Effect of supplemental trace minerals on novel measures of bull fertility[1]

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INTRODUCTION

Trace mineral requirements are specified for growing and finishing cattle, gestating cows, and cows in early lactation, but there are no specific requirements listed for bulls (NRC, 2000). Effects of mineral supplementation on traditional measures of bull fertility have been reported previously (Hidiroglou, 1979; Arthington et al., 2002; Geary et al., 2016). Many of the factors that influence age of puberty in bulls are under direct control of management. Both Zn and Cu are involved in sexual maturity and reproductive development and maintenance of male ruminants (Hidiroglou, 1979). Yearling bulls fed higher concentrations or organic forms of Zn, Cu, and Mn, compared to inorganic forms, tended to reach puberty earlier (Arthington et al., 2002; Geary et al., 2016). Likewise, basic Cu chloride and Zn hydroxychloride, components of IntelliBond minerals, were more bioavailable to bulls than sulfate forms of each mineral (Zezeski et al., 2016).

The breeding soundness evaluation (BSE) is a relatively standard measure of fertility developed by the Society for Theriogenology and is used by veterinarians to assess fertility in beef bulls. The BSE includes a physical examination of body condition score, overall size, feet/leg structure, vision, and palpation of accessory sex glands as well as scrotal shape and circumference and semen evaluation of sperm motility and morphology. Bulls essentially either pass (satisfactory potential breeder) or fail (unsatisfactory potential breeder or classification deferred) this evaluation. However, within each classification, insufficient information is gained to be able to provide a ranking of fertility among a group of bulls. Flow cytometry using fluorescent biomarkers provided a much greater ability to objectively evaluate far more parameters of sperm fertility. Flow cytometry-based semen analysis has multiplied the throughput, speed, sensitivity, accuracy, and informative value of semen analysis by introducing probes for mitochondrial membrane potential, DNA integrity, acrosome integrity, ATP production, ability to withstand oxidative stress, and improved assessment of viability (Evenson and Melamed, 1983; Garner et al., 1994; Mahfouz et al., 2009; Sutovsky and Lovercamp, 2010). Addition of some of these new measures of sperm fertility to a BSE may improve the ability to more accurately predict a bull's fertility. The objective of these studies was to evaluate effects of mineral supplementation on bull fertility using traditional measures of a BSE along with new flow cytometric measures of fertility. Our hypothesis was that bulls fed basic Cu chloride and/or Zn hydroxychloride minerals would have improved trace mineral status and fertility compared with bulls fed sulfate mineral or no mineral supplementation. We report results of a 2-yr study on effect of IntelliBond or no mineral supplementation in peripubertal bulls on liver mineral concentrations and measures of bull fertility.

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MATERIALS AND METHODS

This study was conducted at the USDA-ARS Livestock and Range Research Laboratory, Fort Keogh, Miles City, MT. All procedures were approved by the Fort Keogh IACUC (IACUC No. 121415-1). In year 1, 48 Angus bull calves weaned at approximately 189 ± 2.5 (mean ± SEM) d of age were maintained on a growing diet in Calan gate-equipped pens (n = four bulls per pen; American Calan, Northwood, NH) with four bulls per without mineral supplementation until 264 d of age. At this time, bulls were stratified by body weight (283 ± 4.3 kg) and randomly assigned to one of four trace mineral supplements (one bull per treatment per pen) provided daily to a corn silage/alfalfa hay/corn/wheat growing diet (Table 1): 1) zinc with no copper (ZN; 299 mg/d; n = 12), 2) copper with no zinc (CU; 131 mg/d; n = 12), 3) copper and zinc (ZNCU; n = 12), and 4) no copper or zinc (CON; n = 12; Table 2). In year 2, 80 Angus–Hereford bull calves weaned at 188 ± 1.8 (mean ± SEM) d of age were maintained on a growing diet without mineral supplementation until 307 d of age. At this time, bulls were blocked by sire and stratified by body weight (360 ± 2.5 kg; mean ± SEM) to 1 of 20 pens equipped with Calan gate individual feed bunks. Bulls were randomly assigned within pen to one of same four trace mineral treatments as year 1: 1) ZN (n = 20), 2) CU (n = 20), 3) ZNCU (n = 20), and 4) CON (n = 20). Trace mineral supplements were supplied as basic copper chloride and zinc hydroxychloride (Micronutrients USA LLC, Indianapolis, IN) formulated so that the total mixed ration would meet or exceed NRC requirements for copper and zinc with the assumption that only 50% of the mineral in the basal diet was bioavailable. Mineral supplements were pelletized in a 20% CP, 0.95-cm diameter pellet (with all other nutrients and trace minerals being constant across all treatments) and fed at a rate of 0.91 kg/hd/d prior to being fed the total mixed ration to ensure that all bull calves consume their respective treatments each day (Table 2).

Supplements were fed for 84 d and liver biopsies were collected on d 0 and 85 using the Tru-Cut needle biopsy technique described by Corah and Arthington (1994), and weight, scrotal circumference, semen, and blood collected every 28 d. Liver biopsies (d 0 and 85) were analyzed for mineral status at the Animal Health Diagnostic Laboratory (Michigan State University, East Lansing, MI) using coupled plasma atomic emission spectroscopy techniques (Braselton, 1997). Scrotal circumference was measured at the widest circumference with a manual metal tape (Hammerstedt, 1996). Semen samples were collected by electroejaculation, and two ejaculates were collected from each bull on d 0, 28, 56, and 84, with a 3-min period of rest between ejaculates. All ejaculates were evaluated for sperm concentration using an Accuread spectrophotometer (IMV Technologies, Maple Grove, MN). Ejaculate volume was recorded and 10 µl of raw semen was evaluated microscopically at 100× magnification for gross swirl. Progressive motility and strength of motility (rate of movement across field of view) of diluted semen (1:5 vol:vol, Sperm-TL; Caisson Labs, Smithfield, UT; pH 7.4) were recorded at 400× magnification on a warmed microscope slide. A sample of raw semen was mixed with an eosin/nigrosin morphology stain (Lane Manufacturing Inc., Denver, CO) for morphology and viability evaluation (Lunstra and Echternkamp, 1982). Spermatozoa morphology was evaluated by counting 100 random cells

Table 1. Dietary ingredients and nutrient composition of peripubertal bull diet

Ingredient	Basal diet, % DM
Corn silage	65.0
Alfalfa hay	10.0
Wheat grain	10.0
Corn grain	10.0
Supplement	5.0
Nutrient composition	
DM, %	91.8
CP, % DM	13.7
TDN, % DM	67.6
Ca, ppm	0.8
K, ppm	0.3
Zn, ppm	32.1
Cu, ppm	8.6
Mn, ppm	51.7
Fe, ppm	604.0

Table 2. Trace mineral supplement nutrient composition of peripubertal bull diet

	Supplement[1]			
	CON	ZN	CU	ZNCU
DM, %	88.8	88.8	88.8	88.8
	DM basis			
CP, %	21.3	21.3	21.3	21.3
TDN, %	74.1	74.0	74.1	74.0
	ppm			
Mn, ppm	159	159	159	159
Zn, ppm	50	388	50	389
Cu, ppm	12	12	160	160

[1]Supplements: CON: supplement without Cu and Zn, ZN: supplement with Zn hydroxychloride but no Cu, CU: supplement with basic Cu chloride but no Zn, and ZNCU: supplement with Zn hydroxychloride and basic Cu chloride.

at 1,000× magnification and classifying them as normal or having head or tail abnormalities as described by Barth and Oko (1989). Tail abnormalities and classifications included proximal and distal cytoplasmic droplets, coiled and bent tails, plus any miscellaneous abnormalities. Head abnormalities included abnormal acrosomes and all types of abnormally shaped heads. Puberty was defined as the first collection date at which an ejaculate contained a minimum of 50 × 10^6 total spermatozoa with at least 10% progressive motility (Lunstra et al., 1978). Additional measures evaluated by flow cytometry included energy potential, acrosome integrity, viability, antioxidant capacity, and DNA integrity. Acrosome integrity, sperm viability, mitochondrial membrane potential, and reactive oxygen species were evaluated via flow cytometry using the kits (EASYKIT 2, 3. and 5) and procedures from the manufacturer (IMV Technologies). Semen collections and subjective measures were conducted by the same experienced individual to eliminate variation.

Statistical Analysis

Linear data were analyzed using the MIXED procedure in SAS (SAS v.9.4, SAS Institute) with d 0 measures as covariates. Class variables included bull, pen, year, collection day, treatment, and day × treatment. Preplanned contrasts included comparisons of CON vs. mineral, ZN vs. ANCU, and CU vs. ZNCU. Chi-square analyses of categorical data were analyzed using SAS PROC FREQ (SAS v.9.4, SAS Institute). Pearson correlation coefficients were analyzed using a multivariate analysis.

RESULTS AND DISCUSSION

Bulls in year 1 were not liver Cu deficient (Puls, 1995; Kincaid, 2000) on d 0 and liver Cu concentrations decreased ($P < 0.001$) from d 0 to 84 for bulls in all treatments (Figure 1). On d 84, CU bulls had greater ($P < 0.05$) liver Cu concentrations than CON, ZN, or ZNCU bulls, so it appears that Zn supplementation reduced Cu storage as has been reported previously (Miller et al., 1989; Goff, 2018). In year 2, all bulls were liver Cu deficient on d 0. Bulls receiving CON and ZN supplements were still liver Cu deficient on d 84 whereas CU and ZNCU bulls had greater ($P < 0.05$) liver Cu concentrations than CON and ZN bulls and had adequate liver Cu concentrations (100 to 125 ppm; Puls, 1995; Kincaid, 2000). None of the bulls in year 1 or 2 were liver Zn deficient (<40 ppm; Puls, 1995; Kincaid, 2000) on d 0 or 84, but year 1 bulls had lower d 84 liver Zn concentrations than d 0 and year 2 bulls had greater liver Zn concentrations than d 0 (Figure 2). Liver Zn concentration may not be the best measure of Zn availability to an animal. Very little information is available in the literature regarding the role of trace minerals on reproductive health or on puberty establishment in bulls. Normal liver Zn concentrations (DM) ranged from 83 to 300 ppm (mean, 111 ppm) for aged cows (Mertz, 1986). Trace mineral supplements were removed from bulls in the present study for 75 d (year 1) or 119 d before treatment, yet liver Zn concentrations were all above the adequate liver Zn concentrations (100 ppm) as proposed by Mertz (1986). Liver Cu concentrations were only deficient in bulls from year 2 when bulls were maintained on a mineral deficient diet for 119 d. Previous efforts at making yearling bulls Zn deficient by removing trace minerals from the diet for 81 d were also unsuccessful (Geary et al., 2016). Because CON and ZN bulls remained Cu deficient, but no effect on fertility measures were realized, the role of Cu in bull fertility may be minimal. It is theorized that the body has very strong homeostatic mechanisms

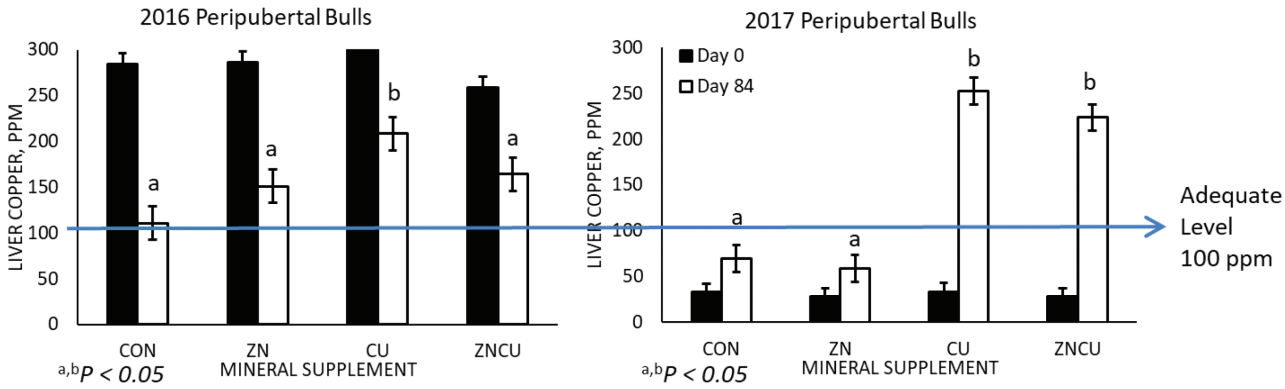

Figure 1. Mean (± SEM) liver copper concentration in peripubertal bulls on d 0 and 84 of supplementation with no mineral (CON), zinc hydroxychloride (ZN), basic copper chloride (CU), or ZNCU mineral treatment ($^{a,b}P = 0.05$). Each bar in 2016 represents 12 bulls per treatment and each bar in 2017 represents 19 bulls per treatment.

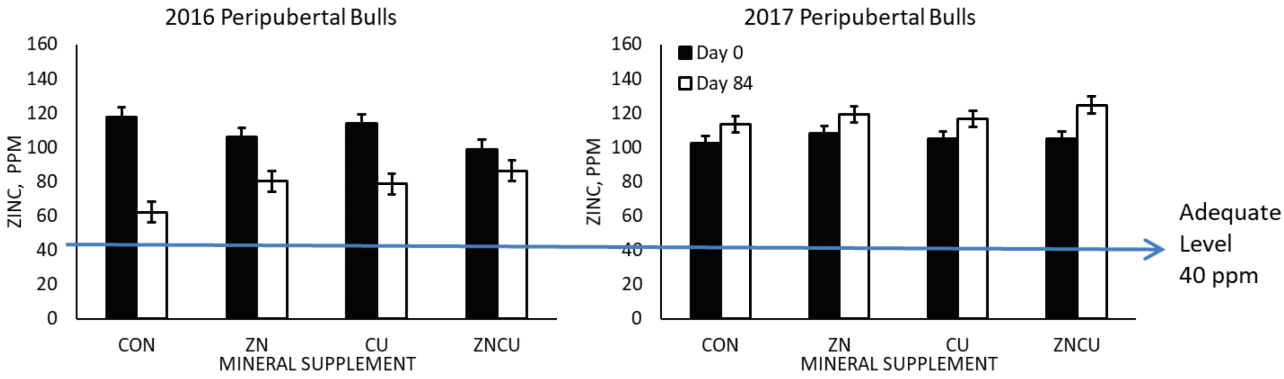

Figure 2. Mean (± SEM) liver zinc concentration in peripubertal bulls on d 0 and 84 of supplementation with no mineral (CON), zinc hydroxychloride (ZN), basic copper chloride (CU), or ZNCU mineral treatment. Each bar in 2016 represents 12 bulls per treatment and each bar in 2017 represents 19 bulls per treatment.

involved in Cu and Zn absorption and metabolism (Kendall et al., 2000). Thus, when rations are deficient in these minerals, the body may become more efficient in maintaining and recycling them within the body.

Across both years, bulls receiving ZNCU had greater ($P = 0.05$) average daily gain (1.69 vs. 1.60 kg) than CU bulls. Mineral supplementation had no effect ($P > 0.10$) on age of puberty. All fertility measures improved from d 0 to 84 in bulls and were not affected ($P > 0.10$) by mineral supplementation. We reported previously (Geary et al., 2016) that organic or sulfate mineral supplementation did not improve routine fertility measures in peripubertal bulls but expected to observe improvements in some of the novel sperm measures with flow cytometry. Age of puberty was not affected ($P > 0.10$) by supplementation and most bulls in year 2 were pubertal by d 0 of the study. There were some minor treatment × day interactions including a trend ($P = 0.06$) on percent normal sperm in the ejaculate. On d 28, ZN supplemented bulls had a greater ($P = 0.02$) percentage of normal sperm in the ejaculate than CON bulls and after 56 d of supplementation, both ZN and CU bulls had a greater ($P \leq 0.03$) percentage of normal sperm in the ejaculate than CON bulls. Before a bull can pass a traditional BSE, he must produce an ejaculate with at least 70% normal sperm in the ejaculate. At d 56, both ZN and CU bulls tended ($P \leq 0.10$) to have a greater percentage of normal sperm in the ejaculate than ZNCU bulls. However, after 84 d of supplementation, there were no differences between treatments. After 28 d of mineral supplementation, ZNCU bulls had greater ($P < 0.02$) live sperm (54 vs. 43%) and sperm with intact acrosome (68 vs. 56%) than CU bulls and tended to have greater ($P = 0.08$) live sperm than ZN bulls (46%). Mineral supplemented bulls had greater percent live, reactive oxygen resistant sperm (39%) than CON bulls (31%). After 56 d of mineral supplementation, sperm from ZNCU bulls had greater ($P = 0.04$) mitochondrial energy potential than CU bulls. None of the fertility measures were different between mineral supplementation treatments by 84 d of supplementation. In summary, the mineral homeostasis mechanisms for peripubertal bulls appear to be extremely efficient. Bulls that were deficient in liver Cu did not have reduced fertility and supplementation of growing diets with Cu and/or Zn did not reveal major improvements in any laboratory or chute-side measures of bull fertility. Bulls with extreme mineral deficiencies or diet antagonists may respond to supplementation differently.

IMPLICATIONS

Use of yearling bulls that are approximately 14 to 16 mo of age for breeding has become commonplace by beef producers. Thus, there is increased pressure on yearling bulls to reach puberty and pass a BSE by 1 yr of age. Ensuring that the bulls receive adequate nutrition to grow and produce adequate spermatozoa for optimal fertility is vitally important to herd fertility. Trace mineral supplementation is believed to be critical for optimal fertility and both copper and zinc have been shown to be important to fertility in other species. However, there are not very good guidelines for the recommended level of these minerals to ensure fertility. In this study, we attempted to make bull calves deficient in copper and zinc by eliminating trace mineral supplementation from their diets after weaning. Providing copper and/or zinc supplementation for 84 d to peripubertal bulls did not affect fertility. Thus, further work to understand the need for these minerals for optimal fertility may be warranted.

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