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Effects of level of dietary copper sulphate and period of feeding on the laying, domestic fowl, with special reference to tissue mineral content

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1. A cereal-based diet containing 7·6 mg copper/kg was fed *ad lib.* to laying hens for up to 48 d. Four other groups were given the control diet to which was added hydrated copper sulphate to provide 250, 500, 1000 and 2000 mg added Cu/kg.

2. Hens were killed on day 0 and after 3, 6, 12, 24 and 48 d. Records were kept of body-weight, food consumption, egg production and egg weight.

3. After slaughter blood haemoglobin, packed cell volume, serum Cu and aspartate aminotransferase (AAT; *EC* 2.6.1.1) were measured. The liver, kidneys, a sample of breast muscle, oviduct, ovary and gizzard were weighed. Gizzard, spleen, liver and kidney tissue were examined histologically.

4. The Cu, zinc and iron concentrations of liver, kidneys and breast muscle and the manganese concentrations of liver and kidneys were determined.

5. Body-weight loss occurred at 500–2000 mg added Cu/kg diet. Egg production was depressed by level of added Cu and period of time on the Cu-containing diets.

6. Mean liver, kidney, oviduct and ovarian weights per unit body-weight were depressed by Cu in the diet and the effect increased with period of time on the diets. Mean gizzard weight per unit body-weight was increased by dietary added Cu and by time.

7. Cu concentrations in the liver were increased by dietary level of added Cu and period of time on the diet. Zn concentration in liver increased at 1000 and 2000 mg added Cu/kg diet and liver Fe concentration was increased at these levels. Histological examination of the gizzard indicated that the Cu content of the gizzard lining increased with dietary added Cu.

Copper has been used as a growth stimulant in pig feeding for many years (Barber *et al.* 1955) but knowledge of its effects on poultry performance is limited (Agricultural Research Council, 1975).

The effects of adding Cu to broiler diets have been investigated (Fisher *et al.* 1972; Fisher, 1973). In more recent studies the effects of short and long term feeding of Cu as sulphate ($CuSO_4.5H_2O$) in the diet of laying hens have been reported (Jackson, 1977; Jackson *et al.* 1979). The longer term studies (Jackson *et al.* 1979) used up to 800 mg added Cu/kg diet and did not cause any obvious pathological effects.

The present experiment was initiated mainly in order to observe the rate at which the performance of laying hens, in terms of egg production, body-weight change, and the mineral content of some specific tissues would be affected by feeding diets supplemented with $CuSo_4.5H_2O$ supplying up to 2000 mg added Cu/kg diet. A brief report of some initial results has already been presented (Stevenson & Jackson, 1979).

EXPERIMENTAL

White light hybrid (Shaver 288) point-of-lay hens (156 birds, 17 weeks old) were placed in galvanized-iron cages fitted with individual feeders and nipple drinkers. The initial lighting regimen of 11 h light and 13 h darkness was changed weekly until 17 h light and 7 h darkness

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was achieved at the commencement of the experiment: this lighting programme was maintained until the experiment ended. At 24 weeks of age, when all the hens had been laying for at least 2 weeks, they were randomly allocated to a pre-experimental group of six birds and to five treatment groups each containing thirty birds. The birds of the pre-experimental group were killed at the start of the experiment.

The diets, fed *ad lib.*, were the control diet, the composition of which has been reported by Jackson (1977), alone or with Cu added as $CuSO_4.5H_2O$ at (mg/kg) 250, 500, 1000 and 2000. The Cu salt was finely ground as specified by Jackson (1977). The control diet contained (/kg): 173 g crude protein (nitrogen $\times$ 6·25), 7·6 mg Cu, 136 mg zinc, 415 mg Fe, 32 g calcium, 5·2 g phosphorus and had a calculated metabolizable energy content of 11·4 MJ/kg.

Six hens, selected at random from each treatment, were killed 3, 6, 12, 24 and 48 d after the start of the experiment. Individual body-weights were recorded at the start of the experiment and at slaughter. Records were kept of food consumption, daily egg production and egg weight.

The birds were killed by decapitation and blood haemoglobin (Hb) and packed cell volume (PCV) determined. Serum Cu was determined by the method of Ichida & Nobuoka (1969) and aspartate aminotransferase (AAT; *EC* 2.6.1.1) by the method of Bergmeyer & Bernt (1965). The results were expressed in international units, where one unit represents the oxidation of 1 μmol NADPH/min at 37 °. The fresh weights of the ovary, oviduct and gizzard were recorded. The liver, kidneys and a portion of breast muscle were weighed and oven-dried at 100 ° before chemical analyses. Portions of the gizzard, spleen, liver and kidneys were examined histologically for the presence of Cu (Lindquist, 1969) and glycogen as previously described (Jackson *et al.* 1979). The Cu, Zn, Fe and manganese concentrations in liver and kidneys were determined by atomic absorption spectrophotometry after dry ashing and solution in dilute hydrochloric acid. These minerals, with the exception of Mn, were also measured in breast muscle. The lipid content of liver samples was measured using the method of Folch *et al.* (1957).

The results were subjected to analysis of variance and log transformations were carried out for those variables which exhibited variance heterogeneity.

RESULTS

Since the determined dietary Cu values were close to the calculated values, the latter values have been used in presenting the results.

Mean daily food intake, body-weight change and egg production results are shown in Table 1. All were significantly depressed ($P < 0.001$) by dietary added Cu level. Body-weight change and egg production were significantly affected by time on the diet (both $P < 0.001$) and showed a significant diet $\times$ time interaction (both $P < 0.001$). Mean egg weight was unaffected either by the level of added Cu or time on the experimental diets. The over-all mean ($\pm$SEM) egg weight was 51·0$\pm$0·03 g.

Table 2 gives the blood Hb and PCV values and the serum Cu and AAT levels. These were significantly affected by the level of Cu supplementation (all $P < 0.001$). The principal effect on the blood Hb, PCV and serum AAT was an increase at the higher levels of addition. There was apparently an increase in serum Cu concentration at the intermediate levels of Cu addition.

The mean fresh weights of liver, kidneys, oviduct, ovary and gizzard, expressed as g/kg body-weight, are presented in Table 3. As the level of Cu supplementation increased liver fresh weight/kg body-weight decreased and the effect became significant ($P < 0.001$) when 1000 and 2000 mg added Cu/kg diet were fed. The period of time on Cu-supplemented diets did not show an over-all significant effect on liver fresh weight/kg body-weight. Kidney

Table 1. *Mean daily food intake, body-weight change and egg number for laying hens given control and Cu-supplemented diets*

(Mean values for six observations)

Dietary added Cu (mg/kg)	Days on experiment					SEM	Statistical significance of effect		
	3	6	12	24	48		Diet	Time	Diet × time
	Daily food intake (g)								
0	107	109	111	105	107	4·8	***	NS	NS
250	117	107	105	106	103				
500	74	87	79	87	90				
1000	35	38	54	51	56				
2000	19	24	31	32	26				
	Daily body-wt change (g)								
0	0·6	3·3	2·7	2·4	1·5	2·94	***	***	***
250	4·7	6·3	1·5	2·1	1·3				
500	−16·1	−6·8	−6·9	−3·5	−1·1				
1000	−37·5	−32·2	−9·2	−7·7	−3·1				
2000	−63·1	−35·8	−19·3	−10·8	−10·3				
	Daily egg number								
0	1·1	1·0	0·9	0·8	0·9	0·06	***	***	***
250	0·9	1·1	0·9	0·9	0·9				
500	1·0	1·0	0·9	0·9	0·7				
1000	0·8	0·8	0·4	0·4	0·2				
2000	0·8	0·7	0·4	0·2	0·1				

NS, not significant. ***, $P < 0·001$.

fresh weight/kg body-weight was significantly depressed by level of added Cu ($P < 0·001$), the period of time on the diets ($P < 0·05$) and the interaction between Cu level and time was also significant ($P < 0·01$).

The oviduct and ovary fresh weight/kg body-weight were depressed and gizzard fresh weight/kg body-weight increased by level of added Cu (all $P < 0·001$) and period of time on the diets. There was also a diet × time interaction for the weights of all three organs (all $P < 0·001$).

The concentrations of Cu in the liver, kidneys and breast muscle are shown in Table 4 together with the liver lipid concentration. The concentrations of Cu in the livers of birds receiving diets supplemented with 500–2000 mg Cu/kg were significantly higher ($P < 0·001$) than those of the birds receiving 0 or 250 mg added Cu. These higher Cu concentrations in the livers were apparent after only 3 d on Cu-supplemented diets and continued to increase up to 12, 24 and 48 d for the 500, 1000 and 2000 mg Cu/kg supplemented diets respectively. The period of time on diet and interaction effects were significant (both $P < 0·001$). The time effect was apparently due to the responses at the 1000 and 2000 mg/kg levels of added Cu. By 48 d, the liver Cu concentration of birds receiving 2000 mg added Cu/kg was 1790 μg/g dry matter compared with 74 μg Cu/g DM after 3 d on this diet. When compared with the pre-experimental control birds this represents an increase in liver Cu of approximately 16000%. The effects of diet on Cu levels in kidneys and breast muscle were less marked although the trends were the same as for liver. The results suggest the breast Cu concentration in DM to be maximum in the region of 500 mg added Cu/kg diet. Liver lipid concentration was significantly reduced by level of added Cu and period of time on the diets (both $P < 0·001$). There was a highly significant diet × time interaction.

Values for liver, kidney and breast muscle mineral concentration, excluding Cu, are given

Table 2. *Blood haemoglobin (Hb), packed cell volume (PCV), serum copper and aspartate aminotransferase (AAT*; EC 2.6.1.1) *of laying hens given control and Cu-supplemented diets*

(Mean values for six observations)

Dietary added Cu (mg/kg)	Days on experiment						SEM	Statistical significance of effect		
	0	3	6	12	24	48		Diet	Time	Diet × time
				Blood Hb (g/l)						
0	87·6	84·2	95·3	83·0	78·4	89·0	3·83	***	•	NS
250		89·0	81·0	79·5	84·7	80·3				
500		90·0	81·5	77·7	76·8	78·8				
1000		88·5	86·5	86·5	78·2	91·8				
2000		97·0	90·0	87·0	93·5	90·0				
				Blood PCV						
0	0·264	0·254	0·283	0·267	0·242	0·275	0·0108	***	•	NS
250		0·273	0·260	0·250	0·263	0·262				
500		0·278	0·257	0·247	0·237	0·258				
1000		0·290	0·295	0·282	0·258	0·272				
2000		0·300	0·277	0·268	0·290	0·270				
				Serum Cu (μg/l)						
0	253	255	257	260	330	268	28·5	***	*	NS
250		267	262	293	380	353				
500		320	272	262	360	285				
1000		213	282	220	253	235				
2000		225	278	252	232	288				
				Serum AAT (i.u.†/l)						
0	178	180	149	187	185	213	15·5	***	NS	**
250		173	164	196	179	178				
500		173	172	186	206	165				
1000		202	229	193	180	207				
2000		204	261	188	208	220				

NS, not significant. • $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
† One unit represents the oxidation of 1 μmol NADPH/min at 37 °.

in Table 5. The effects of diet and time on the levels of liver Zn were significant and there was an interaction (all $P < 0·001$). After 3 and 6 d on diets supplemented with 1000 and 2000 mg Cu/kg, the liver Zn concentrations were significantly higher ($P < 0·001$) than for those in the control and two lower-Cu treatment groups. However, after 6 d the effect was, in most instances, no longer apparent. The Zn concentrations in kidneys and breast muscle did not show either a diet or time effect, and mean values for each treatment are not presented in Table 5. The over-all mean (±SEM) Zn values for kidney and breast muscle were 121·2±5·50 and 31·3±1·13 μg/g DM respectively.

Liver Fe concentrations were increased by Cu treatment ($P < 0·001$). The effect was present by 12 d at the 2000 mg/kg level of Cu addition and by 24 d the effect was also evident at the 1000 mg/kg level. Kidney Fe concentration was affected by dietary added Cu ($P < 0·001$), the tendency being for kidney Fe to be increased at the highest level of dietary Cu addition at 24 and 48 d. Breast muscle Fe showed an increase with the period of time on the diets ($P < 0·001$) but dietary added Cu had no effect.

Liver Mn was increased ($P < 0·001$) and kidney Mn decreased ($P < 0·05$) with level of added dietary Cu.

Table 6 shows the mean total Zn, Fe and Mn contents of liver and the Cu, Zn and Mn contents of the kidneys. Since the statistical effects for total Cu content of liver are the same

Table 3. *Mean fresh weights (g/kg body-weight) of liver, kidneys, oviduct, ovary and gizzard of laying hens given control and copper-supplemented diets*

(Mean values for six observations)

Dietary added Cu (mg/kg)	Days on experiment						SEM	Statistical significance of effect		
	0	3	6	12	24	48		Diet	Time	Diet × time
Liver										
0	22·1	23·9	25·4	25·2	22·7	24·9	1·29	***	NS	NS
250		24·0	22·3	24·5	25·8	22·4				
500		21·5	23·0	24·8	23·9	22·4				
1000		18·2	19·2	18·4	19·9	17·3				
2000		17·5	15·4	15·8	14·2	13·4				
Kidneys										
0	6·3	7·0	6·6	6·6	6·1	6·7	0·31	***	*	**
250		6·6	6·4	6·9	7·2	6·6				
500		7·0	7·1	7·2	7·1	7·3				
1000		8·0	7·1	6·2	6·7	6·3				
2000		6·5	6·9	6·3	6·0	5·3				
Oviduct										
0	34·3	41·2	37·4	38·4	35·8	35·0	2·43	***	***	***
250		34·7	40·8	34·3	36·9	36·2				
500		34·0	36·2	36·7	36·3	35·3				
1000		37·2	28·8	19·1	26·0	6·9				
2000		31·4	18·8	11·4	3·3	1·8				
Ovary										
0	27·1	29·4	25·4	30·6	29·0	27·7	2·53	***	***	***
250		27·1	30·3	29·4	28·8	25·3				
500		26·0	29·5	24·2	25·8	26·7				
1000		36·9	19·1	12·0	17·1	11·1				
2000		25·9	12·5	5·6	2·6	1·1				
Gizzard										
0	12·1	14·0	13·5	13·8	15·2	12·0	0·79	***	**	***
250		14·7	14·9	14·7	13·7	13·2				
500		14·6	16·6	15·4	15·7	14·2				
1000		16·9	17·7	16·7	17·7	18·2				
2000		15·3	16·3	19·4	19·9	26·7				

NS, not significant. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

as those for liver Cu concentration (Table 4) the treatment mean results are not presented. After 12 d of feeding the two highest levels of Cu-supplemented diets, total liver Zn was significantly decreased ($P < 0.01$) and this decrease was maintained up to 48 d. Total liver Fe contents were significantly increased ($P < 0.001$) by feeding diets supplemented with 1000 and 2000 mg Cu/kg for 48 d. Liver Mn was decreased by diet and time ($P < 0.001$ and $P < 0.05$ respectively). Total kidney Cu content was not significantly affected by Cu supplementation. There was a time effect ($P < 0.05$) and this was quite evident at 24 and 48 d. Total Zn contents of kidneys of control birds after 6 d feeding were significantly ($P < 0.05$) higher than those levels for any other birds at any time during the Cu treatment. Kidney Mn was significantly decreased by increasing Cu level in the diet ($P < 0.001$) and period of time on the diets ($P < 0.01$), and there was a diet × time interaction ($P < 0.05$). Total kidney Fe levels were unaffected by Cu supplementation of the diets, the overall mean (±SEM) value being 798±60·0 μg.

Histological changes observed in the liver and kidney of Cu-treated hens were similar

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Table 4. *The mean concentrations (μg/g dry matter) of copper in the liver, kidneys and breast muscle together with the liver lipid concentration g/kg dry matter of laying hens given control and Cu-supplemented diets*

(Mean values for six observations. For liver Cu 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)

Dietary added Cu (mg/kg)	Days on experiment						SEM	Statistical significance of effect		
	0	3	6	12	24	48		Diet	Time	Diet × time
				Liver Cu						
0	11·3 (1·052)	13·0 (1·114)	9·6 (0·984)	11·6 (1·065)	11·9 (1·074)	11·6 (1·063)	(0·0753)	***	***	***
250		13·9 (1·143)	15·8 (1·198)	16·7 (1·223)	12·7 (1·105)	15·5 (1·191)				
500		54·5 (1·736)	64·9 (1·812)	90·0 (1·954)	66·8 (1·824)	45·0 (1·662)				
1000		81·3 (1·910)	184 (2·264)	362 (2·558)	574 (2·759)	555 (2·744)				
2000		74·0 (1·869)	154 (2·187)	641 (2·807)	828 (2·918)	1790 (3·253)				
				Kidney Cu						
0	12·1	13·8	14·9	12·5	13·4	18·8	1·90	***	***	***
250		12·9	13·8	13·9	19·5	15·1				
500		13·9	14·4	14·1	14·5	15·9				
1000		14·4	18·1	19·6	18·6	18·2				
2000		14·7	17·5	18·6	25·2	33·1				
				Breast muscle Cu						
0	1·25	1·59	1·62	1·83	1·98	1·72	0·123	**	***	NS
250		1·45	1·28	1·48	1·96	1·81				
500		1·68	1·70	1·92	2·05	1·93				
1000		1·56	1·20	1·83	1·91	1·85				
2000		1·60	1·52	2·17	1·89	1·80				
				Liver lipid						
0	237·2	210·2	298·5	303·4	258·4	245·9	17·58	***	***	***
250		255·2	187·8	282·1	292·9	226·7				
500		169·8	154·6	192·9	219·5	177·0				
1000		138·5	179·4	168·6	140·3	123·3				
2000		158·2	155·5	124·2	108·6	70·2				

NS, not significant. ** $P < 0.01$, *** $P < 0.001$.

Table 5. *The mean concentration (μg/g dry matter) of zinc in liver, of iron in liver, kidney and breast muscle and of manganese in liver and kidney of laying hens given control and Cu-supplemented diets*

(Mean values for six observations)

Dietary added Cu (mg/kg)	Days on experiment						SEM	Statistical significance of effect		
	0	3	6	12	24	48		Diet	Time	Diet × time
				Liver Zn						
0	131	170	113	120	108	130	31·7	***	***	***
250		151	163	178	120	139				
500		210	207	176	159	140				
1000		366	370	155	152	142				
2000		372	416	184	161	264				
				Liver Fe						
0	375	446	271	316	327	344	86·0	***	***	***
250		371	348	344	379	418				
500		521	451	526	475	475				
1000		525	347	448	690	986				
2000		624	390	589	1068	2025				
				Kidney Fe						
0	402	395	324	339	305	310	34·9	***	NS	NS
250		362	381	356	322	369				
500		377	299	319	309	312				
1000		356	325	330	286	376				
2000		369	396	373	426	544				
				Breast muscle Fe						
0	14·8	14·4	13·7	14·0	12·6	18·9	1·87	NS	***	NS
250		19·7	13·9	13·9	12·2	14·3				
500		15·7	13·5	13·7	13·5	20·7				
1000		16·4	13·8	15·4	13·4	15·7				
2000		15·0	14·7	13·0	14·7	25·8				
				Liver Mn						
0	11·3	10·6	8·9	10·6	10·4	10·1	1·38	***	NS	NS
250		12·5	10·7	10·8	10·5	12·3				
500		14·2	14·1	14·3	16·7	13·6				
1000		18·4	15·1	15·6	16·9	17·7				
2000		17·1	18·2	18·2	18·4	20·5				
				Kidney Mn						
0	13·9	14·9	14·2	12·7	12·9	14·1	0·77	*	**	NS
250		13·7	13·8	13·3	13·1	13·7				
500		13·6	15·0	15·6	13·8	14·0				
1000		15·2	13·0	12·5	12·2	12·6				
2000		14·8	12·7	11·8	11·4	13·1				

NS, not significant. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

to those in control birds. The spleen was apparently unaffected in any of the treatment groups. Birds on the two highest Cu treatments exhibited considerable thickening and damage to the gizzard lining. Microscopic examination of the gizzards revealed focal changes in the koilin layer.

The Cu-staining procedure failed to reveal the presence of Cu in liver, kidney or spleen, but Cu-staining was evident in the koilin layer and this effect increased with the level of added dietary Cu.

Table 6. *The mean total (mg) zinc, iron and manganese contents of liver, and copper (μg), Zn (mg) and Mn (μg) contents of kidneys of laying hens given control and Cu-supplemented diets*

Dietary added Cu (mg/kg)	(Mean values for six observations) Days on experiment						SEM	Statistical significance of effect		
	0	3	6	12	24	48		Diet	Time	Diet × time
				Liver Zn						
0	1·51	1·81	1·49	1·56	1·20	1·52	0·303	NS	***	**
250		1·79	1·66	2·50	1·58	1·43				
500		1·73	1·83	1·78	1·60	1·19				
1000		2·59	2·92	1·22	1·13	0·93				
2000		2·47	2·42	1·06	0·82	0·99				
				Liver Fe						
0	4·23	5·01	3·51	4·05	3·64	4·11	0·558	NS	***	***
250		4·46	3·52	4·52	4·95	4·32				
500		4·30	4·04	5·03	4·87	4·09				
1000		3·67	2·75	3·34	4·81	6·15				
2000		4·14	2·22	3·32	5·28	7·60				
				Liver Mn						
0	0·131	0·112	0·115	0·135	0·117	0·117	0·0108	***	*	NS
250		0·146	0·109	0·137	0·137	0·123				
500		0·117	0·126	0·138	0·172	0·117				
1000		0·130	0·115	0·122	0·124	0·114				
2000		0·114	0·110	0·102	0·093	0·077				
				Kidney Cu						
0	28·8	33·9	35·4	28·7	30·3	49·0	4·55	NS	*	NS
250		31·7	32·5	38·1	50·4	38·0				
500		32·3	33·8	33·2	34·9	36·5				
1000		36·2	39·9	40·7	36·5	34·6				
2000		31·1	38·5	34·6	42·8	51·4				
				Kidney Zn						
0	0·25	0·47	0·59	0·26	0·25	0·30	0·077	•	NS	NS
250		0·29	0·27	0·24	0·29	0·32				
500		0·29	0·25	0·22	0·28	0·25				
1000		0·30	0·26	0·23	0·21	0·21				
2000		0·25	0·31	0·19	0·19	0·20				
				Kidney Mn						
0	32·7	36·6	33·6	30·5	29·9	37·1	2·38	***	**	*
250		33·8	32·3	32·3	34·1	34·3				
500		31·6	35·1	36·3	33·3	31·9				
1000		37·0	28·7	25·9	24·1	24·4				
2000		31·8	27·4	21·9	19·4	20·0				

NS, not significant. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

DISCUSSION

The laying hen has been shown to be extremely tolerant to excessively high levels of added Cu (Jackson, 1977; Jackson *et al.* 1979) and this has been confirmed in the present experiment.

The significant reductions in body-weight gain and food intake at the high levels of added dietary Cu agree with previous results from this laboratory (Jackson, 1977; Jackson *et al.* 1979). The reduced weight gains in the current work were apparent after only 3 d on the Cu-supplemented diets although the improved food intake at about 250 mg added Cu/kg diet reported previously (Jackson, 1977; Jackson *et al.* 1979) was not observed.

The reduction or cessation of egg production in response to high levels of added dietary Cu is in agreement with previous findings (Jackson, 1977; Griminger, 1977; Jackson *et al.* 1979). At the highest level of added Cu, egg laying ceased after 5 d and at 1000 mg added Cu/kg it was only spasmodic after 5 d. Calculation of the metabolizable energy and protein requirements for maintenance and production (Agricultural Research Council, 1975) show that the decreased food intake would explain the reduction in egg numbers. This effect is similar to that in the broiler, where paired-feeding trials showed growth depression to be due to reduced food intake (Fisher *et al.* 1972). However, it is possible in experiments with the laying hen that the added dietary Cu may have influenced the hormonal control of egg production.

In the present experiment serum Cu was increased by 250 and 500 mg added Cu/kg and decreased by the two highest levels compared with the control birds. The Hb and PCV levels were significantly increased by 2000 mg added Cu/kg, and increased serum AAT – which accompanies cellular breakdown and is often indicative of liver dysfunction – was found at the higher levels of dietary added Cu. Jackson (1977) found that giving diets supplemented with up to 1920 mg Cu/kg did not affect blood Cu, but diets with 300 and 400 mg added Cu/kg significantly increased blood Cu levels (Jackson *et al.* 1979). In general the other blood and serum observations agree with those found at high and intermediate levels of $CuSO_4$ addition by Jackson (1977) and Jackson *et al.* (1979) respectively.

The significant decreases in liver, kidney, oviduct and ovary fresh weights, expressed as g/kg body-weight, resulting from added dietary Cu were expected in the light of previous results by the present authors (Jackson, 1977; Jackson *et al.* 1979). In the present experiment fresh liver weights per unit body-weight were reduced as a result of giving diets supplemented with 1000 and 2000 mg Cu/kg. Since, after 48 d on the diet with 2000 mg added Cu/kg, oviduct and ovary weights were extremely small, it is obvious that egg production must have ceased completely by this time.

The increase in gizzard fresh weight/kg body-weight with added dietary Cu has been observed before in the laying hen (Jackson *et al.* 1979). The thickening of the gizzard lining is in accord with the effects reported by Fisher *et al.* (1973). The increase in Cu concentration in the gizzard lining agrees with the quantitative observations of Cunningham (1931), Starcher (1969) and Fisher *et al.* (1973).

Liver Cu concentration (Table 4) was significantly increased by high levels of added dietary Cu and unaffected by 250 mg Cu/kg. The rate of accumulation of Cu in liver was rapid, substantial increases being apparent after only 3 d of Cu treatment. The liver Cu levels continued to increase for 48 d on the highest Cu treatment but, with the 500 and 1000 mg added Cu/kg, a maximum was reached after 12 and 24 d respectively. Jackson *et al.* (1979) have reported that the laying hen appears to exhibit a threshold level of Cu intake between 600 and 800 mg added Cu/kg diet, above which accumulation occurs in the liver. In the current experiment this threshold was found to occur between 250 and 500 mg added Cu/kg diet. This threshold effect has been noted in other species. For example, threshold levels of 200 and 50 mg Cu/kg diet respectively have been reported for the rat and cotton rat (Milne & Weswig, 1968) while the value for the pig lies between 70 and 130 mg/kg (Ritchie *et al.* 1963). On a lipid-free basis the effects of diet and time on liver Cu concentration (not presented) are even more evident. Both dietary added Cu and time on the diets had a highly significant effect on liver lipid concentration. The results suggest that there is a negative linear relationship between liver lipid concentration and the level of added dietary Cu. This effect is similar to that found by Jackson *et al.* (1979) but does not support the observations of Jensen & Maurice (1978) who found 240 mg added Cu/kg diet to cause an increase in liver lipid concentration. The response of liver lipid concentration to time indicates a quadratic relationship over the period of the experiment.

Although added Cu had a significant effect on kidney and breast muscle Cu levels, the increases were of a much lower order than those observed in the liver. Similar results have previously been reported for the laying hen (Jackson *et al.* 1979) and the broiler (Fisher *et al.* 1972; Norvell *et al.* 1974).

Liver Zn levels (Table 5) were significantly affected by Cu treatment. There was an indication of an increase on the three highest treatments at 3 and 6 d, this effect being statistically significant on the two highest Cu treatments. By 12 d and subsequently this effect was no longer evident, except at 48 d for the birds receiving 2000 mg Cu/kg diet. Fisher *et al.* (1972), working with broilers, found that liver Zn levels were increased by diets supplemented with 50 mg Zn/kg together with 500 or 750 mg Cu/kg, but were decreased when dietary Zn concentration was increased to 200 mg/kg. In the current experiment the dietary Zn concentration was 136 mg/kg, and evidence suggests that the contribution from the galvanized cages and fittings would be less than 10 mg Zn/kg diet (Mehring *et al.* 1956). These results in the laying hen and broiler suggest an effect not dissimilar to that in the pig, where dietary Cu had been found to cause a decrease in the incidence of parakeratosis. Also Ritchie *et al.* (1963) have shown a slight, but not statistically significant tendency for storage of liver Zn as dietary Cu level increased. Although Davis (1958) has reported that toxic levels of dietary Cu result in almost complete elimination of Zn from the liver of the pig, there is no evidence of a similar effect in the laying hen.

The increased Fe concentration in the liver as a result of the high Cu treatment is a further indication of differences which exist between the fowl and other species, since in the rat a highly significant inverse correlation has been found between hepatic Fe and Cu (Sourkes *et al.* 1968).

The levels of Mn found in the liver agree with those of Mathers & Hill (1968) for laying hens. The increase in liver Mn concentration is, at first sight, unexpected in light of the dramatic increase in liver Fe associated with increasing dietary Cu, since Fe and Mn show considerable similarity in chemical properties and could reasonably be expected to exhibit antagonism. However, the kidney Mn levels were decreased by increasing Cu level, but the effect was only marginal.

With regard to the total content of Zn in the liver (Table 6) there was no diet effect, and this suggests that the effect on Zn concentration seen in Table 5 was due to reduction in liver weight. However, the effect on liver Fe would appear to be a true increase in total Fe content and not merely a concentration effect. In the instance of liver Mn the increased concentration is apparently due to reduced liver weight, since when the Mn is presented as total content it is significantly decreased by increasing added Cu in the diet.

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