Effects of withdrawal of copper sulphate from the diet of the mature domestic fowl with special reference to production and tissue mineral content

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1. Cereal-based diets containing 0, 500, 1000 or 2000 mg added copper/kg were offered *ad lib.* to laying hens for 8 weeks. All the hens were subsequently offered the control diet (no added Cu, 7·5 mg Cu/kg).

2. Hens from each treatment were killed at 0, 2, 4, 6 and 8 weeks after removal of the Cu-supplemented diets. Records were kept of body-weight, food consumption and egg production.

3. After slaughter, blood haemoglobin, packed cell volume, serum Cu and aspartate aminotransferase (AAT; *EC* 2.6.1.1) were assayed. The liver, kidneys, oviduct, ovary, gizzard, caeca and bile duct were weighed.

4. Mean Cu, zinc and iron concentration of liver, kidneys and caecal contents were determined.

5. The adverse effects of Cu on body-weight, food intake, egg production and liver, oviduct, ovary, gizzard and bile weights were rapidly reversed by removal of added Cu from the diets.

6. Greatly enhanced liver Cu concentration resulted from feeding the high-Cu diets but this effect was rapidly reversed on removal of added Cu from the diets. Liver Fe concentration showed a less marked but similar effect.

7. The Cu concentration of caecal contents was increased by Cu supplementation and rapidly reduced after withdrawal of the Cu-containing diets.

The effects of feeding diets supplemented with copper sulphate on the performance and the mineral content of some tissues of the laying hen have been studied in this Department for some years (Jackson, 1977; Jackson *et al.* 1979; Stevenson & Jackson, 1980).

Stevenson & Jackson (1980) studied the rate at which the performance of laying hens, in terms of egg production and body-weight change and the mineral content of some specific tissues, was affected by up to 2000 mg added copper (as $CuSO_4$)/kg diet. Body-weight loss, which occurred at 500–2000 mg added Cu/kg diet, was apparent after only 3 d of Cu treatment. Egg production was depressed by high levels of added Cu and length of time on the Cu-containing diets. Liver, kidney, oviduct and ovary fresh weights were depressed by Cu in the diet and length of time on the diets.

The liver Cu concentration was increased by dietary Cu level and length of time on the diet. The two highest levels of Cu addition (1000 and 2000 mg Cu/kg diet) increased liver zinc and iron concentrations.

The present experiment was carried out in order to study the effects of the withdrawal of different levels of added $CuSO_4$ from the diet of mature, female, domestic fowl on egg production, body-weight change and the mineral content of some tissues.

EXPERIMENTAL

White light hybrid (Shaver 288) (100, 18 weeks old) pullets were placed in galvanized-Fe cages fitted with individual feeders and nipple drinkers. Initially the lighting programme was 11 h light and 13 h darkness and this was altered by 1 h/week to 17 h light and 7 h

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Table 1. *Mean weekly body-weight change, food intake and egg number of mature, female domestic fowl after withdrawal of Cu-supplemented diets*

(Mean values for five observations)

	Dietary added Cu (mg/kg)	Length of time after withdrawal of added Cu (weeks) 0‡	2	4	6	8	SEM	Statistical significance of effect: Diet	Time	Diet × time
Body-wt change (g)†	0	10·8	10·2	13·8	12·1	10·8	4·91	***	***	***
	500	−6·8	−5·0	2·8	4·7	9·3				
	1000	−17·3	7·8	5·5	5·9	6·1				
	2000	−56·5	−10·0	−3·1	4·9	8·3				
Food intake (g)	0	647	764	733	783	825	44·3	***	***	***
	500	506	737	805	847	795				
	1000	301	791	797	791	888				
	2000	136	663	732	801	808				
Egg no.	0	5·4	5·9	5·9	6·2	6·3	0·45	***	***	***
	500	4·2	5·5	6·2	5·8	6·3				
	1000	1·2	4·8	6·1	6·2	6·3				
	2000	0·5	0·7	3·9	6·0	6·4				

*** $P < 0.001$.

† $\frac{\text{Slaughter weight} - \text{initial weight}}{\text{Total time on experiment}}$ (food intake and egg no. calculated in a similar manner).

‡ Time 0 refers to the end of the initial eight-week feeding period.

darkness at 24 weeks and this maintained throughout the experiment. At 24 weeks of age, when all the hens had been laying for at least 2 weeks, they were randomly allocated to one of four treatment groups. The treatments were the control diet as described by Jackson (1977), and this diet to which was added 500, 1000 or 2000 mg Cu/kg. The Cu was added as $CuSO_4.5H_2O$ the fineness of grinding of which has already been reported (Jackson, 1977). The control diet contained (/kg): 165 g crude protein (nitrogen × 6·25), 7·5 mg Cu, 95 mg Zn, 138 mg Fe, 32 g calcium, 5·2 g phosphorus and had a calculated metabolizable energy content of 11·4 MJ/kg. The diets were offered *ad lib.* for 8 weeks. At the end of this time, five birds from each treatment were randomly selected and killed by decapitation. The remaining birds from each treatment were then offered the control diet *ad lib.* for another 8 weeks. Five hens from each treatment were killed 2, 4, 6 and 8 weeks after placing all the birds on the control diet.

Body-weights were recorded at the start of the experiment, after 8 weeks on the control and Cu-supplemented diets and at two-weekly intervals after withdrawal of the diets containing added Cu. Egg production and food intake were recorded. Blood and blood serum were analysed and organs removed and examined as described by Stevenson & Jackson (1980), except that histological examination of tissues was not carried out. In addition, the caeca and bile ducts were excised and weighed. Caecal contents were removed and a portion taken for Cu analysis. The weights of the empty caeca were recorded.

The methods of chemical and statistical analyses have been described by Stevenson & Jackson (1980).

RESULTS

Mean weekly body-weight change, food intake and egg numbers are presented in Table 1. The Cu supplementation for 8 weeks resulted in significant weight losses and depressed food intake and egg production (all $P < 0.001$). After 2 weeks on the control diet significant food intake differences were no longer apparent. By 6 weeks on the control diet body-weight and egg number differences were no longer significant.

Table 2. *Mean packed cell volume (PCV), serum copper and aspartate aminotransferase (AAT;* EC 2.6.1.1) *of mature, female domestic fowl after withdrawal of Cu-supplemented diets*

(Mean values for five observations)

	Dietary added Cu (mg/kg)	Length of time after withdrawal of added Cu (weeks) 0	2	4	6	8	SEM	Statistical significance of effect: Diet	Time	Diet × time
PCV	0	0·246	0·246	0·278	0·273	0·200	0·0136	NS	**	NS
	500	0·268	0·265	0·288	0·243	0·257				
	1000	0·260	0·264	0·250	0·240	0·233				
	2000	0·268	0·238	0·265	0·242	0·247				
Serum Cu (μg/l)	0	344	300	334	296	316	18·2	NS	**	NS
	500	300	354	322	292	354				
	1000	310	298	326	290	348				
	2000	306	244	330	296	314				
Serum AAT (i.u.†/l)	0	159	325	166	198	165	20·3	NS	***	NS
	500	181	218	209	197	169				
	1000	174	251	182	190	152				
	2000	173	229	185	182	158				

NS, not significant.
** $P < 0.01$, *** $P < 0.001$.
† One international unit refers to the oxidation of 1 μmol NADPH/min at 37°.

Table 3. *Mean fresh weights (g/kg body-weight) of liver, oviduct, ovary, gizzard and bile duct together with the lipid content (g/kg dry matter) of the livers of mature, female domestic fowl after withdrawal of Cu-supplemented diets*

(Mean values for five observations)

	Dietary added Cu (mg/kg)	Length of time after withdrawal of added Cu (weeks) 0	2	4	6	8	SEM	Statistical significance of effect: Diet	Time	Diet × time
Liver	0	21·8	24·3	24·2	22·8	24·5	1·65	**	***	*
	500	19·3	27·4	25·7	26·6	24·6				
	1000	16·7	20·9	22·7	29·3	24·3				
	2000	12·6	23·4	25·3	22·3	21·8				
Oviduct	0	40·5	37·2	32·7	37·8	34·3	3·09	***	***	***
	500	28·7	37·2	34·0	40·1	38·1				
	1000	27·6	30·5	38·6	38·9	35·3				
	2000	2·7	27·6	30·0	34·6	30·4				
Ovary	0	31·7	32·1	26·3	27·9	25·6	3·97	*	***	*
	500	23·7	30·7	33·9	39·5	30·7				
	1000	24·4	30·2	27·0	32·7	29·4				
	2000	4·2	25·4	27·5	35·3	25·2				
Gizzard	0	12·2	12·7	10·6	12·7	12·0	0·80	***	***	***
	500	14·9	15·1	12·3	13·5	13·0				
	1000	16·1	13·0	13·3	14·0	13·2				
	2000	23·1	14·3	13·7	13·2	12·3				
Bile duct	0	0·67	1·22	1·57	1·27	0·95	0·238	*	*	NS
	500	1·17	1·00	0·87	1·44	0·84				
	1000	1·19	1·82	1·12	1·45	0·96				
	2000	1·79	1·96	1·14	1·44	1·12				
Liver lipid	0	223·3	304·5	367·7	325·4	394·0	51·61	*	***	NS
	500	272·1	285·9	426·0	344·8	383·9				
	1000	158·4	211·6	183·8	350·5	372·4				
	2000	114·6	271·3	319·2	255·8	422·9				

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

Table 4. *The mean concentrations of copper (μg/g dry matter) in the liver, kidneys and caecal contents of mature, female domestic fowl after withdrawal of Cu-supplemented diets*

(Mean values for five observations)

	Dietary added Cu (mg/kg)	Length of time after withdrawal of added Cu (weeks) 0	2	4	6	8	SEM	Statistical significance of effect: Diet	Time	Diet × time
Liver Cu†	0	12·9 (1·109)	8·8 (0·946)	7·9 (0·895)	9·9 (0·986)	9·1 (0·960)	(0·0955)	***	***	***
	500	38·4 (1·584)	10·6 (1·019)	9·4 (0·971)	9·8 (0·992)	10·7 (1·031)				
	1000	822 (2·915)	67·5 (1·829)	19·2 (1·284)	8·9 (0·948)	13·8 (1·140)				
	2000	1726 (3·237)	284·4 (2·454)	48·7 (1·687)	20·9 (1·321)	14·6 (1·165)				
Kidney Cu	0	15·6	11·8	12·0	17·6	12·2	1·48	NS	***	*
	500	13·9	12·2	12·6	14·6	14·9				
	1000	15·6	13·0	12·4	14·3	15·7				
	2000	22·7	13·4	13·4	18·2	12·0				
Caecal contents Cu†	0	550 (2·740)	156 (2·192)	151 (2·178)	148 (2·170)	286 (2·456)	(0·0597)	***	***	***
	500	8851 (3·947)	236 (2·372)	316 (2·500)	180 (2·254)	221 (2·344)				
	1000	19680 (4·294)	262 (2·419)	197 (2·294)	151 (2·178)	287 (2·458)				
	2000	13770 (4·139)	305 (2·484)	279 (2·445)	142 (2·152)	299 (2·475)				

NS, not significant.
* $P < 0·05$, *** $P < 0·001$.
† Analysis of variance carried out using log transformations. The mean values presented are the antilogs of the mean of the log transformations. The values in parentheses are the means of the log values.

The blood measurements are shown in Table 2. Cu supplementation and withdrawal did not significantly affect haemoglobin (Hb), packed cell volume (PCV), serum Cu or serum aspartate aminotransferase (AAT; *EC* 2.6.1.1). Length of time after withdrawal of the Cu-supplemented diets significantly affected PCV, serum Cu (both $P < 0·01$) and AAT ($P < 0·001$) but there was no obvious trend in the effects produced. The over-all mean (± SEM) Hb concentration was 78·1 ± 1·02 g/l.

The mean fresh weights of liver, oviduct, ovary, gizzard and bile duct, expressed as g/kg body-weight, together with the lipid content of the livers are given in Table 3. Cu supplementation markedly reduced liver fresh weights expressed as g/kg body-weight. Withdrawal of the 500 and 2000 mg added Cu/kg diet for 2 weeks resulted in an increase in liver weight per unit body-weight ($P < 0·01$ and 0·001 respectively). By 4 weeks, no significant differences between treatments were observed. There was a diet × time interaction ($P < 0·05$).

Cu supplementation markedly reduced oviduct and ovary weight/kg body-weight compared with the control birds. Subsequent feeding of the control diet to all groups resulted in a rapid and significant increase in oviduct and ovary weight/kg body-weight (both $P < 0·001$). Both showed a diet × time interaction.

Gizzard fresh weight (g/kg body-weight) was significantly affected by diet and time and there was a diet × time interaction (all $P < 0·001$). The effect of increasing Cu supplementation of the diet on gizzard weight per unit body-weight was obvious at all Cu levels, the weight increase on 2000 mg Cu/kg being approximately 90% compared to the control. The mean bile duct weight/kg body-weight was significantly increased by the highest level

Table 5. *The total copper content (μg) of the liver, kidneys and caecal contents of mature, female domestic fowl after withdrawal of Cu-supplemented diets*

(Mean values for five observations)

	Dietary added Cu (mg/kg)	Length of time after withdrawal of added Cu (weeks) 0	2	4	6	8	SEM	Statistical significance of effect: Diet	Time	Diet × time
Total liver Cu†	0	132 (2·120)	121 (2·083)	117 (2·069)	119 (2·074)	137 (2·135)	(0·0794)	***	***	***
	500	324 (2·510)	142 (2·153)	140 (2·145)	137 (2·137)	149 (2·174)				
	1000	5105 (3·708)	703 (2·847)	208 (2·317)	157 (2·196)	175 (2·244)				
	2000	6122 (3·787)	3048 (3·484)	705 (2·848)	225 (2·352)	179 (2·253)				
Total kidney Cu	0	37·7	30·6	31·0	45·9	30·3	3·13	NS	***	NS
	500	29·7	30·4	31·3	39·3	35·5				
	1000	26·1	29·8	30·6	37·9	39·6				
	2000	25·6	29·7	34·1	40·8	28·0				
Total caecal contents Cu†	0	389 (2·590)	152 (2·183)	120 (2·078)	158 (2·198)	185 (2·267)	(0·1102)	***	***	***
	500	4765 (3·678)	214 (2·331)	274 (2·437)	150 (2·177)	208 (2·318)				
	1000	13940 (4·144)	218 (2·339)	143 (2·154)	130 (2·115)	244 (2·388)				
	2000	5300 (3·724)	190 (2·278)	160 (2·205)	75 (1·874)	257 (2·409)				

NS, not significant.
*** $P < 0·001$.
† Analysis of variance carried out using log transformations. The mean values presented are the antilogs of the mean of the log transformations. The values in parentheses are the means of the log values.

of Cu supplementation and decreased by the length of time after withdrawal of these diets (both $P < 0·05$). Caecal and kidney weights, expressed as g/kg body-weight, were unaffected by both diet and time, the over-all mean weights (± SEM) being $2·99 \pm 0·057$ and $6·2 \pm 0·09$ g/kg body-weight respectively.

Liver lipid concentration (Table 3) was decreased by the two highest levels of Cu supplementation of the diet ($P < 0·05$). After withdrawing the Cu-supplemented diets for 2 weeks, lipid concentration increased and this trend continued up to 8 weeks.

The concentrations and total Cu present in the liver, kidneys and caecal contents are shown in Tables 4 and 5 respectively. The Cu-supplemented diets offered for the initial 8 weeks caused a highly significant increase in liver Cu concentration while the control birds fed on the basal diet throughout the experiment were unaffected. Replacement of the Cu-containing diets by the control diet caused a significant decrease ($P < 0·001$) in the liver Cu concentration after 2 weeks. By 8 weeks the differences between treatments were not significant.

The total Cu content of the livers of hens (Table 5) given Cu-supplemented diets was significantly higher ($P < 0·001$) than that of hens given the control diet. After 2 weeks withdrawal of the Cu-supplemented diets the hens which had been offered the Cu-containing diets showed a significant reduction in total liver Cu. The trends in the total liver Cu content for the remainder of the experiment generally paralleled those for liver Cu concentrations.

Both kidney Cu concentration and total kidney Cu content were unaffected by dietary-added Cu although the mean values for Cu concentration suggest that feeding 2000 mg

Table 6. *The zinc and iron concentrations (μg/g dry matter) of the liver and kidneys of mature, female domestic fowl after withdrawal of Cu-supplemented diets*

(Mean values for five observations)

	Dietary added Cu (mg/kg)	Length of time after withdrawal of added Cu (weeks) 0	2	4	6	8	SEM	Statistical significance of effect: Diet	Time	Diet × time
Liver Zn	0	137	95	76	111	74	16·3	NS	***	NS
	500	143	112	81	90	95				
	1000	129	142	117	75	104				
	2000	187	103	101	114	100				
Liver Fe†	0	260 (2·415)	230 (2·361)	210 (2·323)	242 (2·384)	173 (2·238)	(0·0785)	***	***	*
	500	442 (2·645)	230 (2·360)	242 (2·383)	234 (2·370)	217 (2·336)				
	1000	687 (2·837)	321 (2·506)	310 (2·492)	166 (2·220)	204 (2·310)				
	2000	1120 (3·051)	525 (2·720)	366 (2·564)	291 (2·464)	254 (2·404)				
Kidney Zn	0	99	107	101	100	100	4·2	NS	NS	**
	500	105	106	105	99	104				
	1000	94	102	106	102	108				
	2000	124	100	98	106	108				
Kidney Fe	0	210	219	206	246	182	27·7	***	***	NS
	500	262	244	223	250	196				
	1000	293	252	248	228	197				
	2000	410	260	295	277	216				

NS, not significant.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
† Analysis of variance carried out using log transformations. The mean values presented are the antilogs of the mean of the log transformations. The values in parentheses are the means of the log values.

added Cu/kg diet for 8 weeks did result in increased kidney Cu concentration. After 2 weeks withdrawal of added Cu, kidney Cu concentration had significantly decreased ($P < 0.001$) in these birds. Over all the total kidney Cu content was increased with time ($P < 0.001$), showing a maximum at 6 weeks.

Feeding the Cu-supplemented diets significantly increased the concentrations and total Cu of the caecal contents (Tables 4 and 5) compared with control hens ($P < 0.001$). After imposing the control diet on all groups of hens the decreases in the Cu concentrations and total Cu of caecal contents were significantly different ($P < 0.001$) compared to the levels found after the initial 8 week period.

The Zn and Fe concentrations and total contents of liver and kidneys are presented in Tables 6 and 7 respectively. Over all, Cu supplementation had no significant effect on liver Zn concentration or content but after withdrawing the Cu-supplemented diets liver Zn concentration decreased ($P < 0.001$) and total Zn increased ($P < 0.05$). Liver Fe concentration and total content were significantly increased by added Cu but after 2 weeks withdrawal of these diets only those birds which had previously been receiving 2000 mg added Cu/kg still showed liver Fe concentrations significantly higher ($P < 0.001$) than the control treatment.

Kidney Zn concentrations were unaffected by added Cu and length of time of withdrawal of this added Cu but there was a diet × time interaction ($P < 0.01$). Total kidney Zn was decreased by Cu supplementation of the diet and after 2 weeks of withdrawal of Cu from the diet the kidney total Zn of the hens offered the two highest levels of added Cu was restored to a value similar to that of the control.

Table 7. *Total zinc and iron contents (μg) of the livers and kidneys of mature, female domestic fowl after withdrawal of Cu-supplemented diets*

(Mean values for five observations)

	Dietary added Cu (mg/kg)	Length of time after withdrawal of added Cu (weeks) 0	2	4	6	8	SEM	Statistical significance of effect: Diet	Time	Diet × time
Total liver Zn	0	1377	1302	1137	1313	1060	140·1	NS	*	NS
	500	1148	1481	1233	1243	1277				
	1000	810	1414	1247	1342	1302				
	2000	669	1068	1435	1247	1191				
Total liver Fe†	0	2673 (3·427)	3148 (3·498)	3141 (3·497)	2972 (3·473)	2582 (3·412)	(0·0670)	**	*	NS
	500	3715 (3·570)	3112 (3·493)	3608 (3·557)	3274 (3·515)	3013 (3·479)				
	1000	4266 (3·630)	3342 (3·524)	3350 (3·525)	2945 (3·469)	2594 (3·414)				
	2000	3990 (3·601)	5635 (3·751)	5310 (3·725)	3126 (3·495)	3097 (3·491)				
Total kidney Zn	0	240	278	260	262	249	16·2	**	***	*
	500	224	264	263	259	250				
	1000	162	234	269	271	273				
	2000	139	219	251	237	251				
Total kidney Fe	0	508	567	537	665	458	63·3	NS	**	NS
	500	551	607	560	680	474				
	1000	486	580	636	604	501				
	2000	454	577	743	617	504				

NS, not significant.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
† Analysis of variance carried out using log transformations. The mean values presented are the antilogs of the mean of the log transformations. The values in parentheses are the means of the log values.

Supplementation of the diet with 2000 mg Cu/kg significantly increased kidney Fe levels ($P < 0.001$) but after 2 weeks withdrawal of added Cu from the diets the Fe levels had significantly decreased ($P < 0.001$) and were no longer statistically different from those of the other treatments. Cu supplementation did not affect total kidney Fe but total kidney Fe increased for up to 6 weeks after Cu withdrawal.

DISCUSSION

The losses in body-weight and the decreases in food intake and egg production with the high levels of added dietary Cu were expected from previous findings (Jackson, 1977; Stevenson & Jackson, 1980). The recovery from body-weight loss and the increases in food intake and egg production after removal of the Cu-supplemented diets are rather similar to the observations of Griminger (1977). The effect of Cu on the reproductive system was readily reversible and, in fact, the egg-laying capacity of the hens previously given 2000 mg Cu/kg was fully restored after 6 weeks on the control diet. The effect on egg production was probably due to the low nutrient intakes resulting from the greatly-reduced food intake of the birds offered the high-Cu diets.

The fact that the intake of high levels of Cu by the hens had no significant effect on Hb, PCV, serum Cu and AAT (Table 2) suggests that the hen has a remarkable ability to adapt to high Cu intakes. This characteristic has been observed before for the mature, domestic fowl (Jackson, 1977; Stevenson & Jackson, 1980). The lack of effect of high levels of dietary

Cu on kidney fresh weight per unit body-weight can be taken as a further indicator of the ability of the domestic fowl to adapt to a high Cu intake.

The significant reductions in liver, oviduct and ovary fresh weights, expressed as g/kg body-weight, in response to Cu supplementation (Table 3), were to be expected since similar results have previously been reported (Jackson, 1977; Jackson *et al.* 1979; Stevenson & Jackson, 1980). The interesting novel information shown by the present work is the rapid restoration of the weights of these tissues to near control values and the accompanying return to normal function of the reproductive system as assessed by egg production.

Pathological effects on the gizzard in response to high dietary Cu have been reported in the broiler (Fisher *et al.* 1973; Poupoulis & Jensen, 1976). In the laying hen the feeding of diets containing up to 800 mg added Cu/kg failed to cause gizzard lesions although gizzard weight was increased (Jackson *et al.* 1979) but feeding higher levels of added Cu caused thickening and damage to the gizzard lining and an associated weight increase (Stevenson & Jackson, 1980). The sex difference in gizzard tolerance to dietary Cu (Fisher *et al.* 1973) may partially explain the lack of severe erosion found in the laying hen in previous experiments reported from this laboratory.

The significant increase of bile duct weight caused by Cu supplementation of the diet and the subsequent decrease when the control diet was given indicates that the bile duct is an important pathway of Cu excretion. This substantiates the observations of Beck (1961) who found that after injection of $CuSO_4$ the biliary Cu level of cockerels was substantially increased. The lack of response of serum Cu to high levels of Cu ingestion in the mature hen is probably related to the fact that the bile duct is an important pathway of Cu excretion.

The functions of the caeca in the digestive processes of the domestic fowl are not clearly understood. Beck (1961) reported that the caeca had some significance in Cu excretion by the cockerel. King (1972) found an effect of dietary Cu on caecal length per unit body-weight in broiler chicks. An increase was observed in the male and a decrease in the female. A decrease in caecal weight per unit body-weight and in weight per unit length has been found for ducklings (King, 1975). The lack of effect in the present experiment indicates that the mature female domestic fowl shows a response which is different from that of the immature bird.

The significant decrease in liver lipid concentration with added dietary Cu is in agreement with the findings of Jackson *et al.* (1979) and Stevenson & Jackson (1980) and is obviously associated with depression of fatty acid and lipid synthesis in the liver (Ranney & Chaikoff, 1951; Goodridge, 1968; O'Hea & Leveille, 1969).

The increases in the concentration and total Cu content of the livers of birds given Cu-supplemented diets were expected in the light of previous results (Jackson, 1977; Jackson *et al.* 1979; Stevenson & Jackson, 1980). A most interesting aspect of the present work was the rapidity with which Cu was eliminated from the liver when the birds were given control diets. After 2 weeks withdrawal of Cu-supplemented diets, the mean Cu concentration and total Cu content of the livers of birds subjected to the highest level of Cu supplementation were only 16% and 50% respectively of the value obtained after 8 weeks of Cu treatment. After 4 weeks of Cu depletion the corresponding values were approximately 3% and 11%. This shows that the domestic fowl has a mechanism for the rapid withdrawal of Cu from the liver. The lack of effect of Cu supplementation on kidney Cu in the present work and the relatively small effect noted previously (Jackson *et al.* 1979; Stevenson & Jackson, 1980) indicates that the kidney is not significantly involved in Cu storage or excretion.

Significant increases in the Cu concentration and the total Cu present in the caeca as a consequence of feeding Cu-supplemented diets as found in the present experiments have also been observed in the broiler (Fisher *et al.* 1973; Jensen & Maurice, 1978). The caecal contents also showed the physical characteristics, namely the dark colour and pastiness,

as described by Jensen & Maurice (1978). After 2 weeks withdrawal of the Cu-supplemented diets, the over-all mean caecal Cu concentration and content were less than 30% and 4% respectively of the values found after giving Cu-supplemented diets for 8 weeks. The concentration and total Cu contents tended to remain fairly constant thereafter. There is no obvious explanation as to why mean Cu concentrations and contents rose after 8 weeks.

Although the concentration and total Zn content of the livers were unaffected by diet, nevertheless the values for zero time of Cu withdrawal show an increase in Zn concentration and a decrease in total Zn content. This agrees with the results of Stevenson & Jackson (1980). The mean liver Zn concentration decreased by approximately 40% over the 8-weeks withdrawal period. The total Zn content increased by 21% over this period this being a reflection of the large increase in liver weight. The increases in liver Fe concentration and total Fe as a result of Cu supplementation are in agreement with previous findings from this laboratory (Jackson *et al.* 1979; Stevenson & Jackson, 1980). The reduction in liver Fe on withdrawal of the dietary Cu appeared to be directly related to the reduction in liver Cu.

As for the liver, the tendency was for Cu supplementation to result in an increase in the kidney Zn concentration and a decrease in total content. This effect on total kidney Zn content was also reported by Stevenson & Jackson (1980). In the present experiment kidney Fe concentration was increased by the highest Cu supplement whereas Stevenson & Jackson (1980) did not find that Fe concentration was significantly increased although there was a trend towards an increase with increasing Cu supplementation. Total kidney Fe was unaffected by Cu treatment but after withdrawal of added Cu there was a significant increase in total kidney Fe and a significant decrease in Fe concentration, which is rather surprising since kidney weight was unaffected by withdrawal of the Cu-supplemented diets.

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