An attempt to distinguish between the direct and indirect effects, in the laying domestic fowl, of added dietary copper sulphate

BY MARY H. STEVENSON AND N. JACKSON

Agricultural and Food Chemistry Research Division, Department of Agriculture for Northern Ireland, and the Queen's University of Belfast, Newforge Lane, Belfast BT9 5PX, Northern Ireland

(*Received 16 October 1980 – Accepted 12 January 1981*)

1. An experiment is reported in which the effects of giving copper sulphate-supplemented diets and control unsupplemented pair-fed diets to laying hens were compared.

2. The level of food intake significantly adversely affected mean body-weight, egg number, egg weight, liver, kidney, oviduct and ovary weights. Gizzard weight/kg body-weight was significantly increased both with decreasing levels of food intake and increased $CuSO_4$ supplementation.

3. There was evidence of a depressing effect of $CuSO_4$ *per se* on egg production and possibly on oviduct and ovary weight.

4. Liver lipid concentration was significantly decreased with decreasing levels of food intake and the results also suggest a depressing effect of $CuSO_4$.

5. The Cu concentrations and total contents in liver and kidneys were significantly increased by dietary added $CuSO_4$. Liver and kidney Zn and Fe concentrations were increased with decreasing levels of food intake rather than by $CuSO_4$ addition.

The effects on laying hens of giving high and moderately high levels of dietary copper sulphate, $CuSO_4 . 5H_2O$ have been studied by Thomas *et al.* (1974), Griminger (1977), Jackson *et al.* (1979), Stevenson & Jackson (1980*a*, *b*) and Jackson & Stevenson (1981).

A consistent finding in all these experiments has been the marked reduction in food intake and egg production which accompanies the consumption of diets containing high levels of added dietary $CuSO_4 . 5H_2O$. Also the water intake has been shown to decrease at levels of dietary $CuSO_4$ providing more than 500 mg copper/kg (Jackson, 1977; Jackson *et al.* 1979). Associated with these effects decreases in liver lipid content and oviduct size and an increase in gizzard weight have been observed. Liver Cu and iron concentrations were elevated at the high levels of dietary $CuSO_4$.

It was decided to carry out a pair-feeding experiment in order to assess if the effects of adding dietary $CuSO_4$ are due to direct toxicity or are indirect and caused by decreased food intake.

EXPERIMENTAL

Thirty-six, white 18-week-old hens (Shaver 288) were placed in galvanized iron cages each fitted with an individual feeder and nipple drinker. The temperature was maintained at 21 ± 2 °. The initial lighting regimen of 11 h light and 13 h darkness was changed once per week until 17 h light and 7 h darkness was achieved. This latter lighting programme was maintained throughout the experiment. At 24 weeks, when all the hens had been laying for at least 2 weeks, they were randomly allocated to one of six treatment groups each containing six birds. The diets, fed *ad lib.* for 6 weeks, were a basal diet supplemented with $CuSO_4 . 5H_2O$ (treatments 2, 4 and 6) at levels equivalent to 500, 1000 and 2000 mg Cu/kg and the same basal diet without added $CuSO_4$ pair-fed to these treatments thus giving three control treatments (nos. 1, 3 and 5). The composition of the basal diet and fineness of grinding of the $CuSO_4 . 5H_2O$ have been given by Jackson (1977) and the control diet analyses reported by Stevenson & Jackson (1980*b*).

The individual daily food intakes of hens on treatments 2, 4 and 6 were measured and treatments 1, 3 and 5 pair-fed to these respective levels, the level of feeding being based on the previous day's mean food intake by the $CuSO_4$-fed birds. Daily egg production, egg weight and initial and final body-weights were recorded. At the end of the 6-week feeding period all the hens were killed by decapitation. Blood was analysed for haemoglobin (Hb) and packed cell volume (PCV) while Cu and aspartate aminotransferase (*EC* 2.6.1.1; AAT) levels were assayed in blood serum. The liver, kidneys, oviduct, ovary and gizzard were removed and weighed and Cu, zinc and Fe concentrations in the liver and kidneys measured after drying at 100 °. The lipid content of the liver was determined by the method of Folch *et al.* (1957).

Chemical and statistical analyses were as described by Stevenson & Jackson (1980*a*).

RESULTS

Mean initial body-weight, body-weight change, food intake, egg number, mean egg weight, absolute tissue weights and these weights expressed as g/kg body-weight are given in Table 1 together with liver lipid concentration. The attempt to equalize the food intake of each paired group was successful. Mean body-weight change and the liver, oviduct and ovary weights and these weights expressed as g/kg body-weight were significantly decreased ($P < 0.001$) with decreasing levels of food intake but the presence of $CuSO_4$ in the diets had no statistically significant effect for each pair-fed group. Mean egg number was significantly decreased with decreasing levels of food intake ($P < 0.001$). Over all, the presence of $CuSO_4$ had no statistically significant effect on egg number but the presence of $CuSO_4$ did depress mean egg number at all levels of addition and in particular at the 1000 mg added Cu/kg diet at which level the effect was statistically significant. Mean egg weight was also significantly decreased ($P < 0.05$) with decreasing levels of food intake. Kidney weight and kidney weight per unit body-weight were significantly decreased with decreasing levels of food intake ($P < 0.001$) and increased by $CuSO_4$ supplementation ($P < 0.05$). The level of food intake did not affect absolute gizzard weight but significantly increased this weight, when expressed as g/kg body-weight ($P < 0.001$). Both absolute gizzard weight and gizzard weight expressed per unit body-weight were significantly increased by $CuSO_4$ supplementation ($P < 0.001$) relative to the appropriate controls. Liver lipid concentration was significantly decreased with decreasing levels of food intake ($P < 0.05$) and although the absolute effect of $CuSO_4$ addition was a decrease in liver lipid concentration this was not significantly different from the corresponding control at any of the three levels used.

The blood and blood serum results and the mean concentrations and total contents of Cu, Zn and Fe in the liver and kidneys are presented in Table 2. Blood Hb, PCV and AAT were significantly increased with decreasing levels of food intake ($P < 0.01$, $P < 0.05$ and $P < 0.01$ respectively) although relative to the controls only AAT was significantly decreased ($P < 0.001$) by $CuSO_4$ supplementation. Serum Cu levels were unaffected by any of the dietary treatments, the values being similar to those reported by Beck (1961) for whole blood Cu.

Liver Cu concentration and total liver Cu content were significantly increased with decreasing levels of food intake and $CuSO_4$ supplementation ($P < 0.001$). The food intake effect was not evident for treatments 1, 3 and 5 and the effect of supplementation was restricted to treatments 2, 4 and 6. Liver Zn and Fe concentrations were significantly increased ($P < 0.01$ and $P < 0.001$ respectively), total liver Zn was significantly decreased and total liver Fe increased ($P < 0.001$) as the level of food intake decreased. Total liver Zn was significantly increased ($P < 0.05$) by the presence of added dietary $CuSO_4$. However, the mean total liver Zn values decreased as the $CuSO_4$ intake increased. When the liver mineral results were expressed on a fat-free basis in general the trends observed were further accentuated and consequently these results are not presented.

Table 1. *Mean initial body-weight (kg), body-weight change (g), food intake (kg), copper intake (mg), egg number, mean egg weight (g), liver, kidney oviduct, ovary and gizzard fresh weights (g) and these weights expressed as g/kg body-weight and liver lipid concentration (g/kg dry matter) of laying hens given copper sulphate-supplemented diets together with their pair-fed controls*

(Mean values for six observations)

	Treatments†							Statistical significance of effect		
	1	2	3	4	5	6	SEM	Level of food intake	With/ without $CuSo_4$	Interaction
Cu in diet (mg/kg)	0	500	0	1000	0	2000	—	—	—	—
Initial BW (kg)	1·40	1·40	1·41	1·41	1·41	1·41	0·051	NS	NS	NS
Body-wt change (g)	−100	−82	−258	−158	−305	−342	33·3	***	NS	NS
Food intake (kg)	3·12	3·20	1·96	1·96	1·14	1·14	0·141	***	NS	NS
Cu intake (mg)	23	1408	15	1803	9	2086				
Egg number	31·8	30·0	17·2	9·3	5·7	3·7	2·63	***	NS	NS
Egg wt (g)	49·9	50·3	48·1	48·5	47·0	45·9	1·31	*	NS	NS
Liver wt (g)	26·6	29·4	22·7	22·8	13·6	16·2	1·79	***	NS	NS
Liver wt (g/kg BW)	20·3	22·2	19·8	18·3	12·4	15·5	1·28	***	NS	NS
Kidneys wt (g)	7·86	9·69	6·72	6·81	5·06	5·71	0·393	***	*	NS
Kidneys wt (g/kg BW)	6·06	7·33	5·88	5·48	4·62	5·38	0·286	***	*	*
Oviduct wt (g)	52·5	47·4	39·0	34·3	20·1	11·2	5·48	***	NS	NS
Oviduct wt (g/kg BW)	40·7	35·7	34·1	27·5	18·8	9·1	4·55	***	NS	NS
Ovary wt (g)	38·9	36·6	22·9	18·3	14·0	6·9	4·50	***	NS	NS
Ovary wt (g/kg BW)	30·5	27·0	20·1	14·9	13·2	6·5	3·80	***	NS	NS
Gizzard wt (g)	19·5	20·7	17·9	24·7	18·8	24·8	1·24	NS	***	NS
Gizzard wt (g/kg BW)	15·0	15·8	15·5	19·9	17·1	23·4	1·01	***	***	*
Liver lipid concentration (g/kg DM)	240·3	184·2	203·9	176·5	141·3	115·2	29·33	*	NS	NS

BW, body-weight; DM, dry matter; NS, not significant.

$^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$, $^{***}P < 0{\cdot}001$.

† Treatments 2, 4 and 6 represent diets supplemented with 500, 1000 and 2000 mg Cu/kg; treatments 1, 3 and 5 are their pair-fed controls.

Determined Cu concentrations were 440, 920 and 1830 mg/kg for treatments 2, 4 and 6 respectively. The value for treatments 1, 3 and 5 was 7·5 mg Cu/kg diet.

Table 2. *Mean blood and serum analytical values, mean concentrations (μg/g dry matter) and total contents (μg) of copper, zinc and iron in liver and kidneys of laying hens given copper sulphate-supplemented diets together with their pair-fed controls*

(Mean values for six observations. For liver Cu, the analysis of variance was carried out using log transformations and the mean values are the antilogs of the mean of the log transformations with the means of the log values given in parentheses)

	Treatments†							Statistical significance of effect		
	1	2	3	4	5	6	SEM	Level of food intake	With/ without $CuSO_4$	Interaction
Cu in diet (mg/kg)	0	500	0	1000	0	2000	—	—	—	—
Blood Hb (g/l)	76·3	73·5	75·5	73·3	90·0	92·3	4·59	**	NS	NS
Blood PCV	0·243	0·222	0·240	0·232	0·260	0·268	0·0106	*	NS	NS
Serum AAT (international units/l)‡	188	113	201	134	248	146	12·8	**	***	NS
Serum Cu (μg/l)	305	313	267	268	212	312	34·4	NS	NS	NS
Liver Cu concentration (μg/g DM)	11·4 (1·058)	44·1 (1·644)	11·7 (1·067)	337·4 (2·528)	13·3 (1·244)	789·0 (2·897)	(0·0920)	***	***	***
Total liver Cu content (μg)	97 (1·988)	404 (2·606)	83 (1·919)	2393 (3·379)	69 (1·836)	3648 (3·562)	(0·0911)	***	***	***
Liver Zn concentration (μg/g DM)	119	118	117	130	147	171	11·4	**	NS	NS
Total liver Zn (μg)	1028	1081	812	914	570	782	60·1	***	*	NS
Liver Fe concentration (μg/g DM)	387	373	678	728	1997	1554	192·3	***	NS	NS
Total liver Fe (μg)	3170	3252	4751	4902	7649	7047	689·1	***	NS	NS
Kidney Cu concentration (μg/g DM)	16·2	16·1	15·6	17·3	16·6	22·4	1·40	*	*	NS
Total kidney Cu (μg)	29·7	36·2	22·5	27·1	18·8	30·8	2·58	**	***	NS
Kidney Zn concentration (μg/g DM)	99	96	108	100	115	114	4·0	***	NS	NS
Total kidney Zn (μg)	183	217	156	157	132	154	9·0	***	•	NS
Kidney Fe concentration (μg/g DM)	291	257	370	315	409	408	31·6	***	NS	NS
Total kidney Fe (μg)	532	572	537	490	466	547	47·4	NS	NS	NS

Hb, haemoglobin; PCV, packed cell volume; AAT, aspartate aminotransferase; DM, dry matter; NS, not significant.
$*P < 0{\cdot}05$, $**P < 0{\cdot}01$, $***P < 0{\cdot}001$.
† Treatments 2, 4 and 6 represent diets supplemented with 500, 1000 and 2000 mg Cu/kg; treatments 1, 3 and 5 are their pair-fed controls.
‡ One international unit refers to the oxidation of 1 μmole NaDPH/min at 37°.

As the level of food intake decreased kidney Cu concentration ($P < 0.05$) and kidney Zn and Fe levels ($P < 0.001$) significantly increased but only the kidney Cu concentration was significantly increased ($P < 0.05$) by increasing dietary $CuSO_4$ supplementation. Total kidney Cu and Zn were decreased with decreasing levels of food intake ($P < 0.01$ and $P < 0.001$ respectively) and both were increased, relative to the control diets, by $CuSO_4$ supplementation ($P < 0.001$ and $P < 0.05$ respectively) although the levels tended to decrease with increasing dietary $CuSO_4$ for treatments 2, 4 and 6. Total kidney Fe was unaffected by either the level of food intake or $CuSO_4$ supplementation, the over-all mean (+SEM) being 524 ± 19.5 μg.

DISCUSSION

The diets with the high levels of added dietary $CuSO_4$ given to laying hens have produced the effects expected in the light of previous work from this Department. From the present experiment, it is apparent that many of the effects, including reduced body-weight gain, egg number, egg weight, liver, oviduct and ovary weights are mainly due to a reduction in food intake rather than to the presence of high levels of dietary added $CuSO_4$. Fisher *et al.* (1971) using 5-week-old broiler chicks given diets containing $CuSO_4$ equivalent to 750 mg Cu/kg diet also found that the decreased body-weight gain could be attributed to the reduction in food intake rather than to the presence of the added Cu salt. However, comparison with their pair-feeding experiment is limited since it was of only 2 weeks duration.

It is not surprising that, in the present experiment, egg production was affected dramatically in both the $CuSO_4$-fed hens and their pair-fed controls since the intakes of protein and energy, especially at the two highest levels of $CuSO_4$ supplementation, were not sufficient to meet their dietary requirements as recommended by the Agricultural Research Council (1975).

Although the results now show that the depressing effect of dietary treatment on egg production can be mainly attributed to reduced food intake nevertheless the evidence also indicates an independent adverse affect of the dietary $CuSO_4$ which attained statistical significance ($P < 0.05$) at the intermediate level of addition.

The depression of water intake at high $CuSO_4$ intake levels (Jackson, 1977; Jackson *et al.* 1979) may be a factor adversely affecting egg production and could also contribute to the increase in blood Hb and PCV at the highest level of $CuSO_4$ addition.

No obvious explanation can be given for the increase in serum AAT with decreasing food intake and the fact that the mean AAT value was, at each level of $CuSO_4$ addition, depressed below the corresponding pair-fed control value does not suggest any tissue damage. In fact, such an effect, although reported in other species, has not been demonstrated in the domestic fowl. In the light of the present results the apparent increase of serum AAT with increasing dietary $CuSO_4$ reported by Jackson (1977) is now believed to be an effect of food intake.

The significant increase in gizzard weight/kg body-weight has resulted from both the lower food intake and the inclusion of $CuSO_4$ at levels equivalent to 1000 and 2000 mg Cu/kg diet. Similar effects of high dietary $CuSO_4$ on gizzard weight in the laying hen have been reported by Jackson *et al.* (1979) and Stevenson & Jackson (1980*a*, *b*). Pathological changes in gizzard integrity of broilers in response to dietary $CuSO_4$ have been observed by Fisher *et al.* (1973) and Poupoulis & Jensen (1976).

The increases in kidney weight per unit body-weight at the lowest and highest levels of $CuSO_4$ supplementation were unexpected since decreases in kidney weight/kg body-weight have been reported previously (Jackson, 1977; Jackson *et al.* 1979; Stevenson & Jackson, 1980*a*).

Although the over-all effects of $CuSO_4$ on oviduct and ovary weights were not significant, the results at 1000 and 2000 mg added Cu/kg indicate a depression of these due to the $CuSO_4$ which is additional to that caused by restriction of intake. It is very possible that these effects

would have been more definite if the experiment had been carried on for a longer period of time.

Although the statistical analyses attribute the reduction in liver lipid content to a decrease in the level of food intake the decrease in the mean value at each level of intake associated with the $CuSO_4$ addition strongly suggests a depressing effect of the additive.

The increase of approximately 2000% in the liver Cu concentration at the highest level of $CuSO_4$ addition substantiates the various results reported previously from this Department.

The slight but statistically significant increase in liver Zn per unit dry weight with reduced food intake appears to be a concentration effect resulting from reduced liver weight since the total Zn content decreased. An increase of liver Zn concentration in the presence of added dietary $CuSO_4$ has been reported by Stevenson & Jackson (1980*b*). This increase of the liver Zn in the presence of added dietary Cu has also been noted in the pig by Ritchie *et al.* (1963) and Suttle & Mills (1966) and does not necessarily indicate a lack of antagonism at the level of the intestine or other tissues since such an antagonist effect has been found by these authors.

The increases in liver Fe concentration and total liver Fe also agree with previous results (Jackson *et al.* 1979; Stevenson & Jackson, 1980*a*, *b*) but the present results associate the differences with the reduction of food intake rather than with the dietary $CuSO_4$. A number of factors may be involved in this effect including the reduced demand for Fe incorporation into the egg.

The increase in kidney Cu concentration with decreasing food intake is similar to the effect found by Stevenson & Jackson (1980*b*) and is obviously attributable to the presence of the $CuSO_4$ rather than level of food intake.

Stevenson & Jackson (1980*b*) suggested that an increase in kidney Fe concentration was related to increasing dietary $CuSO_4$ but the present results indicate that this effect is an indirect one due to decreased food intake and not directly due to dietary $CuSO_4$ concentration.

In this experiment the main effects directly attributable to the $CuSO_4$ are the high liver Cu, increased kidney Cu and kidney weight and increased gizzard weight. However, although the analyses presented attribute the depression of liver lipid and egg production to decreased food intake, it is apparent that the $CuSO_4$ *per se* is also having a deleterious effect.

The authors thank Dr D. J. Kilpatrick for statistical analyses and Mrs R. Park, Mr W. Graham and Mr G. McC. Kirkpatrick for techical assistance.

REFERENCES

Agricultural Research Council (1975). *The Nutrient Requirements of Farm Livestock* no. 1, *Poultry*. London: H.M. Stationery Office.
Beck, A. B. (1961). *Aust. J. agric. Res.* **12**, 743.
Fisher, C., Laursen-Jones, A. P., Hill, K. J. & Hardy, W. S. (1973). *Br. Poult. Sci.* **14**, 55.
Fisher, C., Wise, D. & Filmer, D. G. (1971). *14th Wld's Poult. Congr. Madrid*, p. 759.
Folch, J. Lees, J. & Sloane Stanley, C. H. (1957). *J. biol. Chem.* **226**, 497.
Griminger, P. (1977). *Poult. Sci.* **56**, 359.
Jackson, N. (1977). *Br. J. Nutr.* **38**, 93.
Jackson, N. & Stevenson, M. H. (1981). *Br. J. Nutr.* **45**, 99.
Jackson, N., Stevenson, M. H. & Kirkpatrick, G. McC. (1979). *Br. J. Nutr.* **42**, 253.
Poupoulis, C. & Jensen, L. S. (1976). *Poult. Sci.* **55**, 113.
Ritchie, H. D., Leucke, R. W., Baltzer, B. V., Miller, E. R., Ullrey, D. E. & Hoefer, J. A. (1963). *J. Nutr.* **79**, 117.
Stevenson, M. H. & Jackson, N. (1980*a*). *Br. J. Nutr.* **43**, 205.
Stevenson, M. H. & Jackson, N. (1980*b*). *Br. J. Nutr.* **43**, 551.
Suttle, N. F. & Mills, C. F. (1966). *Br. J. Nutr.* **20**, 135.
Thomas, M. C., Norvell, M. J., Calvert, C. C. & Goatcher, W. D. (1974). *Poult. Sci.* **53**, 1984, Abstr.

Printed in Great Britain