Impact of trace mineral source on beef replacement heifer growth, reproductive development, and biomarkers of maternal recognition of pregnancy and embryo survival

George A. Perry*2,3, Stephanie D. Perkins*, Emmalee J. Northrop*, Jerica J.J. Rich*, Kaithlin M. Epperson*, Taylor N. Andrews*, Adalaide C. Kline*, Lacey K. Quail*, Julie A. Walker*, Cody L. Wright*, and Jason R. Russell†

*Department of Animal Sciences - South Dakota State University, Brookings, SD 57007, USA

†Zinpro Corporation, Eden Prairie, MN, 55344, USA

1 Financial support for this research was provided by Zinpro Corporation, Eden Prairie, MN.

2 Corresponding author: George.Perry@ag.tamu.edu

3 Current address: Texas A&M AgriLife Research and Extension Center, 1710 FM 3053 N, Overton, Tx 75684 Phone: (903) 834-6191 Fax: (903) 834-7140

© The Author(s) 2021. Published by Oxford University Press on behalf of the American Society of Animal Science. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.
ABSTRACT: Trace minerals are known to play important roles in early embryo development. The study objective was to determine effects of trace mineral source on heifer reproductive performance. Beef heifers (n = 129) were randomly assigned to one of two treatments. From weaning through breeding, all heifers were individually fed a basal diet supplemented with Co, Cu, Mn, and Zn either from organic sources (COMP; Cu, Mn, and Zn amino acid complexes and Co glucoheptonate; Availa-4, Zinpro Corporation, Eden Prairie, MN) or inorganic sources (INORG; Cu, Mn, and Zn hydroxychlorides; Intellibond C, M, and Z, Micronutrients, Indianapolis, IN) and Co as CoSO₄. Blood samples and a reproductive tract score (RTS) were collected to determine pubertal status. All animals were synchronized and artificially inseminated (AI). Pregnancy status was determined by lymphocyte gene expression, circulating concentrations of pregnancy associated glycoproteins (PAG), and by transrectal ultrasonography after AI. Embryonic loss was defined as when a previously pregnant animal was subsequently diagnosed not pregnant. Data were analyzed using the MIXED procedure in SAS. Puberty (P = 0.44), pelvic area (P = 0.74), RTS (P = 0.49), and estrus expression (P = 0.82) were not influenced by treatment. There was no effect of treatment (P = 0.37) or treatment by time (P = 0.19) on pregnancy, but there was a tendency (P = 0.13) for decreased embryonic loss among COMP heifers (27 ± 6%) compared to INORG heifers (38 ± 6%). There was a treatment by pregnancy status by time interaction (P < 0.01) on circulating PAG concentrations with PAG concentrations tending (P = 0.08) to be greater on d 25 among heifers in the COMP treatment compared to heifers in the INORG group. In summary, source of trace mineral did not affect puberty, RTS, pelvic area, or overall pregnancy success, but feeding complexed trace minerals tended to increase circulating PAG concentrations and embryo survival.

Keywords: Embryo loss, Reproductive performance, Trace mineral
INTRODUCTION

Trace minerals are necessary for protein synthesis, activation of enzymes, and immune system functions. These trace elements are present in small quantities in the body but can greatly influence reproductive efficiency. Inadequate transfer of these elements can result in fetal mineral deficiency. This can lead to impaired fetal growth and central nervous system development and can also cause skeletal and metabolic abnormalities (Widdowson, 1974). Thus, the effects of biological availability and utilization of trace minerals in livestock diets is critical in reproductive efficiency and early embryo development.

Manganese plays a key role in fetal bone formation (Gamble et al., 1971) and initiates estradiol secretion by the conceptus (Hidiroglou and Shearer, 1976). Zinc is crucial in the binding of steroid-receptor complexes to DNA utilizing zinc finger proteins (Freedman, 1992). Iodine is a necessary precursor for thyroid hormones, and the fetus requires maternal T₄ to support early brain development (Burrow, 1990). Deficiencies lead to reproductive problems such as: fetal resorption, abortion, and stillbirths (Hostetler et al., 2003).

Uterine histotroph is essential for providing nutrients and trace elements to the developing embryo within the maternal environment. More specifically, uterine histotroph is composed of enzymes, factors, cytokines, lymphokines, hormones, amino acids, proteins, lipids, and glucose (Gao et al., 2009). Messenger RNA translation is increased in the developing fetus through nutrient signaling pathways (Martin and Sutherland, 2001), which stimulates cell migration, invasion, growth and proliferation, ribosome synthesis, expression of metabolism related genes, autophagy, and cytoskeleton reorganization in the developing fetus (Liu et al., 2008). Our laboratory has reported that uterine flush fluid from pregnant uteri had decreased mineral concentrations compared to uterine flush fluid from non-pregnant uteri (unpublished data); indicating that minerals are being utilized by the embryo for growth and development.
In addition, the bioavailability of trace minerals may be critical. Peters et al. (2008) reported sows farrowed more total and live born piglets when fed organic compared to inorganic trace minerals. Additionally, a review by Hostetler et al. (2003) stated that conceptuses from sows fed proteinated trace mineral sources had increased mineral concentrations, thereby potentially impacting survival rates of developing conceptuses. Dantas and co-workers (2019) reported that when beef cows were supplemented with inorganic mineral, in vitro embryo production from the cows was decreased compared to cows supplemented with a complexed trace mineral mix. Therefore, the aim of this study was to determine the effects of trace mineral source on replacement heifer reproductive performance.

**MATERIALS AND METHODS**

All procedures were approved by the South Dakota State University Institutional Animal Care and Use Committee.

*Experimental Design*

Data were collected on 129 Angus or Angus × Simmental heifers over the course of two years (Year 1, n = 59; Year 2, n = 70). Heifers were housed at the South Dakota State University Cow-Calf Education and Research Facility for the duration of the study in pens where they were individually fed using the Insentec RIC system (Hokofarm, Marknesse, Netherlands). During each year, heifer calves were weaned and subsequently divided into two pens of 29 and 30 head per pen in Year 1 and 36 and 34 head per pen in Year 2. Heifers were evenly assigned to one of two mineral treatments while accounting for pen, breed, age, and weaning weight. All heifers were individually fed a basal diet (Table 1) supplemented with Co, Cu, Mn, and Zn either from organic sources (COMP; Cu, Mn, and Zn amino acid complexes and Co glucoheptonate; Availa-4, Zinpro Corporation, Eden Prairie, MN) or inorganic sources (INORG; Cu, Mn, and Zn hydroxychlorides; Intellibond C, M, and Z, Micronutrients, Indianapolis, IN) and Co as CoSO₄. In year 1, the initial diet formulation (10.5% CP and 0.882 Mcal/kg DM) for the treatment period resulted in greater than expected ADG. The initial diet formulation was fed from d 0 to d 92. Then on d 93, the diet was adjusted to reduce the ADG of
the heifers. The diet formulation from d 93 to d 166 contained 10.5% CP and 0.743 Mcal/kg DM. In year 2, the heifers were fed the same diet throughout the feeding period (10.5% CP and 0.743 Mcal/kg DM. Heifers were fed twice daily using a slick bunk management system with daily feed allotments adjusted to allow for ad libitum intake. Prior to the start of study diets, heifers were trained to the Insentec RIC individual feeding system over a period of 30 days.

For treatment assignment, heifers were weighed 90 d prior to the start of the experimental diets (d 0 = start of treatment diets). Heifers were then weighed every 15 to 30 days throughout the duration of the study. Heifers were measured for hip height, wither height, and assigned a body condition score (score 1 to 9 by an experienced evaluator; Richards et al., 1986) on day 0, at intermediate weigh day (approximately d 86), during the pre-breeding exam (30-d pre-breeding, approximately d 120), and at the conclusion of the breeding season.

Age at puberty was determined via circulating concentrations of progesterone (1st blood sample to have concentrations > 1 ng/mL). Heifers were synchronized using the 7-d CO-Synch + CIDR protocol and artificially inseminated (Larson et al., 2006). Conception rate and pregnancy rate was determined via blood samples and transrectal ultrasonography. Pregnancy status was determined on days 17 to 21 by increased expression of interferon stimulated genes (ISG15, MX2, and OAS1), on days 22 to 28 by detection of pregnancy-associated glycoproteins (PAG) in plasma, and on days 30 and 60 by transrectal ultrasonography. Calving rate was determined after each respective calving season. Calving date, calf sex, and calf birth weight was recorded during each respective calving season.

Feed Sampling and Analysis

Dietary ingredients were sampled weekly (250 g to 1 kg) during the morning feeding. Samples were labelled and immediately stored at -20°C. Prior to analysis, each sample was dried in a 55°C forced air oven for 24 hours, then ground through a 1-mm screen (Thomas Wiley Mill Model 4; Thomas Scientific USA). Dry matter was measured by drying at 105°C for 16 h, and OM was
determined by combustion (500°C for 16 h). Additionally, N content was analyzed by the Dumas procedure (method no. 968.06; AOAC, 2016; rapid Max N exceed; Elementar, Mt. Laurel, NJ). Neutral detergent fiber was measured as described by Van Soest et al. (1991) and included additions of α-amylase and sodium sulfite; ADF was measured nonsequential to NDF (Van Soest et al., 1991), and acid detergent insoluble ash (ADIA) was calculated by combustion (500°C for 16 h) of ADF residue. Measures of NDF and ADF were corrected for ash content which was measured by combustion (500°C for 8 h). Mineral concentrations in feeds and water were determined by inductively coupled plasma – mass spectroscopy (ICP-MS) at the Michigan State University Veterinary Diagnostic Laboratory (Wahlen et al., 2005; Lansing, MI).

**Blood and Tissue Collection**

Starting at d 90 of the feeding period, blood samples were collected by venipuncture of the jugular vein biweekly to determine age at puberty. Blood samples were collected similarly on d 7, 17, 19, 21, and 28 post-breeding to be used for gene expression analysis, and on d 22, 23, 24, 25, 26, 27, and 28 post-breeding for determination of pregnancy-associated glycoproteins (PAG) concentrations in the blood. Blood samples were centrifuged at 1,200 x g for 30 minutes at 4°C. Plasma was collected and stored at -20°C. Radioimmunoassays (RIA) were performed on plasma samples to determine circulating concentrations of progesterone (Engel et al., 2008). Liver biopsies were collected (Engle and Spears, 2000) to assess liver mineral concentration on d -28 and 28 of the feeding protocol and at the initiation of the synchronization protocol. Liver mineral concentrations were determined by ICP-MS at the Michigan State University Diagnostic Laboratory.

**RT-PCR**

For blood samples collected on d 7, 17, 19, 21, and 28 post-breeding, buffy coats were removed and mixed at a 1:1 ratio with TRI Reagent (MRC, Cincinnati, OH) for subsequent RNA extraction. Ribonucleic acid was extracted using the PROMEGA SV Total RNA Isolation kit (Promega Corporation, Madison, WI) according to manufacturer’s instructions and checked for quality using a NanoPhotometer N60 (Implen, München, Germany). Pure RNA was diluted to 70
ng/µl and stored at -80°C. Real-Time RT-PCR was performed using the BioRad 1-step SYBR green kit (BioRad Laboratories, Hercules, CA) according to manufacturer’s instructions. Samples were run with primers for ISG15, MX2, and OAS1 (Madsen et al., 2015) to determine relative abundance of interferon-stimulated genes, and GAPDH was used as an endogenous reference gene. Day 7 samples were used as a baseline within individual heifers. All samples were run in duplicate on a Stratagene MX3000P (Stratagene California, San Deigo, CA). The SYBR Green reaction was performed with reverse transcription at 42°C for 30 min and 95°C for 10 min to inactivate reverse transcription. Each transcript of interest was amplified for 40 cycles of 30 sec at 95°C for melting; 1 min at 60°C for annealing; and 1 min at 72°C for extension. Base pair size of all PCR products was confirmed through gel electrophoresis and the intra-assay CV for all primer pairs was < 20%.

**Pregnancy-associated glycoproteins**

Pregnancy-associated glycoproteins (PAGs) were assessed in duplicate using the IDEXX Alertys Ruminant Pregnancy Test (IDEXX, Westbrooke, ME) according to manufacturer’s instructions, and analyzed using a SpectraMax 190 microtiter plate reader (Molecular Devices, San Jose, CA) at 450 nm and at 650 nm.

**Statistical Analysis**

Data were analyzed by ANOVA as a completely randomized design using the MIXED procedure in SAS 9.2 (SAS Institute, Inc, Cary, NC). Dietary treatment and year (replicate) were included as fixed effects while heifer was considered a random effect. Day was used as a repeated effect for body weight (BW), dry matter intake (DMI), average daily gain (ADG), and pregnancy success while the subject for the repeated statement was heifer nested in dietary treatment. Initial measurements for blood/tissue and performance measurements were used as covariates. Significance was considered as $P \leq 0.05$ while a tendency was considered $0.06 \leq P \leq 0.15$. 
RESULTS

Heifer Performance

There was no effect of treatment on body weight ($P = 0.90$), hip height ($P = 0.63$), wither height ($P = 0.40$), BCS ($P = 0.42$), or ADG ($P = 0.58$; data not shown). In addition, there was no interaction of treatment by time on body weight ($P = 0.32$), hip height ($P = 0.54$), wither height ($P = 0.88$), or BCS ($P = 0.57$; data not shown). There was a tendency; however, for an interaction of treatment by time on ADG ($P = 0.09$). With heifers in the COMP treatment having increased ($P < 0.02$) ADG from d 81 to 95 compared to heifers in the INORG treatment ($1.06 \pm 0.06$ vs $0.90 \pm 0.06$ kg/d).

Liver Mineral Concentrations

Copper concentrations tended to differ between treatments ($P = 0.15$) and there tended to be a treatment by time interaction ($P = 0.13$). There was no difference in liver Cu concentrations at the start of the study ($P = 0.58$) but by the mid-point concentrations tended ($P = 0.13$) to be greater in COMP heifers compared to INORG heifers. By the final biopsy sample (day of synchronization protocol initiation), Cu concentrations continued to increase and tended ($P = 0.09$) to be greater in heifers supplemented with the complexed mineral (COMP) compared to the inorganic mineral (INORG) supplemented heifers (Figure 1). Cobalt concentrations differed between treatments ($P < 0.01$), and there was an interaction of treatment by time ($P < 0.01$). Heifers supplemented with the COMP mineral had greater Co concentrations on d 28 and on day of synchronization initiation compared to INORG heifers (Figure 2). There tended ($P = 0.13$) to be a difference in overall Zn concentrations between treatments; with INORG heifers ($87.22 \pm 4.6$ mg/kg of dry matter) tending to have greater overall concentrations compared to COMP heifers ($82.22 \pm 4.6$ mg/kg of dry matter), but there was no treatment by time interaction ($P = 0.59$; Figure 3). Likewise, overall Mn concentrations did not differ between treatments ($P = 0.67$) and there was no interaction of treatment by time ($P = 0.99$). Overall concentrations of Cu, Co, Zn, and Mn all increased ($P < 0.01$) over time among both groups.
Reproductive performance

There was no effect of treatment \((P = 0.44)\) or treatment by time \((P = 0.22)\) on when heifers reached puberty, but there was an effect of time \((P < 0.01)\) with more heifers having reached puberty as the study progressed. By the start of the synchronization protocol, greater than 70% of heifers in both treatments had reached puberty. There was no difference between treatments for the percent of heifers that had reached puberty by the start of the breeding season \((P = 0.44)\), pelvic area \((P = 0.74)\), RTS \((P = 0.49)\), or estrus expression \((P = 0.82)\). In addition, there was no effect of treatment \((P = 0.37)\) or an interaction of treatment by time \((P = 0.19)\) on pregnancy success (Figure 4). There was, however, a tendency \((P = 0.13)\) for decreased embryonic loss among COMP heifers (27 ± 6%) compared to those in the INORG treatment group (38 ± 6%). There was no effect of treatment \((P = 0.48)\) or treatment by time interaction \((P = 0.72)\) on circulating PAG concentrations. There was an effect of time \((P < 0.01)\) and a treatment by pregnancy status by time interaction effect \((P < 0.01)\) where PAG concentrations increased over time among heifers that were pregnant compared to heifers that were not pregnant and PAG concentrations tended \((P = 0.08)\) to be greater on d 25 among heifers in the COMP treatment compared to heifers in the INORG group (Figure 5).

DISCUSSION

The histotroph secreted from the uterus plays a vital role in early embryonic development and survival, and thus the uterine environment and conceptus need to be in synchrony in order for successful pregnancy to occur. The secretions that make up the histotroph include enzymes, growth factors, cytokines, lymphokines, hormones, amino acids, proteins, lipids, and glucose (Gao et al., 2009a). These molecules are used by the conceptus for nutrition, homeostasis, and cell signaling. Recently, our laboratory found that in the presence of an embryo, uterine mineral concentrations are reduced, potentially indicating that the embryo may be using minerals for growth and development prior to uterine attachment (unpublished data).
In the present study, trace mineral liver concentrations increased from d -28 through the start of the breeding season, and Cu and Co tended to be or were greater in the COMP heifers compared to the INORG, but Zn and Mn did not differ between treatments. This suggests a potential difference in bioavailability between Cu and Co sources used in this study. Interestingly, relative to an inorganic mineral supplement, Dantas et al. (2019) also reported increased liver Co concentration in beef cows fed a supplement containing Co glucoheptonate as part of an organic complexed mineral supplement. Characterization of trace mineral status was done by liver biopsy, but previous reports indicate that Zn and Mn may be stored in other body tissues such as kidneys and bones (Underwood, 1977; Rojas et al., 1995). Further research is necessary to more accurately determine the relative bioavailability of these trace mineral sources.

Trace minerals are crucial for protein synthesis, activation of enzymes, and the immune system. Fetal deficiency can occur during inadequate transfer from the mother to the uterine environment, resulting in impaired fetal growth, and/or central nervous system, skeleton, and metabolism abnormalities (Widdowson, 1974). In the present study, heifers supplemented with the complexed minerals tended to have reduced embryonic loss compared to those supplemented with inorganic minerals. Therefore, heifers supplemented with complexed trace minerals may provide a more optimal uterine environment for embryo development when compared to heifers supplemented with inorganic trace minerals.

Improvements in cumulus-oocyte complex maturation, reduced apoptosis of cumulus cells, improved in vitro embryo production, and improved embryo development through the blastocyst stage has been reported in mice, cattle, pigs, and humans (Gao et al., 2007; Anchordoquy et al., 2011; Menezo et al., 2011; Picco et al., 2012; Anchordoquy et al., 2014a; Anchordoquy et al., 2014b; Jeon et al., 2014; Geravandi and Azadbakht, 2017; Geravandi et al., 2017; Dantas et al., 2019). Supplementing Cu-free maturation, fertilization, and culture media with 0.46 or 0.68 mg/L of Cu increased the percentage of embryos reaching the 8-cell stage, as well as rate of morula and blastocyst formation and decreased apoptosis (Gao et al., 2007). Even though neither treatment group would be considered deficient in Cu, liver Cu concentrations tended to be increased in heifers supplemented
with complexed minerals, and liver concentrations of Zn tended to be greater in INORG supplemented heifers. Elevated Zn concentrations can cause a secondary copper deficiency, and maternal Zn has been reported to cause fetal Cu deficiencies (Reinstein et al., 1984). Thus, the tendency for decreased Cu and increased Zn in the INORG heifers could have resulted in negative impacts on fetal development. The impact of maternal Cu deficiencies in prenatal development and pregnancy success has been well defined (Keen et al., 1998).

Cobalt was significantly greater in liver biopsies from the complexed mineral group. Cobalt supplementation to deficient ewes resulted in more lambs born and fewer neonatal abnormalities (Fisher, 1991), and Co has been reported to cross the placenta and directly influence cobalt concentrations in the fetus (Szakmary et al., 2001). The impact of Co on early fetal development is likely through the influence of Co on vitamin B$_{12}$ synthesis; previous reports have indicated as Co increased concentrations of vitamin B$_{12}$ also increased (Fisher, 1991; Judson et al., 1997; Stangl et al., 2000). Vitamin B$_{12}$ has been reported to improve the outcome of assisted reproductive technologies in humans (Gaskins et al., 2015) and to have direct effects on the developing embryo/fetus (Molloy et al., 1985; Kirke et al., 1993; Molloy et al., 2002; Molloy et al., 2008).

In a recent review, it was estimated that 28.4% of pregnancy failures occurred before blastocyst formation, another 47.9% of losses occurred during the early embryo stage, and 5.8% occurred during the late embryo and early fetal stages (Reese et al., 2020). In beef cattle, losses after the early fetal stage have been reported to be very minimal (Perry et al., 2005). In the present study, there were no differences in conception rates with 85% and 87% of heifers being pregnant on d 21 after insemination based on ISG expression. The similarities in conception rates are not surprising as there were no differences in puberty status, RTS, BCS, or estrus expression. Furthermore, pregnancy losses were similar to what Reese and other reported in 2020. Pregnancy losses from d 21 to d 28 were 32% for INORG heifers but only 22% for COMP heifers, and from d 28 to d 60 pregnancy losses were 6% and 5% for heifers supplemented with inorganic and complexed minerals, respectively. Overall, heifers supplemented with the inorganic tended to have increased embryonic/fetal losses compared to heifers supplemented with complexed minerals. Thus, the
previously described impacts of trace mineral deficiencies appear to have a greater impact on early embryo survival (before d 28). This is further supported by circulating concentrations of PAGs being decreased in heifers that experienced embryonic/fetal losses. Pregnancy associated glycoproteins are only detectable in the maternal blood supply after the embryo has attached to the uterus when they are released into the maternal blood circulation. Previous studies (Semambo et al., 1992; Gatea et al., 2018) have reported that PAGs may serve as a marker for embryonic survival and may be an indication of pregnancy loss prior to ultrasound because PAGs begin to decrease in circulation the same day as embryonic death. Thus, the tendency for increased circulating concentrations of PAGs on d 25 after insemination among heifers supplemented with complexed minerals further supports the tendency for increased embryonic survival in heifers supplemented with complexed minerals.

In summary, supplementing inorganic or complexed minerals did not affect age at puberty, pelvic area, RTS, estrus expression, or conception rates. Complexed minerals did, however, increase liver cobalt concentrations and tended to increase liver copper concentrations. There was also a treatment by time by pregnancy status interaction where circulating concentrations of PAGs were elevated in the complexed mineral supplementation group on d 25 after insemination, and ultimately there was a tendency for a decrease in embryonic mortality compared to the inorganic mineral group. These results demonstrate the potential importance of trace minerals on early embryo development and survival, and that heifers supplemented with complexed trace minerals potentially provided a better uterine environment for the developing embryo.
DISCLOSURES

Jason R. Russell is employed by the funder of this project (Zinpro Corporation, Eden Prairie, MN) and contributed to the research design. However, the principal investigator (George A Perry) and all other authors of this manuscript have no additional conflicts of interest to report.

ACKNOWLEDGEMENTS

Financial support for this research was provided by Zinpro Corporation (Eden Prairie, MN).
Literature Cited:

Anchordoquy, J. M., J. P. Anchordoquy, M. A. Sirini, S. J. Picco, P. Peral-Garcia, and C. C. Furnus. 2014a. The importance of having zinc during in vitro maturation of cattle cumulus-oocyte complex: role of cumulus cells. Reprod. Domest. Anim. 49(5):865-874. doi: 10.1111/rda.12385

Anchordoquy, J. M., S. J. Picco, A. Seoane, J. P. Anchordoquy, M. V. Ponzinibbio, G. A. Mattioli, P. Peral Garcia, and C. C. Furnus. 2011. Analysis of apoptosis and DNA damage in bovine cumulus cells after exposure in vitro to different zinc concentrations. Cell Biol Int 35(6):593-597. doi: 10.1042/CBI20100507

Anchordoquy, J. P., J. M. Anchordoquy, S. J. Picco, M. A. Sirini, A. L. Errecalde, and C. C. Furnus. 2014b. Influence of manganese on apoptosis and glutathione content of cumulus cells during in vitro maturation in bovine oocytes. Cell Biol Int 38(2):246-253. doi: 10.1002/cbin.10195

Burrow, G. H. 1990. Thyroid status in normal pregnancy. J. Clin. Endocrinol. Metab. 71(2):274-275. doi: 10.1210/jcem-71-2-274

Dantas, F. G., S. T. Reese, R. V. O. Filho, R. S. Carvalho, G. A. Franco, C. R. Abbott, R. R. Payton, J. L. Edwards, J. R. Russell, J. K. Smith, and K. G. Pohler. 2019. Effect of complexed trace minerals on cumulus-oocyte complex recovery and in vitro embryo production in beef cattle1,2. J. Anim. Sci. 97(4):1478-1490. doi: 10.1093/jas/skz005

Engel, C. L., H. H. Patterson, and G. A. Perry. 2008. Effect of dried corn distillers grains plus solubles compared with soybean hulls, in late gestation heifer diets, on animal and reproductive performance. J. Anim. Sci. 86(7):1697-1708.

Engle, T. E., and J. W. Spears. 2000. Effects of dietary copper concentration and source on performance and copper status of growing and finishing steers. J. Anim. Sci. 78(9):2446-2451. doi: 10.2527/2000.7892446x

Fisher, G. E. 1991. Effect of cobalt deficiency in the pregnant ewe on reproductive performance and lamb viability. Res Vet Sci 50(3):319-327. doi: 10.1016/0034-5288(91)90132-8

Freedman, L. P. 1992. Anatomy of the steroid receptor zinc finger region. Endocr. Rev. 13(2):129-145. doi: 10.1210/edrv-13-2-129

Gamble, C. T., S. L. Hansard, B. R. Moss, D. J. Davis, and E. R. Lidvall. 1971. Manganese utilization and placental transfer in the gravid gilt. J. Anim. Sci. 32(1):84-87. doi: 10.2527/jas1971.32184x

Gao, G., J. Yi, M. Zhang, J. Xiong, L. Geng, C. Mu, and L. Yang. 2007. Effects of iron and copper in culture medium on bovine oocyte maturation, preimplantation embryo development, and apoptosis of blastocysts in vitro. The Journal of reproduction and development 53(4):777-784. doi: 10.1262/jrd.18109

Gao, H., G. Wu, T. E. Spencer, G. A. Johnson, X. Li, and F. W. Bazer. 2009. Select nutrients in the ovine uterine lumen. I. Amino acids, glucose, and ions in uterine lumenal flushings of cyclic and pregnant ewes. Biol. Reprod. 80(1):86-93. doi: 10.1095/biolreprod.108.071597

Gaskins, A. J., Y. H. Chiu, P. L. Williams, J. B. Ford, T. L. Toth, R. Hauser, J. E. Chavarro, and E. S. Team. 2015. Association between serum folate and vitamin B-12 and outcomes of assisted reproductive technologies. Am J Clin Nutr 102(4):943-950. doi: 10.3945/ajcn.115.112185

Gatea, A. O., M. F. Smith, K. G. Pohler, T. Egen, M. H. C. Pereira, J. L. M. Vasconcelos, J. C. Lawrence, and J. A. Green. 2018. The ability to predict pregnancy loss in cattle
with ELISAs that detect pregnancy associated glycoproteins is antibody dependent. Theriogenology 108:269-276. doi: 10.1016/j.theriogenology.2017.12.021

Geravandi, S., and M. Azadbakht. 2017. The presence of zinc in the mouse ovary vitrification medium: histological evaluation and follicle growth. Cryo Letters 38(2):108-118.

Geravandi, S., M. Azadbakht, M. Pourmoradi, and F. Nowrouzi. 2017. Zinc supplementation of vitrification medium improves in vitro maturation and fertilization of oocytes derived from vitrified-warmed mouse ovaries. Cryobiology 74:31-35. doi: 10.1016/j.cryobiol.2016.12.007

Hidiroglou, M., and D. A. Shearer. 1976. Concentration of manganese in the tissues of cycling and anestrous ewes. Can J Comp Med 40(3):306-309.

Hostetler, C. E., R. L. Kincaid, and M. A. Miranda. 2003. The role of essential trace elements in embryonic and fetal development in livestock. Vet J 166(2):125-139. doi: 10.1016/s1090-0233(02)00310-6

Jeon, Y., J. D. Yoon, L. Cai, S. U. Hwang, E. Kim, Z. Zheng, E. Lee, D. Y. Kim, and S. H. Hyun. 2014. Supplementation of zinc on oocyte in vitro maturation improves preimplantation embryonic development in pigs. Theriogenology 82(6):866-874. doi: 10.1016/j.theriogenology.2014.06.021

Judson, G. J., J. D. McFarlane, A. Mitsioulis, and P. Zviedrans. 1997. Vitamin B12 responses to cobalt pellets in beef cows. Aust. Vet. J. 75(9):660-662. doi: 10.1111/j.1751-0813.1997.tb15365.x

Keen, C. L., J. Y. Uriu-Hare, S. N. Hawk, M. A. Jankowski, G. P. Daston, C. L. Kwik-Uribe, and R. B. Rucker. 1998. Effect of copper deficiency on prenatal development and pregnancy outcome. Am J Clin Nutr 67(5 Suppl):1003S-1011S. doi: 10.1093/ajcn/67.5.1003S

Kirke, P. N., A. M. Molloy, L. E. Daly, H. Burke, D. G. Weir, and J. M. Scott. 1993. Maternal plasma folate and vitamin B12 are independent risk factors for neural tube defects. Q J Med 86(11):703-708.

Larson, J. E., G. C. Lamb, J. S. Stevenson, S. K. Johnson, M. L. Day, T. W. Geary, D. J. Kesler, J. M. DeJarnette, F. N. Schrick, A. DiCostanzo, and J. D. Arsenault. 2006. Synchronization of estrus in suckled beef cows for detected estrus and artificial insemination and timed artificial insemination using gonadotropin-releasing hormone, prostaglandin F2alpha, and progesterone. J. Anim. Sci. 84(2):332-342.

Liu, L., L. Chen, J. Chung, and S. Huang. 2008. Rapamycin inhibits F-actin reorganization and phosphorylation of focal adhesion proteins. Oncogene 27(37):4998-5010. doi: 10.1038/onc.2008.137

Madsen, C. A., G. A. Perry, C. L. Mogck, R. F. Daly, M. D. MacNeil, and T. W. Geary. 2015. Effects of preovulatory estradiol on embryo survival and pregnancy establishment in beef cows. Anim. Reprod. Sci. 158:96-103. doi: 10.1016/j.anireprosci.2015.05.006

Martin, P. M., and A. E. Sutherland. 2001. Exogenous amino acids regulate trophoderm differentiation in the mouse blastocyst through an mTOR-dependent pathway. Dev. Biol. 240(1):182-193. doi: 10.1006/dbio.2001.0461

Menezo, Y., L. Pluntz, J. Chouteau, T. Gurgan, A. Demirol, A. Dalleac, and M. Benkhalifa. 2011. Zinc concentrations in serum and follicular fluid during ovarian stimulation and expression of Zn2+ transporters in human oocytes and cumulus cells. Reprod Biomed Online 22(6):647-652. doi: 10.1016/j.rbmo.2011.03.015

Molloy, A. M., P. Kirke, I. Hillary, D. G. Weir, and J. M. Scott. 1985. Maternal serum folate and vitamin B12 concentrations in pregnancies associated with neural tube defects. Arch Dis Child 60(7):660-665. doi: 10.1136/adc.60.7.660
Molloy, A. M., P. N. Kirke, L. C. Brody, J. M. Scott, and J. L. Mills. 2008. Effects of folate and vitamin B12 deficiencies during pregnancy on fetal, infant, and child development. Food Nutr Bull 29(2 Suppl):S101-111; discussion S112-105. doi: 10.1177/15648265080292S114

Molloy, A. M., J. L. Mills, J. McPartlin, P. N. Kirke, J. M. Scott, and S. Daly. 2002. Maternal and fetal plasma homocysteine concentrations at birth: the influence of folate, vitamin B12, and the 5,10-methylenetetrahydrofolate reductase 677C--T variant. Am J Obstet Gynecol 186(3):499-503. doi: 10.1067/mob.2002.121105

Perry, G. A., M. F. Smith, M. C. Lucy, J. A. Green, T. E. Parks, M. D. Macneil, A. J. Roberts, and T. W. Geary. 2005. Relationship between follicle size at insemination and pregnancy success. Proc. Natl. Acad. Sci. U.S.A. 102(14):5268-5273.

Peters, J. C., and D. C. Mahan. 2008. Effects of neonatal iron status, iron injections at birth, and weaning in young pigs from sows fed either organic or inorganic trace minerals. J. Anim. Sci. 86(9):2261-2269. doi: 10.2527/jas.2007-0577

Picco, S. J., D. E. Rosa, J. P. Anchordoquy, J. M. Anchordoquy, A. Seoane, G. A. Mattioli, and C. C. Furnus. 2012. Effects of copper sulphate concentrations during in vitro maturation of bovine oocytes. Theriogenology 77(2):373-381. doi: 10.1016/j.theriogenology.2011.08.009

Reese, S. T., G. A. Franco, R. K. Poole, R. Hood, L. Fernandez Montero, R. V. Oliveira Filho, R. F. Cooke, and K. G. Pohler. 2020. Pregnancy loss in beef cattle: A meta-analysis. Anim. Reprod. Sci. 212:106251. doi: 10.1016/j.anireprosci.2019.106251

Reinstein, N. H., B. Lonnerdal, C. L. Keen, and L. S. Hurley. 1984. Zinc-copper interactions in the pregnant rat: fetal outcome and maternal and fetal zinc, copper and iron. J. Nutr. 114(7):1266-1279. doi: 10.1093/jn/114.7.1266

Richards, M. W., J. C. Spitzer, and M. B. Warner. 1986. Effect of varying levels of postpartum nutrition and body condition at calving on subsequent reproductive performance in beef cattle. J. Anim. Sci. 62:300-306.

Rojas, L. X., L. R. McDowell, R. J. Cousins, F. G. Martin, N. S. Wilkinson, A. B. Johnson, and J. B. Velasquez. 1995. Relative bioavailability of two organic and two inorganic zinc sources fed to sheep. J. Anim. Sci. 73(4):1202-1207. doi: 10.2527/1995.7341202x

Semambo, D. K., P. D. Eekersall, R. G. Sasser, and T. R. Ayliffe. 1992. Pregnancy-specific protein B and progesterone in monitoring viability of the embryo in early pregnancy in the cow after experimental infection with Actinomyces pyogenes. Theriogenology 37(3):741-748. doi: 10.1016/0093-691X(92)90153-i

Stangl, G. I., F. J. Schwarz, H. Muller, and M. Kirchgessner. 2000. Evaluation of the cobalt requirement of beef cattle based on vitamin B12, folate, homocysteine and methylmalonic acid. Br J Nutr 84(5):645-653. doi: 10.1017/s0007114500001987

Szakmary, E., G. Ungvary, A. Hudak, E. Tatrai, M. Naray, and V. Morvai. 2001. Effects of cobalt sulfate on prenatal development of mice, rats, and rabbits, and on early postnatal development of rats. J. Toxicol. Environ. Health A 62(5):367-386. doi: 10.1080/152873901300018110

Underwood, E. J. 1977. Trace elements in human and animal nutrition. 4th ed. Academic Press, New York.

Widdowson, E. M. 1974. Trace elements in foetal and early postnatal development. Proc Nutr Soc 33(3):275-284. doi: 10.1079/pns19740050
Figure 1. Liver concentrations of copper (LSMean ± S.E.) over time among heifers supplemented with a complexed mineral source (COMP) or with an inorganic mineral source (INORG). Overall Cu concentrations tended to differ between treatments ($P = 0.15$). There was no difference in liver Cu concentrations at the start of the study ($P = 0.58$) but by the mid-point ($P = 0.13$) and final biopsy sample Cu concentrations tended ($P = 0.09$) to be greater in COMP heifers compared to INORG heifers.

Figure 2. Liver concentrations of cobalt (LSMean ± S.E.) over time among heifers supplemented with a complexed mineral source (COMP) or with an inorganic mineral source (INORG). Cobalt concentrations differed between treatments ($P < 0.01$), and there was an interaction of treatment by time ($P < 0.01$). Concentrations did not differ on d -28, but heifers supplemented with the complexed mineral (COMP) had greater Co concentrations at the midpoint of the study and day of synchronization initiation compared to INORG heifers.

Figure 3. Liver concentrations of Zinc (LSMean ± S.E.) over time among heifers supplemented with a complexed mineral source (COMP) or with an inorganic mineral source (INORG). There tended ($P = 0.13$) to be a difference in overall Zn concentrations between treatments; INORG heifers (87.22 ± 4.6) tended to have greater overall concentrations compared to COMP heifers (82.22 ± 4.6), but there was no treatment by time interaction ($P = 0.59$).

Figure 4. Impact of supplementing heifers with either a complexed mineral source (COMP) or with an inorganic mineral source (INORG) on conception rates and embryo survival.

Figure 5. Circulating pregnancy associated glycoproteins (PAG) concentrations among pregnant and open heifers supplemented with a complexed mineral source (COMP) or with an inorganic source (INORG). There was no effect of treatment ($P = 0.48$) or treatment by time interaction ($P = 0.72$) on circulating PAG concentrations. There was an impact of time ($P < 0.01$) and a treatment by pregnancy status by time interaction ($P < 0.01$) with PAG concentrations increasing over time among heifers that were pregnant compared to heifers that were not pregnant and PAG concentrations tended ($P = 0.08$) to be greater on d 25 among heifers in the COMP treatment compared to heifers in the INORG group (Figure 5).
Table 1. Diet formulations – year 1

| Ingredients       | Adaptation period | Initial period | Slow down period |
|-------------------|-------------------|----------------|------------------|
|                   | d 1 to 14        | d 15 to 28     | INORG | COMP | INORG | COMP |
| Grass hay         | 27.3             | 27.3           | 27.3  | 27.3 | 54.3  | 54.3 |
| Corn silage       | 55.1             | 55.1           | 55.1  | 55.1 | 35.7  | 35.7 |
| Soybean hulls     | 10.84            | 10.84          | 10.81 | 10.81| 6.14  | 6.09 |
| DDGS d\(^d\) \(\) | 5.87             | 5.87           | 5.89  | 5.92 | 3.24  | 3.24 |
| CaCO\(_3\)        | 0.70             | 0.70           | 0.70  | 0.70 | 0.40  | 0.40 |
| NaCl              | 0.17             | 0.17           | 0.17  | 0.17 | 0.19  | 0.19 |
| Rumensin 90       | ---              | 0.00551        | 0.01103| 0.01103| 0.01103| 0.01103|
| Availa-4\(^e\)    | ---              | ---            | 0.06994| ---    | 0.06994| ---    |
| CoSO\(_4\)        | 0.00005          | 0.00005        | 0.00039| ---    | 0.00039| ---    |
| CuSO\(_4\)        | 0.00217          | 0.00217        | ---    | ---   | ---   | ---   |
| ZnSO\(_4\)        | 0.00324          | 0.00324        | ---    | ---   | ---   | ---   |
| Intellibond C\(^f\) | ---             | ---            | 0.00217| ---    | 0.00217| ---    |
| Intellibond M\(^f\) | ---             | ---            | 0.00454| ---    | 0.00454| ---    |
| Intellibond Z\(^f\) | ---             | ---            | 0.00655| ---    | 0.00655| ---    |
| Intellibond Z\(^f\) | ---             | ---            | 0.00493| 0.00493| 0.00493| 0.00493|
| EDM\(^g\)         | 0.00137          | 0.00137        | 0.00137| 0.00137| 0.00137| 0.00137|
| Vitamin A         | 0.00056          | 0.00056        | 0.00056| 0.00056| 0.00056| 0.00056|
| Vitamin D         | 0.00006          | 0.00006        | 0.00006| 0.00006| 0.00006| 0.00006|
| Vitamin E         | 0.00493          | 0.00493        | 0.00493| 0.00493| 0.00493| 0.00493|

\(^a\) All heifers were individually fed a basal diet supplemented with Co, Cu, Mn, and Zn either from organic sources (COMP; Cu, Mn, and Zn amino acid complexes and Co glucoheptonate; Availa-4, Zinpro Corporation, Eden Prairie, MN) or inorganic sources (INORG; Cu, Mn, and Zn hydroxychlorides; Intellibond C, M, and Z, Micronutrients, Indianapolis, IN) and Co as CoSO\(_4\).

\(^b\) d 0 to d 92

\(^c\) d 93 to 166

\(^d\) Dried distillers grains plus solubles

\(^e\) Cu, Mn, and Zn amino acid complexes and Co glucoheptonate; Availa-4, Zinpro Corporation, Eden Prairie, MN

\(^f\) Cu, Mn, and Zn hydroxychlorides; Micronutrients, Indianapolis, IN

\(^g\) Ethylenediamine dihydroiodide
Table 2. Diet formulations – year 2a

| Ingredients         | Adaptation period | Treatment period | INORG | COMP |
|---------------------|-------------------|------------------|-------|------|
| Grass hay           | 66.0              | 27.3             | 27.3  |      |
| Corn silage         | 24.0              | 55.1             | 55.1  |      |
| Soybean hulls       | 1.47              | 10.84            | 10.81 |      |
| DDGSd               | 3.24              | 5.87             | 5.89  |      |
| Soybean meal        | 4.51              |                  |       |      |
| CaCO₃               | 0.30              | 0.70             | 0.70  |      |
| NaCl                | 0.16              | 0.17             | 0.17  |      |
| Urea                | 0.30              |                  |       |      |
| Rumensin 90         | 0.01103           | 0.01103          | 0.01103 |     |
| Availa-4e           | ---               | ---              | 0.06994 |     |
| CoSO₄               | 0.00005           | 0.00039          | ---   |      |
| CuSO₄               | 0.00205           | ---              | ---   |      |
| ZnSO₄               | 0.00238           | ---              | ---   |      |
| Intellibond Cf      | ---               | 0.00217          | ---   |      |
| Intellibond Mf      | ---               | 0.00454          | ---   |      |
| Intellibond Zf      | ---               | 0.00655          | ---   |      |
| EDDIf               | 0.00137           | 0.00137          | 0.00137 |     |
| Vitamin A           | 0.00056           | 0.00056          | 0.00056 |     |
| Vitamin D           | 0.00006           | 0.00006          | 0.00006 |     |
| Vitamin E           | 0.00493           | 0.00493          | 0.00493 |     |

aAll heifers were individually fed a basal diet supplemented with Co, Cu, Mn, and Zn either from organic sources (COMP; Cu, Mn, and Zn amino acid complexes and Co glucoheptonate; Availa-4, Zinpro Corporation, Eden Prairie, MN) or inorganic sources (INORG; Cu, Mn, and Zn hydroxychlorides; Intellibond C, M, and Z, Micronutrients, Indianapolis, IN) and Co as CoSO₄.

bDried distillers grains plus solubles

cCu, Mn, and Zn amino acid complexes and Co glucoheptonate; Availa-4, Zinpro Corporation, Eden Prairie, MN

dCu, Mn, and Zn hydroxychlorides; Micronutrients, Indianapolis, IN

eEthlenediamine dihydroiodide
Figure 1

Liver Copper

Copper, mg/kg of dry matter

Day of the study

-28  28  Synchronization

COMP  INORG
Figure 2

Liver Cobalt

Cobalt: mg/kg of dry matter

Day of the study

-28  28  Synchronization

COMP  INORG
Figure 3

Liver Zinc

Zinc, mg/kg of dry matter

Day of the study

-28  28  Synchronization

COMP  INORG
