Copper sulphate and copper sulphide (CuS) as supplements for growing pigs*

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The growth-promoting effect of the addition of 0·1% copper sulphate (supplying 250 p.p.m. copper) to a growing-pig diet, reported by Barber, Braude, Mitchell & Cassidy (1955), has since been widely confirmed. The mode of action of Cu is unknown, and whether the site of action is enteric, systemic or both remains obscure. In an attempt to throw some light on this problem, a study was made of the effect of supplementing a growing-pig diet with a relatively insoluble Cu salt, as compared with that of the highly soluble copper sulphate.

The present paper gives the results of two experiments in which dietary supplements of Cu were given either as the sulphate or as the sulphide. The results of a preliminary investigation of the number of fungi that could be cultured from samples of faeces taken at intervals from all pigs in the first experiment are also included.

EXPERIMENTAL

Pigs. In both experiments, weaners from the Shinfield, virus pneumonia-free, Large White herd were used and were all individually fed. Each experiment was designed as a randomized block, blocks corresponding to litters, and treatments were allocated at random to the pens, there being no direct communication between the pigs on the different treatments. The number of pigs on each treatment was eight in Expt 1 and twelve in Expt 2. In Expt 1, two replicates began on experiment in June and the rest in September or October 1958. In Expt 2, four replicates began in July and the rest in September 1959. All pigs were given twice daily as much meal as they would consume within 30 min up to a maximum of $6\frac{1}{2}$ lb/day, water at the rate of 3 lb to every 1 lb meal being added immediately before feeding. The pigs were weighed once weekly throughout the experiment and were sent to slaughter individually when their live weight at the weekly weighing exceeded 203 lb. At the factory all carcasses were commercially graded on the basis of carcass length, and shoulder and loin back-fat thickness.

Diets and treatments. The percentage composition of the basal diet used throughout in both experiments was: barley meal 52, fine miller's offal 38 and white-fish meal 10. To each 100 lb of the diet were added 4·5 g Rovimix (containing 50000 i.u. vitamin A and 12500 i.u. vitamin D_3/g, Roche Products Ltd). In Expt 1, there were three treat-

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ments: (1) basal diet only (controls); (2) basal diet with 45·4 g copper sulphate ($CuSO_4.5H_2O$)/100 lb diet, supplying 250 p.p.m. Cu; (3) basal diet with 20·6 g copper sulphide (air-dried CuS)/100 lb diet, supplying 250 p.p.m. Cu. As copper sulphide slowly oxidizes to copper sulphate, each batch was washed with distilled water before use to remove any soluble Cu. In addition, only enough diet to last some 3 weeks was mixed at a time. (Most batches of commercial copper sulphide obtained for these experiments were found to contain appreciable amounts of copper sulphate.)

In Expt 2, a fourth treatment was added in which the basal meal was supplemented with 5·2 g air-dried CuS/100 lb diet, supplying 62·5 p.p.m. Cu.

Estimation of copper content of various organs and tissues. In both experiments a sample of liver tissue adjacent to the bile duct was taken at slaughter from each pig and stored at −20° (see Barber, Braude & Mitchell, 1960). In Expt 2, samples of kidney (from cortex region at anterior end), heart (from left ventricular wall), spleen (from dorsal pole), psoas muscle and back fat were also taken and stored at −20°. The Cu content of all the samples of liver and of kidney, and of samples of heart, spleen, psoas muscle and back fat from four litter-mate replicates on each treatment in Expt 2 were determined by the method of Andrus (1955). The total weight of the liver, kidney, heart and spleen and the dry-matter percentage of the liver and kidney of all pigs on Expt 2 were determined. The dry-matter percentage of the heart and spleen and samples of psoas muscle and back fat from four litter-mate replicates on each treatment was also determined.

Investigation of fungal population in the faeces of the pigs. Samples of freshly voided faeces were collected at approximately fortnightly intervals from all pigs in Expt 1 throughout the duration of the trial.

Portions of 1 g of faeces were dispersed in 9 ml of sterile 0·9% (w/v) sodium-chloride solution and the dispersion was serially diluted with saline. Portions of 1 ml of the 10-fold, 100-fold and 1000-fold dilutions were poured into sterile Petri dishes and covered with 10 ml of warm (45°) medium of the following composition: peptone (Difco) 1 g, diamalt (a commercial starch hydrolysate, British Diamalt Co.) 4 g, beef extract (Oxo Ltd) 0·35 g, sodium chloride 0·75 g, agar 2 g, and water 100 ml. The plates were incubated aerobically at 37° and after 24 h the number of colonies was counted; the counts were confirmed after incubation for a further 24 h.

RESULTS

All standard errors were calculated from randomized block analyses of variance, no adjustments being made for variation in either initial live weight or cold dead weight (see Barber, Braude & Mitchell, 1957).

Daily weight gain, food-conversion efficiency, rate of food consumption, dressing percentage and carcass length

Expt 1 (*Table* 1). None of the treatment differences for any of the variables measured was significant at the 5% level. For daily gain and rate of food consumption, however, the treatment mean squares approached significance at this level and a *t* test showed

Table 1. *Expt 1. Effect of diets supplemented with either copper sulphate or copper sulphide on mean daily weight gain, food-conversion efficiency, rate of food consumption, dressing percentage, carcass length and liver Cu stores*

	Treatment no. and dietary supplement				
	1 None	2 250 p.p.m. Cu as sulphate	3 250 p.p.m. Cu as sulphide	Standard error of the mean†	Significance of treatment mean square‡
No. of pigs	8	8	8§	—	—
Initial weight (lb)	47·2	48·4	48·9	—	—
Final weight (lb)	210·1	208·6	209·0	—	—
Daily gain (lb)	1·53	1·57	1·62	±0·026	N.S.
Food conversion (lb meal/lb live-weight gain)	3·24	3·22	3·21	±0·070	N.S.
Rate of food consumption (lb/day)	4·94	5·03	5·19	±0·082	N.S.
Dressing percentage	73·4	74·1	72·5	±0·46	N.S.
Carcass length (mm)	791	784	783	±4·8	N.S.
Concentration of Cu in liver (mg/kg dry tissue)‖:					
Derived mean	46	328	43	—	***
95 % confidence limits	38–55	273–394	36–51	—	—

† Based on 13 degrees of freedom.
‡ N.S., $P > 0.05$; *** $P < 0.001$.
§ One pig died shortly after the beginning of the experiment, and missing values, calculated by the missing-plot technique (Yates, 1933), were substituted.
‖ Logarithmic transformation was used.

Table 2. *Expt 2. Effect of diets supplemented with either copper sulphate or copper sulphide on mean daily weight gain, food-conversion efficiency, rate of food consumption, dressing percentage and carcass length*

	Treatment no. and dietary supplement					
	1 None	2 250 p.p.m. Cu as sulphate	3 250 p.p.m. Cu as sulphide	4 62·5 p.p.m. Cu as sulphide	Standard error of the mean†	Significance of treatment mean square‡
No. of pigs	12§	12§	12§	12	—	—
Initial weight (lb)	41·3	40·8	39·9	40·9	—	—
Final weight (lb)	209·3	207·3	207·3	207·8	—	—
Daily gain (lb)	1·40	1·58	1·47	1·41	±0·031	**
Food conversion (lb meal/lb live-weight gain)	3·26	3·13	3·23	3·27	±0·063	N.S.
Rate of food consumption (lb/day)	4·53	4·93	4·73	4·60	±0·064	***
Dressing percentage	73·0	74·4	73·0	73·5	±0·28	**
Carcass length (mm)	802	784	799	788	±4·1	*

† Based on 29 degrees of freedom.
‡ N.S., $P > 0.05$. * $0.05 > P > 0.01$. ** $0.01 > P > 0.001$. *** $P < 0.001$.
§ Two pigs on treatment 1 and one each on treatments 2 and 3 died or were taken off experiment for reasons unconnected with the trial, and missing values, calculated by the missing-plot technique (Yates, 1933), were substituted.

that the differences in daily gain and rate of food consumption resulting from supplementation of the basal diet with copper sulphide were just significant at the 5 % level. Values for the Cu content of liver samples are also given in Table 1.

Expt 2 (*Table* 2). Supplementation of the basal diet with copper sulphate resulted in a significant increase of 12·9 % in rate of gain, 8·8 % in rate of food consumption and 1·9 % in dressing percentage, and a significant decrease of 2·2 % in carcass length. Efficiency of food utilization was also improved by 4·0 %, but the difference was not statistically significant. Supplementation of the basal diet with either level of copper sulphide, on the other hand, had no significant effect on any of the variables measured with the exception that the rate of food consumption of the pigs receiving the higher level of copper sulphide was significantly greater than that of the unsupplemented control pigs.

Table 3. *Commercial grading of carcasses of pigs in Expts* 1 *and* 2

Expt no.	Dietary supplement	No. of pigs: Total	AA+	AA	A	Total A and above	B+	B	C	F	Grade A on: Loin	Shoulder
1	None	8	2	2	1	5	2	0	1	0	5	7
	250 p.p.m. Cu as sulphate	8	1	2	1	4	4	0	0	0	4	5
	250 p.p.m. Cu as sulphide	7	2	2	2	6	0	1	0	0	6	6
2	None	10	5	4	0	9	1	0	0	0	9	10
	250 p.p.m. Cu as sulphate	11	1	1	0	2	7	0	2	0	5	6
	250 p.p.m. Cu as sulphide	11	3	3	0	6	3	0	2	0	6	8
	62·5 p.p.m. Cu as sulphide	12	3	4	1	8	3	0	1	0	9	8

The rate of gain, rate of food consumption and dressing percentage of the pigs given copper sulphate were significantly greater than of the pigs given copper sulphide at either level. In addition, the mean carcass length of the former pigs was significantly less than that of the pigs receiving the higher level of copper sulphide.

Commercial grading (*Table* 3)

In the first experiment there were no marked differences in the commercial grading between any of the treatments. In Expt 2, on the other hand, supplementation of the diet with copper sulphate and, to a lesser extent, with the higher level of copper sulphide, resulted in a reduction in the proportion of grade A carcasses and a corresponding increase in grade B carcasses, reflecting an increase in the thickness of fat along the back.

Copper stores in the liver, kidney, heart, spleen, psoas muscle and back fat (Tables 1 and 4)

In both experiments there was a large, significant increase in the Cu content of the liver as a result of supplementing the diet with copper sulphate, but no such increase occurred when copper sulphide was used. Similarly, there was a significant increase in the Cu content of the kidneys of pigs given copper sulphate but not in those of pigs given copper sulphide. There was some indication that the Cu content of the psoas muscle was slightly higher in pigs given the supplemented diets, but no treatment differences were apparent with the heart, spleen or back fat.

Table 4. *Expt 2. Concentrations of Cu (mg/kg dry tissue) in samples of liver, kidney, heart, spleen, psoas muscle and back fat*

		Treatment no. and dietary supplement				
		1 None	2 250 p.p.m. Cu as sulphate	3 250 p.p.m. Cu as sulphide	4 62·5 p.p.m. Cu as sulphide	Significance of treatment mean square
		Mean values for twelve pigs†				
Liver:	derived mean‡	59	246	57	58	***
	95 % confidence limits	48–72	202–301	47–70	48–71	—
Kidney:	derived mean‡	38	64	39	37	***
	95 % confidence limits	33–43	56–73	34–44	33–42	—
		Mean values for four pigs				
Heart		30	27	28	24	—
Spleen		6	6	6	7	—
Psoas muscle		3	5	4	5	—
Back fat		1	1	1	1	—

*** $P < 0·001$.
† See Table 2 for note on pigs withdrawn from test.
‡ Logarithmic transformation was used.

Total weight and moisture content of liver, kidney, heart, spleen, psoas muscle and back fat (Table 5)

None of the treatments caused significant differences in the weight or moisture content of any of the organs or tissues studied. However, there were indications from the mean figures that supplementation with Cu caused some reduction in the weights of the liver and heart, and some increase in the moisture content of all the organs.

Faecal fungi

There was a wide variation in the counts of fungi in the faeces of the pigs receiving the same diet. None the less it was apparent that the mean counts for the pigs receiving the supplement of copper sulphate were appreciably lower than those of either the control pigs or those receiving copper sulphide. In fact from the 5th to the 11th week of the experiment no fungi could be cultured from the faeces of the pigs given copper sulphate. The addition of copper sulphide did not cause any decrease in counts and there was some indication that it increased the faecal count during the later stage of the experiment.

Table 5. *Expt 2. Total weight of liver, kidney, heart and spleen (g) and percentage moisture content of these organs and of samples of psoas muscle and back fat. (Mean values for twelve pigs* except for moisture content of heart, spleen, psoas muscle and back fat, which are mean values for four pigs)*

		Treatment no. and dietary supplement				
		1 None	2 250 p.p.m. Cu as sulphate	3 250 p.p.m. Cu as sulphide	4 62·5 p.p.m. Cu as sulphide	Standard error of means†
Liver:	weight	1679	1590	1662	1661	±42·0
	moisture	63·6	65·1	66·2	65·5	0·71
Kidney:	weight	139	150	148	140	±14·0
	moisture	77·7	77·9	78·1	78·0	0·52
Heart:	weight	284	277	275	280	± 4·7
	moisture	75·0	76·4	76·6	76·0	—
Spleen:	weight	112	113	103	100	± 4·8
	moisture	76·5	77·7	78·2	76·1	—
Psoas muscle:	moisture	62·9	74·0	73·5	70·9	—
Back fat:	moisture	8·7	8·2	7·1	9·4	—

* See Table 2 for note on pigs withdrawn from test.

† Based on 29 degrees of freedom. None of the treatment mean squares reached significance at the 5 % level.

DISCUSSION

Whereas in the second experiment the response of the pigs to a supplement of copper sulphate agreed in all respects, except for commercial grading, with the results consistently obtained previously (see Barber *et al.* 1960), the pigs in the first experiment failed to show the expected response. Comparison of the performance of the pigs given copper sulphate shows that it was very similar in all respects in both experiments, whereas the performance of the control animals in the first experiment was markedly superior to that in the second The reason for this difference in the performance of the control animals in the two experiments is unknown. Recently, King (1960) reported that supplementation of a growing-pig diet with 0·1 % copper sulphate had no significant effect on the growth rate of pigs kept in a 'warm' environment, whereas both the growth rate and efficiency of food utilization of pigs kept in a 'cold' environment were significantly improved. The performance of the unsupplemented control animals was significantly better in the 'warm' than in the 'cold' environment. It would not appear, however, that any seasonal effects could explain the different results in the two experiments reported here.

When the results of the two experiments are taken together, a not unreasonable interpretation of the effects of copper-sulphide supplementation would appear to be that, when 250 p.p.m. Cu are supplied in the form of the sulphide, some improvement in growth rate may be expected but that it is appreciably less than that normally obtained by giving the same amount of Cu in the form of the sulphate, and that when only one-quarter of this amount of Cu is given as sulphide no response at all occurs. It is also quite clear that supplementing the diet with either level of copper sulphide has no effect on the level of the Cu stores in the liver or other organs, which is in marked contrast to the large increase in both liver and kidney Cu that consistently occurs when 250 p.p.m. Cu as copper sulphate are given. Allen, Barber, Braude & Mitchell (unpublished) showed that whereas some response was obtained when a growing-pig diet was supplemented with 62·5 p.p.m. Cu given as copper sulphate, it was less than that obtained when the level of copper sulphate was such as to supply 250 p.p.m. Cu. In addition, the lower level of copper sulphate had no effect on liver Cu stores, in contrast to the marked increase resulting from supplementation with the higher level. These results are in general agreement with those previously reported by Lucas & Calder (1957) and by Dammers & van der Grift (1959).

It appears that the results of supplementing a growing-pig diet with 250 p.p.m. Cu as the sulphide are very similar in many respects to those of supplements of 62·5 p.p.m. Cu as the sulphate. Recently, Bowland, Braude, Chamberlain, Glascock & Mitchell (1961) showed that though significant amounts of labelled Cu were absorbed by pigs given copper sulphide (1–2% of the total dose), about three times as much was absorbed when the same amount of Cu was given as the sulphate. This finding, together with the observation on the effects of lower levels of copper sulphate, and of the results, presented in this paper, of copper-sulphide supplementation, suggest that the effectiveness of Cu in promoting growth is related to the amount of soluble Cu in the gut, but whether the site of action is systemic, enteric, or both, remains unknown.

No conclusions are possible from the observations made on faecal fungi, in view of their very preliminary nature, but they do appear to illustrate one way in which the activity of copper might depend on the amount of soluble Cu present in the gut. The effect of Cu on gut fungi clearly merits further study.

The effect of copper-sulphate supplementation in increasing dressing percentage and reducing carcass length has been previously observed (see Barber *et al.* 1960) and the evidence now available indicates that both are true treatment effects. The marked down-grading of the carcasses of pigs given copper sulphate in Expt 2, although not in Expt 1, due to an increase in thickness of back fat, was not apparent in previous trials (Barber *et al.* 1960). It would appear most likely that such differences between experiments are due to inherent differences in grading potential between the groups of pigs used, but no definite conclusion on this aspect of Cu supplementation of practical pig rations can be made until the results of further studies, now in progress, are available. The figures for the Cu content of various organs and tissues agree well with those of Barber, Braude, Mitchell, Rook & Rowell (1957), although the values for the Cu content of the kidney were rather higher in the present work. In addition to the significant effects of copper-sulphate supplementation on Cu stores in liver and kidney these authors also reported a small, but statistically significant, increase in the Cu content of the psoas muscle, and the trend, apparent in the present study, adds support to this finding. Further confirmation of these results is provided by the results of feeding trials reported by Bowland *et al.* (1961), who found that 24 h after administration of labelled copper sulphate the concentration of labelled Cu was highest in the liver, next highest in the kidney, and in all other organs was relatively low. Of the tissues examined, the muscle contained more Cu than the fat, skeleton or skin.

The trend for copper-sulphate supplementation to reduce liver weight, seen in the figures in Table 5, was also reported by Barber, Braude, Mitchell, Rook & Rowell (1957), and the possible correlation between the observed increase in dressing percentage in pigs given diets supplemented with copper sulphate and the apparent reduction in the weights of the liver and gut was discussed by Barber *et al.* (1960).

Little is known of the chemical composition of the carcasses of pigs given Cu-supplemented diets and whether it differs in any respect from that of carcasses of pigs given unsupplemented diets. The indication (Table 5) of a trend for Cu supplementation to cause some increase in moisture content of most of the organs and tissues studied is of interest in this connexion, but much more work is needed before any definite conclusions can be drawn.

SUMMARY

1. Seventy-two individually fed, virus pneumonia-free Large White weaners were used in two experiments to compare the effects of dietary supplements of copper sulphate and copper sulphide on the performance of growing pigs from weaning to bacon weight.

2. A supplement of 250 p.p.m. Cu given as copper sulphate had no significant effect on performance in the first experiment, but in the second it significantly increased growth rate by 12·9%, rate of food consumption by 8·8% and dressing percentage by 1·9%, and decreased carcass length by 2·2%. It also had an adverse effect on the commercial grading of the carcasses. The better performance of the unsupplemented control pigs in the first experiment compared with that in the second experiment is discussed.

3. Small, not significant increases in mean growth rate were apparent in both experiments when 250 p.p.m. Cu was given as copper sulphide. It was concluded that some improvement in growth rate was probably to be expected when this level of Cu was given as the sulphide, but that it was appreciably less than that normally obtained with 250 p.p.m. Cu given as copper sulphate. A supplement of 62·5 p.p.m. Cu given as sulphide had no effect on the performance of the pigs.

4. It is suggested that the effectiveness of Cu in promoting growth in pigs is related to the amount of soluble Cu in the gut, but whether the site of action is systemic, enteric, or both, remains unknown.

5. Copper sulphate, but not copper sulphide, in the diet significantly increased liver and kidney stores of Cu.

6. Values for total weight and moisture content of various organs and tissues are presented.

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