Nutrient Profiling – Mineral Supplementation

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Trace minerals (TM) are defined as a nutrient that is required in small amounts in the body and include cobalt, copper, iron, manganese, molybdenum, selenium and zinc; with each having roles in physiological, biochemical, and immune, processes throughout the animal's body. The TM are necessary for proper growth and development, and for immune and reproductive functions of both animals and humans.

An animal's requirements for TM begin while it is *in utero,* as TM are essential for proper embryonic development and survival (Ashworth and Antipatis, 2001; Hostetler et al., 2003). Moreover, TM deficiencies of the developing fetus carry over into the postnatal period with lesser TM storage in tissues of the neonates, further limiting neonatal growth, development, performance and immunity. During late gestation, the fetus undergoes a rapid phase of growth and increases its nutrient demands from the gestating dam to meet the needs of its developing organs. As gestation progresses, the fetal stores of TM in the body as a whole and particularly the liver increase (Hansard et al., 1968; Hidiroglou, 1980; Gooneratne and Christensen, 1989). Increased fetal and subsequent lactation TM demands increase the TM and nutrient requirements of late gestating and lactating animals. An animal's requirement for TM will ultimately vary depending upon its age, stage of production, and breed or genotype; necessitating the development of supplementation strategies that vary in response to the animals' level/stage of production (McDowell, 2003).

Bioavailability, refers to the proportion of mineral able to be utilized by the animal Various factors affect TM bioavailability and include the amount of TM in the diet, pH of the rumen and abomasum, antagonistic interactions with other TM, and breed and genetic variations in TM absorption and metabolism (Ashmead, 1993; McDowell, 2003). Available TM supplements can be delivered as a free choice mineral, a concentrated feed pellet, a soluble bolus, an injectable, or be added to animal's drinking water; where the form of TM delivery can also contribute to an animals' TM consumption (McDowell, 2003). The TM source, as either inorganic or organic is theorized to affect their bioavailability to the animal; with organic TM proposed to be more bioavailable than their inorganic counter parts (Spears, 1996). Moreover, the TM source may affect the ability of the TM to be utilized by the animal; with varying effects on post-absorption physiology. Trace minerals are involved in numerous enzymes related to cellular proliferation, carbohydrate and lipid metabolism, bone formation, and hormone production; all of which have the potential to impact animal growth, performance, and body composition.

Role of Trace Minerals in Male Reproduction

Proper spermatogenesis is necessary for successful reproduction and fertility. Poorer quality semen with decreased sperm motility, and increased sperm abnormalities and DNA damage can reduce fertility by inhibiting the ability of sperm to fertilize the oocyte or by generating poorer quality embryos (Saacke et al., 2000). Collectively, poor semen quality negatively affects reproductive potential of the male by reducing the number of offspring they are able to sire, and can reduce female production if they are not able to successfully fertilize a viable embryo. Spermatogenesis is known to be adversely affected by heat or cold stress, and poor nutrition, though, the effects of TM on male reproduction and fertility has received minimal attention in cattle. A majority of research on the effects of TM supplementation and source on male reproduction has been carried out in laboratory species, chickens, pigs, and humans. Furthermore, the effects of TM source on sexual development in bulls are even scarcer. One study conducted in peripubertal Hereford crossbred bulls demonstrated that supplementation with organic

amino acid complexed compared to inorganic sulfate sources of Co, Cu, Mn, and Zn tended to reach puberty 15 d earlier during the experiment (Geary et al., 2016). This study implies potential effects of TM source on bull sexual development may exist; however, additional research is needed to clarify the effects on puberty and sexual maturity in beef bulls. The following section will now discuss the roles of individual TM in male reproduction.

Role of Trace Minerals in Female Reproduction

The level of nutrition (i.e. protein, energy, and/or nutrients), physiological, psychological, or environmental stress, and the endocrine milieu affect female reproduction, which is the essential to livestock production. While the effects of nutrition are accepted, the effects of TM and in particular, TM source need further clarification. Research has yielded conflicting results on the effects of TM supplementation and TM source on reproductive performance. No difference in AI pregnancy rate, AI first service conceptions rate, and number of inseminations per female was reported between beef cows that received two 20 g Cu oxide boluses and control cows that received no bolus (Arthington et al., 1995). A 2 yr experiment provided no supplemental Cu (control), inorganic Cu sulfate, or organic amino acid complexed Cu to 2 yr old crossbred beef cows from 45 d pre-calving through 60 d post-calving. In yr 1, the 30 d pregnancy rates were greater in control (86 %) and organic (75 %) compared to inorganic (57 %) cows and no difference in 60 d pregnancy rates occurred between any treatment, while in yr 2 organic (85 and 93 %) and inorganic (80 and 87 %) cow 30 d and 60 d pregnancy rates did not differ, respectively (Muehlenbein et al., 2001). Similarly, another 2 yr experiment that utilized 2 yr old crossbred beef cows that were fed no TM (control), inorganic (sulfates and Co carbonate), or organic (Co glucoheptonate, Cu lysine, and Mn and Zn methionine) sources of Co, Cu, Mn, and Zn from the time of calving to breeding did not observe differences in pregnancy rates between the control or TM sources in either yr, however, in yr 1 the ING cows tended to conceive earlier than the ORG cows (Olson et al., 1999). Supplementation of crossbred multiparous beef cows with no TM (control), inorganic sulfate or organic proteinate sources of Cu, Mn, and Zn during the 3rd trimester through 110-135 d post-calving did not result in differences in estrus response to PGF2α, or AI pregnancy rate in yr 2; however overall pregnancy rate tended to be greater in supplemented than control cows (Ahola et al., 2004). Additionally, no differences in AI, bull, and overall pregnancy rate were observed between crossbred beef cows not supplemented and supplemented with inorganic sulfate or organic amino acid complexes of Co, Cu, Mn, and Zn (Marques et al., 2016).

In contrast, several studies have reported positive results of TM supplementation or source on reproduction. Mature beef cows that received TM (Cu, Mn, Se, and Zn) injections 105 d pre-calving and 30 d prior to fixed-time AI had greater AI pregnancy rates (60.2 %) compared to controls (51.2 %) which received saline injection; however, final pregnancy rate did not differ between treatments (Mundell et al., 2012). First calf heifers supplemented with organic amino acid chelate sources of TM became pregnant earlier in the breeding season compared to first calf heifers supplemented with inorganic TM sources (Kropp, 1990). Likewise, dairy cows supplemented with organic amino acid complexes of Cu, Mn, and Zn and Co glucoheptonate had fewer days to first service, services to conception, and days to conception compared to dairy cows that received inorganic TM in their total mixed ration (Uchida et al., 2001). Beef cows supplemented with a low or high level of inorganic (Co carbonate, Cu and Zn sulfate, and Mn oxides) or high level of amino acid complex organic sources of Co, Cu, Mn, and Zn did not differ in overall pregnancy rates, however, the high level of organic treatment had a greater AI pregnancy rate (Stanton et al., 2000). Interestingly, young (3 and 4 year olds) but not mature Braford cows (> 4 years old) supplemented with organic TM had greater pregnancy rates and a reduced calving interval compared to young and old cows supplemented with inorganic TM sources (Arthington and Swenson, 2004). Similarly, in nulliparous and primiparous crossbred beef heifers supplemented with inorganic sulfate or 50 % organic proteinate sources of Cu, Mn, and Zn, estrous cyclicity in yr 2 tended to be greater in organic compared to inorganic heifers; however, overall pregnancy rate tended to be greater in inorganic compared to organic heifers (Ahola et al., 2005a). Collectively, these studies suggest that the results of

TM supplementation and/or source on reproduction may be occurring during early establishment of pregnancy or on early embryonic development evidenced by greater AI, early pregnancy, and fewer services to conception. Moreover, most studies have utilized synchronization protocols at the time of breeding, which can potentially mask any effect of TM source on pubertal development in heifers, and on length of postpartum interval in cows. Research to determine whether TM source affects heifer sexual development is warranted.

We undertook a number of studies using inorganic and organic source mineral two of the project objectives related to nutritional profiling on sexual development.

1. Study the effects of prenatal and postnatal TM source on sexual development in bulls in relation to puberty and sexual maturity.

2. Explore the effects of prenatal and postnatal TM source on sexual development in heifers with respect to puberty and pregnancy.

Effects of prenatal and postnatal trace mineral supplement source bull growth, performance, and sexual development

A study was conducted to evaluate breed (Angus, AN vs. Brangus, BN) and prenatal/postnatal TM source (inorganic, ING vs. organic, ORG) on bull growth, performance, and sexual development. Bulls (241 ± 2 d, 548 ± 9 lb, *n* = 32, 8 per TM × breed) born to dams that were supplemented with either Co, Cu, Mn, Se, and Zn as ING (Na selenite or salt sulfates) or ORG (Se-yeast and proteinates) TM sources were stratified by sire, age, and weaning BW. Bull diet included cracked corn, cottonseed hulls, a protein pellet, wet brewer's grains, and TM supplement pellet (1.0 lb/1,000 lbBW/day). Weekly BW, 28-d hip height, and bi-weekly semen collection, scrotal circumference (SC), and BCS (scale 1-9) were recorded. Serum and liver biopsies to determine TM status every 56 d. At puberty, there was no effect of TM source or breed except for sperm concentration which was greater in BN (172.4 ± 28.2 × 10^6 cells/mL) compared to AN (96.9 ± 23.3 × 10^6 cells/mL) bulls. At sexual maturity, except for ADG which tended to be greater in ING (2.76 ± 0.18 lb/d) compared to ORG (2.32 ± 0.18 lb/d) bulls and secondary abnormalities which were lesser in BN (9.6 ± 1.67 %) compared to AN (15.0 ± 1.18 %) bulls; no effect of TM source, breed or TM source × breed occurred for performance or seminal traits. Liver and serum TM concentrations were not affected by TM source. Mean liver Cu, Mn, and Se concentrations were greater in BN compared to AN bulls, while mean liver Co, Fe, Mo, and Zn did not differ by breed. Performance, body composition, serum and liver TM concentrations, and seminal traits were all affected by day of the experiment. Age at puberty did not differ by TM source, breed or TM source × breed. Although not significant, ORG bulls were numerically 41 d younger than ING bulls at sexual maturity. Bull TM source had minimal effects on pubertal parameters, but ORG TM supplementation may hasten the age bulls reach sexual maturity.

Table 1. Effect of inorganic (ING) or organic (ORG) prenatal and postnatal trace mineral (TM) supplement source on Angus (AN) and Brangus (BN) bull sexual parameters at puberty[1]

	TM source × Breed (B)					*P*-value		
Item	ING-AN	ING-BN	ORG-AN	ORG-BN	SEM	TM	B	TM×B
Puberty, *n*	8	6	8	5				
Age, d	338	342	346	313	15	0.52	0.34	0.23
BW, kg	730^{x}	856^{y}	842^{xy}	730^{xy}	53.0	0.89	0.88	0.04
ADG, kg/d[2]	2.43^{ab}	2.71^{a}	2.45^{a}	1.83^{b}	0.22	0.05	0.42	0.04
BCS	4.7	4.8	4.9	4.8	0.14	0.59	0.80	0.44
SC, cm[3]	29.9^{a}	33.5^{b}	31.2^{a}	30.3^{a}	0.82	0.26	0.11	0.01
Sperm conc., 10^6 cells/mL	102.2	218.3	91.6	126.5	36.4	0.17	0.05	0.28
Gross motility[4]	1.75	1.83	1.88	1.80	0.20	0.82	0.98	0.70
Normal sperm, %	34.0	51.5	51.0	61.0	8.93	0.15	0.14	0.68

[1]Puberty was defined as the date at which the bull's ejaculate with a sperm concentration ≥ 50×10^6 cells/mL and ≥ 10% motility.
[2]ADG was calculated based on difference from d 0 and when bull reached puberty.
[3]SC = scrotal circumference.
[4]Gross motility scale of 0 to 4, (0 = none, 1 = poor, 2 = fair, 3 = good, and 4 = very good).
[a, b] Row means with different superscripts differed, ($P \leq 0.05$).
[x, y] Row means with different superscripts differed, ($P \leq 0.10$).

Table 2. Effect of inorganic (ING) or organic (ORG) prenatal and postnatal trace mineral (TM) supplement source on Angus (AN) and Brangus (BN) bull sexual parameters at sexual maturity[1]

	Trace mineral (TM) source × breed (B)					*P*-value		
Item	ING-AN	ING-BN	ORG-AN	ORG-BN	SEM	TM	B	TM × B
Sexual maturity, *n*	6	2	4	3				
Age, d	412	370	371	332	24.3	0.14	0.13	0.94
BW, kg	972	955	911	789	87.1	0.23	0.45	0.57
ADG, kg/d[2]	2.89	2.65	2.40	2.21	0.22	0.07	0.38	0.93
BCS	5.3	5.3	5.1	5.0	0.21	0.41	0.78	0.78
SC[3], cm	34.1	35.0	33.5	32.0	1.44	0.25	0.85	0.43
Sperm conc., 10^6 cells/mL	172.2	222.5	116.9	134.2	55.3	0.23	0.56	0.77
Gross motility[4]	2.5	2.5	2.3	2.7	0.38	0.92	0.61	0.61
Normal sperm, %	75.5	76.0	77.0	77.7	2.4	0.53	0.82	0.97

[1]Sexual maturity was defined as the first date at which a passed to consecutive biweekly BSE.
[2]ADG was calculated based on difference from d 0 and when bull reached puberty.
[3]SC = scrotal circumference.
[4]Gross motility scale of 0 to 4, (0 = none, 1 = poor, 2 = fair, 3 = good, and 4 = very good).

Effects of prenatal and postnatal trace mineral supplement source on heifer growth, performance, and sexual development

A study was designed to examine the effects of prenatal and postnatal trace mineral (TM) source on heifer performance, body composition, and sexual development across 2 yrs in Angus (AN, yr 1 = 40, yr 2 = 30) and Brangus (BN, yr 1 = 40, yr 2 = 31) heifers supplemented with inorganic (ING, sulfate salts; yr 1 = 40, yr 2 = 31) or organic (ORG, Se-yeast and proteinates; yr 1 = 40, yr 2 = 30) trace mineral (TM) sources of Co, Cu, Mn, Se, and Zn. Heifers were stratified by maternal TM source, age, sire, and weaning BW, and

randomly assigned to pens for a 168 d development period and combined into 4 breeding groups (1 per TM source × breed) for natural service breeding. The TM supplement was pen fed as a pellet 3 d/wk at 1.0 lb/1,000 lb BW/day. The TM source did not affect BW, BCS, or ADG at the end of development for both yrs. Mean liver Se concentrations were greater in ORG compared to ING both yrs. Liver Mn concentrations were greater in BN than AN both yrs, and liver Cu was greater in BN compared to AN at breeding in yr 1 and overall in yr 2. Pubertal status at the start of breeding in yr 1 did not differ by TM source, breed, and TM source × breed. In yr 2, more ORG (47% = 14/30) heifers were pubertal compared to ING (23% = 7/31). The interval to puberty in yr 1 was affected by TM source × breed, as ORG-BN heifers were pubertal 12 d earlier than ING-AN. In yr 2, ORG (405 ± 7 d) heifers were 29 d younger than ING (434 ± 8 d) heifers based on survival analysis of age to puberty. The interval to pregnancy in yr 1 was less in ORG and BN compared to ING and AN heifers, respectively. There was no effect of TM source, breed, and TM source × breed on age or interval to pregnancy in yr 2. Final breeding season pregnancy rates did not differ by TM source, breed, or TM source × breed for either yr. The results suggest that breed has a greater influence than TM source on performance and body composition traits. However, ORG prenatal and postnatal TM supplementation may hasten the time/age to puberty and pregnancy in heifers.

Table 3. Year 1 characteristics of Angus (AN) and Brangus (BN) heifers supplemented prenatally and postnatally with inorganic (ING) or organic (ORG) sources of trace minerals (TM)

	TM source × Breed (B)					*P*-value		
Item	ING-AN	ING-BN	ORG-AN	ORG-BN	SEM	TM	B	TM × B
Trial start, *n*	20	20	20	20				
Age, d	233	233	234	239	5	0.45	0.60	0.54
BW, lb	475	485	492	496	11	0.18	0.40	0.71
BCS	4.3[a]	4.6[b]	4.6[b]	4.7[b]	0.1	0.001	0.001	0.02
End Dev. (d 168)								
BW, lb	714	730	736	759	15	0.13	0.26	0.83
ADG, lb/d	1.43	1.43	1.41	1.52	0.07	0.49	0.31	0.48
BCS	5.4	5.4	5.4	5.5	0.1	0.38	0.56	0.77
RTS[1], (1-5)	2.8	3.2	3.0	3.6	0.2	0.09	<0.01	0.54
PA[2], cm^2	353	375	380	401	8.9	< 0.01	0.02	0.97
Cycling status start of breeding, *n* (%)[3]	4/20 (20)	4/20 (20)	2/20 (10)	3/20 (15)	--	0.36	0.71	0.71

[1]RTS = reproductive tract score, scale 1-5.

[2]PA = pelvic area, calculated from pelvic height and pelvic width measurements.

[3]Cycling status determined by progesterone concentrations, where concentrations ≥ 1.5 ng/mL was considered cycling.

[a-b]Means within a row with different superscripts differed, ($P < 0.05$).

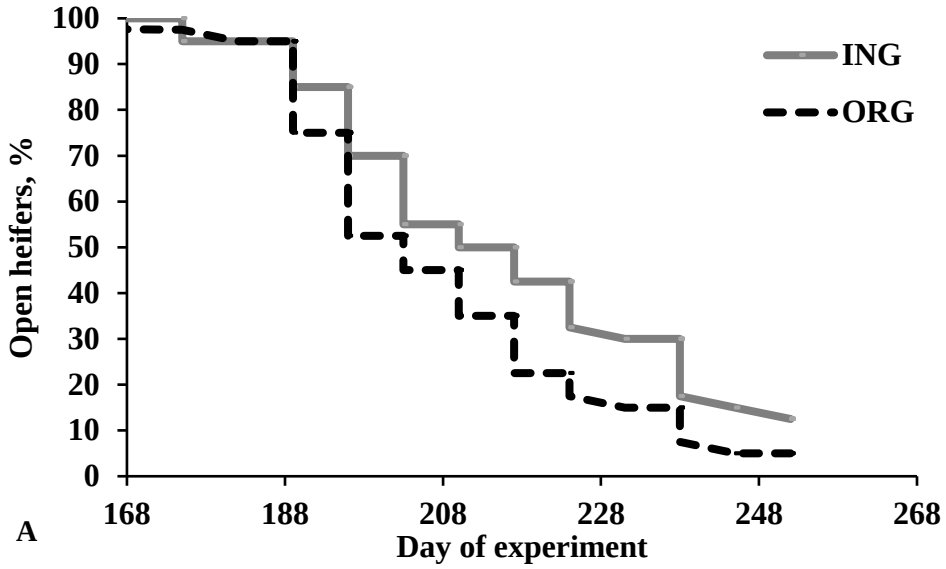

Figure 1. Effect of prenatal and postnatal inorganic (ING) or organic (ORG) trace mineral supplement source in year 1 in heifers on survival analysis interval to pregnancy. Mean (± SE) of interval to pregnancy: ING = 216 ± 4 d, ORG = 207 ± 3 d, (*P* = 0.02).

Table 4. Year 2 characteristics of Angus (AN) and Brangus (BN) heifers supplemented prenatally and postnatally with inorganic (ING) or organic (ORG) trace mineral (TM) sources

	TM × breed (B)					*P*-value		
Item	ING-AN	ING-BN	ORG-AN	ORG-BN	SEM	TM	B	TM × B
Trial start, *n*	16	15	14	16				
Age, d	245	243	238	231	4	0.03	0.28	0.52
BW, lb	476	525	474	523	14	0.91	0.001	1.00
BCS	4.4	4.7	4.3	4.6	0.08	0.40	<0.001	0.56
End Dev. (d 168)								
BW, lb	675	770	695	772	18	0.55	<0.001	0.66
ADG, lb/d	1.19	1.46	1.28	1.48	0.07	0.34	<0.001	0.48
BCS	5.0	5.6	5.2	5.6	0.1	0.51	<0.001	0.36
RTS[1]	2.9	3.4	3.6	3.6	0.3	0.16	0.40	0.38
PA[2], cm^2	444	481	440	481	11.3	0.86	0.001	0.87
Cycling status start of breeding[3], *n* (%)	2/16 (26)	5/15 (33)	7/14 (50)	7/16 (44)	--	0.05	0.40	0.21

[1]RTS = reproductive tract score, scale 1-5.

[2]PA = pelvic area, calculated from pelvic height and pelvic width measurements.

[3]Cycling status determined by progesterone concentrations, where concentrations ≥ 1.5 ng/mL was considered cycling.

[a-c]Means within a row with different superscripts differed, ($P < 0.05$).

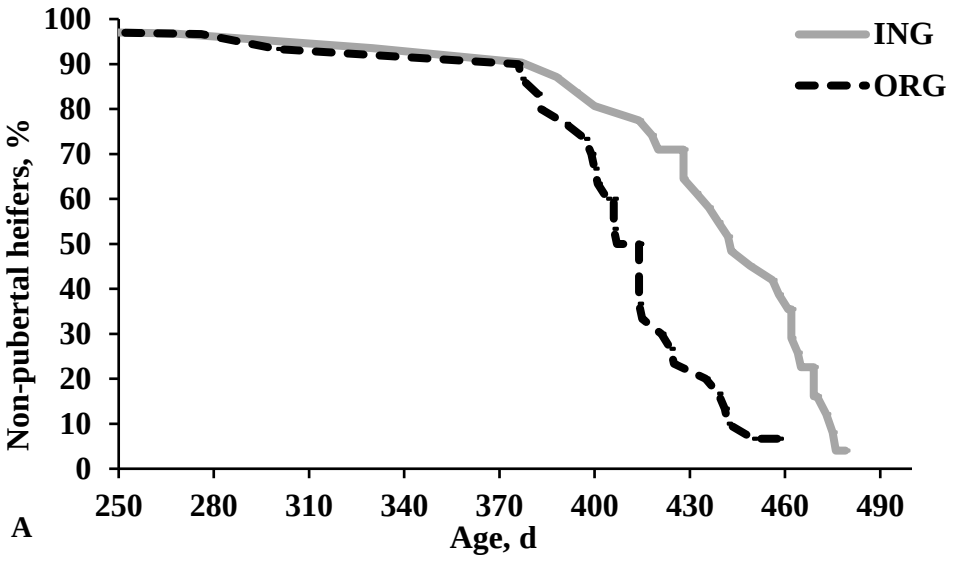

Figure 2. Effect of prenatal and postnatal inorganic (ING) or organic (ORG) trace mineral supplement source in year 2 in heifers on survival analysis of age to puberty. Mean (± SE) age to puberty: ING = 434 ± 8 d, ORG = 405 ± 7 d, ($P < 0.001$).

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