New Frontiers in Mineral Nutrition

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Agenda

- Overview of trace minerals
  - Forms of trace minerals
- Nutrient management & the environment
- Antagnists
- Bioavailability estimation and effect on determination of dietary supply of trace minerals
- Determination of animal trace mineral status
  - Useful biomarkers of trace mineral status
  - Experimental determination of trace mineral status
- Cow/calf (pre-calving, pre-weaning and receiving programs)

Agenda (cont)

- The role of minerals on responses to immunization, function of chelates on better immunity
- Antagonists and the function of chelates
- Summary

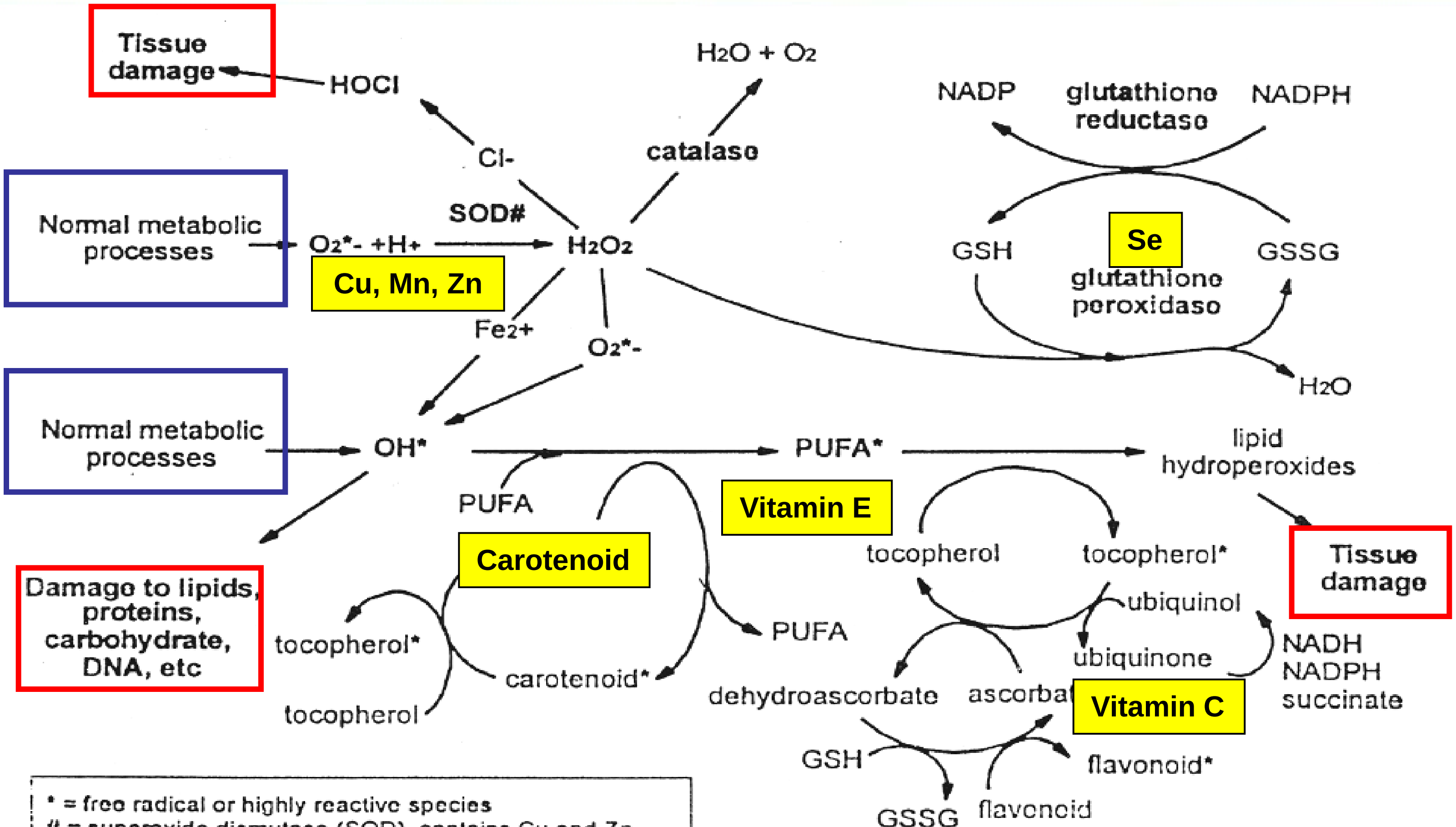
Overview of minerals in nutrition

- Structural
  - Can form important structural components in body organs and tissues
  - Ca and P in bone
  - P and S in muscle
- Physiological
  - Can occur in body fluids and tissues as electolytes
  - Na, K, Cl, Ca in blood
- Catalytic
  - Can act as catalysts or cofactors for enzyme and hormone systems
  - Fe, Cu, Zn, Mn, Se in an enormous range of enzymes
- Regulatory
  - Can regulate cell replication and development
  - Ca with hormonal signaling
  - Zn with gene expression

Catalytic and regulatory functions of some trace minerals

Trace mineral	Metalloprotein	Protein Role	Function
Copper	Cytochrome oxidase	Terminal link in the electron transport chain; permits formation of ATP	Energy metabolism
	Lysyl oxidase	Lysine oxidation in elastin and collagen crosslinking	Connective tissue formation and integrity
	Superoxide dismutase	converts superoxide into oxygen and hydrogen peroxide	Protects against oxidative damage
Manganese	Glycosyl-transferase	Proteoglycan synthesis	Bone development and wound healing
	Superoxide dismutase	As above	Protects against oxidative damage
Selenium	Glutathione peroxidase	Reduces hydrogen peroxide and lipid peroxides	Protects against oxidative damage
	Thioredoxin reductase	Reduces thioredoxin	Protects against oxidative damage
Zinc	Collagenase	Breaks the peptide bond in collagen	Tissue remodeling, bone development and wound healing
	Superoxide dismutase	As above	Protects against oxidative damage
	Zinc finger transcription factors	Protein-DNA interactions	Regulate gene transcription

Trace minerals as components of antioxidants



* = free radical or highly reactive species
# = superoxide dismutase (SOD), contains Cu and Zn

Forms of Trace Minerals

- Oxides
- Sulfates
- Proteinates
- Amino Acid Complexes
- Chelates

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Nutrient management and the environment

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Efficient nutrient management

- Ration balancing at a completely different level
- Estimating production of wastes and nutrient composition
- Establishing pollutant production
- Mitigating environmental issues

Role of environment on nutrient utilization

- Stress
  - Heat
  - Cold
  - Drought
- Forages
  - Maturity
  - Type
  - Utilization

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Bioavailability

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Inorganic trace mineral bioavailability, %

	Absorption Coefficients (Bioavailability), %				
Mineral	Feeds	Sulfates	Chlorides	Carbonates	Oxides
Cu	4	5	5	-	1
Mn	1	1.2	1.2	0.15	0.25
Zn	15	20	20	10	12

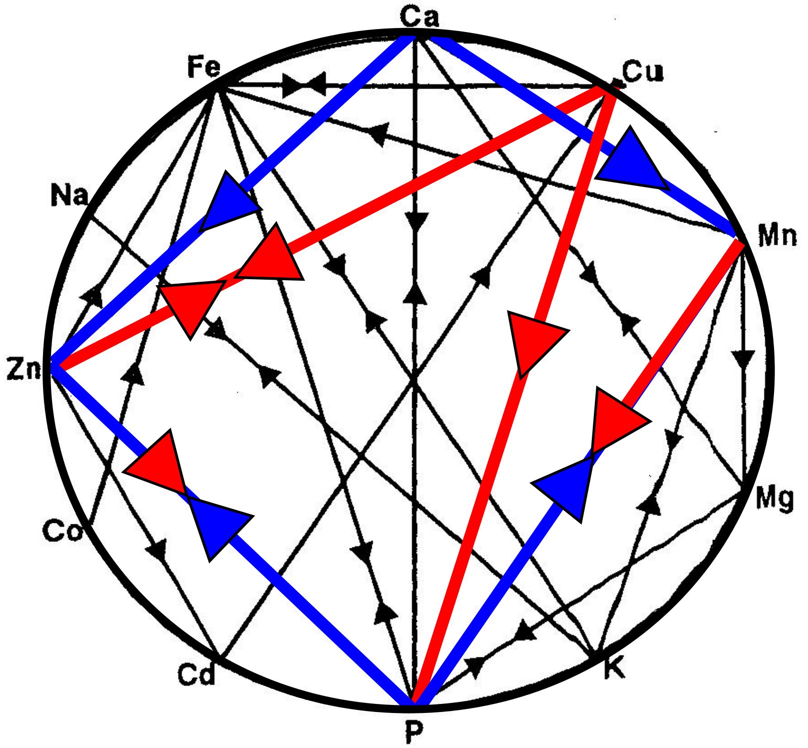
NRC, 2001

Mineral antagonisms affect bioavailability

Dietary Antagonisms

- Copper:
  - S, Fe, Mo
  - High sulfate water
  - DDGS contain high and variable levels of Sulfates
- Manganese:
  - Ca, P, Fe
- Zinc:
  - Ca, P, Cu

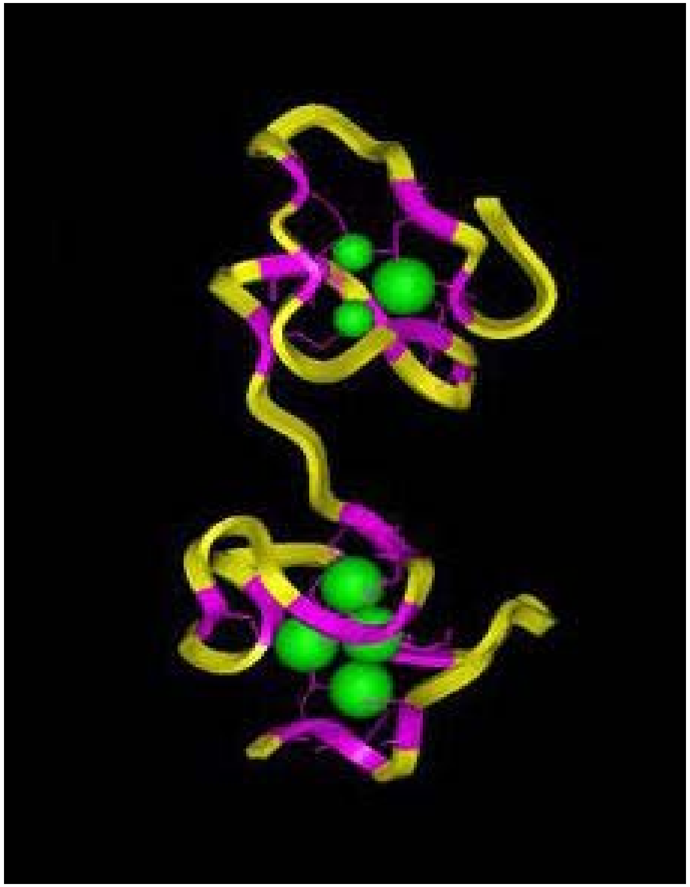
Just feeding more inorganic trace minerals does not cure deficiencies



Biomarkers of trace mineral status

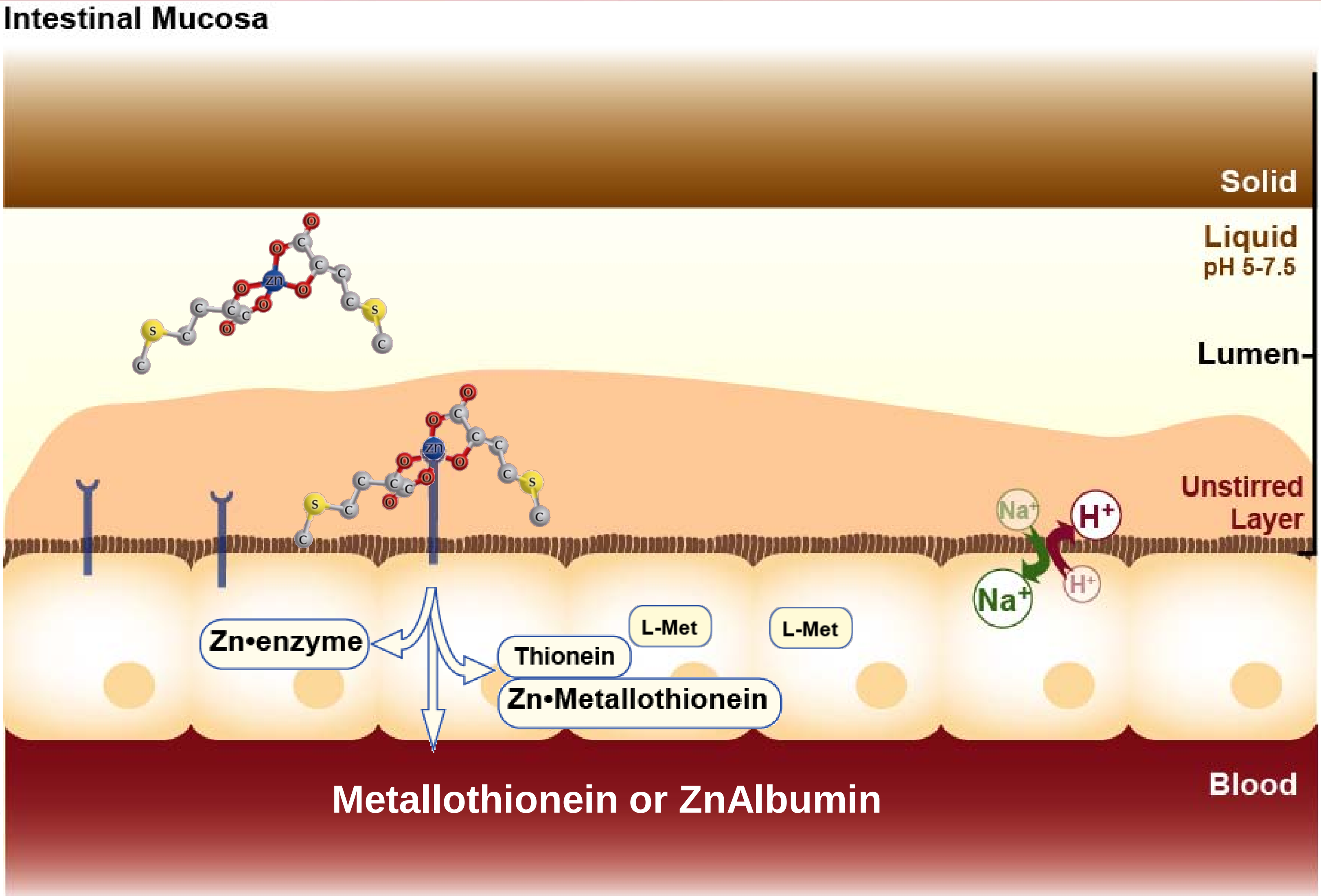
- Zinc
  - Plasma Zinc
  - Activity of Zinc dependent enzymes
  - Liver Zinc
  - Bone Zinc
  - Metallothionein expression
- Copper
  - Plasma copper
  - Plasma ceruloplasmin
  - Liver copper
- Manganese
  - Plasma manganese
  - Bone manganese
- Selenium
  - Plasma selenium
  - Whole blood selenium
  - Milk selenium
  - Plasma glutathione peroxidase

Molecular Assay for Zn Bioavailability: Metallothionein (MT) mRNA Expression



- Metallothionein (MT) proteins bind to Zn and other metals
- One MT protein can bind up to 7 cations, such as Zn
- MT expression is a marker of Zn uptake by cells
  - As Zn absorption increases → MT mRNA and protein increase

Model for Mineral Delivery and Absorption



MT expression is a well-accepted biomarker of zinc absorption & status

Selected Publications

- Chickens
  - Lu et al (1990) *J. Nutr.* 120: 389-397.
  - Cao et al (2002) *Anim. Feed. Sci. Tech.* 101: 161-170.
  - Huang et al (2009) *J. Anim. Sci.* **87:** 2038-2046.
- Pigs
  - Martínez et al (2004) *J. Nutr.* 134: 538-544.
  - Carlson et al (2007) *J. Anim. Phys. & Anim. Nutr.*, **91:** 19-28.
- Sheep
  - Rojas et al (1995) *J. Anim. Sci.* 73: 1202-1207.
- Rodents
  - McCormick et al. (1981) Am. J. Physiol. 240: E414-E421.
  - Blalock et al (1988) *J. Nutr.* 118: 222-228.
  - Reeves (1995) *J. Nutr. Biochem.* 6: 48-54.
  - Blanchard et al (2001) *Proc. Natl. Acad. USA* Sci. 98: 13507-13513.
- Humans
  - Sullivan et al (1998) *J. Nutr.* 128: 707-713.
  - Cao and Cousins (2000) *J. Nutr.* 130: 2180-2187.
  - Aydemir et al (2006) *Proc. Natl. Acad. Sci. USA* 103: 1699-1704.

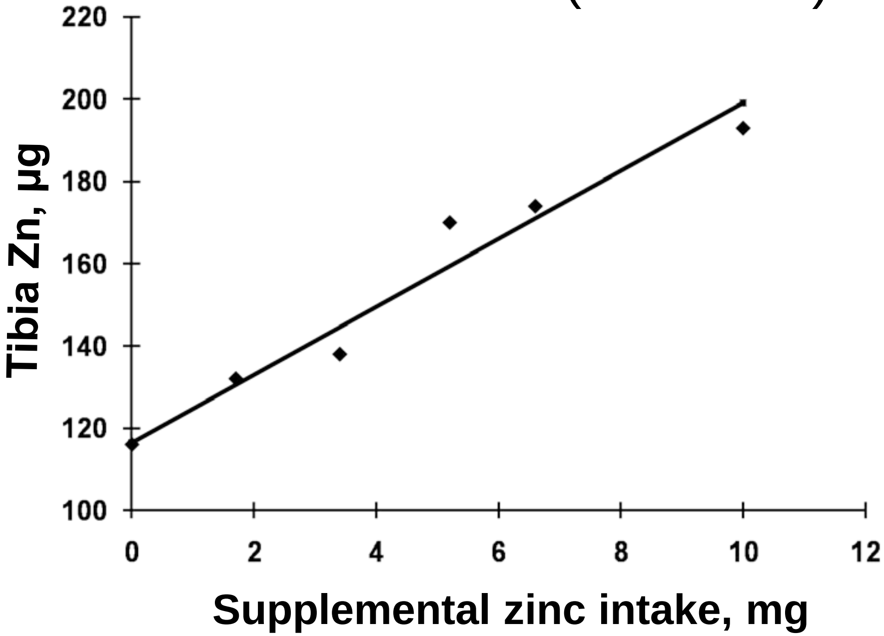
HMTBa Zn bioavailability trial in broilers

- Birds were fed a common low zinc starter, and then switched to treatments (right) on day 8
- Small intestinal metallothionein (MT) measured on day 11
- Tibia zinc was measured on day 14

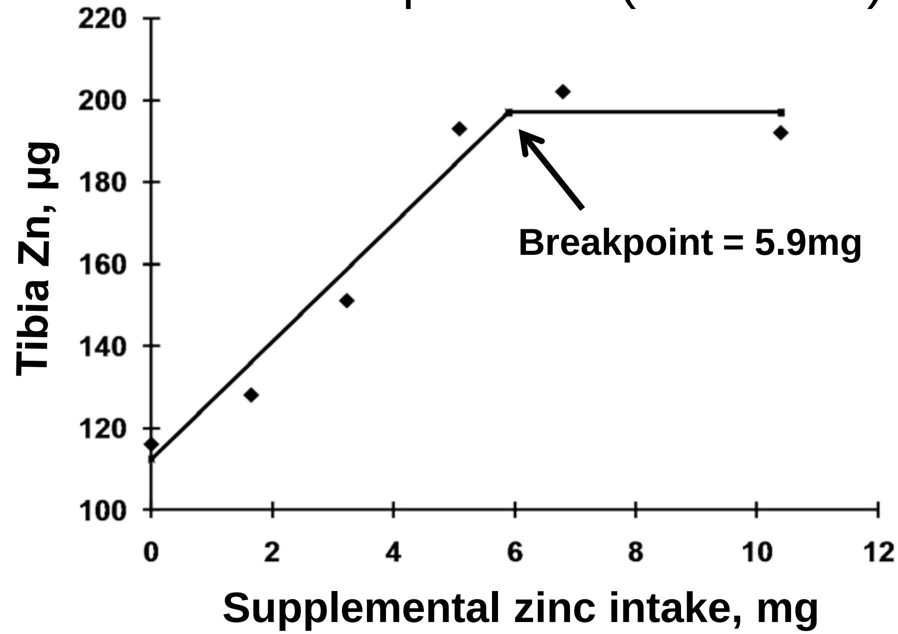
Trt	# pens	Source	Suppl. Zn (ppm)
1	12	None	0
2	6	Sulfate	5
3	6	Sulfate	10
4	6	Sulfate	15
5	6	Sulfate	20
6	6	Sulfate	30
7	6	HMTBa	5
8	6	HMTBa	10
9	6	HMTBa	15
10	6	HMTBa	20
11	6	HMTBa	30

Tibia zinc demonstrates greater bioavailability of HMTBa Zn

Zn Sulfate: linear (P<0.0001)



HMTBa Zn: quadratic (P=0.0006)



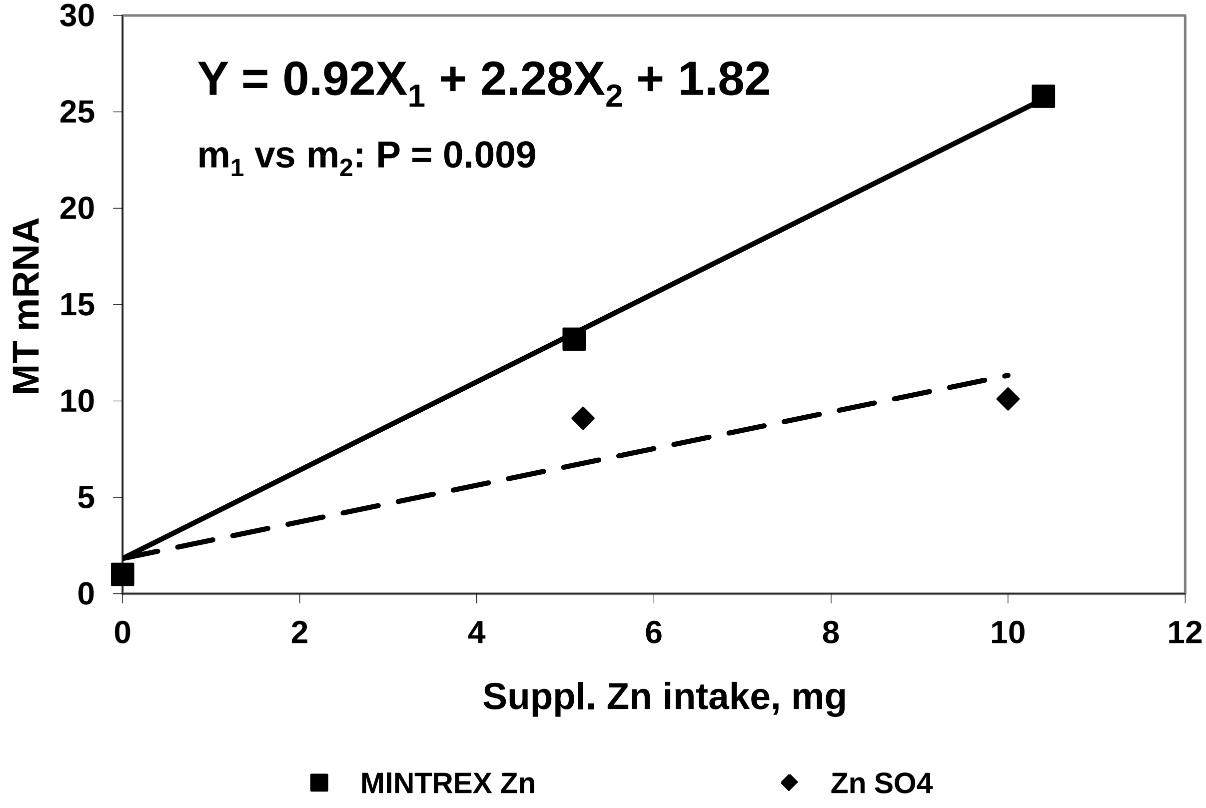
$$Y = 8.66X_1 + 13.93X_2 + 113.7$$

m_1 vs m_2: P = 0.001

Slope ratio = 161%

Novus, Data on file

MT assay also demonstrates greater bioavailability of HMTBa Zn



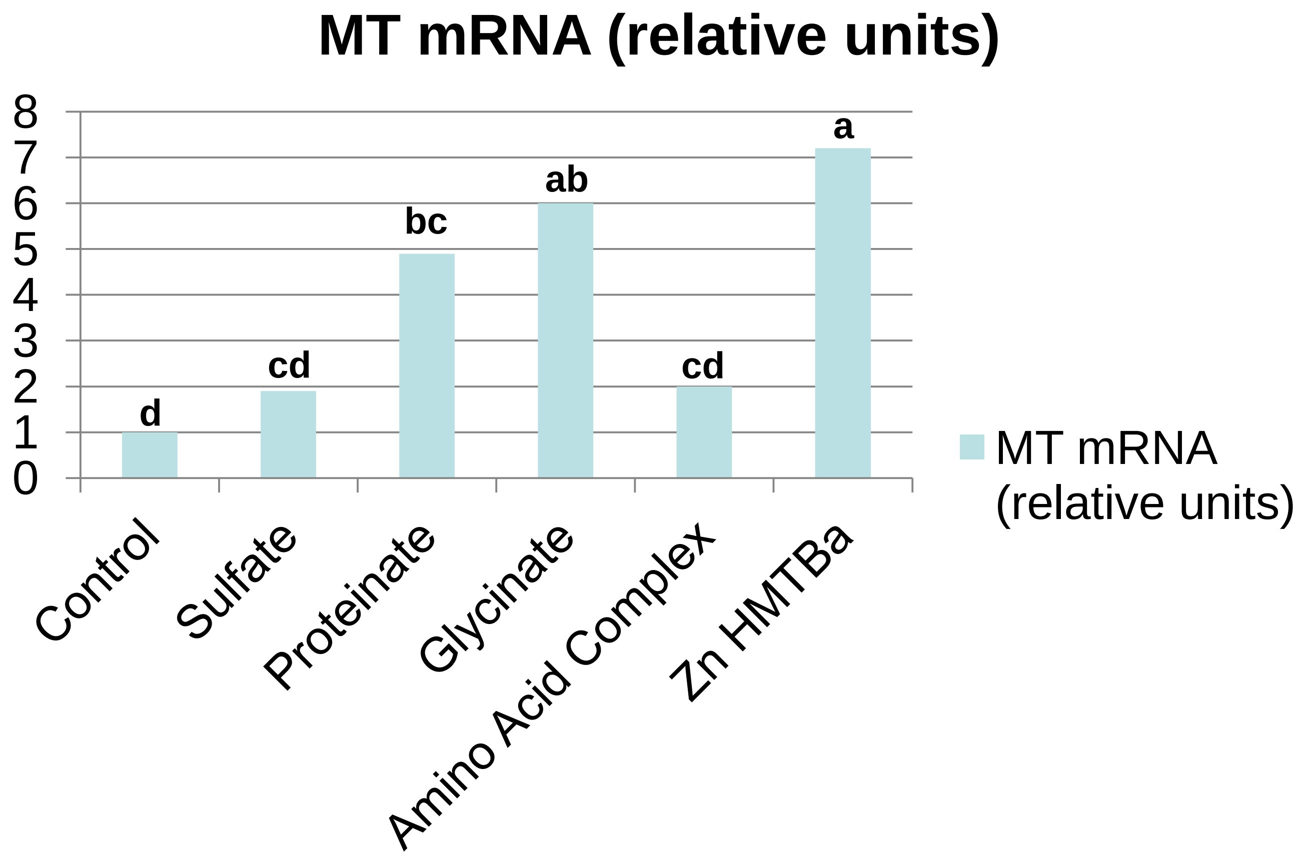
Slope ratio = 248%

Novus, Data on file

Zinc Bioavailability Trial in Broilers

- Broilers on a milo-soy diet for 13 days
- Placed on corn-soy treatment diets on day 14:
  - Control 35 ppm Zn from ingredients
  - Zinc Sulfate +70 ppm Zn
  - Zinc Proteinate +70 ppm Zn
  - Zn Glycine +70 ppm Zn
  - Zn Amino Acid Complex +70 ppm Zn
  - Zn HMTBa +70 ppm Zn
- Jejunum samples collected on day 16 for MT assay

MT Assay Shows Zn Absorption



Novus, Data on file

Effects of trace mineral sources on bioavailability and function in dairy cattle.

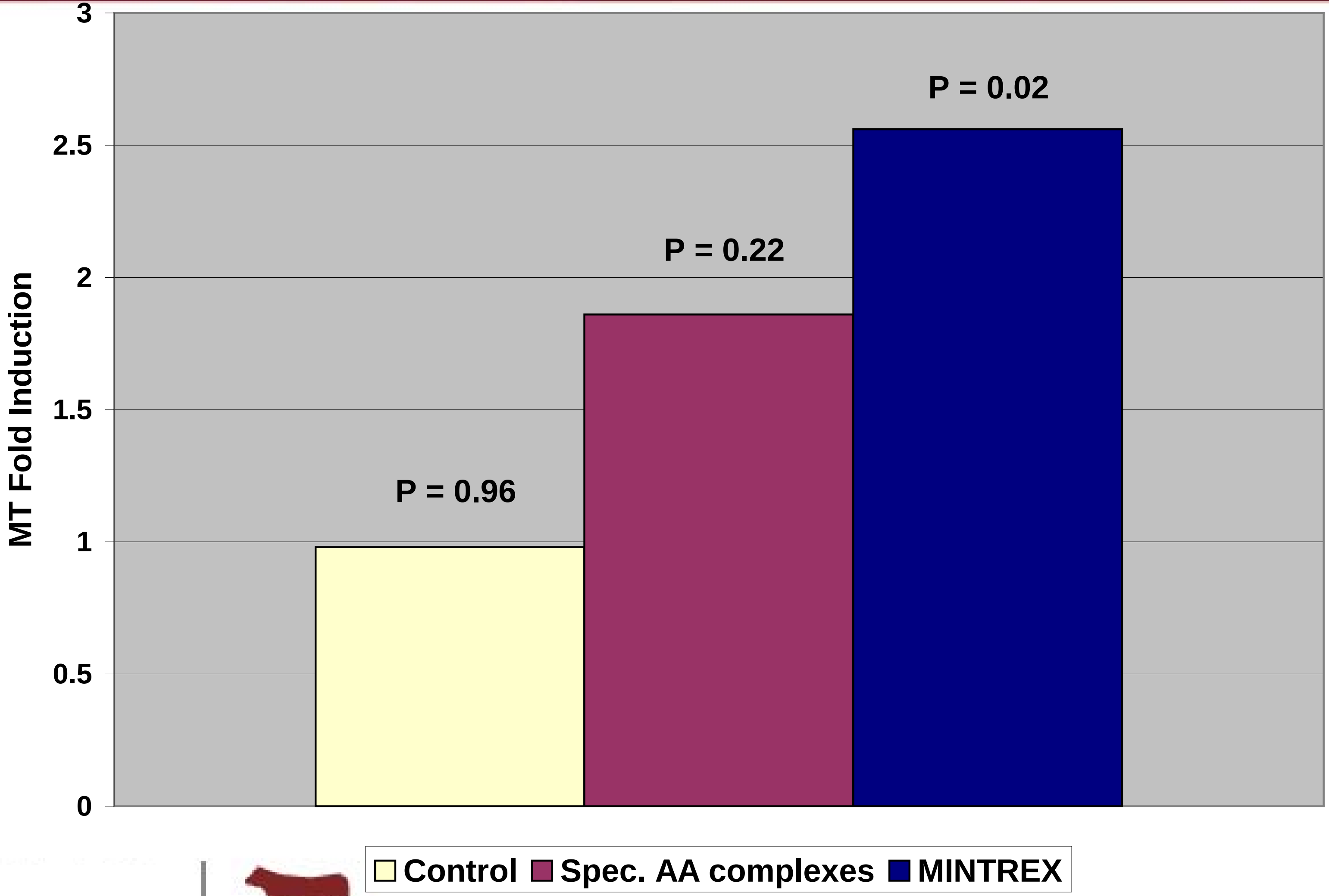
- 30 lactating multiparous dairy cows fed basal diet for 3 weeks
- Split into 3 groups (10 per) for 4 weeks
  - Basal control diet
  - Basal plus additional 320 mg Zn, 150 mg Cu, 130 mg Mn
    - HMTBa Chelate (14 g/d of HMTBa blend with 2g Zn, 1g Cu, 1g Mn) (also supplies 3.14 g HMTBa)
    - Metal specific amino acid complex blend (ZnMet, CuLys, MnMet—also supplies 0.93 g methionine and 0.69 g lysine)

- Basal diet: 53 ppm Zn, 11 ppm Cu, 46 ppm Mn
- Supplemented diets: 66 ppm Zn, 17 ppm Cu, 51 ppm Mn

- Collect liver for MT analysis
  - Prior to treatment diets (Week 0)
  - After one week on treatment diets (Week 1)

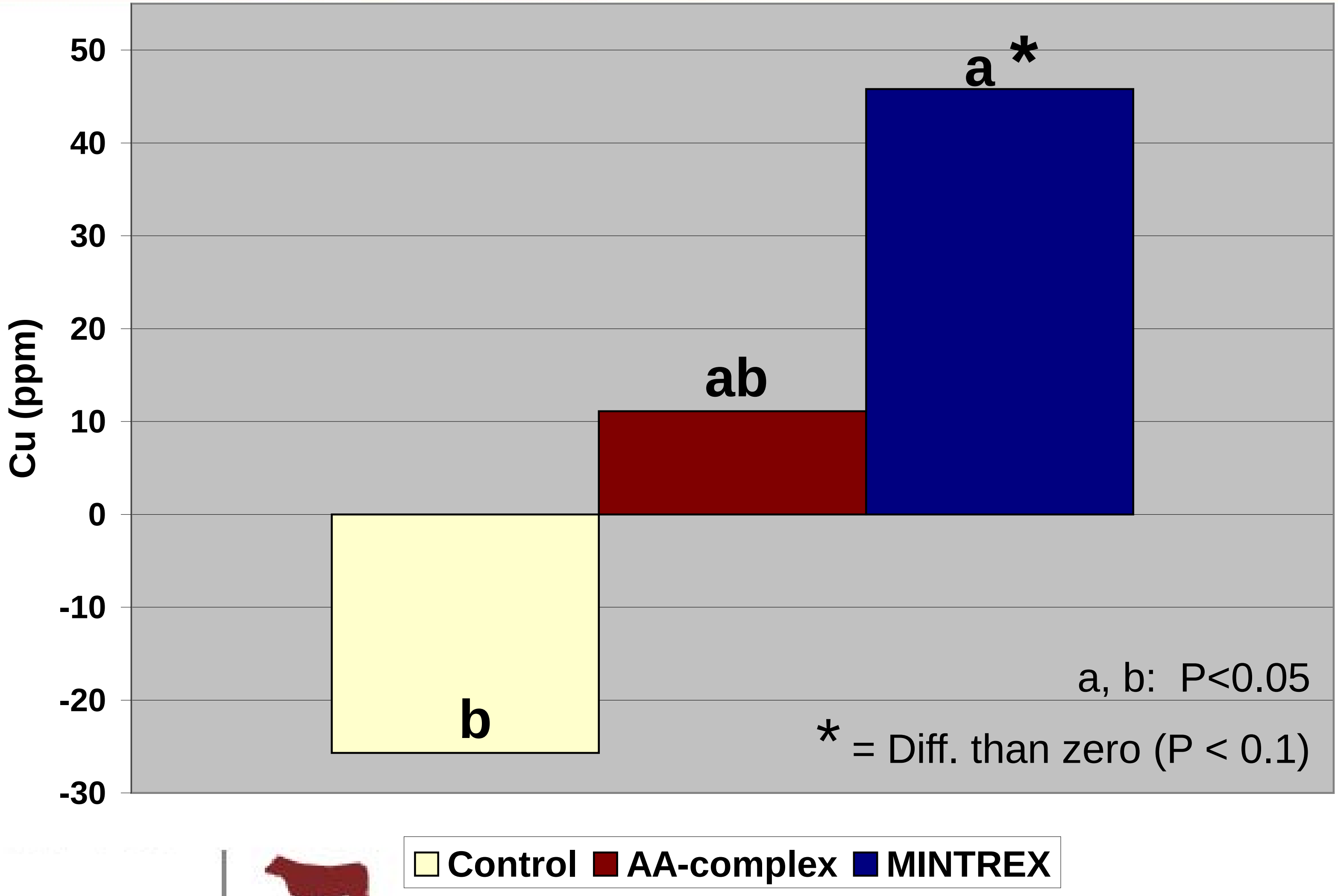
Thering et al., 2007

Only the cows on HMTBa OTMs had a significant increase in MT expression



Thering et al., 2007

Delta Liver Copper—(Week 4 minus Week 0, ppm)



Thering et al., 2007

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Cow/calf (pre-calving and pre-weaning)

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Effect of maternal trace mineral source on cow/calf performance and the subsequent feedlot performance of beef calves from high and low marbling lines

- Objective:
  - evaluate the performance of beef calves supplemented with chelate or inorganic trace minerals as a component of a free-choice mineral supplement starting in late gestation of the dam through weaning of the calves over two years.

- Treatments:
  - Inorganic trace minerals, Zn, Cu, and Mn supplemented as sulfates at NRC levels (assuming 4 oz AF intake [113 g] per hd per d of supplement and 11 kg/d of DMI; 30 ppm Zn, 15 ppm Cu, and 40 ppm Mn) and MFP supplemented to balance the methionine provided by the HMTBa in treatment 2
  - HMTBa Zn, Cu, and Mn supplemented at an equal mineral intake to the inorganics in treatment 1

Calf weaning weight per cow exposed was greater in cows fed chelate

Reproductive Performance	Inorganic	Chelate	S.E.	*P* Value
Calf BW, kg	34.4	34.5	0.4	0.90
Adjusted 205-d WW, kg	242.7	249.1	3.6	0.25
Calf Weaning, %	94.2	96.5	1.2	0.21
kg of Calf Weaned/cow exposed	228.5	240.1	2.2	0.008
Conception Rate, %	87.7	87.6	4.7	0.98

Novus, Data on file

Comparison of chelated versus inorganic trace minerals on rate and efficiency of gain and pregnancy rates in beef heifers

- Objective:
  - evaluate any differences in rate and efficiency of gain and conception rates in heifers supplemented with either a methionine chelated form of Cu, Zn, and Mn (chelate) or a SO_4 form of Cu, Zn, and Mn.

- The trial was replicated across 3 ranches:
  - Dillon, MT (498 Angus heifers)
  - Terry, MT (236 Red Angus x Charolais x Tarentaise heifers)
  - Ranchester, WY (1742 Angus x Composite heifers).

Whitehurst et al., 2012

% Bred was increased for heifers fed Chelate, particularly on Ranch 3

							SE	P-value		
	Ranch 1		Ranch 2		Ranch 3			Trt	Ranch	Ranch X Trt
Treatment	**Sulfate**	**Chelate**	**Sulfate**	**Chelate**	**Sulfate**	**Chelate**				
No. heifers	251	246	120	119	870	872				
Days on Test	181	181	149	149	77	77				
Initial BW, kg	250	251	269	270	289	295	3			
End BW, kg	341	340	390	391	347	349	4			
Gain, kg	91	90	121	121	59	55	3	0.6	<0.01	0.88
ADG, kg/d	0.505	0.495	0.814	0.814	0.764	0.705	0.027	0.57	<0.01	0.76
F:G	13.74	13.65	8.4	8.41	9.44	8.98	1.06	0.91	<0.01	0.85
% Bred	85	86	92	91	59	66	0.02	0.05	<0.01	0.47
% Bred 1^{st} Service	58	57	54	51	59	66	0.02	0.12	<0.01	0.54

Whitehurst et al., 2012

Receiving and Immunity

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The effect of chelates (Cu, Zn, Mn) on the health and growth performance of high-risk calves during a 42 day receiving study

- Objective:
  - To evaluate the effects of feeding Glycine Zn, Cu, Mn, Se-Yeast, MOS, and antioxidants on growth performance, feed efficiency, morbidity, mortality and drug costs in highly stressed, newly weaned calves.
- Collaborator: Eric Larson, Larson Nutrition Services and Apishapa Ranch

Treatments

- **Control** – calves will receive a diet with inorganic minerals as the only source of minerals and at levels defined by the feedlot nutritionist.

- **Starter Pack** - Calves will be fed chelates (Cu, Zn, Mn), Se-Yeast, MOS, and antioxidants. Supplements will be formulated to contain iso-mineral levels of Cu, Zn, Mn, and Se as the control supplement.

Rations

	Control	Starter Pack
Rolled Corn	47	47
Ground Sorghum Hay	11.6	11.6
Ground Alfalfa Hay	24.5	24.5
Sorghum Silage	8.2	8.2
Suspension Supplement	5	5
Control Mineral Meal	3.7	-
Novus Mineral Meal	-	3.7

Composition

	Control	Starter Pack
DM, %	74.31	74.66
Cu, ppm	27.87	28.17
Zn, ppm	99.39	103.54
Mn, ppm	62.44	65.73
Se, ppm	0.43	0.42
Nem, Mcal/lb	0.81	0.82
NEg, Mcal/lb	0.49	0.49
CP, %	12.7	12.27
ADF, %	17.51	17.08

Cattle performance did not differ

Item	Control	Starter Pack	SEM	P-Value
Day 0	514	513	0.89	0.93
Day 14	547	548	1.31	0.67
Day 28	573	574	1.47	0.74
Day 42	609	611	1.8	0.66

Item	Control	Starter Pack	SEM	P-Value
ADG, lbs/d	2.34	2.37	0.06	0.73
DMI, lbs/d	13.17	13.32	0.14	0.45
Feed/Gain	5.67	5.69	0.12	0.92

Times pulled did not differ

Treatment	Pens	Head	Pulled 1 Time	Pulled 2 Times	Pulled 3 Times	Pulled 4 times	Pulled 5 times
Control	8	478	325	110	31	12	2
Starter	8	477	328	97	31	14	2

The starter pack did not impact morbidity, but reduced mortality

Item	Control	Starter Pack	SEM	P-Value
Morbidity, % of pen				
Pulled 1x	68.08	68.78	2.56	.85
Pulled 2x	23.05	20.33	1.95	.36
Pulled 3x	6.47	6.49	.83	.99
Pulled 4x	2.51	2.93	.80	.72
Days Between Re-pulls	6.35	6.26	.25	.80
Medicine cost, $/hd	16.18	15.88	.78	.80
Deads, %	2.09	1.04	.31	**.05**
Total Out, % (Realizers+Deads)	3.14	2.93	.61	.95

Conclusion

- Performance did not differ between treatments
- Morbidity did not differ between treatments
- Mortality was halved by the starter pack

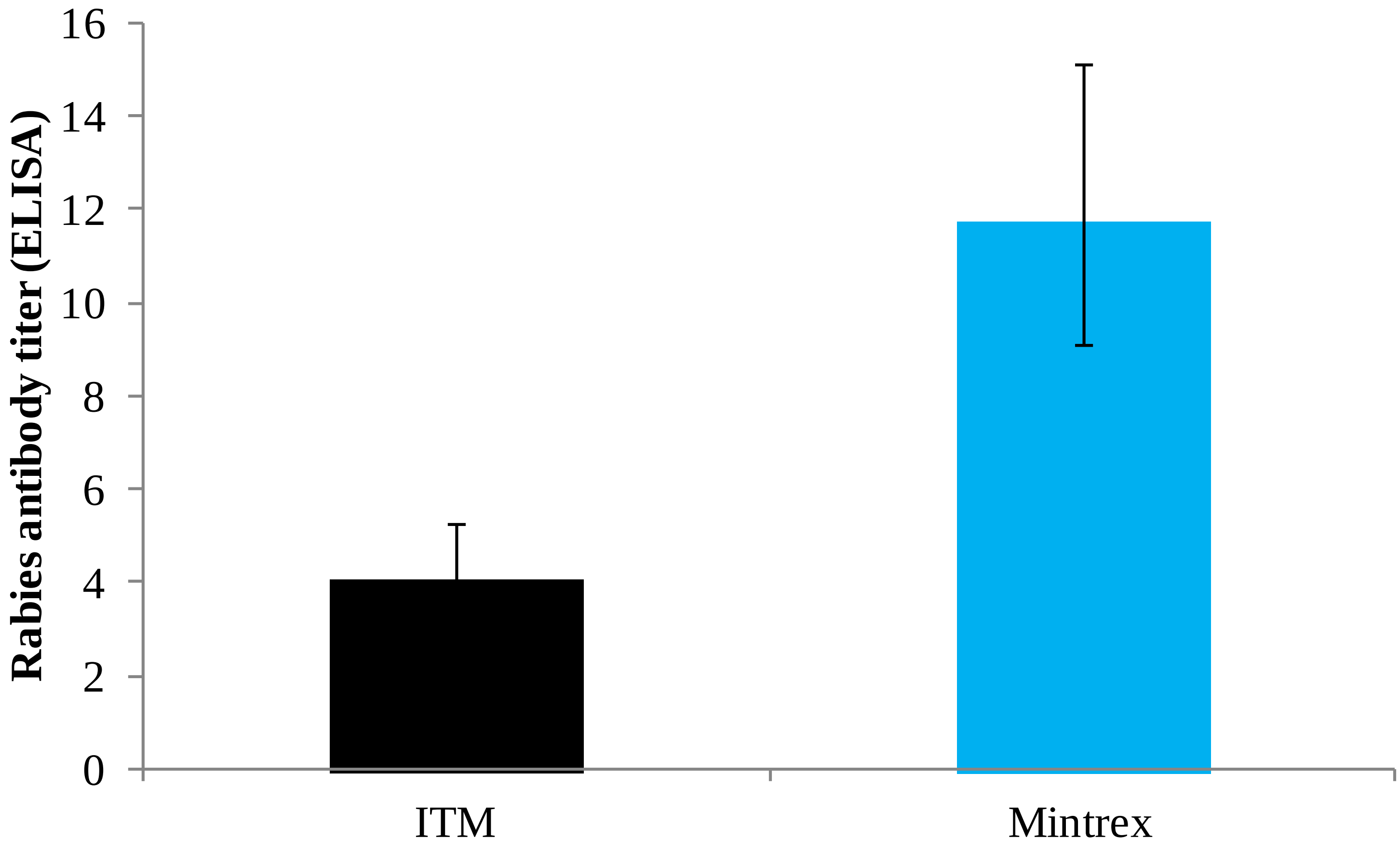
Immune responses in lactating Holstein cows supplemented with Cu, Mn, and Zn as sulfates or chelates

- Objective:
  - compare performance, plasma and milk minerals, and measures of innate and adaptive immune function in early lactation cows fed Cu, Mn, and Zn supplied by either inorganic or chelated organic sources for 12 wk.
- Materials and Methods:
  - 26 Holstein cow (all parities) in early lactation
  - Diets supplemented with sulfates or chelates to NRC
  - Vaccinated for rabies on week 8

	ITM	Chelates
Zn	73	94
Cu	21	23
Mn	42	46

Nemec et al., 2012

Cows fed Mintrex had a greater antibody titer to rabies than cows fed ITM



Nemec et al., 2012

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Antagonists

Performance in the face of antagonists

Iowa Feedlot

- **Retained Ownership/Custom Finish**
- **Purchased $H_2$0 from Town**
  - **6-8cents/hd/d 16.1 ppm S**
  - **Morbidity 10% Mortality 2 % (400-525# calves)**
- **Reduce Production Costs- Well $H_2$0**
  - ***No other management changes***
  - **1,700 ppm Sulfate**
  - **Morbidity 30% Mortality 5-7% (most attributed to respiratory disease)**
  - **↑ Dark Cutting Carcasses**

High Sulfur content in drinking water and feedstuffs causes antagonisms with Cu and other trace minerals

Cu deficiency leads to Immune Function disorders

Vázquez-Añón et al., 2007

Case study:

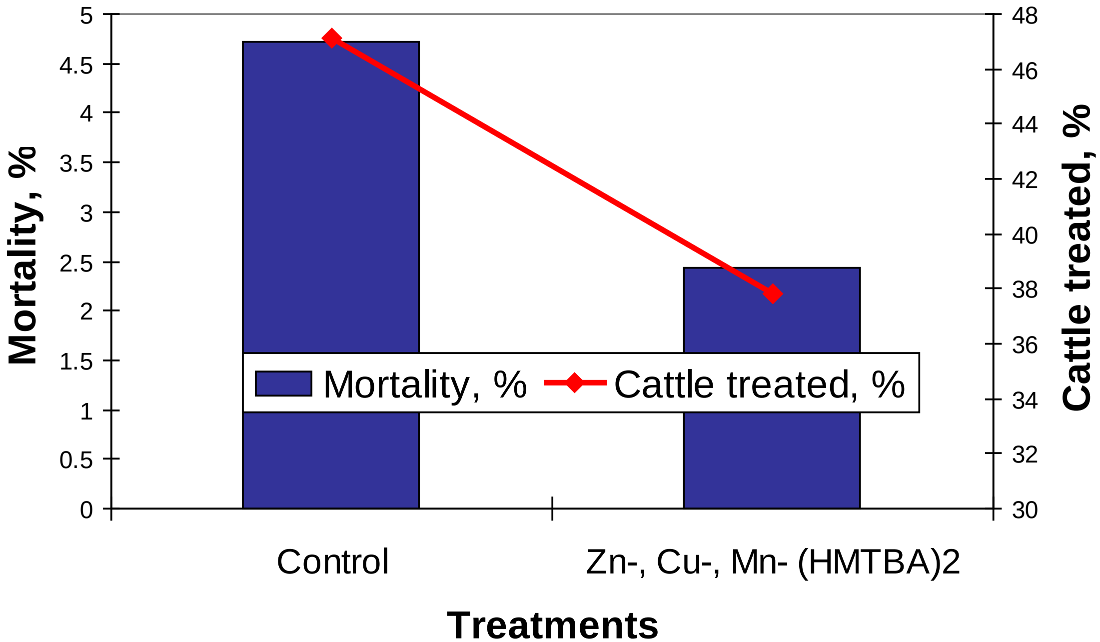
Iowa Feedlot

- Design
  - 4 pens/trt - 80hd/pen
  - Identical handling, growth promotant, vaccination regimen, feeding phases
- Treatments:
  - Control: Standard mineral program (79 ppm Zn, 27 ppm Cu, 35 ppm Mn)
  - Chelate: Control+ Chelate (116 ppm Zn, 44 ppm Cu, 50 ppm Mn)

Vázquez-Añón et al., 2007

Sulfate Antagonism Was Alleviated with Organic Trace Minerals

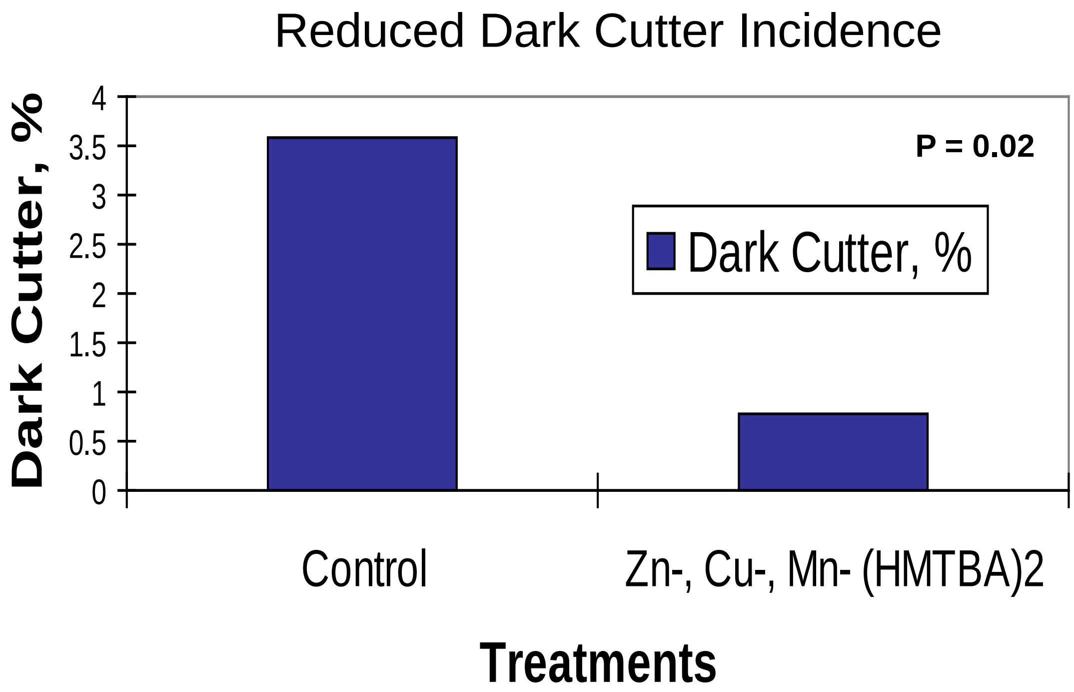
Improved Health of the Herd by Reducing Mortality and Morbidity



Mortality P=0.05; % First Treated P=0.06

Vázquez-Añón et al., 2007

Sulfate Antagonism Was Alleviated with Organic Trace Minerals



Vázquez-Añón et al., 2007

Take home message

- Trace mineral absorption is reduced by antagonisms
- Chelated trace minerals can reduce the impact of antagonisms and are more bioavailable
- Greater bioavailability leads to improved functional benefits in immunity and reproduction

Summary

- Trace minerals are essential for wide ranging biological functions.
- Chelated minerals are more bioavailable than other ITM forms.
  - Tissue mineral experiments
  - Gene expression experiments
- This translates into a variety of benefits:
  - Immune benefits
  - Performance in the face of antagonists
  - Similar or increased performance at lower levels of supplementation
  - Oxidative balance benefits
  - Structural (bone, footpad, eggshell, intestinal breaking, etc) benefits

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Thank you

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