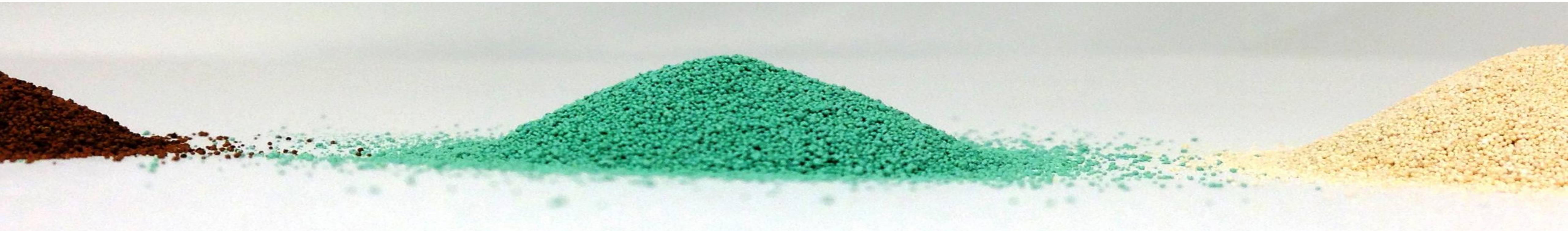
# Copper Hydroxychloride as a beneficial tool in broiler production

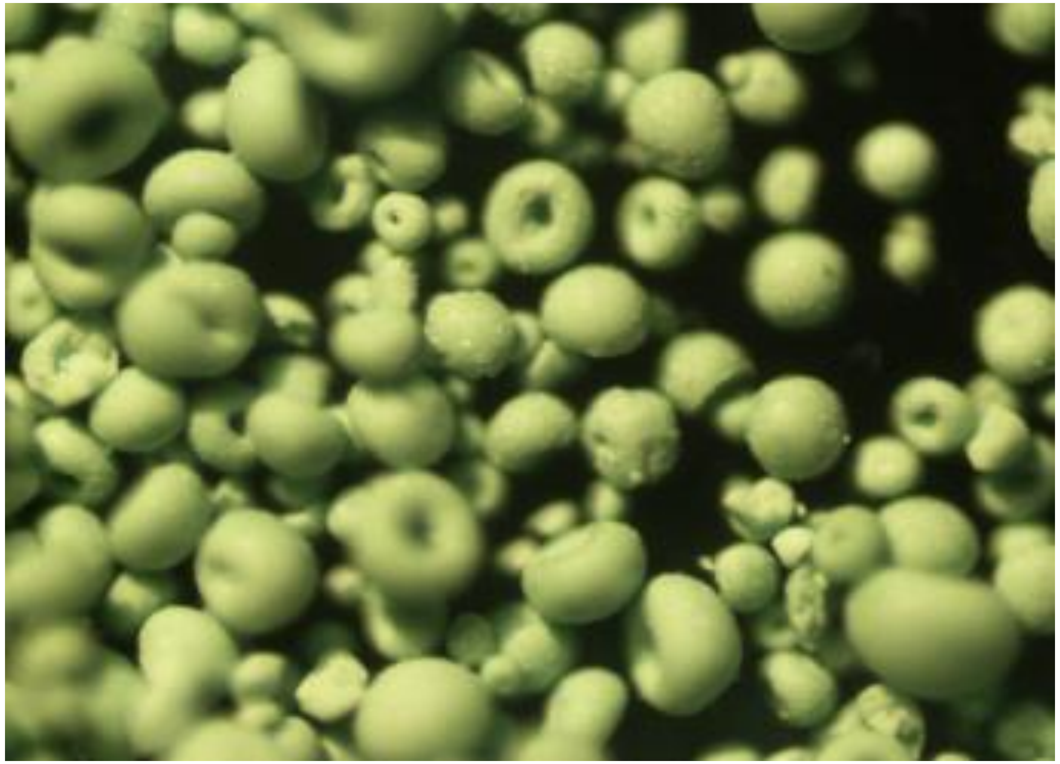
**AFMA, 2020**



Kurt Perryman, PhD

# Introduction:

- **Feeding high levels of copper**
- **Mode of action**
- **MIC work *in vitro/in vivo***
- **Necrotic Enteritis Model Development**



# All natural copper limits microbial growth

Spoiler Alert: Super Bug MRSA Has No Chance against

**In the US, 95% of broilers are fed high levels of Cu (>100mg/kg) at some point in their life-cycle**

Paging Doctor Copper: Metal Wins Fans in Health Care

Copper is used in everything from automobiles to air conditioners, but it has one property that makes it especially attractive for medical uses: It kills bacteria

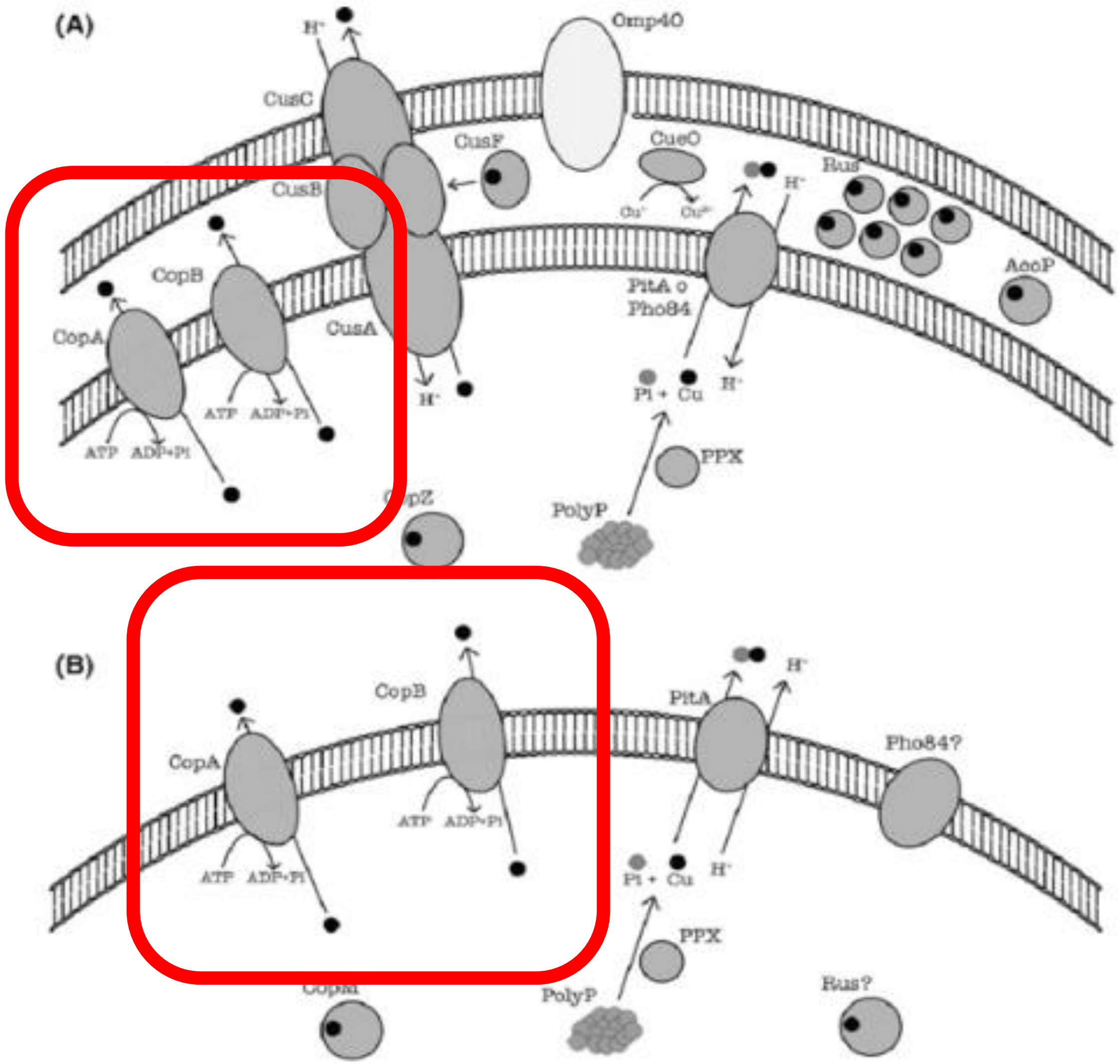
Dr. Copper Turns Bug-Killer as Hospitals Seen Boosting Demand

By Mark Burton

April 26, 2017, 10:28 AM EDT *Updated on* April 26, 2017, 7:01 PM EDT

# Copper prevents colony formation via 'exhaustion'

- **CopA and CopB proteins are the primary means of maintaining Cu homeostasis**
  - **Requires ATP**
- **Copper build-up in cell results in cell death**
- **Because not targeting a single critical 'process', resistance risk is low**




Micronutrients
a Nutreco company

*Bussenius et al., 2016*

# Copper Source Differences

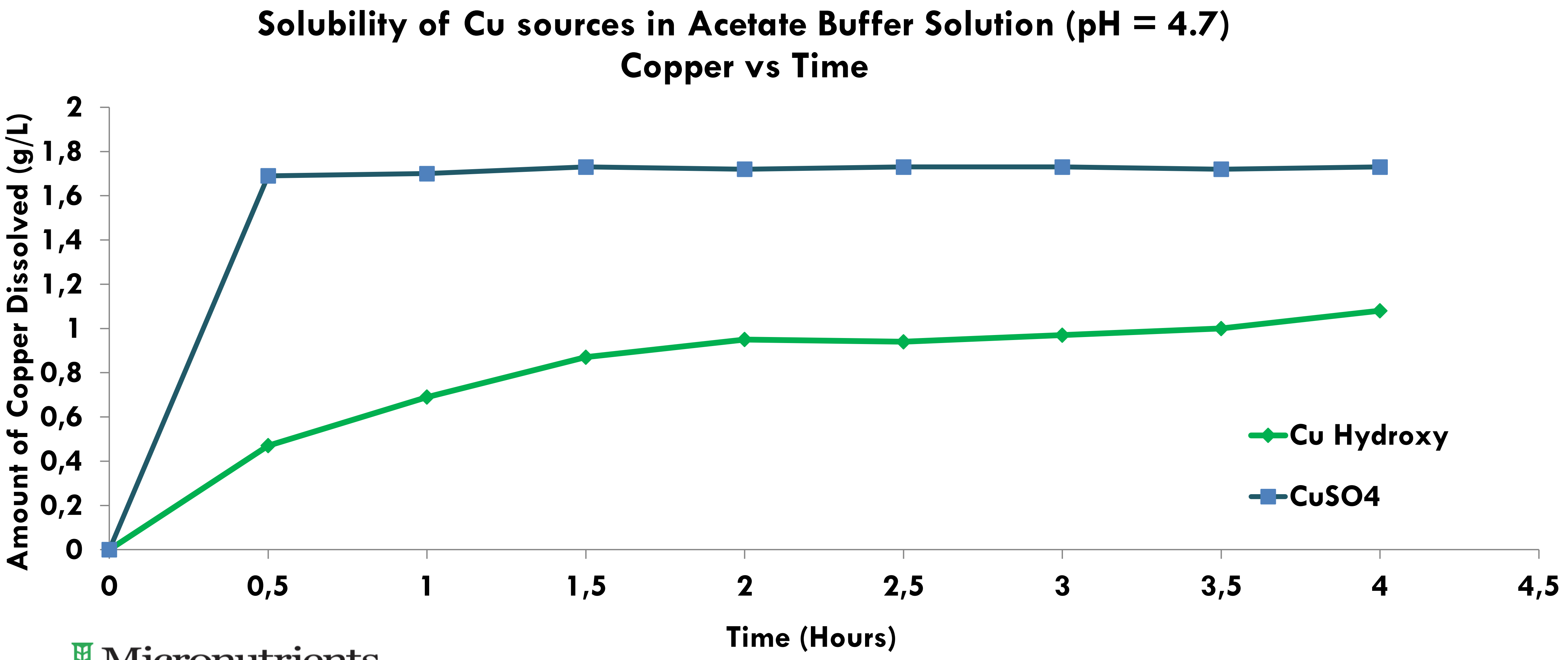
# Covalent vs. Ionic Bonds

- **Ionic Bonds**
  - No sharing electrons

**Better classification than simply Organic vs Inorganic trace minerals**

- **Covalent Bonds**
  - Share electrons (much stronger)
  - Not broken by polar water (Need low pH or molecules with much more favorable electronegative properties)
  - Cu Hydroxychloride, Most Organic Trace minerals

# Solubility Differences

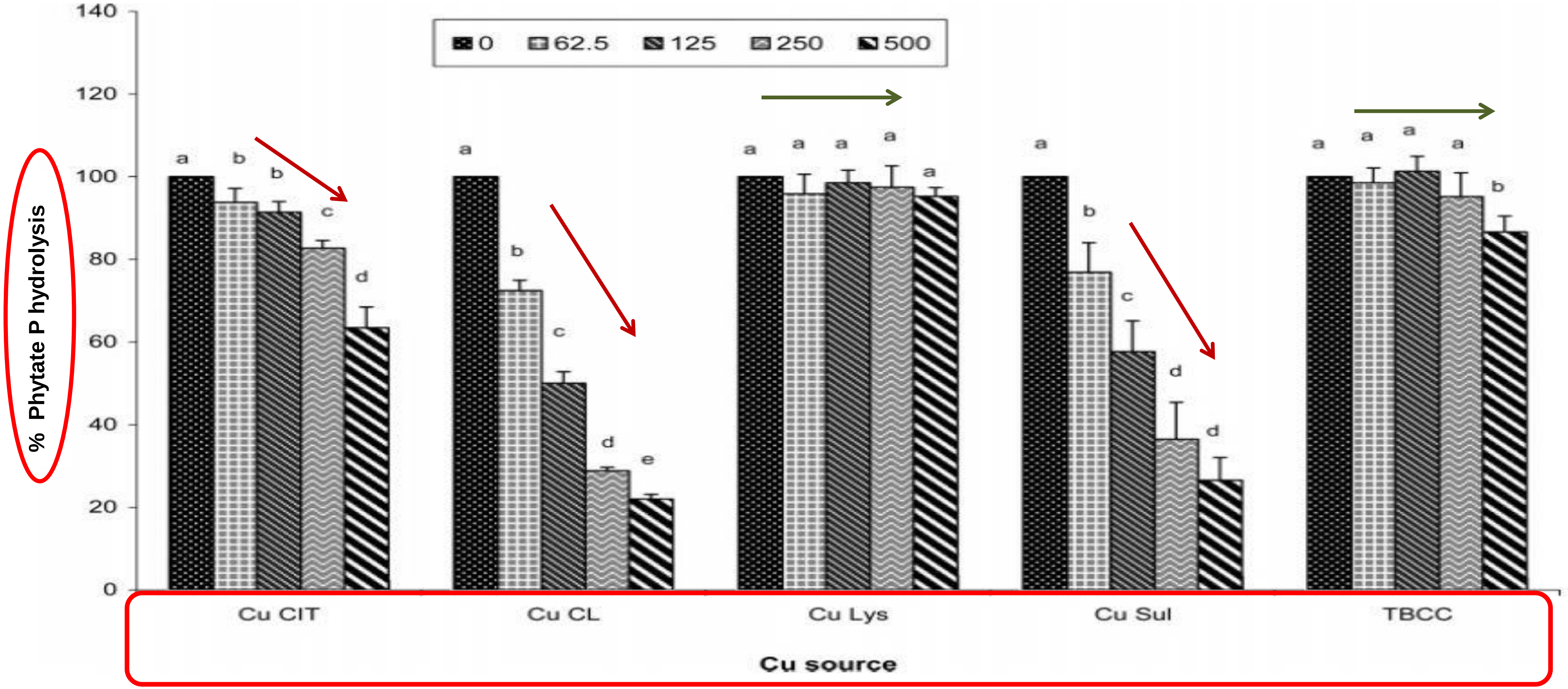


# Copper hydroxychloride is fundamentally different than $CuSO_4$

**<u>Cu Hydroxychloride</u>**
- **Low Solubility**
- **Low Reactivity**

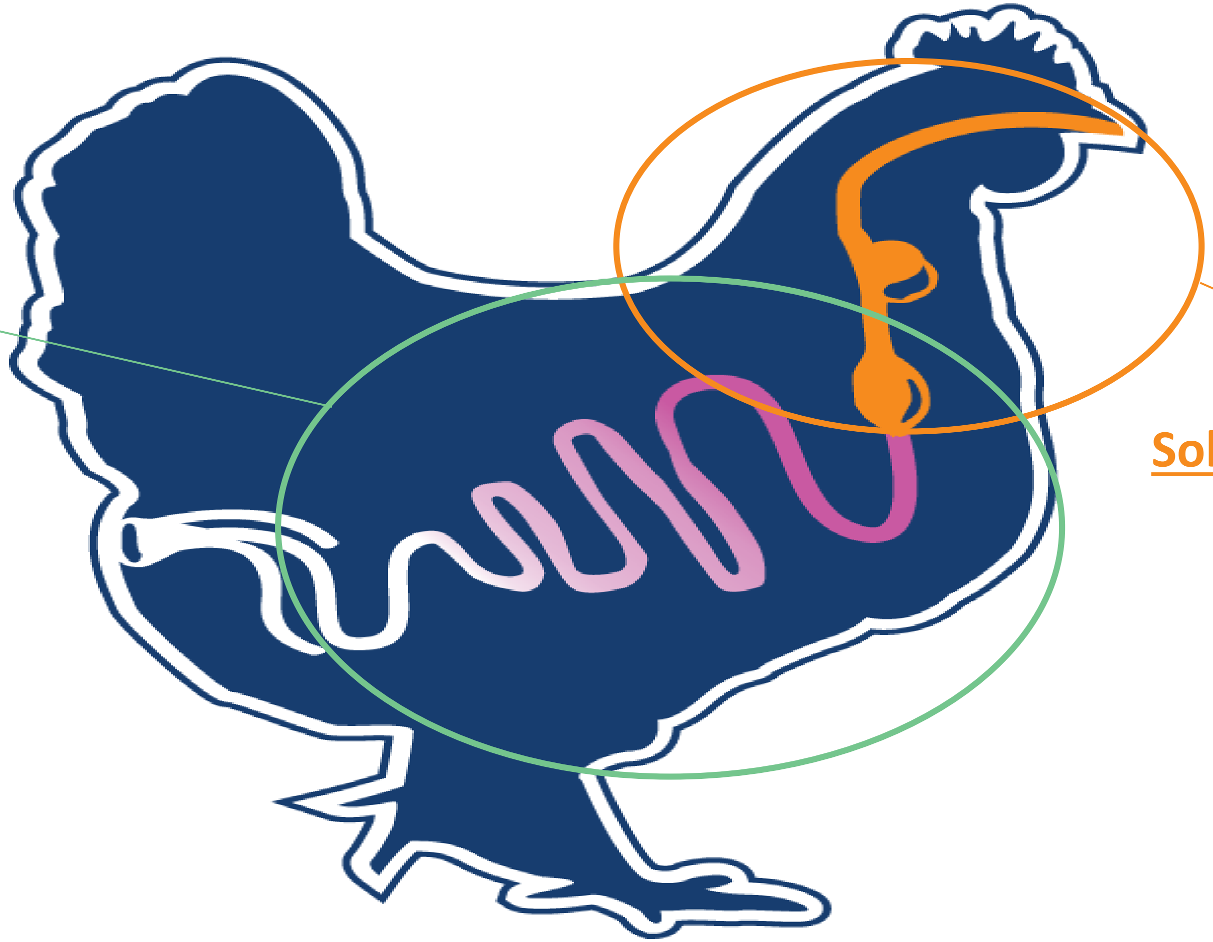
**<u>Cu Sulfate</u>**
- **High Solubility**
- **High Reactivity**

# Copper Antagonisms with Nutrients




*Pang and Applegate, 2006*

# Reactivity Affects Dissociation Location



Cu Hydroxychloride

- Less antagonism
- More bacteria for Cu

Soluble, Ionic Minerals

- Antagonisms
- Less bacteria

# Experimental Data

## A series of trials by Dr. Kirk Klasing, UC Davis

# Minimum Inhibitory Concentration *In Vitro*

- **Nutrient agar supplemented with $CuSO_4$ or Cu Hydroxychloride**
  - 0, 10, 25, 50, 75, 100, 150, 200, 400 or 600 mg/kg

- **Agar with inoculated with approximately 500 bacteria**
  - *E. coli, Clostridia perfringens, Salmonella gallinarum,* or *Salmonella enteritidis* per plate (n = 4) and incubating at 41 C.
  - MIC was defined as the lowest concentration that resulted in no visible colony formation after 20 hrs of incubation

- **Excysed sporozoites from *E. maxima***
  - Viability determined at the end of 16 hrs
  - Dubelcos media.
  - Uptake of 0.5% trypan blue into cells.

# *In Vitro* Minimum Inhibitory Concentration

## Copper Sulfate

| Level mg/kg | E. coli | C. perfringens | S. gallinarum | S. enteritidis | Eimeria % viable* |
|---|---|---|---|---|---|
| 0 | +++ | +++ | +++ | +++ | 88 |
| 10 | +++ | +++ | +++ | +++ | 94 |
| 25 | +++ | +++ | +++ | +++ | 92 |
| 50 | +++ | +++ | +++ | +++ | 86 |
| 75 | +++ | +++ | +++ | +++ | 95 |
| 100 | 135 | +++ | +++ | 150 | 89 |
| 150 | 69 | +++ | 119 | 114 | 94 |
| 200 | 33 | 133 | 106 | 44 | 85 |
| 400 | 4 | 56 | 57 | 9 | 89 |
| 600 | 0 | 19 | 0 | 0 | 88 |
| MIC | 600 | >600 | 600 | 600 | |

## Copper Hydroxychloride

| Level mg/kg | E. coli | C. perfringens | S. gallinarum | S. enteritidis | Eimeria % viable* |
|---|---|---|---|---|---|
| 0 | +++ | +++ | +++ | +++ | 92 |
| 10 | +++ | +++ | +++ | +++ | 95 |
| 25 | +++ | +++ | +++ | +++ | 94 |
| 50 | +++ | +++ | +++ | +++ | 88 |
| 75 | +++ | +++ | +++ | +++ | 84 |
| 100 | +++ | +++ | +++ | +++ | 91 |
| 150 | 102 | 188 | +++ | 91 | 88 |
| 200 | 29 | 38 | 129 | 13 | 88 |
| 400 | 0 | 11 | 6 | 0 | 93 |
| 600 | 0 | 0 | 0 | 0 | 87 |
| MIC | 400 | 600 | 600 | 400 | |

- +++ Too Many to Count
- *ANOVA P values: Source = 0.63; Level = 0.31; Source x Level = 0.70

Micronutrients
a Nutreco company

# *In Vivo/In Vitro* Materials and Methods

- **Chicks (4 pens of 3 chicks each) were fed one of 4 diets:**
  - No supplemental copper
  - 150 mg/kg $CuSO_4$
  - 150 mg/kg Cu Hydroxychloride
  - 150 mg/kg Cu-amino acid chelate
- **After 2 weeks, birds were humanely euthanized and digesta from upper ileum were collected.**
- **Thawed digesta were spiked with 500 bacteria or sporozoites per mL and incubated at 41 C.**
- **Bacteria number (qPCR signal) was analyzed by one way ANOVA.**

# Copper as a microbial growth inhibitor (150mg/kg)

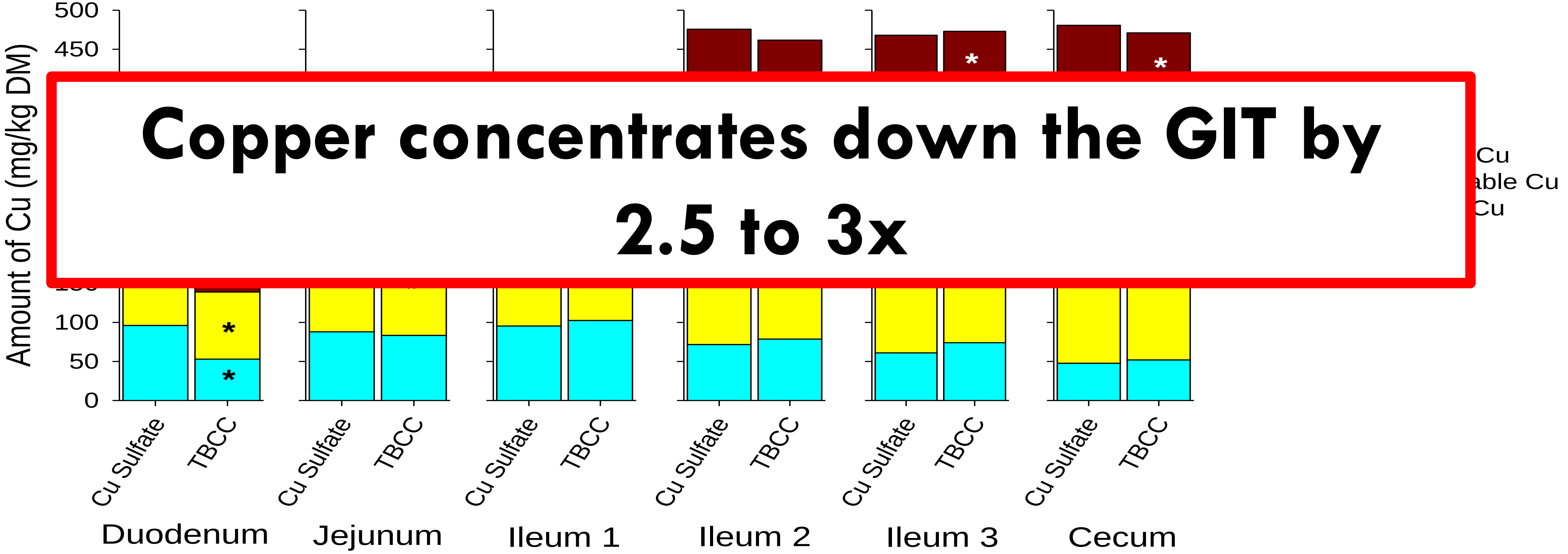
| Copper source | *E. coli* | *C. perfringens* | *S. gallinarum* | *S. enteritidis* | Eimeria % viable* |
|---|---|---|---|---|---|
| 0 added Cu | 1.0 [a] | 1.0 [a] | 1.0 | 1.0 | 79 |
| $CuSO_4$ | 0.47 [b] | 0.88 [b] | 1.12 | 0.93 | 76 |
| Cu Hydroxychloride | 0.31 [c] | 0.78 [bc] | 1.18 | 0.90 | 75 |
| Cu-amino acid | 0.33 [c] | 0.75 [c] | 1.09 | 1.11 | 81 |
| SEM | 0.02 | 0.03 | 0.05 | 0.03 | 2.6 |
| ANOVA P value | 0.001 | 0.015 | 0.20 | 0.33 | 0.51 |

* For bacteria, the data are expressed in units of DNA normalized to 0 mg/kg added copper. For E. maxima, the data are expressed as % viability.

# GIT Cu: Materials and Methods

- **6 pens/treatment – 3 birds/pen**
- **Chicks fed 150 mg/kg Cu from either $CuSO_4$ or Cu Hydroxychloride**
- **Fed from d 3 – 14**
- **Luminal contents were collected from 6 GIT locations to determine:**
  - Total Cu
  - Water extractable Cu
  - Cu that could be extracted using the strong complexing agent, ethylene bis-hydroxyphenylglycine (EHPG)
  - Cu that could not be extracted (presumed to be unavailable for nutritional and microbicidal purposes)

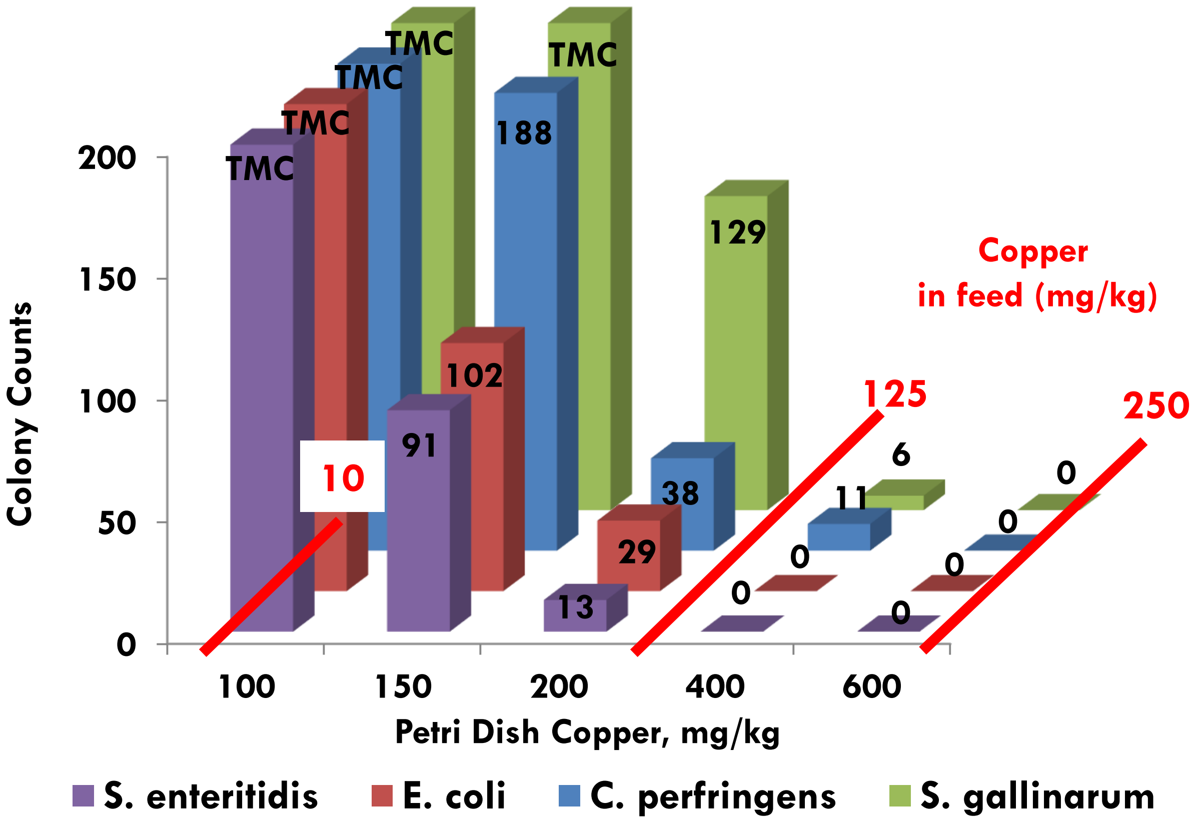
# Cu x Source in the GIT




**Copper concentrates down the GIT by 2.5 to 3x**

* Within a tissue and Cu type, means are different ($p<0.05$).

# Feeding Higher Cu as Microbial Growth Inhibitor




*TMC = Too many colonies to count

**Feeding high Cu sets up a GIT environment that limits microbial growth.**

- **Feed 10mg/kg (no inhibition of bacterial growth)**
- **Feed 125mg/kg (start of inhibition)**
- **Feed 250mg/kg (significant inhibition)**

# Necrotic Enteritis Data Model Validation

## Work conducted at Colorado Quality Research

# Necrotic Enteritis Model Validation

- **Cobb-500 males (21 birds/pen; 8 pens/treatment)**
- **Starter Feed (Day 0-17); Grower Feed (17-35)**

- **Day-of-Age Full Dose Coccidiosis Vaccination**
- ***Clostridium perfringens*** **challenge on Day 17 for first two cycles**
  - **3 ml/bird $10^8$ Cp broth on 25 g/bird Grower Feed**
- **Natural Exposure third cycle**

- **Weight Gain & Feed Conversion**
  - **Day 0-17, Day 17-28, Day 0-28 and 0-35**
- **NE lesion scoring Day 21 (5 birds/pen)**
- **NE Mortality**

# Necrotic Enteritis Model Validation

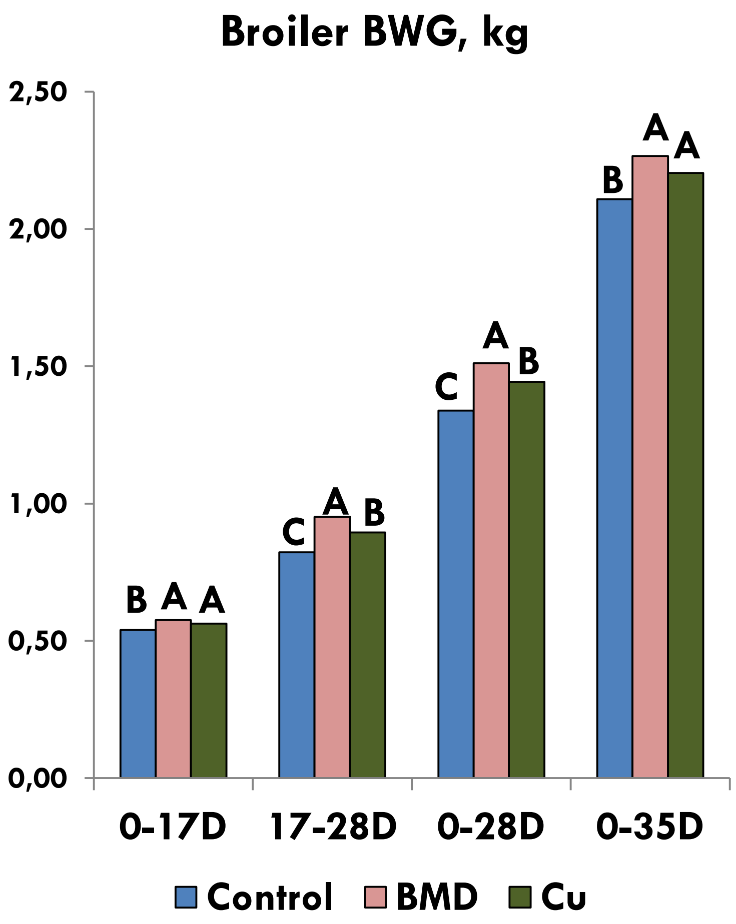
- **Clean pine shavings for first cycle and then same treatments on used litter the next two cycles**
  - **~2 weeks between flocks**

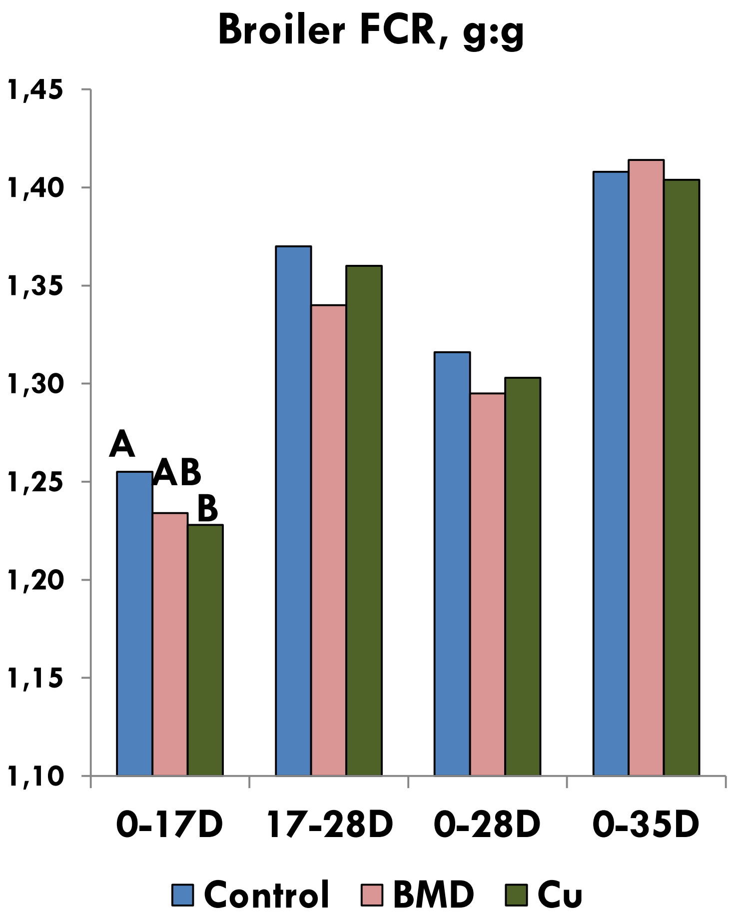
- **Corn, Soy, DDGs and MBM diets**

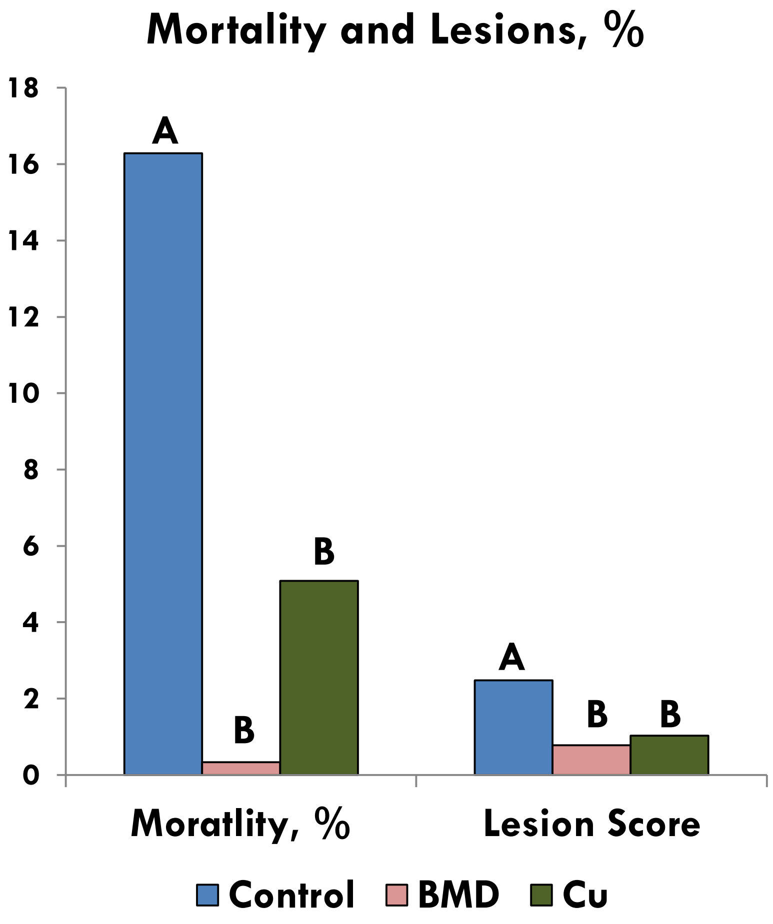
**Treatments**

1) **Challenged, no treatments**
2) **Challenged, 50g/ton BMD**
3) **Challenged, Copper Hydroxychloride, 275 mg/kg Cu**

# Trial 1 Performance



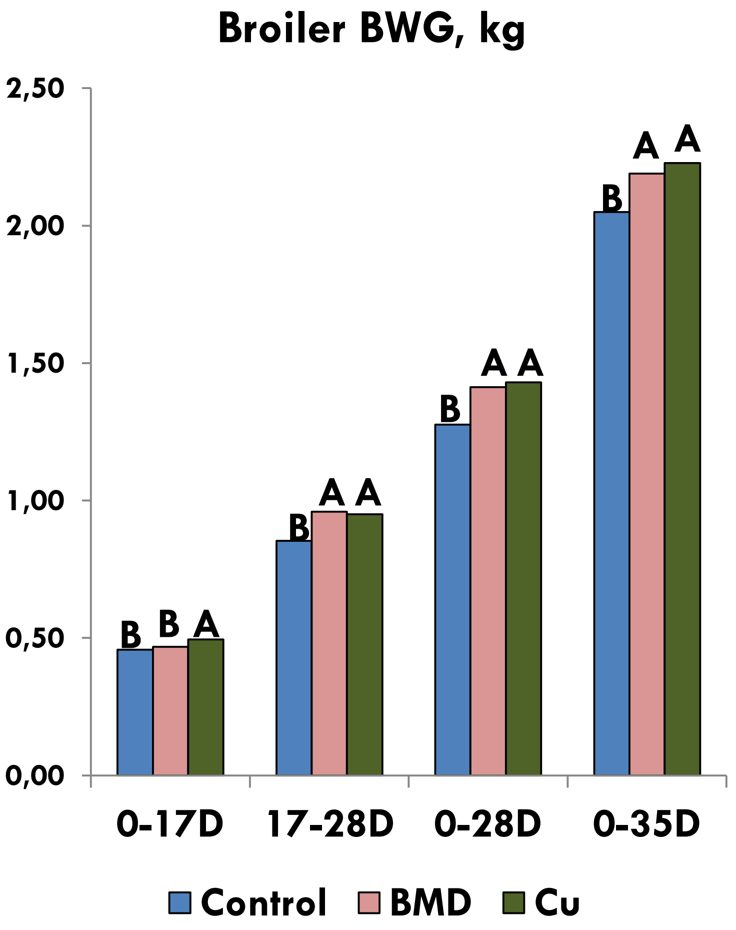



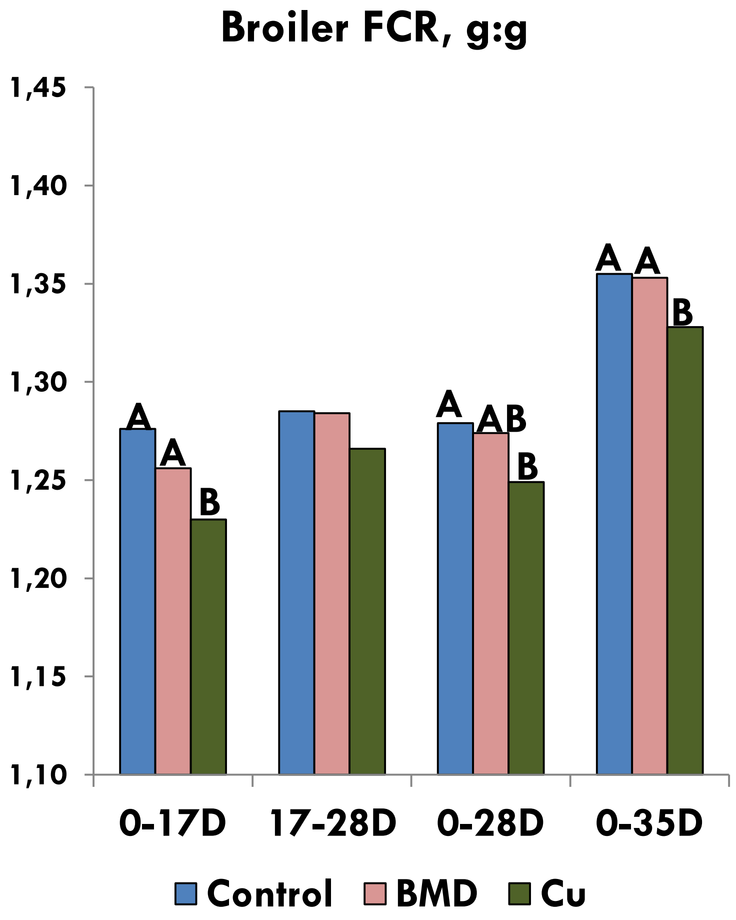



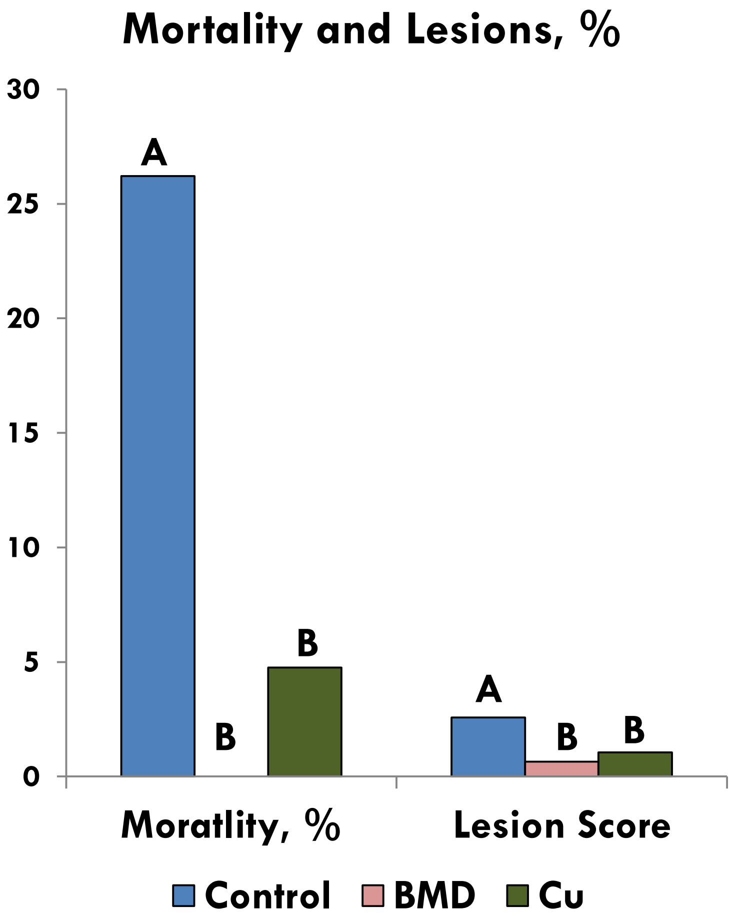



A,B denotes significance @ P <0.05

# Trial 2 Performance



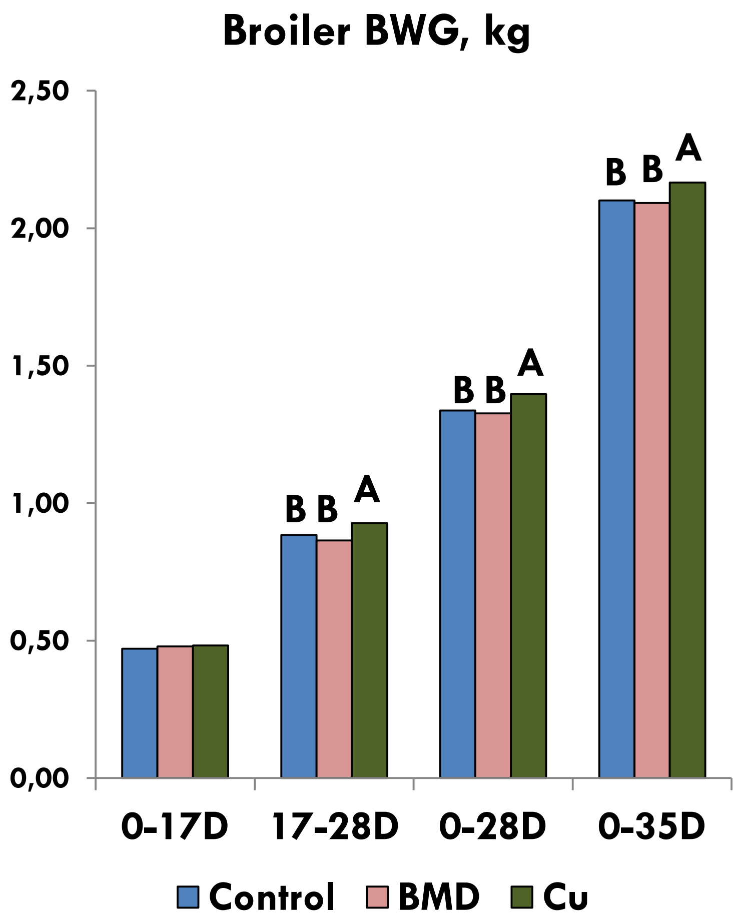



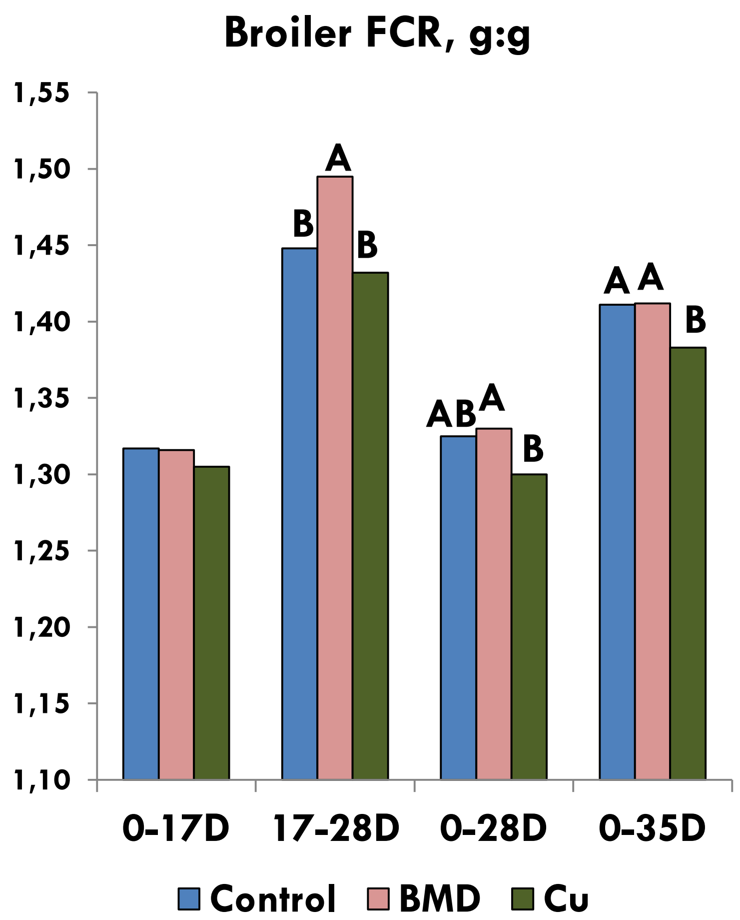



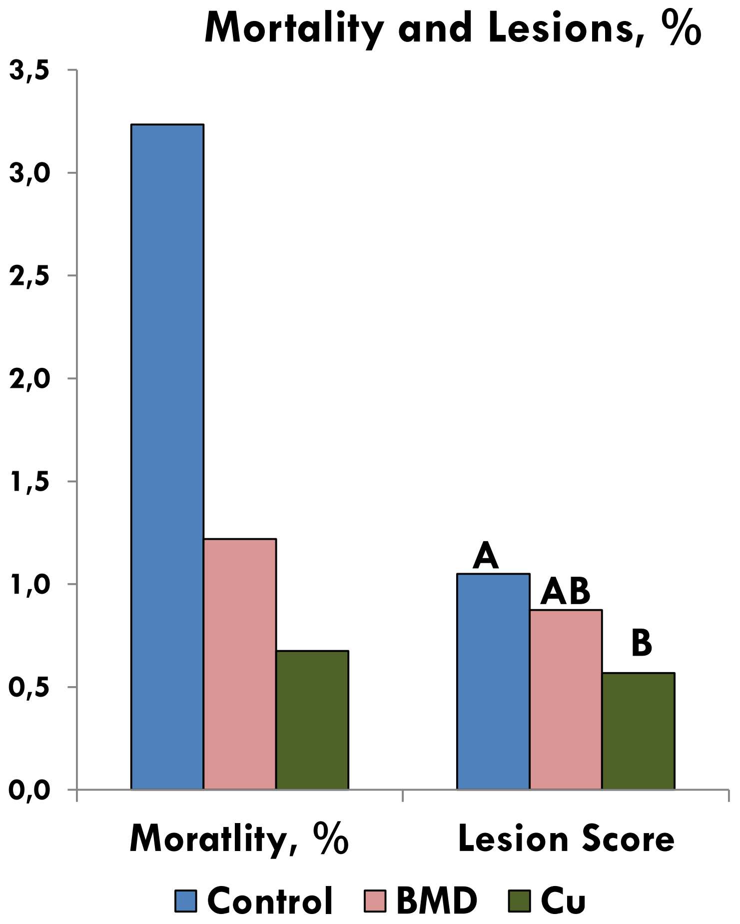

Micronutrients
a Nutreco company

**A,B denotes significance @ P <0.05**

# Trial 3 Performance



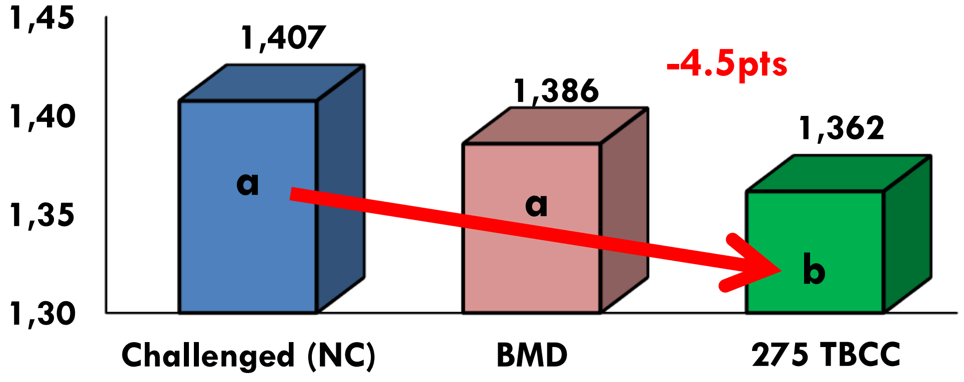




Micronutrients
a Nutreco company

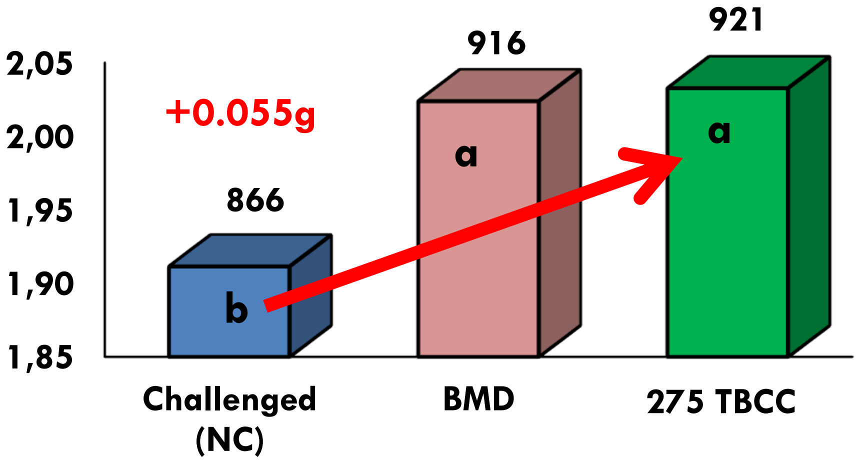
A,B denotes significance @ P <0.05

# Meta Analysis of 3 CQR trials

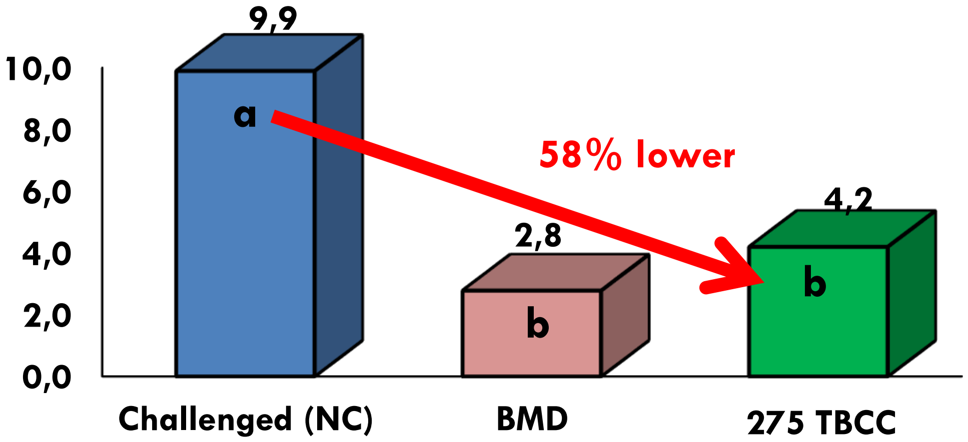
### FCRc 0-35, g:g



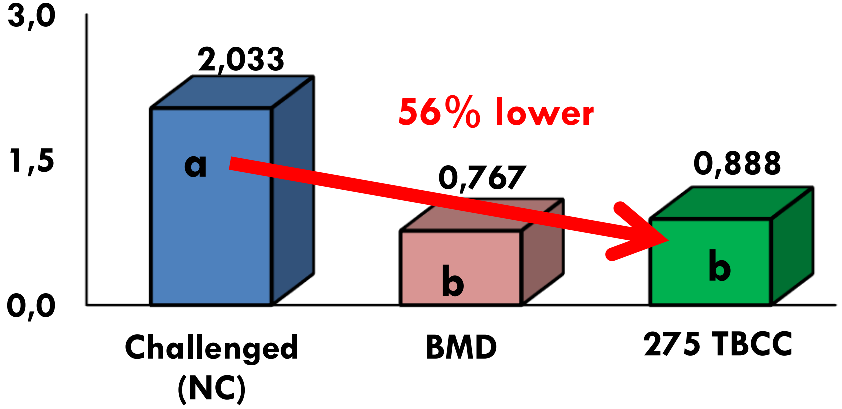

### BWG 17-28d, g




### Mort. 0-35d, %




### Lesion Scores




A,B denotes significance @ P <0.05

# Conclusions

- **Copper is commonly used, where authorized, to improve broiler growth performance.**
- **Chemical bonds give copper source unique properties**
  - **Covalent vs. ionic >>> inorganic vs. organic**
- **Copper concentrates down GIT (2.5-3x)**
- **>400-600 mg/kg Cu has strong bacteriostatic effect (feed 125-275mg/kg)**
- **Feeding 275 mg/kg Cu hydroxychloride performed consistently and similar to an AGP during the development of a NE model.**