

**Improved Stabilities of Vitamins A, D₃, E, and
Riboflavin with Tribasic Copper Chloride
(TBCCtm) Compared to Copper Sulfate
Pentahydrate in Crumbled Broiler Starter Feed**

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Review of Previous Research

Miles *et al.* (1998; Poultry Sci. 77:416-425):

- Copper sulfate or TBCC added to broiler starter at 25, 100, or 300 mg copper/kg.
- Storage 20 d at 37° C, tested at 4-d intervals
- Lipids were solvent extracted, analyzed for peroxide and anisidine (aldehyde) values.
- Oxidation promotion lower ($P < 0.001$) with copper at 300 mg/kg from TBCC.

Miles *et al.*, 1998 (PS 77:416)
20-Day Feed Storage (Lipids)

	Peroxide, mEq/kg	Anisidine Value
CuSulf 25	29.4	151
CuSulf 100	44.2	195
CuSulf 300	99.5	303
TBCC 25	26.5	151
TBCC 100	37.9	162
TBCC 300	62.5	208

Experiment 1 – Ross x HiY Chicks

- Equal mixed-sexes
- Battery brooders
- TBCC vs Cu sulfate
- Cu sulfate Cu ~10 mg/kg in control vs TBCC Cu 250 mg/kg
- Mash or crumbles
- 6 Pens of 10 chicks
- 21-d Performance
- Pooled blood or liver samples / pen

Experiment 1 – Analyzed Vitamins Per Ton of Feed

	Feed Form	Vitamin A, IU	Vitamin D₃, IU
Control 10	Mash	6,463,451	2,746,387
Control 10	Crumble	5,911,213	2,718,374
TBCC 250	Mash	6,514,136	2,723,458
TBCC 250	Crumble	6,314,136	2,699,764

Experiment 1 – Analyzed Vitamins Per Ton of Feed (Relative)

	Feed Form	Vitamin A, IU	Vitamin D₃, IU
Control 10	Mash	100.0%	100.0%
Control 10	Crumble	91.5%	99.0%
TBCC 250	Mash	100.8%	99.2%
TBCC 250	Crumble	97.7%	98.3%

Experiment 1 – Analyzed Vitamins Per Ton of Feed

	Feed Form	Vitamin E, IU	Ribofla- vin, mg
Control 10	Mash	14,873	7,236
Control 10	Crumble	13,115	6,794
TBCC 250	Mash	14,976	7,197
TBCC 250	Crumble	14,485	7,068

Experiment 1 – Analyzed Vitamins Per Ton of Feed (Relative)

	Feed Form	Vitamin E, IU	Riboflavin, mg
Control 10	Mash	100.0%	100.0%
Control 10	Crumble	88.2%	93.9%
TBCC 250	Mash	100.7%	99.5%
TBCC 250	Crumble	97.4%	97.7%

Experiment 1 -- 21-Day Vitamin E Levels in Serum and Liver (Wet)

	Feed Form	Serum E, mcg/ml	Liver E, mcg/g
Control 10	Mash	5.68 a	4.47 ab
Control 10	Crumble	4.15 c	3.02 b
TBCC 250	Mash	5.54 a	4.04 ab
TBCC 250	Crumble	5.02 b	4.68 a

Experiment 1 -- Conclusions

- Pelleting/crumbling control feed *lowered* levels of all vitamins examined vs mash.
- Vitamins A, E, and riboflavin were *higher* in TBCC than in control crumbles.
- Serum E was *lower* ($P < 0.05$) on crumbled versus mash feed, but TBCC crumbles gave higher serum E than control crumbles.
- Control mash, or either TBCC diet, had *higher* ($P < 0.05$) liver E than control crumbles.

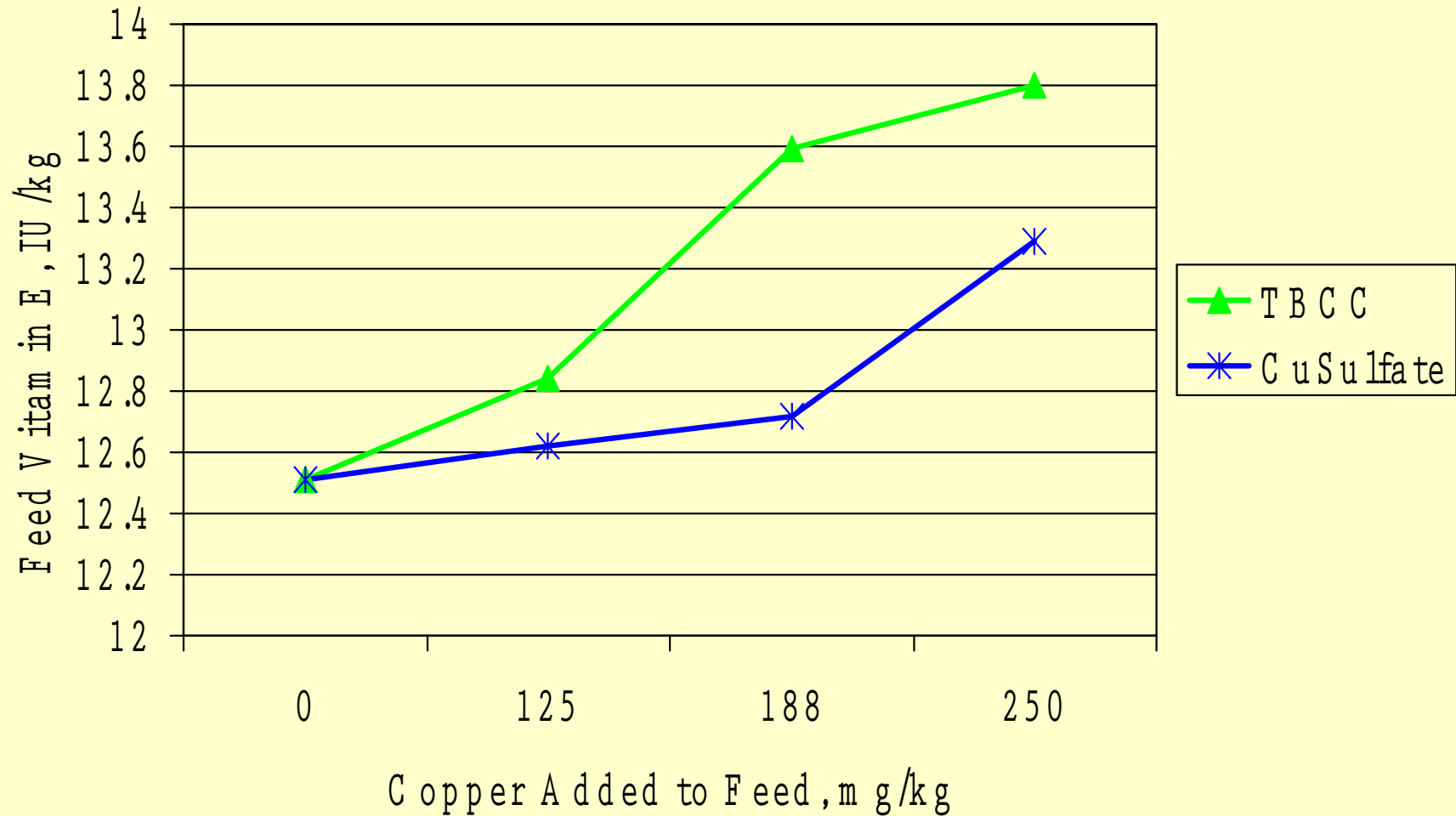
Experiment 2 – Ross x Cobb Chicks

- Day-old male chicks
- Battery brooders
- TBCC vs Cu sulfate
- Added Cu at 0, 125, 187.5, or 250 mg/kg
- Mash or crumbles
- 8 Pens of 10 chicks
- 21-d Performance
- Pooled blood or liver samples / pen

Feed Vitamin E Levels (P<0.05 All)

Feed Vitamin E, IU/kg:	<i>Mash</i>	<i>Crumbles</i>
Control	14.14 a	12.51 e
CuSulfate 125	14.13 a	12.62 e
CuSulfate 187.5	13.96 ab	12.72 e
CuSulfate 250	14.15 a	13.29 cd
TBCC 125	14.00 ab	12.84 de
TBCC 187.5	13.84 ab	13.59 bc
TBCC 250	14.12 a	13.80 ab

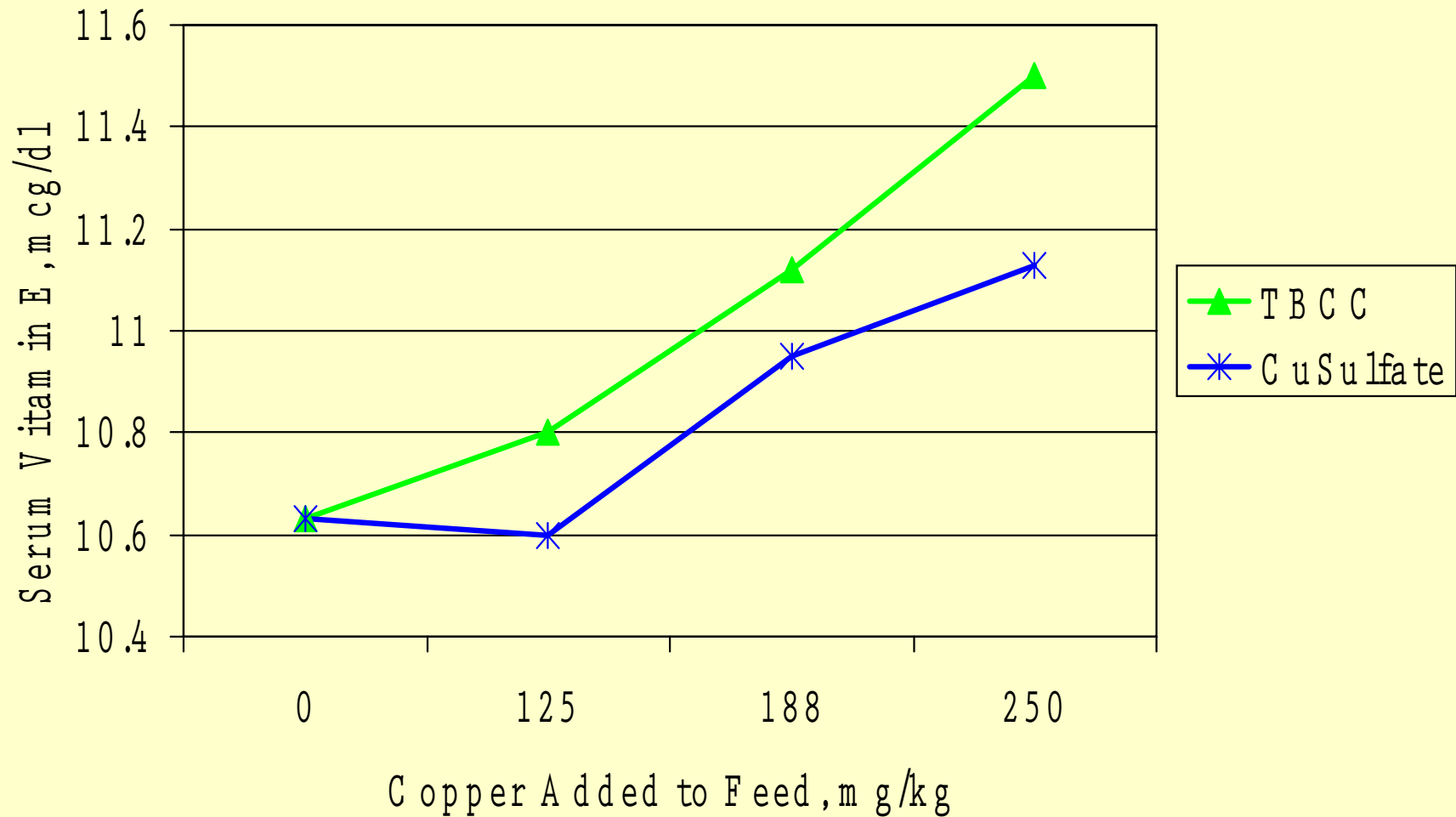
Vitamin E in Crumbled Feed by Copper Sources



Serum Vitamin E Levels (P<0.05 All)

Serum Vitamin E, mcg/dl	<i>Mash</i>	<i>Crumbles</i>
Control	10.74 bcde	10.63 cd
CuSulfate 125	11.02 bc	10.60 d
CuSulfate 187.5	10.83 bcde	10.95 bcd
CuSulfate 250	10.95 bcd	11.13 ab
TBCC 125	10.86 bcde	10.80 bcd
TBCC 187.5	10.89 bcd	11.12 ab
TBCC 250	11.02 bc	11.50 a

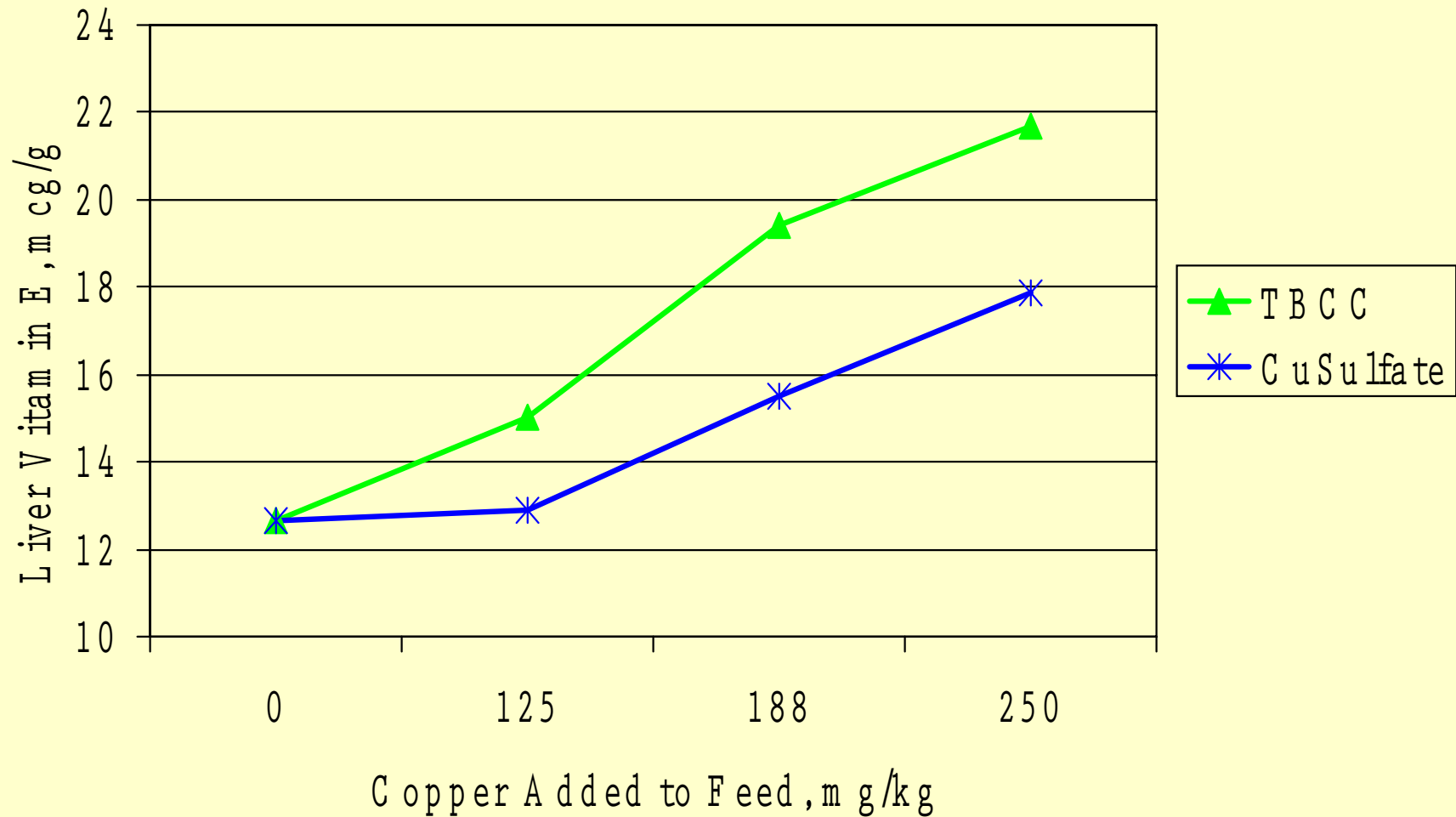
Serum Vitamin E by Copper Sources (Crumbled Feed)



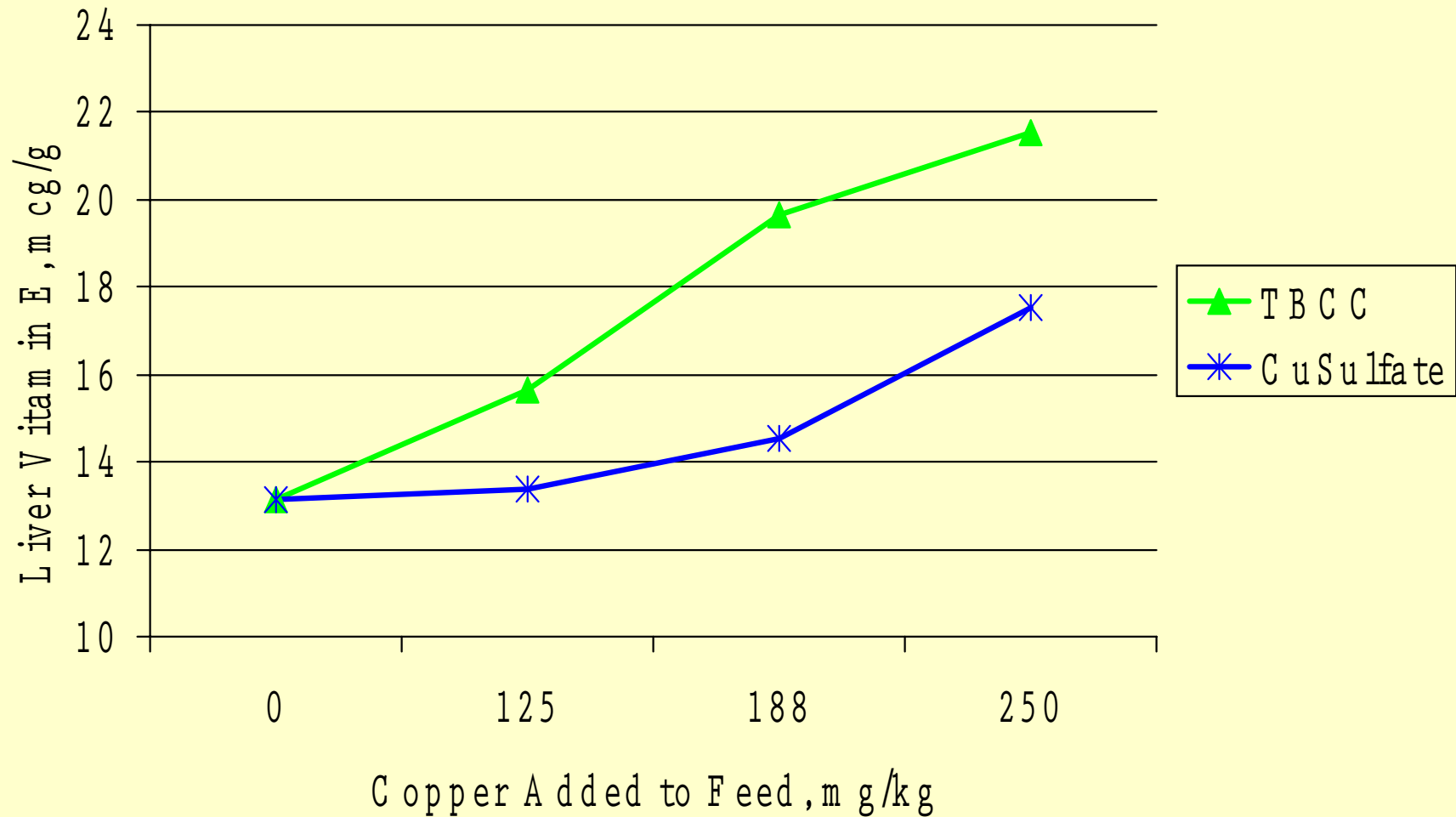
21-D Liver Vitamin E (P<0.05 All)

Liver Vitamin E, mcg/g	<i>Mash</i>	<i>Crumbles</i>
Control	12.64 g	13.13 efg
CuSulfate 125	12.90 fg	13.40 efg
CuSulfate 187.5	15.52 cdef	14.53 efg
CuSulfate 250	17.87 bc	17.55 bcd
TBCC 125	15.00 defg	15.67 cde
TBCC 187.5	19.39 ab	19.65 ab
TBCC 250	21.67 a	21.55 a

Liver Vitamin E by Copper Sources (Mash Feed)



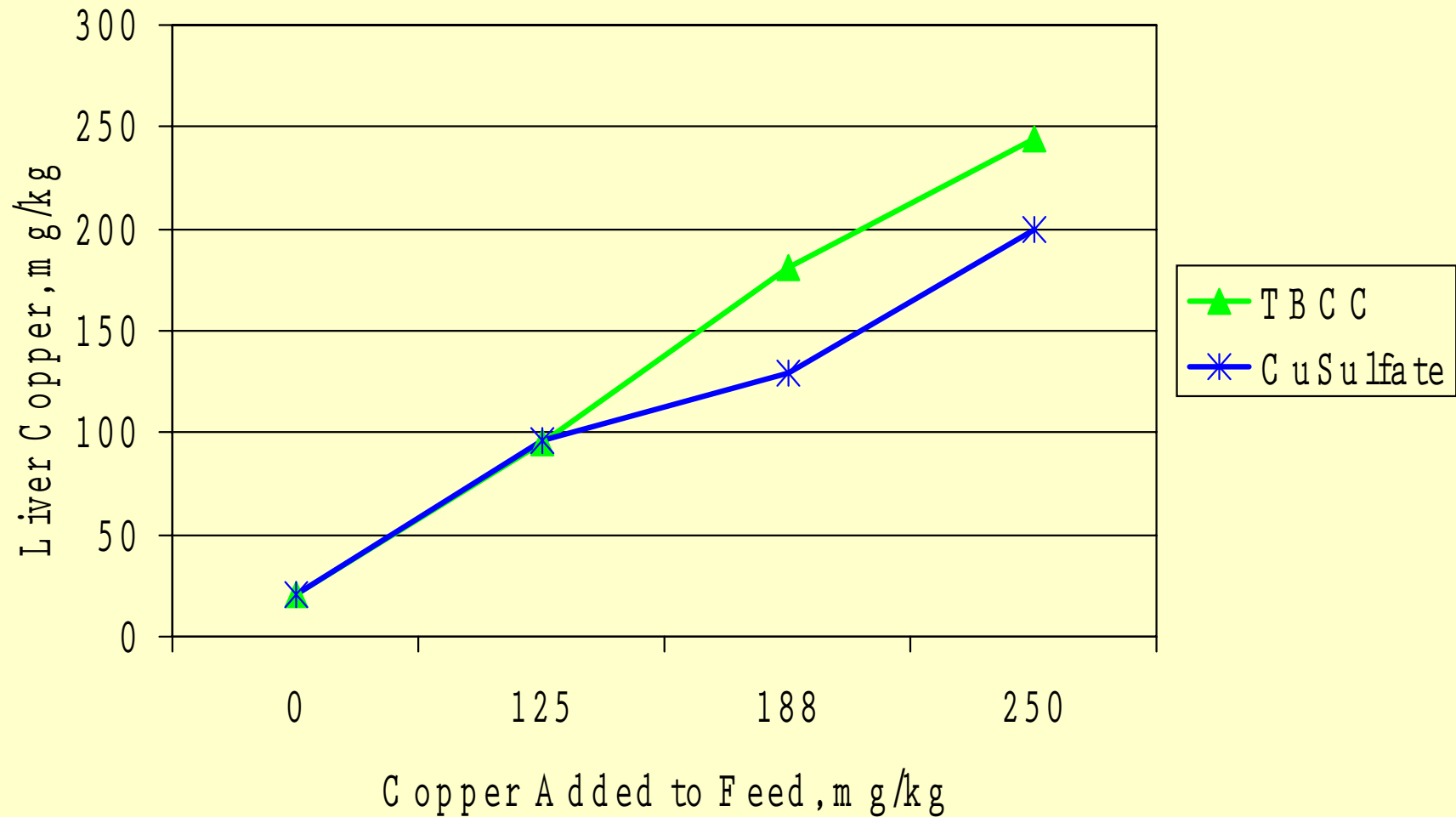
Liver Vitamin E by Copper Sources (Crumbled Feed)



21-D Liver Copper (P<0.05 All)

Liver Copper, mg/kg	<i>Mash</i>	<i>Crumbles</i>
Control	20.2 f	20.6 f
CuSulfate 125	95.4 e	96.2 e
CuSulfate 187.5	129.8 d	128.3 d
CuSulfate 250	199.4 b	200.3 b
TBCC 125	94.3 e	95.3 e
TBCC 187.5	180.5 c	181.5 c
TBCC 250	243.5 a	244.3 a

21-D Liver Copper by Copper Sources (Mash and Crumbles Averaged)



Experiment 2 -- Conclusions

- Pelleting/crumbling *lowered* vitamin E levels in all feeds *except* those with the highest levels of TBCC.
- In crumbles, feed vitamin E *improved* more with TBCC than with Cu sulfate.
- Serum vitamin E levels on crumbled feed and liver E levels on both mash and crumbles were improved more with increasing Cu from TBCC than from Cu sulfate.

Experiment 2 – Conclusions p. 2

- Liver Cu was about *equal* for mash or crumbled feeds by treatment.
- Averaging mash and crumbles, TBCC *increased* liver Cu more at 188 and 250 mg added Cu/kg than Cu sulfate.

Experiment 3 –Antimicrobial Screening Procedure *in vitro*

- 3 x 3 factorial design in 10 ml test tubes
- DI water acidified to pH 2
- Three “sources”
 - Control (no Cu)
 - TBCC (250 ppm Cu)
 - CuSulfate (250 ppm Cu)

Experiment 3 –Antimicrobial Screening Procedure *in vitro*

- Inoculated with a DelMarVa strain of one:
E. coli, Salmonella or Camplobacter
- Incubated at 37⁰ C for one hour
- 0.2 ml from each tube plated on both MacConkey and Blood Agar, dried 30 min., incubated 24 hours at 37⁰ C then counted.

Number of Bacterial Colonies Counted

Source (Cu conc. mg/l)	Bacterial Inoculum		
	<i>E. Coli</i>	<i>Salmonella</i>	<i>Camplobacter</i>
Control (0)	>500	>500	>100
CuSulfate (250)	200 < x < 300	400 < x < 500	60 < x < 80
TBCC (250)	50 < x < 100	200 < x < 300	50 < x < 80

Overall Conclusions

- Miles *et al.* 1998: TBCC had a *lower* pro-oxidant activity than Cu Sulfate.
- In every case, crumbled feed had *lower* vitamin levels than mash.
- Elevated copper *decreased* losses of vitamins during pelleting.
- Liver vitamin E levels showed that elevated copper decreased losses on *unpelleted mash*.

Overall Conclusions – Cont'd

- TBCC displayed stronger antimicrobial activity *in vitro* than Cu sulfate
- Modes of action of TBCC in sparing vitamins may be both
 - reduced pro-oxidant activity, and
 - increased antimicrobial effectiveness
- TBCC was *more effective* than Cu sulfate at reducing losses of vitamins as measured both in feed and *in vivo*.

Overall Conclusions – Cont'd

- Liver copper levels were *higher* with TBCC than with copper sulfate indicating higher bioavailability.