 to 42.

³Steer BW obtained before feeding every 28 d from d 84 to 252.

mental unit for the analyses of BW, ADG (pre- and postpreconditioning), carcass characteristics, plasma, and serum measurements. Body weight (pre- and postpreconditioning), plasma, and serum measurements (except for seroconversion) were analyzed as repeated measures and tested for the fixed effects of treatment, day, and treatment × day using steer (treatment × pen) as the subject and random effect. The autoregressive 1 covariance structure was used for the analysis of plasma and serum measurements, whereas compound symmetry covariance structure was used for the BW analysis as it generated the lowest Akaike information criterion. Body weight did not differ among treatments on d -14 ($P = 0.99$) but was included as a covariate ($P < 0.0001$) to adjust BW from d 0 to 56. Seroconversion to IBRV and BVDV-1a and -2 were analyzed as repeated measures using the GLIMMIX procedure of SAS and steer(treatment × pen) as subject. All results are reported as least squares means. Data were separated using PDIFF if a significant preliminary F test was detected. Significance was set at $P \leq 0.05$, and tendencies were considered if $P > 0.05$ and ≤ 0.10 .

RESULTS

Preweaning (d -14 to 0) and Preconditioning (d 0 to 56) Phases. A treatment × day effect was de-

Table 3. Growth performance of steers that received no estrogenic implant (NOIP) or 36 mg of zeranol on d -14, 0, or 14, relative to weaning (IP-14, IP0, and IP+14, respectively; $n = 12$ steers/treatment)

Item	Treatment				SEM	P-value, treatment
	NOIP	IP-14	IP0	IP+14		
ADG, ¹ kg/d						
d -14 to 0	0.38 ^a	0.58 ^b	0.35 ^a	0.31 ^a	0.079	0.09
d 0 to 14	1.53 ^a	1.95 ^b	1.97 ^b	1.75 ^{a,b}	0.137	0.09
d 14 to 28	1.41	1.48	1.31	1.28	0.128	0.68
d 28 to 42	1.30	1.49	1.61	1.42	0.123	0.36
d 42 to 56	1.42	1.43	1.42	1.44	0.167	0.99
d 0 to 56	1.43	1.60	1.58	1.49	0.061	0.14
d -14 to 56	1.20 ^a	1.38 ^c	1.33 ^{b,c}	1.24 ^{a,b}	0.049	0.05
DMI, ² kg/d						
d 0 to 13	4.80	4.76	4.71	4.67	0.163	0.94
d 14 to 27	6.55	6.82	6.67	6.83	0.195	0.70
d 28 to 41	6.67	7.02	6.90	6.76	0.188	0.61
d 42 to 56	8.19	8.33	8.34	7.91	0.322	0.76
d 0 to 56	6.58	6.72	6.67	6.54	0.171	0.85
G:F ³	0.215	0.235	0.235	0.222	0.0088	0.34

^{a-c}Within a row, means without a common superscript differ ($P \leq 0.05$).

¹Average daily gain was calculated using the average BW of 2 consecutive measurements obtained every 14 d from d -14 to 56.

²Steers were provided daily free-choice access to a preconditioning TMR from d 0 to 56. Daily DMI was determined by subtracting the weekly DM refused from the weekly DM offered and then divided by 7 d.

³Estimated by dividing total BW gain by total DMI (d 0 to 56).

tected ($P = 0.03$) for BW from d 0 to 56 (Table 2) using BW on d -14 as a covariate ($P < 0.0001$). Body weight on d 0 did not differ among treatments ($P \geq 0.29$) but was greater for IP-14 and IP0 than NOIP and IP+14 steers on d 14, 42, and 56 ($P \leq 0.05$). Treatment effects were detected for ADG from d -14 to 56 ($P = 0.05$), whereas ADG from d -14 to 0 and 0 to 14 tended to differ among treatments ($P = 0.09$; Table 3). Steers implanted on d -14 had greater ($P \leq 0.05$) ADG from d -14 to 0 compared to NOIP, IP0, and IP+14 steers. Average daily gains from d -14 to 56 were greater for IP-14 than NOIP steers ($P \leq 0.05$) and intermediate for IP0 and IP+14 steers, whereas ADG from d 0 to 14 was greater for IP-14 and IP0 compared to NOIP steers ($P \leq 0.04$) and intermediate for IP+14 steers. Treatment effects were not detected for G:F and DMI from d 0 to 56 ($P \geq 0.34$) and ADG from d 14 to 28, 28 to 42, 42 to 56, and 0 to 56 ($P \geq 0.14$; Table 3)

A treatment × day effect was detected for plasma IGF-1 concentrations ($P = 0.05$). Plasma IGF-1 concentrations were greater for IP-14 than NOIP steers on d -7 and 21 ($P \leq 0.05$) and greater for IP-14 compared to IP0 steers on d 0 and 14 ($P \leq 0.05$) but did not differ among treatments on d -14 and 7 ($P \geq 0.28$; Fig. 1). On d 21, plasma IGF-1 concentrations were similar between IP0 and IP+14 steers ($P = 0.94$), but both had greater

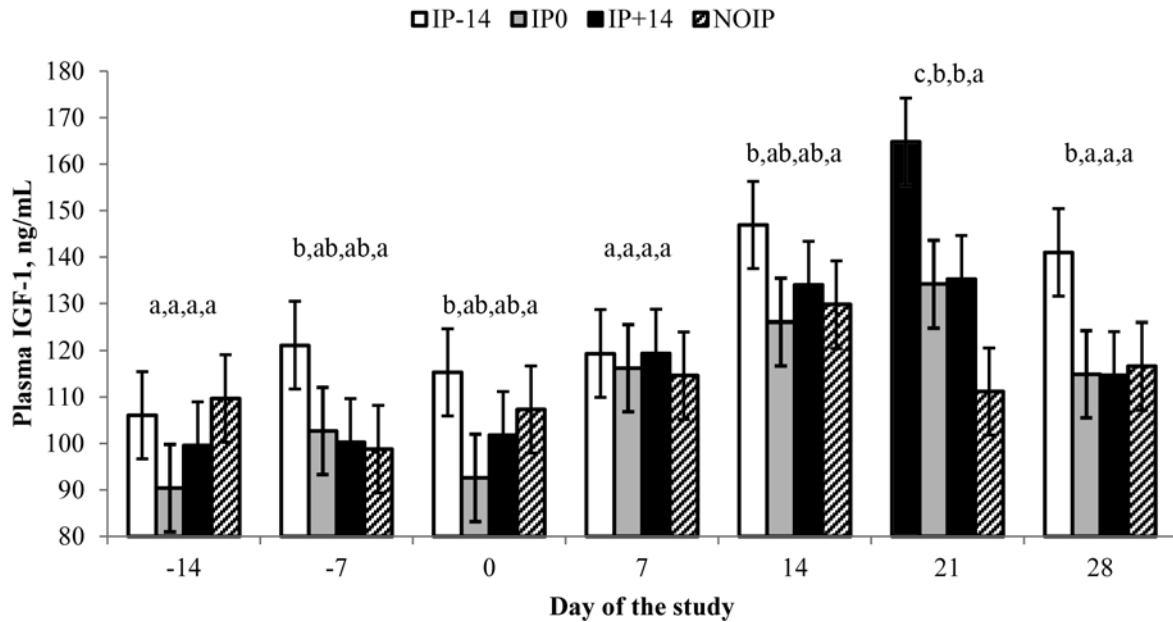


Figure 1. Plasma concentrations of IGF-1 of steers assigned to receive no estrogenic implant (NOIP) or 36 mg of zeranol on d -14, 0, or 14, relative to weaning (IP-14, IP0, and IP+14, respectively; $n = 12$ steers/treatment). A treatment \times day effect was detected for plasma concentrations of IGF-1 ($P = 0.05$). ^{a-c}Within day, means without a common superscript differ ($P \leq 0.05$).

concentrations than NOIP steers ($P \leq 0.05$). Effects of day, but not treatment \times day and treatment ($P \geq 0.37$), were detected for plasma concentrations of cortisol and haptoglobin ($P < 0.0001$; Fig. 2). Effects of treatment \times day and treatment were not detected ($P \geq 0.37$) for seroconversion and serum titers against BVDV-1a and -2 (Table 4). However, effects of treatment \times day were detected ($P = 0.05$) for serum IBRV titers but not for seroconversion to IBRV ($P = 0.22$). Serum IBRV titers did not differ among treatments on d -14 ($P \geq 0.24$) but were greater ($P \leq 0.02$) for IP+14 compared to NOIP, IP-14, and IP0 steers on d 56 (Table 4).

Postpreconditioning Phase (d 56 to 252). Body weight of steers did not differ among treatments from d 84 to 168 ($P \geq 0.18$; Table 2). On d 196, IP-14 steers were heavier ($P \leq 0.05$) than NOIP and IP+14 steers, whereas IP0 steers were intermediate ($P \geq 0.16$). On d 224, IP-14 and IP0 steers were heavier ($P \leq 0.05$) than NOIP and IP+14 steers. On d 252, IP-14 and IP0 steers were heavier ($P \leq 0.05$) than NOIP, whereas IP+14 steers were intermediate ($P \geq 0.13$).

Effects of treatment \times day were not detected for ADG measured every 28 d from d 56 to 252 ($P \geq 0.12$), and hence, ADG and DMI results during the postpreconditioning period were analyzed and reported as overall ADG and DMI from d 56 to 167 (growing phase) and d 168 to 252 (finishing phase; Table 5). Effects of treatment were not detected ($P \geq 0.17$) for ADG and G:F from d 56 to 167, 168 to 252, and 56 to 252 or DMI from d 56 to 167. Effects of treatment were not detected for HCW, yield grade, LM area, back fat thickness, marbling, and KPH ($P \geq 0.18$; Table 5).

DISCUSSION

Prewaning (d -14 to 0) and Preconditioning (d 0 to 56) Phases. As expected and in agreement with others (Selk, 1997), preweaning ADG of IP+14 steers was increased by implant insertion 14 d before weaning. During the preconditioning phase, DMI, G:F, and ADG from d -14 to 56 did not differ among treatments. This lack of detection of treatment effects on G:F and ADG during the preconditioning phase could be associated with gut fill effects. As previously mentioned, shrunk BW was not obtained from d 0 to 42 in an effort to not disturb feeding behavior and avoid shrink-induced physiological stress response that could interfere with plasma measurements and vaccine response (Marques et al., 2012). Nonetheless, ADG and G:F from d 0 to 56 were, on average, 4.2% to 11.9% and 3.3% to 9.3%, respectively, greater for implanted compared to nonimplanted steers. Likewise, data summarized by Duckett et al. (1996) indicated that ADG and feed efficiency (reported as feed to gain) were, on average, 18% and 8%, respectively, greater for implanted compared to nonimplanted feedlot steers. The relatively greater growth performance of steer after implant insertion was expected and often reported in the literature and can be partially associated with differences in plasma IGF-1 concentrations, as will be discussed below.

Overall ADG from d -14 and 56 (using shrunk BW collected on d -14 and 56) was 7.2% to 15% greater for IP-14 and IP0 steers than NOIP and IP+14 steers. Consequently, IP-14 and IP0 steers were 8 to 14 kg heavier on d 56 compared to IP+14 and NOIP steers.

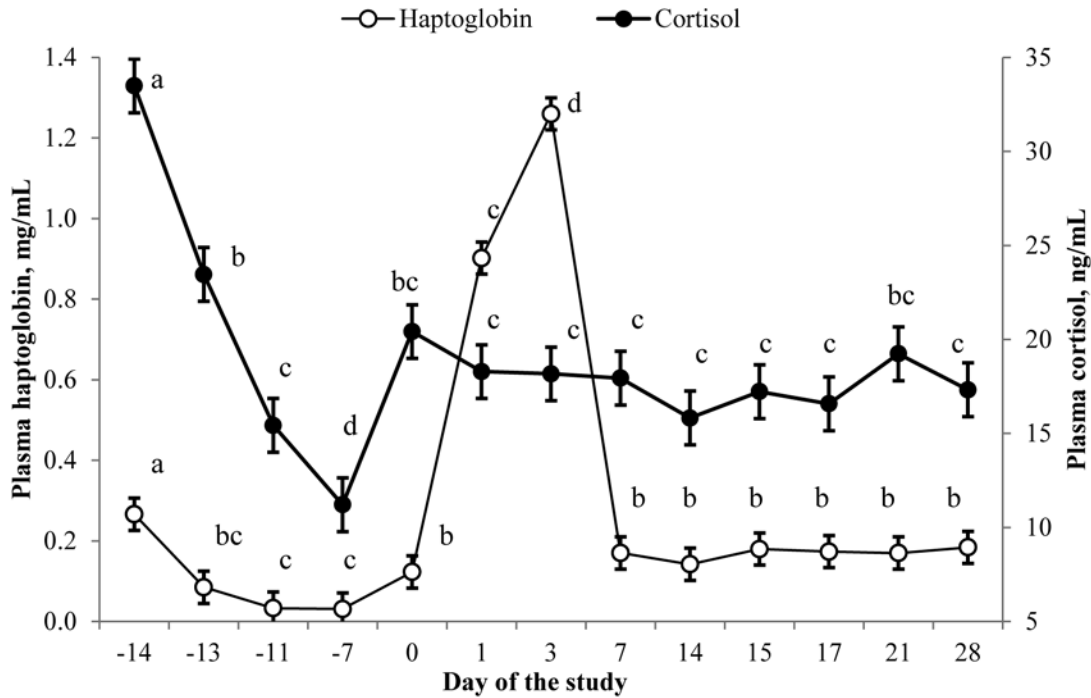


Figure 2. Plasma concentrations of haptoglobin and cortisol of steers assigned to receive no ear implant (NOIP) or 36 mg of zeranol on d -14, 0, or 14, relative to weaning (IP-14, IP0, and IP+14, respectively; $n = 12$ steers/treatment). Effects of day, but not treatment and treatment \times day ($P \geq 0.39$), were detected for plasma concentrations of haptoglobin and cortisol ($P < 0.0001$). ^{a-d}Within plasma measurement, means without a common superscript differ ($P \leq 0.05$).

Similar to steers in this trial, steers fed a corn silage-based TMR and implanted with 36 mg of zeranol at the start of the growing period had an 8.5% greater ADG for 80 d after implant insertion compared to nonimplanted steers (Madder, 1994). Despite the differences in preweaning ADG, BW of IP-14 and IP0 steers did not differ on d 56. This is primarily because the increment on ADG from d -14 to 0 (+0.23 kg/d for IP-14 vs. IP0 steers) and the interval of implant insertion among treatments (14 d) were relatively small to result in substantial differences in BW. It is possible that significant differences in BW of IP-14 steers compared to IP0 steers would have been observed at the end of preconditioning phase if the timing of preweaning implant insertion was greater than 14 d. Thus, for preconditioning steers, it is questionable if the additional animal handling 14 d before weaning is economically justifiable and recommended compared to the convenience of implanting steers at weaning.

Surprisingly, we also demonstrated that delaying the implant insertion to 14 d after weaning to avoid multiple existing inflammatory responses did not improve preconditioning growth performance of IP+14 steers compared to nonimplanted steers to a level that is economically justifiable and recommended to cow-calf operations. This response was unexpected, but one reasonable explanation is the relatively shorter postimplant period to improve BW than implanting 14 d before or at weaning (42 vs. 70 and 56 d, respectively). In support of this rationale, the BW difference between IP+14 and NOIP

steers gradually increased from 3 kg at the end of preconditioning phase (d 56) to 16 kg immediately before slaughter (d 253), despite the similar management, ADG, and DMI from d 56 to slaughter. Likewise, Richeson et al. (2015) also reported no differences in ADG during a 21-d receiving period among auction market-purchased steers that were not implanted or administered 200 mg progesterone and 20 mg estradiol benzoate implant (Synovex S; Zoetis, Florham Park, NJ) on d 14 of a 42-d receiving/stocker grazing system. Hence, anticipating or delaying implant insertion by 14 d, relative to weaning, did not improve growth of steers during a 56-d preconditioning period compared to the convenience of inserting implants at the time of weaning.

The somatotrophic axis is a critical component of multiple systems controlling growth (Le Roith et al., 2001). Although liver is the major source of circulating IGF-1 (Yakar et al., 1999), IGF-1 can also be synthesized by most body tissues (Le Roith et al., 2001). Anabolic implants increase the synthesis of local and systemic IGF-1 (Dayton and White, 2008), promoting proliferation and differentiation of myogenic cells, as well as enhancing protein synthesis and suppressing protein degradation in myogenic cells (Dayton and White, 2008). Together, these responses could alleviate the negative effects of APR on growth performance (O'Connor et al., 2008). For instance, implanting yearling steers with trenbolone acetate and estradiol-17 β increased IGF-I mRNA expression in LM (Johnson et al., 1998b), leading to the proliferation of satellite cell population in semimem-

Table 4. Seroconversion and serum titers against bovine viral diarrhoea virus (BVDV) types 1a and 2 and infectious bovine rhinotracheitis virus (IBRV) of steers that received no estrogenic implant (NOIP) or 36 mg of zeranol on d -14, 0 or 14, relative to weaning (IP-14, IP0, and IP+14, respectively; $n = 12$ steers/treatment)¹

Item	Treatment				SEM	<i>P</i> -value	
	NOIP	IP-14	IP0	IP+14		Treatment	Treatment × day
BVDV type 1a							
Titers, ² log ₂	2.79	3.53	3.09	2.67	0.379	0.38	0.37
Seroconversion, ³ %	58.3	70.8	79.2	50.0	9.61	0.16	0.16
BVDV type 2							
Titers, ² log ₂	3.97	4.24	4.19	4.10	0.257	0.90	0.27
Seroconversion, ³ %	79.2	75.0	70.8	70.8	7.55	0.85	0.85
IBRV							
Titers, log ₂							
d -14	0 ^a	0.22 ^a	0.38 ^a	0.17 ^a	0.228	0.08	0.05
d 56	0.13 ^a	0.30 ^a	0.43 ^a	1.23 ^b	0.228		
Seroconversion ³ , %	4.2	12.5	20.8	29.2	7.85	0.15	0.22

^{a,b}Within a row, means without a common superscript differ ($P \leq 0.05$).

¹Steers were treated with doramectin for internal and external parasites on d -27 (5 mL subcutaneous [s.c.]; Dectomax injectable, Zoetis Animal Health, Kalamazoo, MI) and received 2 mL s.c. of Bovi Shield Gold FP5 VL5 and Ultrabac 7 (Zoetis Animal Health) on d -27 and 0.

²Overall serum titers obtained on d -14 and 56.

³Percentage of steers with positive serum neutralization (titer value ≥ 4).

branosus muscles, compared with that of nonimplanted steers (Johnson et al., 1998a). Also, Pampusch et al. (2003) reported that steers administered 24 mg estradiol and 120 mg trenbolone acetate (Revalor-S; Merck Animal Health) had greater serum IGF-1 concentrations 7 d postimplant insertion and, consequently, greater ADG and feed efficiency (36% and 34%, respectively) after 26 d of implant insertion than nonimplanted steers. Except for IP0 steers, plasma IGF-1 concentrations of IP-14 and IP+14 steers increased 7 d after implant insertion, which is in agreement with Pampusch et al. (2003). However, plasma IGF-1 concentrations of IP0 steers increased only 21 d after implant insertion (approximately 14 d after the end of APR). Proinflammatory cytokines released after APR may induce a state of IGF-1 resistance, inhibiting the anabolic effects of IGF-1 and facilitating energy and protein mobilization from body stores (O'Connor et al., 2008). Thus, the delayed rise in plasma IGF-1 concentrations of IP0 steers was likely due to the APR occurring between d 0 to 14 that prevented plasma IGF-1 synthesis to ensure nutrient partitioning toward the support of the immune system. Nevertheless, this lack of earlier rise in plasma IGF-1 concentrations of IP0 steers did not prevent improvements in growth performance compared to that of NOIP steers and likely suggests that muscle growth of IP0 steers was improved by the actions of local rather than systemic IGF-1.

Furthermore, IP+14 steers had similar plasma IGF-1 concentrations throughout the entire preconditioning phase, except on d 21, when plasma IGF-1 concentrations were greater for IP+14 than NOIP steers. As will be discussed below, the APR was not

affected by timing of implant insertion, suggesting that the demand for nutrients to support the APR also did not differ among treatments. Hence, another plausible explanation for the lack of positive effects on growth performance between NOIP and IP+14 steers is the similar inflammatory responses and the fact that plasma IGF-1 concentrations differed only on d 21; consequently, the duration of elevated plasma IGF-1 concentrations was not sufficient to enhance ADG of IP+14 steers compared to that of NOIP steers.

Weaning, feedlot entry, and vaccination induce APR that persists for approximately 14 d and leads to increased concentrations of acute-phase proteins (i.e., haptoglobin) and cortisol (Cooke et al., 2011; Arthington et al., 2013; Moriel and Arthington, 2013). During an immunological challenge, the metabolic demand for acute-phase protein synthesis is increased (Reeds and Jahoor, 2001). Consequently, the multiple APR experienced by preconditioning calves substantially increases nutrient demand, which, coupled with suppressed feed intake, can further decrease growth (Moriel et al., 2015, 2016). In agreement, DMI of all steers was least during the first 14 d of the preconditioning period, which corresponds to the period of APR. Haptoglobin prevents Fe utilization for bacterial growth (Wassell, 2000) and may be used as an indicator of inflammatory conditions in cattle when plasma concentrations are ≥ 0.11 mg/mL (Tourlomoussis et al., 2004). Cortisol may induce immune suppression effects (Salak-Johnson and McGlone, 2007), weaken the innate immune response (Dai and McMurray, 1998), and block the cytokine secretion involved in antibody production (Salak-Johnson

Table 5. Growth performance during growing (d 56 to 167) and finishing (d 168 to 252) phases and carcass characteristics at slaughter (d 253) of steers that received no estrogenic implant (NOIP) or 36 mg of zeranol on d -14, 0, or 14, relative to weaning (IP-14, IP0, and IP+14, respectively; $n = 12$ steers/treatment)

Item	Treatment				SEM	<i>P</i> -value, treatment
	NOIP	IP-14	IP0	IP+14		
ADG, ¹ kg/d						
d 56 to 167	1.51	1.53	1.53	1.53	0.058	0.99
d 168 to 252	1.57	1.74	1.58	1.61	0.069	0.34
d 56 to 252	1.53	1.63	1.55	1.57	0.039	0.48
DMI, ² % BW						
d 56 to 167	2.36	2.42	2.41	2.42	0.044	0.74
d 168 to 252	1.80	1.98	1.86	1.90	0.053	0.17
d 56 to 252	2.03	2.14	2.08	2.10	0.034	0.20
G:F ³						
d 56 to 167	0.168	0.163	0.163	0.165	0.0045	0.83
d 168 to 252	0.164	0.154	0.156	0.160	0.0056	0.64
d 56 to 252	0.167	0.161	0.160	0.164	0.0039	0.58
HCW, kg	351	376	354	363	8.8	0.18
Back fat thickness, cm	1.75	1.80	1.89	1.86	0.041	0.75
LM area, ⁴ cm ²	86.6	89.2	87.0	89.2	1.82	0.60
KPH, %	2.02	1.79	1.93	2.23	0.135	0.23
Yield grade	3.32	3.40	3.50	3.39	0.137	0.82
Marbling ⁵	672	663	623	592	29.9	0.26

¹From d 56 to 252, steers remained in the same facility and pen distribution assigned on d 0.

²Dry matter intake reported as the percentage of average BW from d 57 to 168, 168 to 252, and 57 to 252.

³Estimated by dividing total BW gain by total DMI of the respective period.

⁴Actual measurement obtained after slaughter measured between the 12th and 13th ribs.

⁵Marbling score: small = 500 to 590; modest = 600 to 690.

and McGlone, 2007). These factors can be used as indicators of calf immune protection (Bolin and Ridpath, 1990), vaccine efficacy (Richeson et al., 2008), and disease prevention (Howard et al., 1989). Thus, we hypothesized that an anabolic implant insertion at the time of weaning, vaccination, and feedlot entry would exacerbate the APR, leading to immune suppression and reduced vaccine response.

Although we observed differences among treatments in plasma IGF-1 concentration, plasma concentrations of haptoglobin and cortisol did not differ among treatments. These results demonstrated that the insertion of a single 36-mg zeranol implant did not elicit a new APR, nor did it exacerbate the existing APR caused by weaning, feedlot entry, and vaccination, which is contrary to our hypothesis. This lack of inflammatory response due to implant insertion suggests that the act of implant insertion does not elicit a psychological stress response on calves. Also, the animal body did not recognize the implant as a foreign object, and consequently, it

did not induce a physiological stress response. The lack of effects on APR also likely explains the similar seroconversion and serum titers against BVDV-1a and -2 at the end of the preconditioning phase. The ability of an animal to respond to vaccination differs from animal to animal and depends on environmental and genetic factors, maternal antibody concentrations, ADG (Downey et al., 2013), timing of vaccination after feedlot entry (Richeson et al., 2008), metabolizable protein supply (Moriel et al., 2015), frequency of energy supplementation (Artioli et al., 2015), and energy concentration of maternal diet offered during late gestation (Moriel et al., 2016). Our results are in agreement with those of Richeson et al. (2015), who reported no differences in serum BVDV-1a titers among auction market-purchased steers that were not implanted or received a Synovex S implant on d 0, 14, or 28 of a 42-d receiving/stocker grazing system. Together, these findings indicate that estrogenic implants could be administered simultaneously to other calf management procedures without causing or enhancing the inflammatory response or impairing the overall vaccine response to BRD-associated pathogens.

In the current study, however, serum IBRV titers at the end of the preconditioning phase were increased when implant insertion was delayed by 14 d after weaning compared to the remaining treatments. Munson et al. (2012) reported a tendency toward lower morbidity rates (25% vs. 29%, respectively) for auction market-derived steers that received a Revalor XS implant immediately at or 45 d after feedlot entry. Our results on serum IBRV titers could provide a plausible explanation for the results observed by Munson et al. (2012). However, serum IBRV titers were substantially lower than the values observed for serum BVDV-1a and -2 titers. Hence, it is questionable whether the slightly greater serum IBRV titers of IP+14 steers would result in improved immune protection and reduced susceptibility to BRD. Downey et al. (2013) observed that calves that experienced greater ADG after vaccination had an increased initial response to the vaccination. For every 1 kg/d increase in ADG, serum BVDV-2 titers increased by 0.068 units. However, those authors analyzed only serum BVDV-2 titers. Also, in our study 1) only serum IBRV titers differed among treatments, and 2) ADG was never greater for IP+14 steers than the other treatments. Thus, the observed differences in serum IBRV titers cannot be attributed to differences in growth performance. At this point, we are unaware of potential reasons that could explain the observed effects on IBRV titers.

Postpreconditioning Phase (d 56 to 252). In the current study, ADG during the entire postpreconditioning phase did not differ among treatments, and hence, BW differences observed among treatments at the end

of the preconditioning phase remained until the end of the study. Postpreconditioning growth and carcass characteristics at slaughter did not differ among treatments. However, the numerical differences in HCW and LM area among treatments mirrored the numerical differences observed for BW of steers at the end of the study (d 252). It is important to highlight that the sample size utilized in this study was adequate for all of the analyses of DMI, ADG, and blood parameters but not sufficient to obtain a power test of 80% for the analyses of carcass characteristics. According to PROC POWER of SAS ($\alpha = 0.05$), 19 steers per treatment would have been necessary to obtain a power test of 80% for carcass characteristics analyses, which could not be achieved in our study because of physical limitations of the feedlot (maximum of 16 pens with 3 steers/pen). Therefore, interpretation of results observed for carcass characteristics needs to be done with care. Nonetheless, our results support previous findings reporting that carryover effects of suckling and/or stocker implants are minimal for feedlot performance and carcass quality and that the increased gain with implanting is generally additive throughout all phases of beef production (Kunkle et al., 1980; Kuhl et al., 1997; Schaneman and Pritchard, 1998; Duckett and Andrae, 2001). For instance, Kunkle et al. (1980) reported no carryover effects on steer ADG when implanting 36 mg of zeranol during suckling, at weaning, and 84 to 120 d postweaning. Similarly, Munson et al. (2012) reported no differences in ADG and G:F during a 187-d feedlot period and carcass characteristics at slaughter between auction market-derived steers administered a Revalor XS implant immediately at or 45 d after feedlot arrival.

In summary, our results indicate that a 36-mg zeranol implant could be administered simultaneously with other calf management procedures, such as weaning, vaccination, and feedlot entry, without causing or enhancing the inflammatory response or impairing the overall vaccine response to BRD-associated pathogens. Under the conditions of the present study, delaying or anticipating zeranol implant insertion by 14 d had no benefit for the innate and humoral immunity of recently weaned beef calves (except for serum IBRV titers). Furthermore, preconditioning growth performance of steers could be enhanced by a zeranol implant insertion 14 d before or at weaning compared to nonimplanted steers, without causing carryover effects on growing and finishing growth performance and carcass characteristics at slaughter.

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