

Effects of Organic or Inorganic Co, Cu, Mn, and Zn Supplementation to Late-Gestating Beef Cows on Productive and Physiological Responses of the Offspring

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Abstract text: Eighty-four pregnant Angus × Hereford cows were ranked by BW and BCS, and allocated to 21 drylot pens at the end of their 2nd trimester of gestation (d 0). Pens were assigned to receive: 1) diet supplemented with sulfate sources of Cu, Co, Mn, and Zn (INR), 2) diet supplemented with an organic source of Cu, Mn, Co, and Zn (ORG), and 3) no Cu, Co, Mn, and Zn supplementation (CON). From d 0 until calving, cows were offered a forage-based diet formulated to meet requirements for energy, protein, macrominerals, Se, I, and vitamins. The INR and ORG diets were formulated to provide the same daily amount of Cu, Co, Mn, and Zn. Cow BCS was recorded, and liver samples were collected on d -10 and 2 wk (d 75) before the calving season. Within 3h after calving, calf BW was recorded, liver samples were collected, and the placenta was retrieved for cotyledon collection. All liver and cotyledon samples were analyzed for Cu, Co, Mn, and Zn. After calving, cow-calf pairs were assigned to the general management of the herd that included inorganic mineral supplementation. Calves were weaned a 6 mo of age and preconditioned for 45 d. Cows receiving CON had less ($P \leq 0.05$) BCS gain during the last trimester of gestation compared with INR and ORG cows, although cows from all treatments had similar ($P = 0.61$) and adequate pre-calving BCS. On d 75, liver concentrations of Co, Cu, and Zn were greater ($P \leq 0.05$) for INR and ORG compared with CON, whereas INR cows had reduced ($P = 0.04$) liver Co but greater ($P = 0.03$) liver Cu compared with ORG cows. In the cotyledons, Co concentrations were greater ($P \leq 0.05$) in ORG and INR compared with CON cows, whereas Cu concentrations were increased ($P = 0.05$) in ORG compared with CON cows. Calves from INR and ORG cows had greater ($P < 0.01$) liver Co concentrations compared with calves from CON cows. Liver Cu and Zn concentrations were also greater ($P \leq 0.05$) for calves from ORG cows compared with cohorts from CON cows. Calf BW and value at weaning and upon preconditioning were greater ($P \leq 0.04$) for calves from ORG cows compared with calves from CON cows, and similar ($P \geq 0.18$) between calves from INR cows compared with the other treatments. Therefore, supplementing late-gestating beef cows with organic Co, Cu, Zn, and Mn is an alternative to enhance offspring productivity and economic returns in cow-calf systems.

Keywords: beef cows, minerals, offspring, pregnancy

INTRODUCTION

Energy and protein intake of beef cows during late-gestation influence performance responses of the offspring (Bohnert et al., 2013). However, little is known about the impacts of trace mineral status of late-gestating cows, which are essential for fetal development in livestock species (Hostetler et al., 2003), on offspring productivity.

The fetus depends completely on the dam for proper supply of trace minerals to support metabolic processes required for fetal growth (Hidioglou and Knipfel, 1981). If maternal supply is inadequate, fetal development and postnatal performance can be impaired (Weiss et al., 1983). As examples, Zn, Cu, Mn, and Co (as component of vitamin B₁₂; NRC, 2000) are required for adequate development of the fetal nervous, reproductive, and immune systems (Hostetler et al., 2003; Pepper, 2011). Moreover, Cu concentrations in bovine fetal liver are greater than maternal liver Cu concentrations, suggesting that the maternal system shunts Cu to support fetal development (Gooneratne and Christensen, 1989). Therefore, we hypothesized that supplementing Cu, Mn, Zn, and Co to late-gestating cows is indispensable for optimal offspring productivity. One strategy to enhance trace mineral status in cattle is to feed organic sources (Spears, 1996). Hostetler et al. (2003) reported that Cu, Mn, and Zn concentrations in tissues of fetuses collected from sows supplemented with organic sources of these elements were greater compared with fetuses from sows supplemented with inorganic sources, which translated into reduced fetal degeneration. Hence, we also theorized that supplementing organic sources of Cu, Mn, Zn, and Co to beef cows during late gestation is an alternative to further optimize postnatal offspring productivity.

Based these hypotheses, the objective of this experiment was to evaluate the effects of organic and inorganic Cu, Mn, Zn, and Co supplementation to beef cows during late gestation on productive and physiological responses of the offspring.

MATERIALS AND METHODS

Animals and treatments. Eighty-four multiparous, non-lactating, pregnant Angus × Hereford cows (BW 512 ± 6 kg, age = 5.8 ± 0.3 yr, BCS = 5.11 ± 0.04 according to Wagner et al., 1988) were assigned to the experiment at the end of their 2nd trimester of gestation. Cows were pregnant to AI using semen from a single Angus sire (n = 56) or

pregnant to Hereford bulls (n = 28), according to the breeding management and pregnancy diagnosis described by Cooke et al. (2014). Cows were ranked by pregnancy type (AI or natural service), BW, and BCS, and allocated to 21 drylot pens (4 cows/pen) in a manner that pens had equivalent BW and BCS, and either 3 or 2 cows pregnant to AI. Pens were ranked by proportion of cows pregnant to AI or natural service, and randomly assigned to receive 1 of 3 treatments: 1) diet supplemented with sulfate sources of Cu, Co, Mn, and Zn (**INR**), 2) diet supplemented with an organic source of Cu, Mn, Co, and Zn (**ORG**; Availa®4; Zinpro Corporation, Eden Prairie, MN), and 3) no dietary supplementation of Cu, Co, Mn, and Zn (**CON**).

During the treatment feeding period (d 0 until calving), all cows were offered the diet described in Table 1, which was consumed within 6 h after feeding and formulated to meet requirements for energy, protein, macrominerals, Se, I, and vitamins (Table 1) of pregnant cows during the last trimester of gestation (NRC, 2000). Treatments (INR and ORG) were mixed into the corn, and formulated to provide the same daily amount of Cu, Co, Mn, and Zn (based on 7 g/cow daily of Availa®4). Samples of all feed ingredients were collected prior to the beginning of the experiment, and analyzed for nutrient content by a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY, USA)

Sampling. Cow BW and BCS (Wagner et al., 1988) were recorded, and liver samples were collected from all cows prior to the beginning of the experiment (d -10; initial measurement), and 2 wk before the expected beginning of the calving season (d 75; pre-calving measurement). Within 3 h after calving, calf BW was recorded, a liver sample was collected, and the placenta was retrieved. Cow and calf liver samples were collected via needle biopsy. The placenta was rinsed with distilled water for 5 min, and the 5 largest cotyledons were collected. Cotyledons from each placenta were pooled and dried for 24 h at 65°C. Liver and cotyledon samples were stored at -80°C, and analyzed for concentrations of Cu, Co, Mn, and Zn (Michigan State Animal Health Diagnostic Laboratory; Lansing, MI).

After calving, cow-calf pairs were removed from their respective pen, and assigned to the general management of the research herd that included inorganic trace mineral supplementation (described by Cooke et al., 2014). At weaning (d 283 relative to beginning of experiment), calf BW was determined and 205-d adjusted weaning BW was calculated according to BIF (2010). Weaned calves were preconditioned as a single group for 45 d, and received mixed alfalfa-grass hay (14% CP, 56% TDN; DM basis) for *ad libitum* consumption. During preconditioning, calves were observed daily for bovine respiratory disease (**BRD**) symptoms, and treated when clinical symptoms were observed. Calf BW was recorded again at the end of the preconditioning period when cattle were shipped to a commercial feedlot.

Blood samples were collected immediately prior to weaning, as well as 1, 3, 5, and 7 d after weaning, via jugular venipuncture into commercial heparinized blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ, USA), placed on ice immediately, and centrifuged at 2,400 × g for 30 min at 4°C temperature for plasma collection. Plasma was stored at -80°C, and

analyzed for concentrations of cortisol and haptoglobin (Guarnieri Filho et al. 2014).

Table 1. Ingredient composition and nutrient profile of diets containing or not (**CON**) inorganic (**INR**) or organic (**ORG**) sources of Cu, Co, Mn, and Zn, as well as nutrient requirements (**REQ**; as % of diet, DM basis) of pregnant cows during last trimester of gestation

Item	CON	INR	ORG	REQ ¹
Ingredients, kg/d (as-fed basis)				
Alfalfa hay	6.8	6.8	6.8	-
Grass-seed straw	2.7	2.7	2.7	-
Cracked corn	2.3	2.3	2.3	-
Mineral mix	0.060	0.060	0.060	-
Inorganic trace mix ²	-	0.004	-	-
Organic trace mix ³	-	-	0.007	-
Nutrient profile (DM basis)				
NE _m , Mcal/kg	1.45	1.45	1.45	1.10
CP, %	14.4	14.4	14.4	7.8
Co, ppm	1.03	2.18	2.14	0.10
Cu, ppm	9.10	20.8	20.6	10
Mn, ppm	55.9	74.0	74.3	40
Zn, ppm	30.6	63.9	63.7	30

¹ Based on NRC (2000) and actual DMI.

² Containing (DM basis) 462 g/kg ZnSO₄, 294 g/kg MnSO₄, 228 g/kg CuSO₄, and 16 g/kg of CoSO₄.

³ Availa®4 (Zinpro Corporation, Eden Prairie, MN).

Statistical analysis. Data were analyzed with pen as the experimental unit, and pen(treatment) and cow(pen) as random variables. Quantitative data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC), and binary data were analyzed using the GLMMIX procedure of SAS (SAS Inst. Inc.). Model statements for cow-related responses included the effects of treatment. Model statements for calf-related responses included the effects of treatment, calf sex as independent variable, in addition to day and the treatment x day interaction for plasma variables. The specified term used in the repeated statement for plasma variables was day, the subject was cow(pen), and the covariance structure used was autoregressive, which provided the best fit for these analyses according to the Akaike information criterion. Results are reported as least square means and separated using PDIF. Significance was set at $P \leq 0.05$, and tendencies were determined if $P > 0.05$ and ≤ 0.10 .

RESULTS AND DISCUSSION

Nutrient composition and profile of diets offered to CON, INR, and ORG cows are described in Table 1. The CON diet provided adequate amounts of all nutrients and trace minerals, based on the requirements of pregnant cows during last trimester of gestation (NRC, 2000). As expected, including the inorganic or organic sources of Cu, Co, Mn, and Zn similarly increased the concentration of these trace elements in INR and ORG diets (Table 1).

Length of treatment administration was similar ($P = 0.36$) among CON, INR, and ORG cows (Table 2). As expected based on the experimental design, initial cow BW and BCS were similar ($P \geq 0.41$) among treatments (Table 2). No differences were also detected ($P \geq 0.61$) for BW change or pre-calving BW (Table 2). Cows receiving CON had less ($P \leq 0.05$) BCS gain during the last trimester of

gestation compared with INR and ORG cows (Table 2), although cows from all treatments had similar ($P = 0.61$) and adequate (Bohnert et al, 2013) pre-calving BCS (Table 2). Hence, providing supplemental Co, Cu, Mn, and Zn to pregnant cows increased BCS gain during the last trimester of gestation, independent if supplemental source was organic or inorganic

Table 2. Performance of beef cows receiving diets containing or not (CON) inorganic (INR) or organic (ORG) sources of Cu, Co, Mn, and Zn during the last trimester of gestation.^{1,2}

Item	CON	INR	ORG	SEM	P =
Days receiving diets, d	99	94	93	3	0.36
BW, kg					
Initial (d -10)	520	511	505	11	0.60
Pre-calving (d 75)	643	645	634	14	0.85
BW change	127	134	134	6	0.61
BCS					
Initial (d -10)	5.19	5.10	5.04	0.08	0.41
Pre-calving (d 75)	5.75	5.93	5.94	0.14	0.61
BCS change	0.55 ^a	0.83 ^b	0.82 ^b	0.09	0.10

¹ INR and ORG received the same amount of Cu, Co, Mn, and Zn from sulfate sources or Availa[®]4 (Zinpro Corporation, Eden Prairie, MN).

² Means with different superscripts differ ($P \leq 0.05$).

No differences were detected ($P \geq 0.38$) among CON, INR, and ORG cows for initial liver concentrations of Co, Cu, Mn, and Zn (Table 3), indicating that cows from all treatment had similar Co, Cu, Mn, and Zn status prior to the experiment. In the pre-calving liver samples, liver concentrations of Co, Cu, and Zn were greater ($P \leq 0.05$) for INR and ORG compared with CON, whereas INR cows had reduced ($P = 0.04$) liver Co but greater ($P = 0.03$) liver Cu compared with ORG cows (Table 3). No treatment differences were detected ($P = 0.67$) on pre-calving liver Mn concentration (Table 3). These results indicate that the INR and ORG diets successfully increased liver content of Co, Cu, Zn, but not Mn.

Table 3. Liver concentrations of Co, Cu, Mn, and Zn of pregnant beef cows receiving diets containing or not (CON) inorganic (INR) or organic (ORG) sources of these trace minerals during the last trimester of gestation.^{1,2,3}

Item	CON	INR	ORG	SEM	P =
Co, ppm					
Initial	0.29	0.28	0.27	0.01	0.38
Pre-calving	0.21 ^a	0.40 ^b	0.44 ^c	0.01	< 0.01
Cu, ppm					
Initial	93	106	95	10	0.68
Pre-calving	69 ^a	155 ^b	129 ^c	9	< 0.01
Mn, ppm					
Initial	12.8	12.8	12.2	0.5	0.58
Pre-calving	8.7	9.0	8.7	0.3	0.67
Zn, ppm					
Initial	171	176	171	5	0.70
Pre-calving	211 ^a	230 ^b	235 ^b	7	0.05

¹ INR and ORG received the same amount of Cu, Co, Mn, and Zn from sulfate sources or Availa[®]4 (Zinpro Corporation, Eden Prairie, MN).

² Samples collected prior to the beginning of the experiment (d -10; initial samples), or 2 wks prior to calving (d 75; pre-calving samples).

³ Means with different superscripts differ ($P \leq 0.05$).

In the placental cotyledons (Table 4), Co concentrations were greater ($P \leq 0.05$) in ORG and INR

compared with CON cows, whereas Cu concentrations were only increased ($P = 0.05$) in ORG compared with CON cows. Upon calving, calves from INR and ORG cows had greater ($P < 0.01$) liver Co concentrations compared with calves from CON cows. Liver Cu and Zn concentrations were greater ($P \leq 0.05$) for calves from ORG cows compared with cohorts from CON cows, but similar ($P \geq 0.17$) between calves from INR and CON cows. Hence, supplementing inorganic and organic Co, Cu, Mn, and Zn sources to beef cows during late gestation increased the concentration of Co in the cotyledon and newborn calf liver, indicating increased passage of this trace mineral through the placenta to the fetus (Pepper, 2011). Similar outcomes were detected for calf liver Cu and Zn only when comparing ORG and CON cows, suggesting enhanced transfer of these elements from maternal to fetal tissues when the organic source was offered.

Cows assigned to CON cows gave birth to and weaned a reduced ($P \leq 0.05$) proportion of male calves compared with INR and ORG cows (Table 5). Calf sex was not controlled in the present design because cows were assigned to treatments without knowledge of their fetal gender. For this reason, calf performance variables were analyzed using calf sex as independent covariate, whereas the treatment \times sex interaction was not tested because calf sex was not blocked on the experimental unit level. Nevertheless, steers and heifers had similar ($P \geq 0.26$) weaning age (182 vs. 183 d; SEM = 3), weaning BW (223 vs. 224 kg; SEM = 5), 205-d adjusted weaning BW (254 vs. 252 kg; SEM = 5), and BW at the end of preconditioning (259 vs. 251 kg; SEM = 5).

No treatment differences were detected ($P = 0.27$) for calving rate (cows that calved a live calf; Table 5). Calf birth BW was similar ($P \geq 0.44$) among treatments (Table 4), suggesting that INR and ORG did not impact fetal growth despite differences detected for calf liver and cotyledon trace mineral concentrations. At weaning, no treatment differences were detected ($P \geq 0.17$) for weaning rate (cows that weaned a live calf; Table 5) and age. However, weaning BW and 205-d adjusted weaning BW (BIF, 2010) were greater ($P \leq 0.04$) for calves from ORG cows compared with calves from CON cows, and similar between calves from INR cows compared with the other treatments ($P \geq 0.18$; Table 5). Hence, supplementing pregnant beef cows during late gestation with Co, Cu, Zn, and Mn increased weaning BW by nearly 20 kg when ORG was fed. To our knowledge, these results are novel and evidence the advantages of providing supplemental Co, Cu, Zn, and Mn, particularly an organic source, to late-gestating beef cows in order to optimize offspring performance. Therefore, further research is warranted to understand the physiological mechanism underlying these outcomes.

A treatment \times day interaction was detected ($P < 0.01$) for plasma cortisol concentrations, which were greater for calves from ORG and INR cows compared with CON cohorts 3 d after weaning (Figure 1). In addition, mean plasma cortisol concentrations upon weaning were greater ($P \leq 0.02$) in calves from ORG and INR cows compared with calves from CON (23.7, 23.3, and 20.8 ng/mL, respectively; SEM = 0.8). Accordingly, Long et al. (2010) reported that maternal nutrition during gestation influences

adrenal steroidogenesis and health-related stress reactions of the offspring. No treatment effects were detected for plasma haptoglobin concentrations (Figure 1), which sharply increased for all treatment upon weaning (day effect; $P < 0.01$). Collectively, these results suggest that Co, Cu, Zn, and Mn supplementation to late-gestating cows impacts the steroidogenesis required to cope with the weaning stress in the offspring, without impacting the resultant haptoglobin response (Carroll et al., 2007)

During the 45-d preconditioning, no treatment effects were detected ($P \geq 0.42$) for incidence of calves that required treatment for BRD, mortality rates, and ADG (Table 5), suggesting that treatment differences detected for plasma cortisol (Figure 1) did not influence preconditioning performance and health parameters. At the end of preconditioning, BW was still greater ($P = 0.03$) for calves from ORG cows compared with calves from CON cows, and similar between calves from INR cows compared with the other treatments ($P \geq 0.25$). These outcomes further corroborate that supplementing an organic source of Co, Cu, Zn, and Mn to late-gestating beef cows enhance offspring performance. Nevertheless, kg of preconditioning calf produced/cow assigned to the experiment were similar ($P = 0.35$) among treatments, which can be attributed, at least in part, to the unexpected numerical increase ($P = 0.27$) in overall calf loss of INR cows (Table 5).

Based on the 2013-2014 U.S. average for weaned cattle prices (US\$ 3.1/kg of BW across genders, USDA-Agricultural Marketing Service, 2015), differences in actual weaning BW would result in \$32 value increase per calf born from INR cows, or \$70 value increase per calf born from ORG cows when compared to calves from CON cows, although only the ORG vs. CON comparison was statistically significant ($P = 0.01$; Table 5). A similar outcome was detected for calf value at the end of preconditioning ($P = 0.01$; Table 5). Based on actual feed prices, the INR source cost was \$12.81/kg and the ORG source was \$4.73/kg. According to cow DMI (Table 1) and feeding days (Table 2), the INR treatment increased total feeding cost by \$4.81/cow, and the ORG treatment by \$3.08/cow. These feeding costs, associated with the increase in calf BW and value upon weaning and preconditioning (Table 5) suggests an economical advantage of providing supplemental Co, Cu, Mn, and Zn, particularly an organic source, to late-gestating beef cows. Yet, economical return based on preconditioned calf value/cow assigned to the experiment were similar between treatments, which can also be associated with the numerical increase ($P = 0.27$) in calf loss of INR cows (Table 5).

IMPLICATIONS

Supplementing beef cows during late gestation with Co, Cu, Zn, and Mn increased concentrations of these trace minerals in the placental cotyledon as well as maternal and newborn calf liver, particularly when an organic source of Co, Cu, Zn, and Mn was offered. However, calf BW and calf value upon weaning and following a 45-d preconditioning were only increased when late-gestating cows were supplemented with an organic source of Co, Cu, Zn, and Mn. Hence, supplementing late-gestating beef cows

with an organic Co, Cu, Zn, and Mn is an alternative to optimize offspring productivity and economic returns in cow-calf systems.

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Table 4. Cotyledon and calf liver concentrations of Co, Cu, Mn, and Zn from beef cows receiving diets containing or not (CON) inorganic (INR) or organic (ORG) sources of these trace minerals during the last trimester of gestation.^{1,2,3}

Item	CON	INR	ORG	SEM	P =
Co, ppm					
Cotyledon	0.13 ^a	0.20 ^b	0.24 ^b	0.03	0.02
Calf	0.09 ^a	0.12 ^b	0.13 ^b	0.01	< 0.01
Cu, ppm					
Cotyledon	3.88 ^a	4.75 ^{ab}	5.11 ^b	0.39	0.10
Calf	362 ^a	428 ^{ab}	450 ^b	33	0.18
Mn, ppm					
Cotyledon	22.0	18.2	22.9	4.5	0.73
Calf	5.82	5.22	5.83	0.36	0.43
Zn, ppm					
Cotyledon	65	66	68	4	0.87
Calf	456 ^a	562 ^{ab}	660 ^b	57	0.01

¹ INR and ORG received the same amount of Cu, Co, Mn, and Zn from sulfate sources or Availa[®]4 (Zinpro Corporation, Eden Prairie, MN).

² Cotyledon and calf liver samples were collected within 3 h after calving.

³ Means with different superscripts differ ($P \leq 0.05$).

Figure 1. Concentration (ng/mL) of plasma cortisol (1A) and haptoglobin (1B) in calves weaned (d 283 of the experiment) from beef cows receiving diets containing or not (CON) inorganic (INR) or organic (ORG) sources of Cu, Co, Mn, and Zn during the last trimester of gestation. A treatment × day interaction was detected ($P < 0.01$) for cortisol, whereas a day effect was detected for haptoglobin ($P < 0.01$). Within days, letters indicate treatment differences ($P < 0.01$); a = INR vs. CON, b = ORG vs. CON.

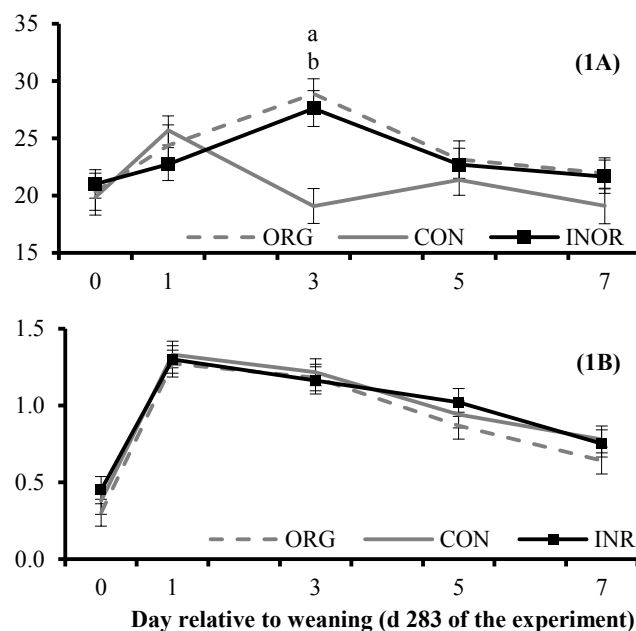


Table 5. Calving, weaning, and preconditioning outcomes from beef cows receiving diets containing or not (CON) inorganic (INR) or organic (ORG) sources of Cu, Co, Mn, and Zn during the last trimester of gestation.^{1,2}

Item	CON	INR	ORG	SEM	P =
Calving results					
Calving rate, %	95.5	84.6	95.5	5.4	0.27
Calf birth BW, kg	42.1	43.0	41.7	1.0	0.68
% of male calves born	25.9 ^a	61.6 ^b	48.2 ^b	9.5	0.05
Weaning results					
Weaning rate, %	92.9	82.1	89.3	6.2	0.46
% of male calves weaned	23.1 ^a	58.3 ^b	52.0 ^b	9.4	0.02
Calf weaning age, d	178	183	186	3	0.17
Calf weaning BW, kg	212 ^a	223 ^{ab}	236 ^c	6	0.04
Calf value, ³ US\$	660 ^a	692 ^{ab}	730 ^b	20	0.04
Calf 205-d adjusted weaning BW, ⁴ kg	244 ^a	252 ^{ab}	263 ^b	5	0.05
Calf value, ³ US\$	757 ^a	781 ^{ab}	815 ^b	18	0.09
Preconditioning results					
Preconditioning ADG, kg/d	0.59	0.51	0.55	0.05	0.49
Treated for bovine respiratory disease symptoms, %	34.9	36.4	31.5	11.7	0.95
Mortality rate, %	0.0	7.5	0.0	4.5	0.42
End of preconditioning BW, kg	245 ^a	255 ^b	265 ^c	6	0.05
Calf value, ³ US\$	756 ^a	793 ^{ab}	822 ^b	19	0.05
Overall calf loss, %	7.1	21.4	10.7	6.4	0.27
Kg of preconditioned calf produced /cow assigned to experiment ⁵	226	202	236	18	0.35
Value of preconditioned calf/cow assigned to experiment, ³ US\$	702	582	734	55	0.15

¹ INR and ORG received the same amount of Cu, Co, Mn, and Zn from sulfate sources or Availa[®]4 (Zinpro Corporation, Eden Prairie, MN).

² Means with different superscripts differ ($P \leq 0.05$).

³ Calculated based on the latest U.S. average for weaned cattle prices (US\$ 3.1/kg of BW, 2103 and 2014; USDA-Agricultural Marketing Service, 2015).

⁴ Calculated according to BIF (2010).

⁵ Calculated based on shipping rate (% of live calves that were transferred to feedlot) and calf BW at the end of preconditioning