Understanding Trace Minerals: Diagnostics, Reproduction & Immunity

Brad De Groot, DVM, PhD
Dillon, MT
Multimin USA

Part 1 - Physiological Functions of Trace Minerals

Part 2 - Trace Mineral Functions in Immunity

Part 3 - Trace Mineral Functions in Reproduction

Part 4 - Trace Mineral Diagnostics

Part 5 - Trace Mineral Patterns in Cattle Production

Part 6 - Injectable Trace Mineral Supplementation for Cattle

Nutrition

- Fat
- Carbohydrate
- Protein
- Vitamins
- Minerals
  - Macrominerals
  - Microminerals (Trace minerals)

Raw Materials

Nutrition

- Fat
- Carbohydrate
- Protein
- Vitamins
- Minerals
  - Macrominerals
  - Microminerals (Trace minerals)

Tools

MACRO vs MICRO(TRACE) MINERALS

Macromineral Requirements in Beef Cattle

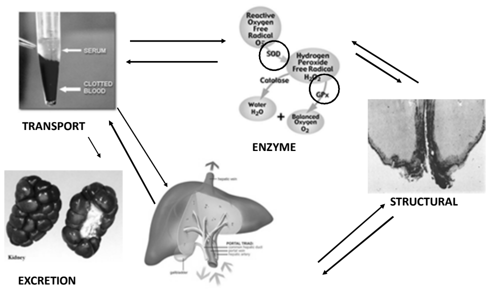
	Requirement in Total DM Intake			
Mineral, %	Growing and Finishing Cattle	Stressed Calves	Dry, Gestating Cows	Lactating Cows
Calcium	0.31	0.6-0.8	0.18	0.58
Magnesium	0.10	0.2-0.3	0.12	0.20
Phosphorus	0.21	0.4-0.5	0.16	0.26
Potassium	0.60	1.2-1.4	0.60	0.70
Sodium	0.06-0.08	0.2-0.3	0.06-0.08	0.10
Sulfur	0.15	0.15	0.15	0.15

Trace Mineral Requirements in Beef Cattle

	Requirement in Total DM Intake			
Mineral, ppm	Growing and Finishing Cattle	Stressed Calves	Dry, Gestating Cows	Lactating Cows
Cobalt	0.10	0.1-0.2	0.10	0.10
Copper	10.00	10.0-15.0	10.00	10.00
Iodine	0.50	0.3-0.6	0.50	0.50
Iron	50.00	100.0-200.0	50.00	50.00
Manganese	20.00	40.0-70.0	40.00	40.00
Selenium	0.10	0.1-0.2	0.10	0.10
Zinc	30.00	75.0-100.0	30.00	30.00

Part 1
Physiological Functions of Trace Minerals

TRACE MINERAL METABOLISM



Manganese

Fe

Enzyme	Functions	Significance
Hemoglobin	Oxygen transport	Aerobic life
Myoglobin	Oxygen utilization by muscle	Anything that moves
Cytochrome C Oxidase	Electron transport (with Cu)	Aerobic life
Myeloperoxidase	$Cl^- + H_2O_2 \Leftrightarrow OCl^- + H_2O$	Bactericidal, Virucidal
Catalase	$H_2O_2 \Leftrightarrow H_2O + O_2$	Antimicrobial, Antioxidant

Trace Elements and Host Defense: Recent Advances and Continuing Challenges[1,2]
Mark L. Failla[3] J. Nutr. 133: 1443S–1447S, 2003.

Kretsinger, RH. 2013
Encyclopedia of Metalloproteins.
Springer 978-1-4614-1533-6, pp. 939-963

Zinc

Zn

- ***"Zinc is required for the structural and functional integrity of over 2000 transcription factors and almost every signaling and metabolic pathway is dependent on one or more zinc-requiring proteins."*** *(Beattie and Kwun,2004; Cousins et al., 2006).*

Zinc-dependent enzymes (5, 21, 22)

Cu_2Zn_2SOD	Disproportionation of superoxide
Aminopeptidase	Protein hydrolysis
Aldehyde hydrase	Aldehyde hydration
Esterase	Ester hydrolysis
Methylmalonyl-oxaloacetate transcarboxylase	Transcarboxylation
Carboxypeptidases A and B	Protein hydrolysis
NAD-dependent dehydrogenases	Oxidations
Carbonic anhydrase	Dehydration of carbonic acid
α-Hydroxyacid dehydrogenase	Oxidation of α-hydroxy acids
Alkaline phosphatase	Phosphorylation
Purine and pyrimidine nucleoside kinases	Phosphorylation of nucleosides
DNA polymerase and gyrase	DNA synthesis
"Zinc-finger" proteins	Transcription regulating proteins

Manganese

Mn

Enzyme	Functions	Significance
Glycosyltransferase	Cartilage proteoglycans, prothrombin	Skeletal malformations ,swollen joints, dwarfism, prolonged clotting time
Pyruvate Carboxylase	Lipid and Carbohydrate Metabolism	Gluconeogenesis
Mn-Superoxide Dismutase	$O2^{-} \Leftrightarrow H2O2$	Mitochondrial oxidation control

---

Copper

(suttle, Neville R. Mineral Nutrition of Livestock 4th edition)

Cu

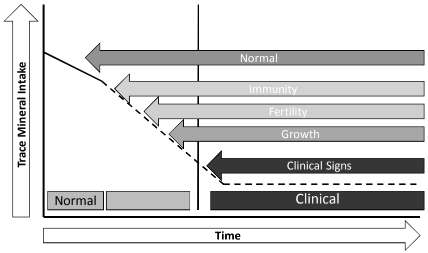
Enzyme	Function	Significance
Caeruloplasmin (ferroxidase)	Iron Transport	Anemia, Antioxidant
Cytochrome c Oxidase	Cellular Respiration	Anoxia, Impaired cellular immunity
Dopamine-B-mono oxygenase	Catecholamine metabolism	Behavior?
Hephaestin	Export Fe from intestine	Anemia
Ferroxidase II	Iron Oxidation	Anemia
Lysyl Oxidases	Desmosine cross linkages in connective tissue	Aortic Rupture, joint disorders, osteoporosis
Monamine Oxidase	Oxidative deamination of monoamines	Cell signaling, Leukocyte trafficking
Superoxide Dismutases e.g. ZnCuSOD	$O2^{-} \Leftrightarrow H2O2$	Lipid Peroxidation, Vascular Tone, Impaired cellular immunity
Thiol Oxidase	Disulfide bond formation	Loss of wool and hair strength
Tyrosinases	Tyrosine to melanin	Depigmentation

Selenium

Se

Enzyme	Functions (location)	Abbrev.	Pathognomic Sig.
Cytosolic glutathione peroxidases	Antioxidant, storage (tissue cytosol, RBC,)	GPX1	Impaired Immunity, White Muscle Disease,
Phospholipid hyperoxide	Antioxidant (intracellular memebranes, testes)	GPX2	cellular damage due to oxidative stress.
Plasma GPX	Antioxidant (plasma,kidney, lung)	GPX3	
Gastrointestional GPX	Antioxidant (intestinal mucosa)	GPX4	
Epidydimal GPX	Antioxidant (epididymus)	GPX5	
iodthyronine (deiodinases)	Conversion T4-T3 (liver, kidney, muscle) Ruminants	ID1	Reduce basal metabolic rate
	Conversion T4-T3 (BAT, brain)	ID2	Neonate resistance to cold stress
	Conversion T4-rT3 (Placenta)	ID3	
Selenoprotein N	Cell proliferation (Muscle)	SePN	
Selenoprotein P	Transport, Metal detox. (Plasma)	SePP	

Trace Mineral Functions



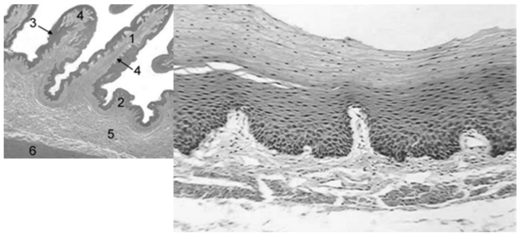
Wikse, 1992 Texas A&M Beef Cattle Short Course

Part 2
Trace Mineral Functions in Immunity

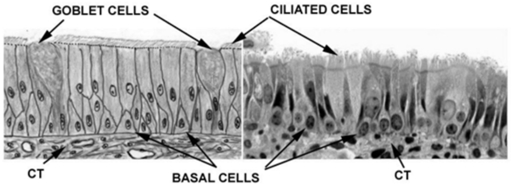
Immune System Parts

- Innate
  - How the body is built
    - Anatomic or Physical Barriers (skin, mucous membranes)
    - Physiological Barriers (pH, bile acids, mucus, organic acids)
  - How the body responds
    - Inflammation (vasodilators, complement, fibrin)
    - Natural Killer Cells
    - Phagocytic Cells (neutrophils and monocytes)
- Acquired
  - Humoral
  - Cell-Mediated

Immune System – Physical Barriers



Immune System – Physiological



Immune System – Inflammation

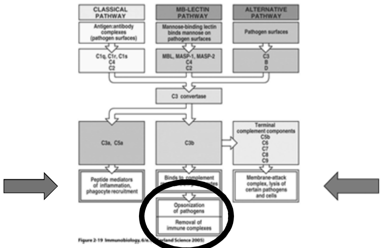
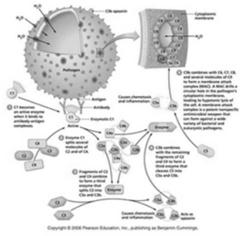
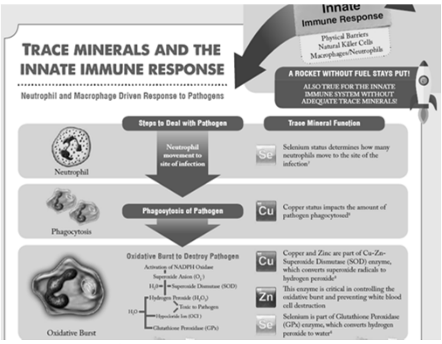


Figure 2-19 Immunobiology, 6/e. (Garland Science 2005)

Immune System – Inflammation





TRACE MINERALS AND THE INNATE IMMUNE RESPONSE

Neutrophil and Macrophage Driven Response to Pathogens

Innate Immune Response

Physical Barriers
Natural Killer Cells
Macrophages/Neutrophils

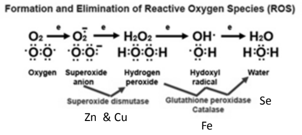
A ROCKET WITHOUT FUEL STAYS PUT!

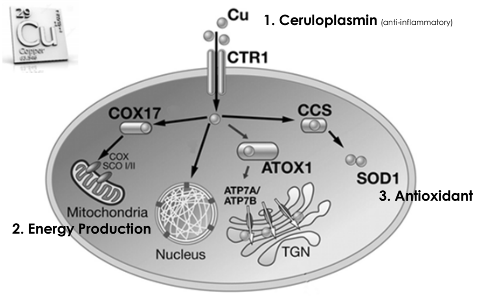
ALSO TRUE FOR THE INNATE IMMUNE SYSTEM WITHOUT ADEQUATE TRACE MINERALS!

Steps to Deal with Pathogen		Trace Mineral Function
Neutrophil movement to site of infection	Se	Selenium status determines how many neutrophils move to the site of the infection[7]
Phagocytosis of Pathogen	Cu	Copper status impacts the amount of pathogen phagocytosed[8]
Oxidative Burst to Destroy Pathogen	Cu	Copper and Zinc are part of Cu-Zn-Superoxide Dismutase (SOD) enzyme, which converts superoxide radicals to hydrogen peroxide[4]
	Zn	This enzyme is critical in controlling the oxidative burst and preventing white blood cell destruction
	Se	Selenium is part of Glutathione Peroxidase (GPx) enzyme, which converts hydrogen peroxide to water[4]

OXIDATIVE STRESS :

PRODUCTION OF ROS >>>> ANTIOXIDANT CAPACITY





Reduced T-cell response & Reduced Interferon production
Decreased Neutrophil Phagocytosis & Killing

http://theredish.com/imgs/showimg

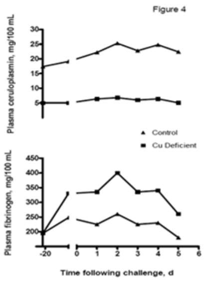


Figure 4. Effect of copper status on the acute phase protein response following IBR challenge (d=0) in growing heifers (Arthington et al. JAS. 1996. 74:2759).

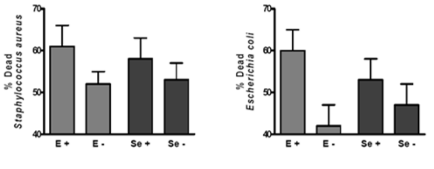
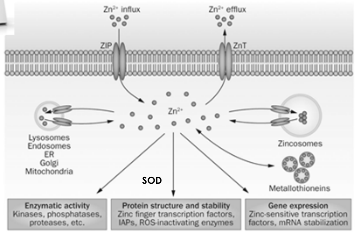


Figure 2. Percent intracellular killing by bovine neutrophils. Means represented (± SE); E = vitamin E and Se = selenium supplemented cows. There were no significant vitamin E x selenium interactions. Intracellular *S. aureus* killing was greater for vitamin E ($P < 0.01$) and selenium ($P < 0.05$) supplemented cows. Intracellular *E. coli* killing was greater for vitamin E ($P < 0.01$), but not selenium ($P > 0.05$) supplemented cows. Figure adapted from Hogan et al. (1990).

Vitamin A – Transport and utilization (Epithelium)



SOD

Ribonucleotide reductase

Zn deficiency: Reduction in T cell in number and maturation
Decrease in Interferon-γ , TNF-α , Interleukins
Affects appetite, cortisol, balance between cell-mediated and humoral immunity

http://www.nature.com/nrurol/journal/v10/n4/fig_tab/nrurol.2013.43_F1.html

---

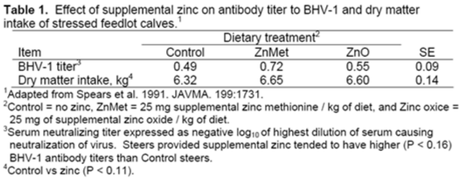


Table 1. Effect of supplemental zinc on antibody titer to BHV-1 and dry matter intake of stressed feedlot calves.[1]

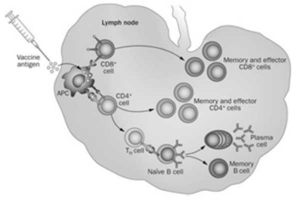
	Dietary treatment[2]			
Item	Control	ZnMet	ZnO	SE
BHV-1 titer[3]	0.49	0.72	0.55	0.09
Dry matter intake, kg[4]	6.32	6.65	6.60	0.14

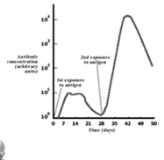
[1] Adapted from Spears et al. 1991. JAVMA. 199:1731.

[2] Control = no zinc, ZnMet = 25 mg supplemental zinc methionine / kg of diet, and Zinc oxice = 25 mg of supplemental zinc oxide / kg of diet.

[3] Serum neutralizing titer expressed as negative $\log_{10}$ of highest dilution of serum causing neutralization of virus. Steers provided supplemental zinc tended to have higher (P < 0.16) BHV-1 antibody titers than Control steers.

[4] Control vs zinc (P < 0.11).





Immune System – Acquired Action

- Quick overview of innate/acquired interaction
- https://www.youtube.com/watch?v=Bf2t8n1ibwQ

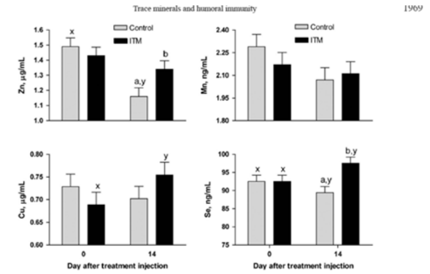


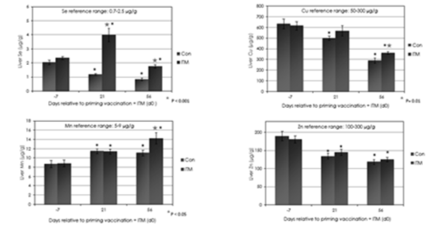
Figure 1. Serum mineral concentrations at d 0 and 14 relative to vaccination and treatment administration. Samples analyzed from a random sub-sample of steers in each treatment (n = 15 steers/treatment). Steers provided a 7-mL injection of trace minerals (ITM) or 7 mL of sterile saline (Control) on d 0. Treatment × time, $P \leq 0.05$, 0.33, 0.01, and 0.001 for Zn, Mn, Cu, and Se, respectively. Values with unlike letters differ ($P < 0.05$) between treatments within sampling days (a,b) and within treatments across sampling days (x,y).

ace minerals on the humoral immune response to multivalent vaccine administration in beef calves
J. D. Arthington and L. J. Havenga

J ANIM SCI 2012, 90:1966-1971.
2011-4024 originally published online December 16, 2011

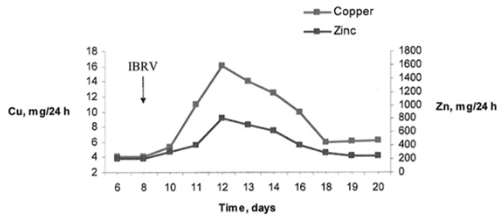
Vaccine Response and Mineral Levels

Liver concentration of Se, Cu, Mn & Zn on days -7, 21 & 56 relative to priming vaccination (day 0)



Palomares, AABP 2015

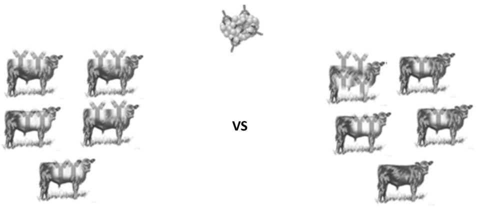
Infectious Challenge and Minerals



Orr, CL, et al, 1990 JAS v68 p2893

Multimin Improves Immune Response

- Teixeira JDS 2014 v. 97 p.4216 – Phagocytosis
- Arthington JAS 2012 v. 90 p. 1996 – Humoral
- Arthington JAS 2014 v. 92 p. 2630 – Humoral
- Roberts PNC 2015 poster – Humoral
- Palomares Vet Imm & Immunopath 2016 – Humoral and CMI



VS

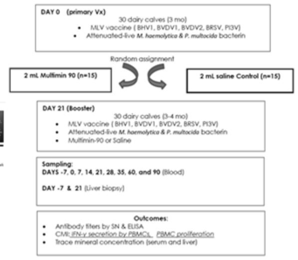
Improved Humoral & CMI – Palomares

Veterinary Immunology and Immunopathology

Effects of injectable trace minerals on humoral and cell-mediated immune responses to *Bovine viral diarrhea virus*, *Bovine herpes virus 1* and *Bovine respiratory syncytial virus* following administration of a modified-live virus vaccine in dairy calves

R.A. Palomares, D.J. Hurley, J.H.J. Bittar, J.T. Saliki, A.R. Woolums, F. Moliere, L.J. Havenga, N.A. Norton, S.J. Clifton, A.B. Sigmund, C.E. Barber, M.L. Berger, M.J. Clark, M.A. Fratto

Methods:



DAY 0 (primary Vx)
30 dairy calves (3 mo)
- MLV vaccine (BHV1, BVDV1, BVDV2, BRSV, PI3V)
- Attenuated-live *M. haemolytica & P. multocida* bacterin

Random assignment

2 mL Multimin 90 (n=15)

2 mL saline Control (n=15)

DAY 21 (Booster)
30 dairy calves (3-4 mo)
- MLV vaccine (BHV1, BVDV1, BVDV2, BRSV, PI3V)
- Attenuated-live *M. haemolytica & P. multocida* bacterin
- Multimin-90 or Saline

Sampling:
DAYS -7, 0, 7, 14, 21, 28, 35, 60, and 90 (Blood)

DAY -7 & 21 (Liver biopsy)

Outcomes:
- Antibody titers by SN & ELISA
- CMI: IFN-γ secretion by PBMCs, PBMC proliferation
- Trace mineral concentration (serum and liver)

The University of Georgia

BVDV-1 on day 28

CONTROL

53% had 4x titer response

The University of Georgia

❷ *"ITM enhanced the CMI response elicited by MLV vaccine in dairy calves".*

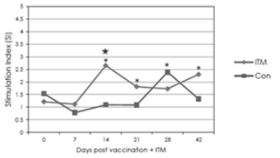
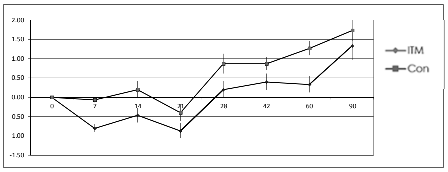


Figure 3. PBMC proliferation response to BVDV (expressed as stimulation index; SI) in dairy calves treated with ITM concurrently with MLV vaccine. Proliferation response using counts per minutes (CPM). SI= CPM in response to virus / CPM in response to media. * Value differs significantly from the value on day 0 ($P < 0.05$). * Difference between groups ($P= 0.08$).

Mannheimia haemolytica titers



Palomares 2015 AVC

Part 3
Trace Mineral Functions in Reproduction

Zinc

Decrease growth, hypogonadism

Fetal teratogenesis, prolonged gestation, difficult labor.

Zn crucial in PROSTAGLANDIN synthesis

PGF2ά → parturition

↓

Initiate uterine contractions

Tucker HF, Salmon WD Parakeratosis or zinc deficiency disease. Proceedings of the society for experimental biology in medicine 88, 613-616

Favier AE 1992, The role of zinc in reproduction: Hormonal mechanisms, Biological Trace element research 32 363-382

MANGANESE

Testicular Atrophy

HurleyWL Doane RM , 1989, Recent developments in the roles of vitamins and minerals in reproduction , Journal of Dairy Science 72,784-804

Mn transport -> ά-macroglobulin into tissue into cell into mitochondria

Maynard LS Cotzias GC The partition of manganese among organs and intracellular organelles, Journal Of Biological Chemistry 214, 489-495

BONE – chondroitin sulfate

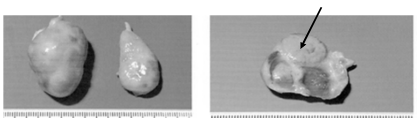
LeachRM Metabolism and function of manganese Prasad AS Academic press New York p235-248

Manganese (Regulates Cholesterol Synthesis)

Irregular estrus cycle – Impaired ovulation

High level in Pituitary and Ovary

Progesterone secretion- increase in Mn in CL



Trace element deficiencies and fertility in ruminants : A review ,Hidiroglou M, J Dairy Sci 62: 1195-1206

Hidroglou M Shearer DA, Concentration of manganese in the tissues of cycling and anestrous ewes, Canadian Journal of comperative Medicine 40, 306-309

COPPER

Cytochrome c oxidase – mitochondrial energy production

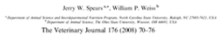
Superoxide dismutase

Ceruloplasmin

Lysyl oxidase

Reduced T-cell response & Reduced Interferon production

Decreased Neutrophil Phagocytosis & Killing



Role of antioxidants and trace elements in health and immunity of transition dairy cows

Jerry W. Spears, William P. Weiss

The Veterinary Journal 176 (2008) 70–76

Trace Elements and Host Defense: Recent Advances and Continuing Challenges[1,2]

Mark L. Failla[3] J. Nutr. 133: 1443S–1447S, 2003.

Engle, Terry E., "Effects of Mineral Nutrition of Immune Function and Factors That Affect Trace Mineral Requirements of Beef Cattle" (2001). *Range Beef Cow Symposium.* Paper 87.

Selenium deficiency

- Reduced recognition and implantation of fetus
- Degeneration of ovaries and atresia of follicles
- Retained placenta
- Reduced neutrophil function (diapedesis and killing ability)
- Cystic ovarian disease from reduced GPx activity on follicle development, steroid hormone synthesis.

Vitamin E and Selenium for Reproduction of the dairy cow, Harrison J H , Hancock D D , Conrad HR, J Dairy Sci 1984, 67: 123-132

Selenium,the thyroid,and the Endocrine System,Kohrle J,Jakob F,ContempreB and Dumont JE , Endocrine Reviews, 2005 ,26(7) 944-984

Literature Review

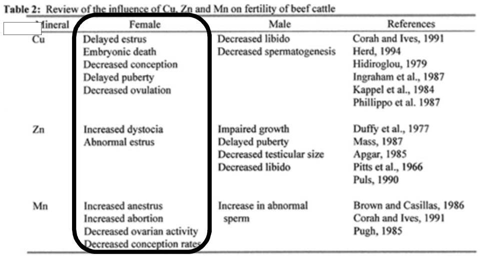


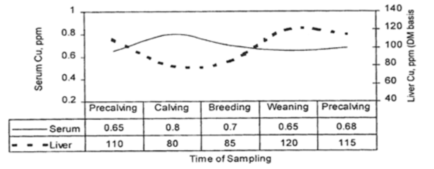
Table 2: Review of the influence of Cu, Zn and Mn on fertility of beef cattle

Mineral	Female	Male	References
Cu	Delayed estrus Embryonic death Decreased conception Delayed puberty Decreased ovulation	Decreased libido Decreased spermatogenesis	Corah and Ives, 1991 Herd, 1994 Hidiroglou, 1979 Ingraham et al., 1987 Kappel et al., 1984 Phillippo et al. 1987
Zn	Increased dystocia Abnormal estrus	Impaired growth Delayed puberty Decreased testicular size Decreased libido	Duffy et al., 1977 Mass, 1987 Apgar, 1985 Pitts et al., 1966 Puls, 1990
Mn	Increased anestrus Increased abortion Decreased ovarian activity Decreased conception rates	Increase in abnormal sperm	Brown and Casillas, 1986 Corah and Ives, 1991 Pugh, 1985

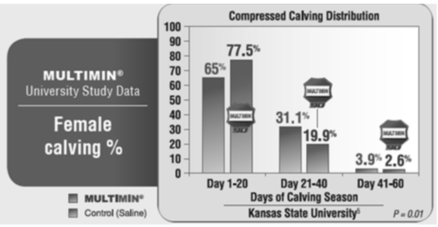
Assessing the role of Copper and Zinc in the cow-calf production cycle. Paterson J,Swenson C,Johnson B,Ansotegui R,Montana State University

Trace Mineral Status Not Static

Figure 3: Changes in liver and serum copper concentrations for beef cows (Swenson, 1998).



	Precalving	Calving	Breeding	Weaning	Precalving
Serum	0.65	0.8	0.7	0.65	0.68
Liver	110	80	85	120	115



L.R. Mundell, J.R. Jaeger, J.W. Waggoner, J.S. Stevenson, D.M. Grieger, L.A. Pacheco, J.W. Bolte, N.A. Aubel, G.J. Eckerle, M.J. Macek, S.M. Ensley, L.J. Havenga, K.C. Olson, **"Effects of prepartum and postpartum bolus injections of trace minerals on performance of beef cows and calves grazing native range."** The Professional Animal Scientist 28 (2012):82-88

Literature Review

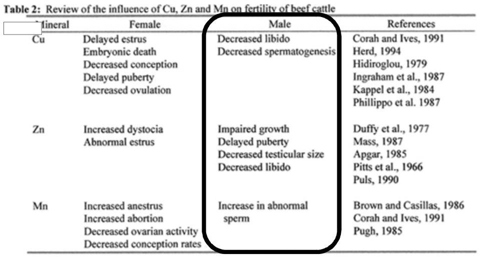


Table 2: Review of the influence of Cu, Zn and Mn on fertility of beef cattle

Mineral	Female	Male	References
Cu	Delayed estrus Embryonic death Decreased conception Delayed puberty Decreased ovulation	Decreased libido Decreased spermatogenesis	Corah and Ives, 1991 Herd, 1994 Hidiroglou, 1979 Ingraham et al., 1987 Kappel et al., 1984 Phillippo et al. 1987
Zn	Increased dystocia Abnormal estrus	Impaired growth Delayed puberty Decreased testicular size Decreased libido	Duffy et al., 1977 Mass, 1987 Apgar, 1985 Pitts et al., 1966 Puls, 1990
Mn	Increased anestrus Increased abortion Decreased ovarian activity Decreased conception rates	Increase in abnormal sperm	Brown and Casillas, 1986 Corah and Ives, 1991 Pugh, 1985

Assessing the role of Copper and Zinc in the cow-calf production cycle. Paterson J,Swenson C,Johnson B,Ansotegui R,Montana State University

Part 4
Trace Mineral Diagnostics

TRACE MINERAL DIAGNOSTICS

Mineral testing - The approach depends on what you want to find out

(New Zealand Veterinary Journal 41, 98–100, 1993.)

- <u>Clinical:</u> Poor Performance/Disease
  vs.
- <u>Sub-Clinical:</u> Deficiency

- Serum vs. Liver Tissue
- Sensible Reference Range

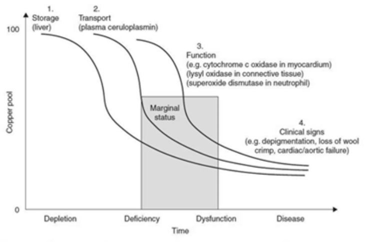


Fig. 11.11. The sequence of biochemical changes leading to the appearance of clinical signs of copper deprivation.

Farm Deficiency (Sub-Clinical)

- Sample: Blood (serum, plasma), Liver Tissue
- Sample Time:
  - ***<u>Before anticipated deficiency:</u>***
    - Late pregnancy, early lactation, or growing calves (>6 months).

  - Results:
  - Mean or average and individual results

Recommendations

- Number: 10-12 samples
  - Blood or Liver Tissue

• Serum or Whole blood	Liver
<u>Royal blue-top Vacutainer® tubes</u>	Falcon tubes

Liver Biopsy videos

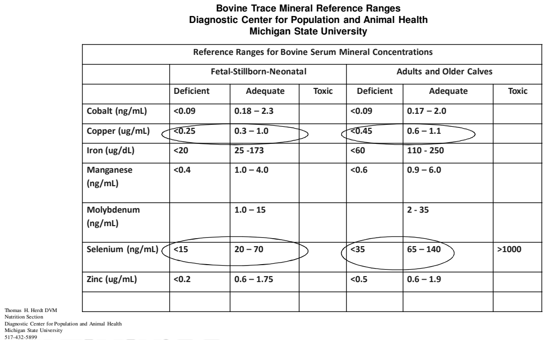
- Dr. Steve Ensley – IA State
  <u>https://www.youtube.com/watch?v=yqFS0OwBRMk</u>
- Dr. Jeff Hall - UT State
  https://www.youtube.com/watch?v=3ZhezywrN8U

Tru-Cut 14 ga. x 6" biopsy punch

Manufacturer:	Allegiance
MWI SKU:	001093
Manf Code:	BXT2N2704X
Price:	$40.63

Veterinary Toxicologists/Nutritionists

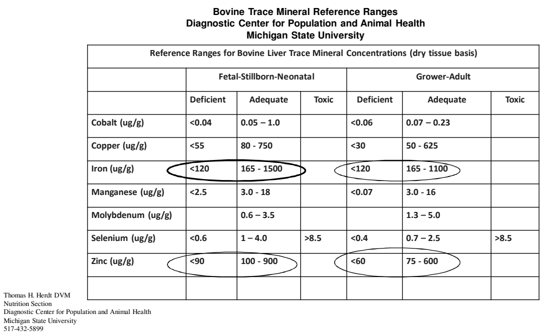
- **Jeff Hall – Utah State University**
  - http://www.usu.edu/uvdl/
- **Steve Ensley – Iowa State University**
  - http://vetmed.iastate.edu/diagnostic-lab/diagnostic-services/diagnostic-sections/chemistry-/-toxicology
- **Thomas Herdt – Michigan State University**
  - http://animalhealth.msu.edu/



Bovine Trace Mineral Reference Ranges
Diagnostic Center for Population and Animal Health
Michigan State University

Reference Ranges for Bovine Serum Mineral Concentrations						
	Fetal-Stillborn-Neonatal			Adults and Older Calves		
	Deficient	Adequate	Toxic	Deficient	Adequate	Toxic
Cobalt (ng/mL)	<0.09	0.18 – 2.3		<0.09	0.17 – 2.0	
Copper (ug/mL)	<0.25	0.3 – 1.0		<0.45	0.6 – 1.1	
Iron (ug/dL)	<20	25 -173		<60	110 - 250	
Manganese (ng/mL)	<0.4	1.0 – 4.0		<0.6	0.9 – 6.0	
Molybdenum (ng/mL)		1.0 – 15			2 - 35	
Selenium (ng/mL)	<15	20 – 70		<35	65 – 140	>1000
Zinc (ug/mL)	<0.2	0.6 – 1.75		<0.5	0.6 – 1.9	

Thomas H. Herdt DVM
Nutrition Section
Diagnostic Center for Population and Animal Health
Michigan State University
517-432-5899



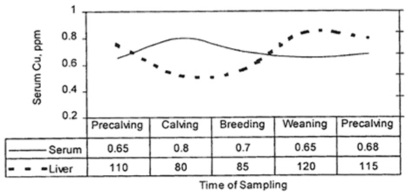
Bovine Trace Mineral Reference Ranges
Diagnostic Center for Population and Animal Health
Michigan State University

Reference Ranges for Bovine Liver Trace Mineral Concentrations (dry tissue basis)						
	Fetal-Stillborn-Neonatal			Grower-Adult		
	Deficient	Adequate	Toxic	Deficient	Adequate	Toxic
Cobalt (ug/g)	<0.04	0.05 – 1.0		<0.06	0.07 – 0.23	
Copper (ug/g)	<55	80 - 750		<30	50 - 625	
Iron (ug/g)	<120	165 - 1500		<120	165 - 1100	
Manganese (ug/g)	<2.5	3.0 - 18		<0.07	3.0 - 16	
Molybdenum (ug/g)		0.6 – 3.5			1.3 – 5.0	
Selenium (ug/g)	<0.6	1 – 4.0	>8.5	<0.4	0.7 – 2.5	>8.5
Zinc (ug/g)	<90	100 - 900		<60	75 - 600	

Thomas H. Herdt DVM
Nutrition Section
Diagnostic Center for Population and Animal Health
Michigan State University
517-432-5899

Part 5
Trace Mineral Patterns in Cattle Production

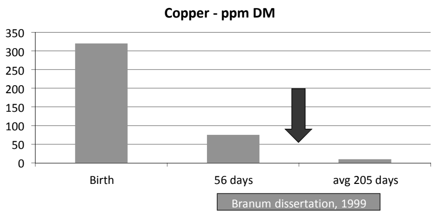
Figure 3: Changes in liver and serum copper concentrations for beef cows (Swenson, 1998).



	Precalving	Calving	Breeding	Weaning	Precalving
—— Serum	0.65	0.8	0.7	0.65	0.68
- - -Liver	110	80	85	120	115

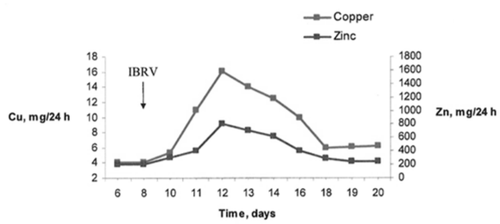
Time of Sampling

Liver copper depletion



Branum dissertation, 1999

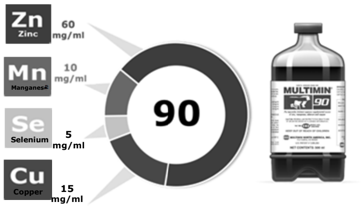
Infectious Challenge and Minerals

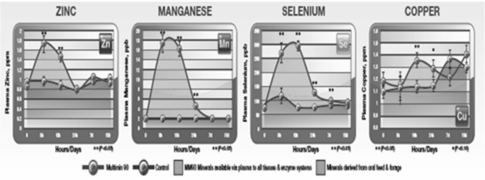


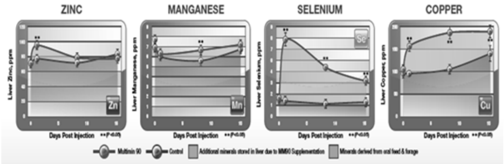
Orr, CL, et al, 1990 JAS v68 p2893

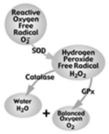
Part 6
Injectable Trace Mineral Supplementation for Cattle

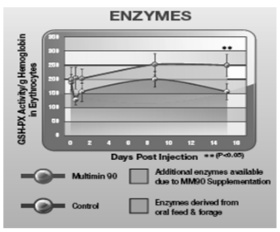
MULTIMIN 90 provides **Zinc**, **Copper**, **Manganese** and **Selenium** in a readily available injection











With Injectable Multimin 90

- You supplement every animal you inject
- You provide supplementation quickly
- You by-pass any antagonists
- You target periods of high challenge and demand for your cattle