

***EFFECT OF COPPER SOURCE ON CROP MYCOSIS in vivo IN BROILERS
AND 21-day BROILER PERFORMANCE WHEN CHALLENGED WITH
Coccidia AND Candida albican (21 day floor pen study).***

STUDY NUMBER: PARC STUDY NUMBER: 99-MIC-03-B

SIGNATURES:

Approved by:

SPONSOR:

_____ Date: _____

Fred Steward
Micronutrients, Inc.
Indianapolis, IN 46231-3350
Telephone: (317) 486-5882
Fax: (317) 486-5888

Acknowledged by:

STUDY

DIRECTOR:

_____ Date: _____

James L. McNaughton, Ph.D.
Director Of Research
PARC Institute, Inc.

OBJECTIVE, INTRODUCTION AND STUDY OVERVIEW:

- **OBJECTIVES:** The objective of this study is to evaluate the effect of feeding various copper sources (TBCC and feed grade copper sulfate) on broiler performance and *in vivo* crop mycosis (caused by *Candida albicans*) activity, at 21 days of age when broilers are challenged with coccidia and candida at 7 days of age in floor pens. Coccidal will also be added at 7 days of age to aid in bacterial proliferation. *Candida albicans* will be infected as a model of curing and/or reducing the effect by test materials.
- **INTRODUCTION:** *Candida albicans* is well-known to cause crop mycosis in broilers; consequently causing gizzard erosion. Copper is an essential mineral for broilers. Historically (at least 50% of all broiler feeds), in addition to the normal level of added copper in trace mineral premixes, copper sulfate has been added at 1 to 2 pounds per ton for an additional benefit of mold reduction and for prevention of crop mycosis. This study will be conducted to determine *in vivo* the effect of copper sources in reducing crop mycosis.
- **STUDY OVERVIEW:** The test period will begin on Trial Day 0 (day of hatch of chicks), which will be fed a commercial-type CRUMBLED FEED (starter feed after pelleting). Each of the six test groups will contain 50 mixed-sex broilers randomly assigned into five replicates per group containing 10 mixed-sex broilers per replicate for a total number of 300 animals on study. Chicks will NOT be replaced during the course of the trial. The chicks will be observed daily for signs of unusual growout patterns or health problems. Body weights and food consumption will be measured on trial day 21. Body weight and mortality will also be evaluated.
- **REGULATORY STATUS:** This study will be conducted as a marketing study without regard to or in compliance with the Food and Drug Administration's Good Laboratory Practices regulations 21 CFR Part 58, 21CFR Part 514.111(a)(5)(ii) Essentials of Adequate and Well-Controlled Clinical (Field) Investigations and FDA/CVM Guidelines for Conduct of Clinical Investigations: Responsibilities of Clinical Investigators and Monitors for Investigational New Animal Drug Studies, October, 1992, except as otherwise noted.

SPONSOR, TESTING FACILITY AND TEST SITES

LOCATION	COMPANY (Individual)	ADDRESS
<i>SPONSOR:</i>	Micronutrient, Inc. Fred Steward	Micronutrient, Inc. 1550 Research Way Indianapolis, IN 46231-3350 Tel.: (317) 486-5882 Telefax: (317) 486-5888
<i>INVESTIGATOR:</i>	James L. McNaughton, Ph.D. PARC Institute, Inc.	PO Box 1161 30 North Harrison Street Easton, Maryland 21601
<i>FARM LOCATION:</i>	PARC Farm #1	1701 Starr Road Queen Anne, MD 21657
<i>FEED MILL LOCATION:</i>	PARC Feeds, Inc.	PO Box 340 6202 Nagel Road (Harmony, MD) Preston, Maryland 21655
<i>RAW DATA AND FINAL REPORT:</i>	PARC Institute, Inc.	PO Box 1161 30 North Harrison Street Easton, Maryland 21601
<i>NUTRIENT ANALYSIS SITE (where required)</i>	Woodson-Tenet, Inc.	4207 Delaware Av. Des Moines, IA 50313 (Attention: Marilyn Nair)

PROPOSED STUDY DATES:

- Animal Receipt: To be determined (document in final report)
- Initiation of Dosing: Day 0 to Day 21.
- Schedule: A detailed schedule is contained in Attachment A.

STUDY PERSONNEL

- Study Director: James L. McNaughton, Ph.D. (phone: 410-820-5661 and fax: 410-822-0429)
- Study Veterinarian and Pathologist: Ed Odor, DVM
- Operations Manager: William J. Graves
- Study Assistants: Cliff Harris and Rick Salter

TEST SYSTEM

Treatment	Total Chicks Placed on Day 0 Per Replicate	Density, ft ² per chick	Number Of Rep	Chicks Treated Per Trt Group ¹
MIC3-1	10 mixed-sex	0.68	5	50
MIC3-2	10 mixed-sex	0.68	5	50
MIC3-3	10 mixed-sex	0.68	5	50
MIC3-4	10 mixed-sex	0.68	5	50
MIC3-5	10 mixed-sex	0.68	5	50
MIC3-6	10 mixed-sex	0.68	5	50
TOTAL				300

¹ Six treatment groups will be fed for 21 days.

TEST GROUP	TEST MATERIAL	COPPER source Lb/ton(ppm)	Candida Inoculation via water @ 7 days
MIC3-1	Control Basal	None	No
MIC3-2	Control Basal	None	Yes
MIC3-3	TBCC	0.43(125)	Yes
MIC3-4	TBCC	0.86(250)	Yes
MIC3-5	Copper Sulfate	1.00(125)	Yes
MIC3-6	Copper Sulfate	2.00(250)	Yes

NOTE: Copper sources will be added to all ration types equally. The following will be added at the rate of 50,000 per bird (*Candida albicans*). A total of 50 birds will be used for each treatment.

Candida albicans INOCULATION:

1. *Candida* (from a natural source) will be placed in starter feed.
2. Birds will be starved for 4 hr.
3. The birds will be given 25 g inoculation feed containing high levels of *candida* per bird on Day 3 of the trial fed *ad libitum*.
4. Sugar (1% by weight) will be placed in drinking water to aid in *candida* proliferation.
5. Birds will be allowed to consume all the inoculation feed.
6. After all feed has been consumed, regular feed consumption from treatment feeds will continue.

CROP MYCOSIS AND INTESTINAL LESION SCORING:

Scores are based on inspection of internal membrane surfaces using a scale of 0-5.

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|-------------------------------|------------------|
| 0 = no observed effect | 3 = 50% coverage |
| 1 = slight browning of ridges | 4 = 75% coverage |
| 2 = 25% coverage | 5 = moribund |

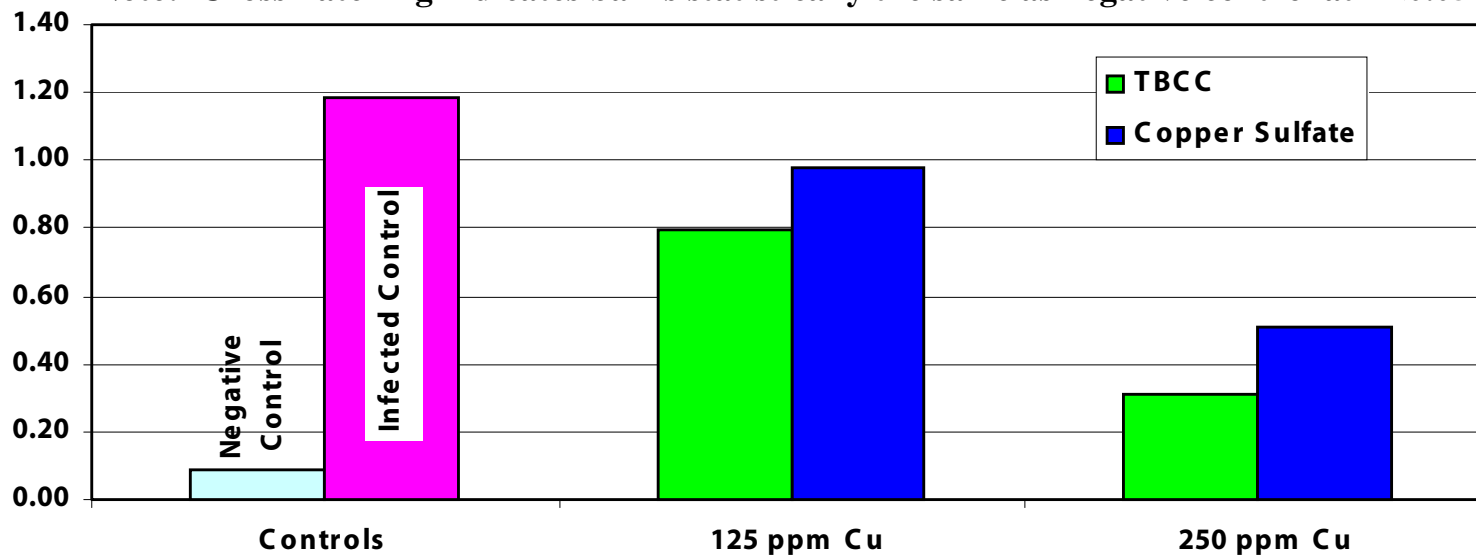
Crop Mycosis Score

Data: Trial days 0 – 21 (Broiler)

Trial 99-MIC-03-BB

Rep	Control		Treatment		Sulfate	
	No Cand.	Candida	TBCC 125 ppm	TBCC 250 ppm	125 ppm	250 ppm
1	0.00	1.22	0.89	0.11	0.89	0.11
2	0.10	1.00	1.00	0.40	1.10	0.50
3	0.00	1.30	0.67	0.20	1.00	0.67
4	0.10	0.89	0.80	0.50	0.89	0.70
5	0.22	1.50	0.63	0.33	1.00	0.56
Mean	0.084	1.182	0.796	0.309	0.976	0.507
STAT*	a	d	c	ab	cd	b
S.D.	0.082	0.217	0.139	0.139	0.080	0.211
C.V.	97.262	18.365	17.426	44.951	8.162	41.577

Note: Cross hatching indicates bar is statistically the same as negative control at P<0.05



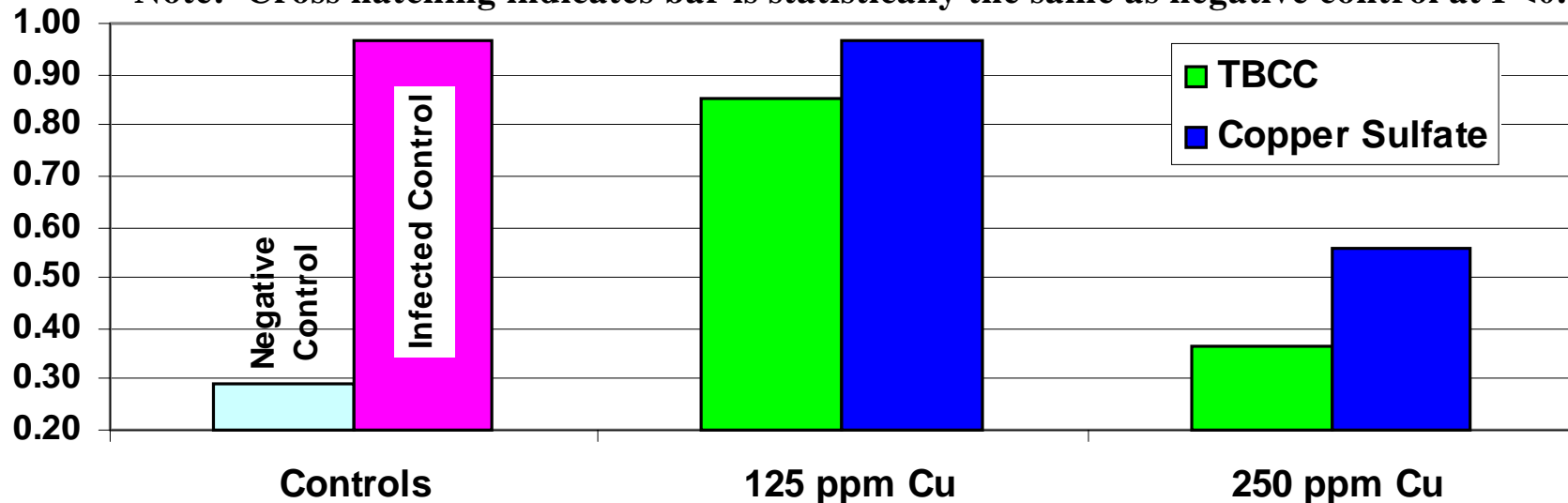
Intestinal Lesion Score

Data: Trial days 0-21 (Broiler)

Trial 99-MIC-03-BB

Rep	Treatment					
	Control No Cand.	Control Candida	TBCC 125 ppm	TBCC 250 ppm	Sulfate 125 ppm	Sulfate 250 ppm
1	0.00	0.78	0.56	0.89	0.78	0.89
2	0.70	1.00	1.22	0.00	0.90	0.60
3	0.11	1.30	1.11	0.10	1.25	0.44
4	0.30	0.89	1.00	0.40	1.22	0.30
5	0.33	0.88	0.38	0.44	0.67	0.56
Mean	0.29	0.97	0.85	0.37	0.96	0.56
STAT*	a	c	bc	a	c	ab
S.D.	0.24	0.18	0.33	0.31	0.23	0.20
C.V.	82.81	18.61	38.59	84.98	24.37	35.01

Note: Cross hatching indicates bar is statistically the same as negative control at $P < 0.05$



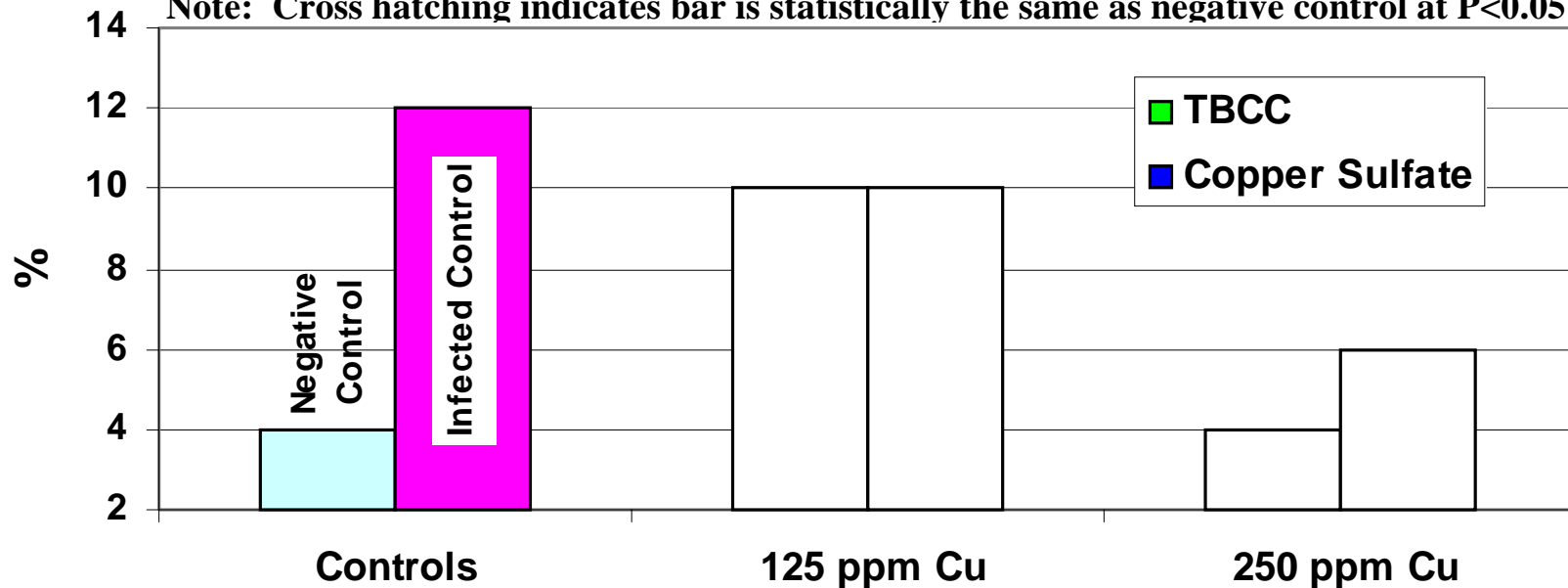
Mortality (%) Days 0-21

Data: Trial days 0-21 (Broiler)

Trial 99-MIC-03-BB

Rep	Treatment					
	Control No Cand.	Control Candida	TBCC 125 ppm	TBCC 250 ppm	Sulfate 125 ppm	Sulfate 250 ppm
1	0.00	10.00	10.00	10.00	10.00	10.00
2	0.00	20.00	10.00	0.00	0.00	0.00
3	10.00	0.00	10.00	0.00	20.00	10.00
4	0.00	10.00	0.00	0.00	10.00	0.00
5	10.00	20.00	20.00	10.00	10.00	10.00
Mean	4.00	12.00	10.00	4.00	10.00	6.00
STAT*	a	b	ab	a	ab	ab
S.D.	4.9	7.5	6.3	4.9	6.3	4.9
C.V.	122.5	62.4	63.2	122.5	63.2	81.6

Note: Cross hatching indicates bar is statistically the same as negative control at P<0.05

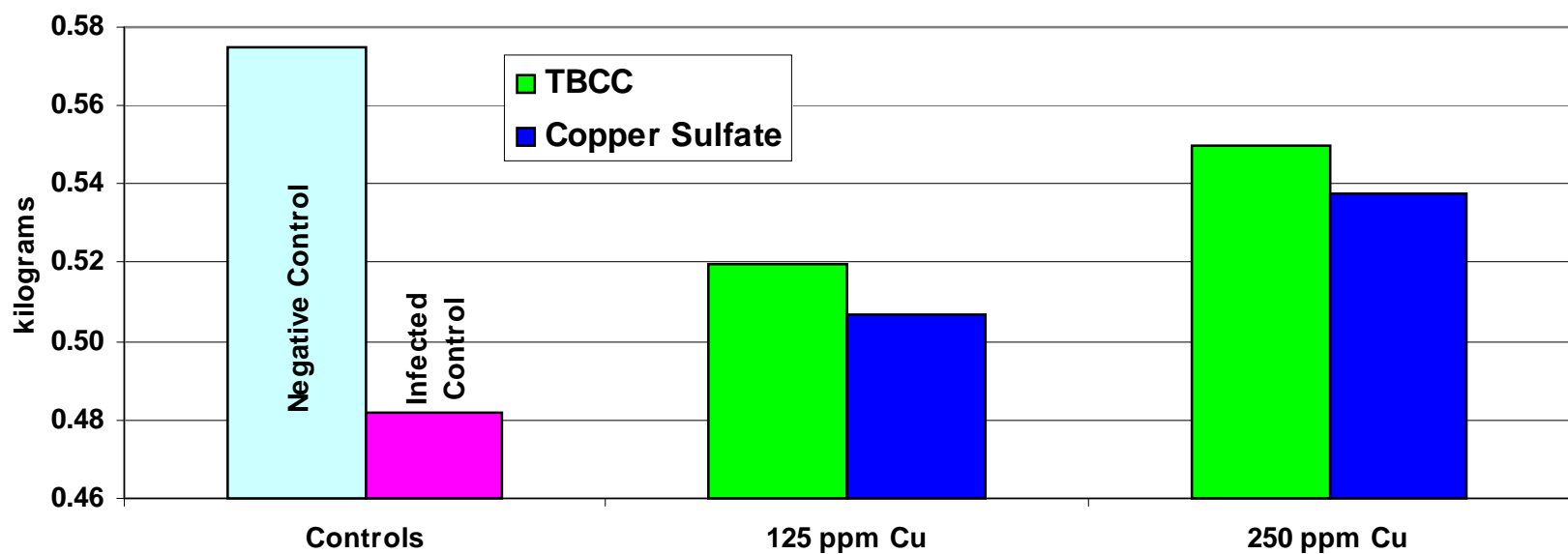


Body Weight (kg)

Data: Trial days 0-21 (Broiler)

Trial 99-MIC-03-BB

Rep	Control		Treatment		Sulfate	
	No Cand.	Candida	TBCC 125 ppm	TBCC 250 ppm	125 ppm	250 ppm
1	0.594	0.461	0.508	0.563	0.503	0.563
2	0.566	0.494	0.533	0.535	0.528	0.541
3	0.552	0.478	0.529	0.543	0.495	0.533
4	0.587	0.493	0.511	0.554	0.496	0.529
5	0.576	0.481	0.518	0.554	0.512	0.521
Mean	0.575	0.482	0.520	0.550	0.507	0.538
STAT*	a	e	cd	b	d	bc
S.D.	0.015	0.012	0.010	0.010	0.012	0.014
C.V.	2.588	2.467	1.837	1.745	2.368	2.666

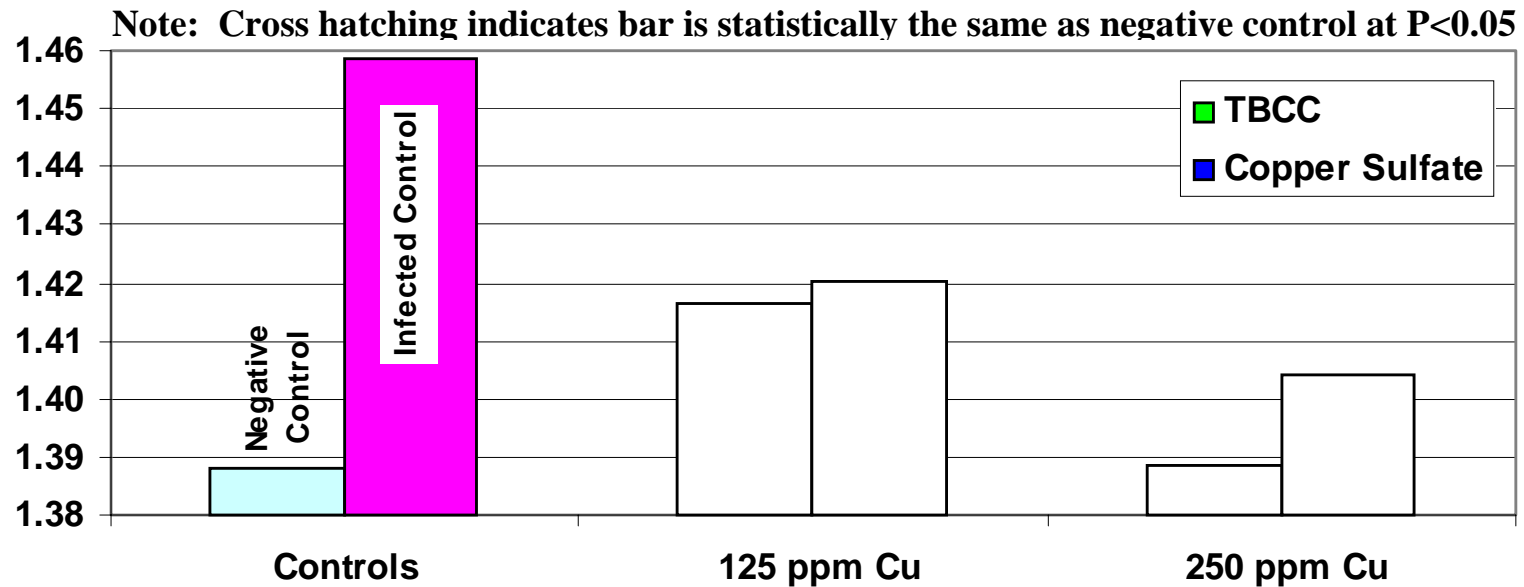


Feed:Gain (Body Weight Corrected)

Data: Trial days 0-21 (Broiler)

Trial 99-MIC-03-BB

Rep	Control		Treatment		Sulfate	
	No Cand.	Candida	TBCC 125 ppm	TBCC 250 ppm	125 ppm	250 ppm
1	1.403	1.516	1.396	1.411	1.376	1.402
2	1.356	1.432	1.462	1.438	1.418	1.358
3	1.398	1.388	1.452	1.348	1.401	1.465
4	1.382	1.496	1.388	1.325	1.421	1.432
5	1.401	1.462	1.385	1.423	1.486	1.366
Mean	1.388	1.459	1.416	1.389	1.420	1.404
STAT*	a	b	ab	a	ab	ab
S.D.	0.018	0.046	0.033	0.044	0.036	0.040
C.V.	1.287	3.133	2.347	3.201	2.561	2.859

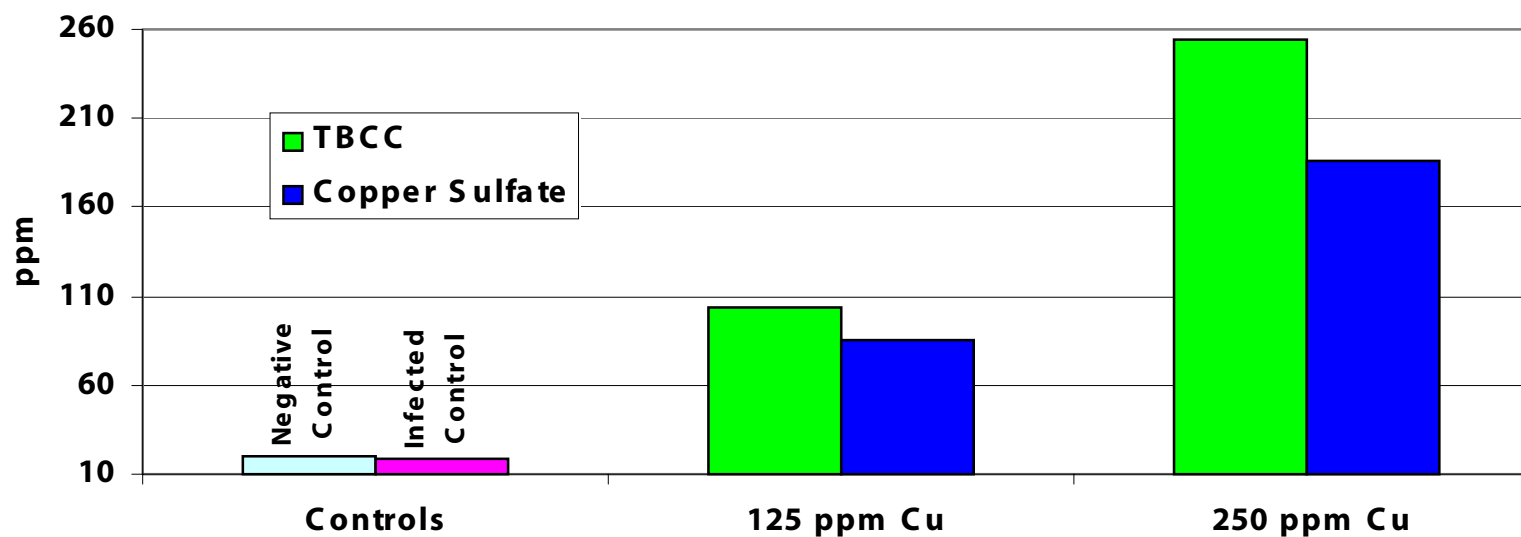


Liver Copper (ppm)

Data: Trial days 0-21 (Broiler)

Trial 99-MIC-03-BB

Rep	Control		Treatment		Sulfate	
	No Cand.	Candida	TBCC 125 ppm	TBCC 250 ppm	125 ppm	250 ppm
1	11.91	11.20	121.94	238.56	68.04	182.72
2	15.95	26.55	83.83	239.68	73.54	186.32
3	25.40	11.02	85.71	253.49	112.82	163.86
4	17.66	9.72	126.51	256.78	105.61	209.03
5	26.86	31.67	99.46	284.42	66.30	186.06
Mean	19.556	18.033	103.490	254.586	85.262	185.596
STAT*	d	d	c	a	c	b
S.D.	5.7	9.2	17.8	16.6	19.8	14.4
C.V.	29.2	51.0	17.2	6.5	23.3	7.7



Conclusions

- **Copper was effective against crop mycosis.** As measured by disease scores for spread of infection in crop membranes and for number of observed intestinal lesions, both copper sources at both treatment levels gave a significant improvement over untreated, infected birds. 250 ppm copper was statistically better than 125 ppm, and for TBCC gave similar results as the uninfected birds.
- **TBCC performed better than copper sulfate.** At 250 ppm there was no difference between the TBCC treated birds and the uninfected birds on crop mycosis, while copper sulfate treated birds did significantly worse. In addition, TBCC gave numerically better results than copper sulfate at both doses on weight gain, feed efficiency, crop mycosis and intestinal lesion scores and at the 250 ppm level on mortality.
- **TBCC has better bioavailability.** Copper concentration in liver was numerically higher at both levels of added copper for the TBCC birds, and was significantly higher at 250 ppm. A linear regression of the data shows TBCC to be 112% as bioavailable as copper sulfate.