COPPER AND IRON SUPPLEMENTATION IN THE TROPICAL ENVIRONMENT - EFFECTS ON HAEMATOLOGICAL MEASUREMENTS, ORGAN WEIGHTS, TISSUE COPPER AND IRON LEVELS IN LAYERS

J.A. AGUNBIADE¹ and G.M. BABATUNDE²

¹ Department of Animal Production, Ogun State University, P.M.B. 2002, Ago-Iwoye, Nigeria.

² Department of Animal Science, University of Ibadan, Ibadan, Nigeria.

Received 15 March, 1994; Accepted 11 August, 1995

ABSTRACT

The study which lasted 3 months was a factorial arrangement in which nine dietary treatments resulting from supplementation of a commercial layers diet with 0, 200 or 400 mg/kg Copper and 0, 100 or 200 mg/kg Iron were fed to 135 fifty two-week old laying hens of the Golden Hubbard Connet Strain. After 12 weeks on the diets, three birds, randomly selected from each treatment, were slaughtered and their blood, liver, spleen and heart analysed for effect of supplementary copper and iron on haemoglobin content, packed cell volume and weights of the liver, spleen and heart and their copper and iron concentrations. No significant effects of supplementary copper or iron or their interaction were observed on haemoglobin, packed cell volume or weights of the liver, spleen and heart. However, dietary iron supplementation produced significant ($P < 0.05$) effects on iron storage in the liver and spleen but not on copper storage. On the other hand, copper addition significantly ($P < 0.05$) increased liver storage of copper and spleen storage of iron. Supplementary copper and iron interaction effects which were not significant for all other measurements, were observed to be significant ($P < 0.05$) for liver copper and iron concentrations and spleen copper concentration and highly significant ($P < 0.01$) for spleen iron concentration.

Key Words: Copper, Iron, Diets, Laying Hens, Blood, tissue.

INTRODUCTION

Copper (Cu) and iron (Fe) are essential nutrients required in small amounts for numerous physiological functions in the animal body particularly in the production of

haemoglobin to form red blood cells. Hence, Mitchell and Miller (1931) and Charles and Gennard (1961) have indicated that as either dietary Cu or Fe was increased, the concentration in the haemoglobin increased. However, as high as 1920 mgCu/kg diet has been fed to laying hens without any significant effect on blood haemoglobin (Jackson, 1977).

The effects of dietary Cu supplementation on organ weights of birds have been reported by many workers. Almost all the work investigating the effect of dietary Cu on liver weight found a depression in the organ weight at high level of supplementation. Other organs on which the effect of added dietary Cu has been reported include; oviduct, kidney, ovary, caecum and gizzard (King, 1972; Jackson, 1977; Jackson, Stevenson and Kirkpatrick, 1979 and Stevenson and Jackson, 1980). However, results of the effects of Cu on organ weights are contradictory. While some workers (Mehring, Brumbaugh, Sutherland and Titus, 1960) have reported increased organ weight at the lower level of Cu addition, others (Jackson *et al.*, 1979) have found no significant effect. The general observation by most researchers, however, was a depression in the weights of most organs at very high levels of Cu inclusion. Very limited investigation has been conducted on the effect of dietary Cu on spleen and heart of laying hens.

A number of authors have noted a threshold effect of Cu intake on liver Cu concentrations. While Jackson *et al.* (1979) observed the effect at between 600 and 800 mg added Cu/kg diet, Stevenson and Jackson (1980) found the effect to occur at between 250 and 500 mg/kg levels. Most of the

TABLE 1. PROPORTIONS OF SUPPLEMENTARY COPPER AND IRON IN EXPERIMENTAL DIETS.

Diet	Supplementary Copper (mg/kg)	Supplementary Iron (mg/kg)
1	0	0
2	200	100
3	400	200
4	0	200
5	200	0
6	400	100
7	0	100
8	200	200
9	400	0

previous works have concentrated mainly on liver storage of Fe to the exclusion of spleen which is also known to play a significant role in Fe storage (Maynard, Loosli, Hintz and Warner, 1979). There is a dearth of information on the effect of supplemental Fe either alone or in conjunction with supplemental Cu on the performance of laying hens. In a previous study by Agunbiade and Babatunde (1988), egg production, feed intake, efficiency of feed conversion, mortality and body weight changes of laying hens were observed not to be significantly affected by either supplemental Cu or Fe or by their interaction.

The present study was undertaken to investigate how haematological measurements, weights and mineral contents of specific organs of mature laying hens are influenced by dietary Cu and/or Fe supplementation under the tropical environment.

MATERIALS AND METHODS

A total of 135 one-year-old hybrid Golden Hubbard layers, obtained from the Poultry unit of the Teaching and Research Farm, University of Ibadan, were used in the study which lasted for 12 weeks. The hens which were initially fed standard starter, grower and layers diets compounded at the University of Ibadan were subsequently randomly assigned in a 3 x 3 factorial design to each of the nine treatment diets (Table 1) arising from the

basal diet (the composition of which has been reported previously by Agunbiade and Babatunde, 1988) incorporated with 3 levels of Cu; 0, 200 and 400 mgCu/kg diet and 3 levels of Fe; 0, 100 and 200 mgFe/kg diet. Crystalline copper sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) and ferrous sulphate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) were used as sources of supplemental Cu and Fe, respectively. The basal diet contained (/kg): 176g crude protein (nitrogen x 6.25), 38mg Cu, 761mg Fe, 35g calcium, 6g phosphorus and had a calculated metabolizable energy content of 10.66 MJ/kg.

The study was preceded by a one-week adjustment of the chickens on the control diet following which, each of the nine experimental diets was fed to triplicate groups of five birds for three months. At the end of the feeding trial, random samples of three birds per dietary treatment were slaughtered by decapitation and blood samples were collected into sample bottles containing di-sodium salt of ethylene diamine tetra acetic acid (E.D.T.A) and analysed for packed cell volume (PCV) and blood haemoglobin (Hb). The modified alkaline-acid hematin method of Bankowski (1942) was used for haemoglobin determination. The values for PCV were obtained using a microcapillary centrifuge.

From the slaughtered birds, livers, spleens and hearts were removed, weighed, processed and analysed for contents of Cu and Fe by atomic absorption spectroscopy subsequent to

wet ashing with concentrated nitric acid and perchloric acid.

The data collected were analysed by analysis of variance (Steel and Torrie, 1960) and significant differences among treatment means were determined with the Duncan's (1955) multiple range test.

RESULTS

The means and standard errors for blood haemoglobin, PCV and liver, spleen and heart weights are presented in Table 2. Blood haemoglobin concentration, PCV, liver, spleen and heart weights (fresh weights per unit body weight and dry weights) were not significantly affected by added dietary Cu or Fe or by the interaction between the two. The mean

Contrary to the observed treatment effects on the haematological measurements, dietary Cu addition, on the average, increased the fresh weights (expressed as g/kg body weight) and dry weights of liver, spleen and heart of birds fed the Cu supplemented diet over and above those on the control diet. In almost all the organs, the 200mgCu/kg diet treatment produced a greater increase in fresh and dry weights than the 400mgCu/kg diet level over the control. The depression of the dry weights of the spleen by the 200mg/kg dietary level of Cu below the control value was the only exception. The influence of added dietary Fe on organ fresh and dry weights followed an inconsistent pattern. The means and standard errors for Cu and Fe concentrations in the

TABLE 2: HAEMATOLOGICAL MEASUREMENTS AND ORGAN WEIGHTS OF LAYING HENS GIVEN CONTROL AND Cu AND/OR FE-SUPPLEMENTED DIETS

Copper Level (mg/kg)	Iron level (mg/kg)	Blood haemoglobin (g/l)	PVC (%)	Liver Fresh wt. (g/kg body wt)	Spleen Fresh wt. (g/kg body wt)	Heart Fresh wt. (g/kg body wt)	Liver dry wt. (g)	Spleen dry wt. (g)	Heart dry wt. (g)
0	0	75.1	29.8	16.7	0.87	3.91	7.00	0.50	3.52
200	0	74.9	29.0	19.3	0.97	4.11	7.70	0.48	4.30
400	0	69.3	28.8	16.3	0.94	4.09	6.90	0.59	3.50
	SEM	2.05	0.84	1.39	0.07	1.18	0.64	0.05	0.39
0	0	73.8	29.8	18.7	0.94	4.34	7.20	0.44	4.31
0	100	72.0	29.0	17.1	0.92	3.95	6.80	0.57	3.40
0	200	73.6	28.8	16.5	0.92	4.04	7.60	0.57	3.40
	SEM	2.05	0.84	1.39	0.07	0.18	0.64	0.05	0.39
0	0	70.7	28.7	14.6	0.75	3.70	7.30	0.40	4.83
0	100	78.0	30.7	19.0	0.88	3.89	6.37	0.61	2.63
0	200	76.7	30.0	16.4	0.97	4.12	9.00	0.48	3.11
200	0	75.3	28.7	23.4	1.12	4.52	8.47	0.44	5.33
200	100	72.7	28.3	15.7	0.93	3.77	6.63	0.45	4.33
200	200	76.7	30.0	19.0	0.88	4.03	8.00	0.56	3.23
400	0	75.3	31.0	18.0	0.95	4.13	5.97	0.47	2.77
400	100	65.3	28.0	16.5	0.95	4.19	7.40	0.64	3.87
400	200	67.3	27.3	14.3	0.92	3.96	7.33	0.66	3.87
	SEM	3.56	1.45	2.41	0.13	0.31	1.11	0.08	0.68

Sem = Standard error of means

haemoglobin and PCV values ($P > 0.05$) across the various levels of added dietary Fe appeared to be decreased with increased added dietary Cu. A similar trend in the haematological measurements was observed for added dietary Fe across the various Cu levels, except that a greater reduction in haemoglobin concentration was recorded at the 100mg/kg added dietary Fe than at the 200mg/kg level.

liver, spleen and heart of laying hens fed the control and Cu and/or Fe supplemented diets are shown in Table 3. Dietary Cu supplementation significantly ($P < 0.05$) increased only liver storage of Cu and spleen Fe concentration, while Fe addition in the diet produced significant effects on liver and spleen Fe concentrations but not on Cu storage of the organs. As the level of Cu supplementation increased, liver Cu

TABLE 3. COPPER AND IRON CONCENTRATIONS (ON DM BASIS) IN LIVER, SPLEEN AND HEART OF LAYING HENS GIVEN Cu AND / OR Fe-SUPPLEMENTED DIETS.

Copper levels (mg/kg)	Iron levels (mg/kg)	Liver Cu con. (mg/kg)	Liver Fe con. (ug/g)	Spleen Cu con. (ug/g)	Spleen Fe con. (ug/g)	Heart Cu con. (ug/g)	Heart Fe con. (ug/g)
Copper main effects							
0	0	43.2 ^a	875.9	25.7	1165 ^a	31.6	453
200	0	49.2 ^a	930.9	28.7	1371 ^{ab}	30.7	475
400	0	82.2	800.2	28.0	1581 ^b	31.7	609
	Sem	9.75*	51.21	1.60	87.8*	1.75	60.8
Iron main effects							
0	0	59.9	763.1 ^a	28.3	1576 ^b	32.8	551
0	100	60.4	969.9 ^b	25.6	1310 ^a	29.6	457
0	200	54.3	874.0 ^{ab}	28.5	1231 ^a	31.6	529
	Sem	9.8	51.2*	1.6	87.8*	1.8	60.8
Copper and Iron interaction effects							
0	0	48.0 ^{ab}	825 ^{abc}	28.1 ^{abc}	1139 ^a	34.7	439
0	100	36.7 ^a	798 ^{ab}	21.2 ^a	1210 ^a	28.7	492
0	200	45.0 ^a	1005 ^{bc}	27.6 ^{abc}	1146 ^a	31.3	428
200	0	43.0 ^a	894 ^{bc}	25.5 ^{ab}	1344 ^a	31.0	447
200	100	41.3 ^a	1110 ^c	25.4 ^{ab}	1475 ^a	28.7	431
200	200	63.3 ^{ab}	789 ^{ab}	35.0 ^c	1293 ^a	32.3	546
400	0	88.7 ^{ab}	571 ^a	31.1 ^{bc}	2244 ^b	32.7	767
400	100	103.3 ^b	1001 ^{bc}	30.2 ^{abc}	1243 ^a	31.3	448
400	200	54.7 ^{ab}	828 ^{bc}	22.8 ^{ab}	1254 ^a	31.0	611
	Sem	16.9*	88.6*	2.77*	152.1**	3.0	105

Treatment means with different superscripts differ significantly, * ($P < 0.05$); highly significantly, ** ($P < 0.01$).

concentration increased and the effect became significant ($P < 0.05$) when 400mg added Cu/kg diet was fed.

The concentration of Fe in the spleen of birds receiving diets supplemented with 400mgCu/kg diet (1518ug/g) was significantly ($P < 0.05$) higher than those of birds receiving the control diet (1165ug/g). The difference in spleen Fe concentration of birds fed the 0 or 200mg added Cu was, however, not significant. Although statistical significance was not attained on the effect of dietary Cu on liver Fe and spleen Cu concentrations, the intermediate level of Cu addition increased liver Fe and spleen Cu concentrations, while the 400mg added Cu/kg diet caused a depression in the mineral concentrations of the organs. While dietary Fe supplementation brought about an increase in the liver storage of Fe, the effect was a significant drop in spleen Fe storage. Whereas the 100mgFe/kg diet produced significantly higher liver Fe storage over the control, there was no added Fe storage brought about by supplementing up

to 200mg added Fe/kg diet.

There were significant ($P < 0.05$) interaction effects between dietary Cu and Fe with respect to liver Cu and Fe concentrations and spleen Cu concentration. In the absence of supplemental Cu, dietary Fe supplementation alone reduced liver Cu storage. This reduction was, however, not significant. With the 200mg Cu/kg diet treatment, there was a drop and then a rise in liver Cu storage at the 100 and 200mg/kgFe supplementations respectively. Contrary to the observation at the intermediate Cu level, the 400 mgCu/kg supplement resulted in a significantly higher liver Cu storage with 100mgFe/kg supplementation. Although the liver Cu storage of birds receiving diets supplemented with 0mgFe/kg or 200mgFe/kg together with 400mgCu/kg supplements was lower than that of birds receiving diet supplemented with 100mgFe/kg, the difference was not significant.

In the absence of supplemental Cu, liver Fe storage increased with increasing added dietary Fe. However, with dietary Cu

supplementation (both at 200 and 400mg/kg levels) Fe supplementation at 100mg/kg level increased Fe storage in the liver. Further increase in added dietary Fe to 200mg/kg level together with Cu supplementation caused a depression in liver Fe storage. The highest and the lowest storage of Fe in the liver were obtained at the 200mgCu/kg together with 100mgFe/kg supplement and 400mgCu/kg together with 0mgFe/kg supplements respectively. Dietary Fe supplementation either in the absence of Cu addition (0mg/kg) or along with high level (400mg/kg) of added dietary Cu caused a reduction in spleen Cu storage (Table 3). However, at an intermediate (200mg/kg) level of added dietary Cu, an increase in spleen Cu storage which was significantly greater than the values for 0mgCu, 100mgFe; 200mgCu, 200mgCu, 100mgFe; and 400mgCu, 200mgFe supplements occurred. The highest value was, however, not significantly different from the other values. With spleen Fe storage, the 400mgCu/kg together with 0mgFe/kg supplements produced significantly higher values than all other combinations among which there were no significant differences.

DISCUSSION

The effect of Cu intake on blood haemoglobin and PCV observed in the present study is in agreement with the findings of Kline, Hays and Cromwell (1972); Jackson (1977) and Stevenson and Jackson (1980) who all observed no significant effect of dietary Cu on haemoglobin at levels of Cu supplementation ranging from below 500 mg/kg in pigs to as high as 1920 mg/kg level in laying hens. A significant effect was, however, noticed at 2000 mg added Cu/kg diet in hens (Stevenson and Jackson, 1980). The results of this experiment, as those of the earlier workers regarding the effect of supplemental Cu intake on haemoglobin and PCV, could be explained from the reports of Mitchell and Miller (1931) and Charles and Gennard (1961) who have indicated that as either the Cu or Fe was increased, the concentration of haemoglobin increased. It could be that increasing level of

either Fe or Cu is capable of increasing the haemoglobin and PCV only when the intake has not reached the minimum necessary for haemoglobin formation or at a very high level beyond which the ability of the liver for storage or excretion is exceeded. This would then mean that at fairly low level of supplemental Cu or Fe intake, the blood haemoglobin and PCV will remain unaffected as obtained in this study.

The non-significant increase in the fresh and dry weights of liver as a result of dietary Cu addition observed in this study is in line with the report of Jackson *et al.* (1979) at 200 mgCu/kg diet level and observed a significant depression at the higher levels (400, 600 and 800 mg/kg) of dietary Cu supplementation. A probable similar depression in organ weights was already initiated in this study (Table 1) and it could be that any further increase in the levels of added Cu above 400 mg/kg will result in greater depression in fresh and dry weights until significance is attained at the very high levels of Cu addition. The increase in liver Cu concentration with added dietary Cu observed in the present study has been reported earlier for different species and classes of poultry and for the rat (Mehring *et al.*, 1960 on chicks; Beck, 1961 on cockerels and ducks; Norvell, Calvert, Thomas and Goatcher, 1974 on laying hens and Milne and Weswig, 1968 on the rat). Liver Cu concentration (Table 2) was significantly increased by the highest (400 mgCu/kg) level of added dietary Cu and unaffected by 200 mgCu/kg. This observation appears to be in line with the threshold effect of Cu intake previously reported in laying hens. But while Jackson *et al.*, (1970) observed the threshold level of Cu intake (above which accumulation occurs in the liver) to occur between 600 and 800 mg added Cu/kg diet, Stevenson and Jackson (1980) found the same to occur between 250 and 500 mg added Cu/kg diet. In the present work, this threshold was observed to occur between 200 and 400 mg added Cu/kg diet.

The lack of significant effect of added Cu on liver Fe noted in the current study seems not to disagree totally with the results of

earlier researcher (Jackson, 1977 and Stevenson and Jackson, 1980) who although observed increased liver Fe with high dietary Cu (1000 to 2000mg/kg) found no remarkable effect of dietary Cu on liver Fe concentration at the lower levels of dietary Cu (120 to 500 mg/kg) addition which are similar to the levels used in this study. The results of the present study, indicating liver Fe of laying hens to be transiently increased by lower levels of Cu, further corroborate the reports of previous workers (Jackson, 1977; and Stevenson and Jackson 1980) who postulated the difference existing between fowl and other species and in particular the rat where highly significant inverse correlation has been observed between hepatic Fe concentration and dietary Copper levels (Sourkes, Lloyd and Birnbaum, 1968).

The significant increase in spleen Fe concentration with added dietary Cu observed in this study suggests a positive relationship between added dietary Cu level and spleen Fe concentration. There seems not to be any previous record of the influence of added dietary Cu on spleen Fe concentration of laying hens. It is known that both liver and spleen are repository sites for Fe, but the capacity of the liver in this respect is greater (Maynard *et al*, 1979). However, the greater Fe concentration in the spleen (even in those birds receiving the control diets) observed in this experiment could be an indication of greater Fe mobilization from the liver. The Fe status of the spleen therefore may have been contributory to significant increase noted with added dietary Cu. Further work is needed to confirm and establish the basis for these observations.

The observed increased storage of Fe in the liver with added dietary Cu which did not attain significance is in line with the observations on chicks by Mehring *et al*. (1960). The increase in Fe accumulation in the liver with added dietary Fe noted in this study agrees with the results obtained on pigs (Gublers, 1951; Mercedes, *et al* (1967). While in the present work added dietary Fe significantly reduced spleen Fe accumulation, Mercedes *et al*. (1967) reported a lack of

effect of dietary Fe on spleen Fe concentrations when as high as 573 mg added Fe/kg diet was used. Except for specie difference that may be implicated, it is difficult to explain the discrepancy in the observations. It would appear that different dietary Cu and Fe ratios would be required to bring about substantial storage of the two mineral elements in the liver and spleen of mature laying hens. These ratios as obtained in the present study are 4:1 for liver Cu, between 2:1 and 4:1 for liver Fe and 1:1 for spleen Cu storage.

There is a suspicion of a sparring effect of supplemental Cu on liver Fe storage at the intermediate level (100 mg/kg) of added dietary Fe. This effect was, however, lost at higher level (200mg/kg) of Fe supplementation regardless of the level of Cu addition in the diet. No explanation seems available for this observation. The observation with spleen Fe storage where the 400 mg added Cu/kg together with 0 mg added Fe/kg supplement produced significantly higher spleen Fe storage than all other combinations, among which there was no significant difference, seems to indicate that at higher level of Cu supplementation (400 mg/kg), dietary Fe addition serves as a protection for spleen Fe storage.

In this study, both heart Cu and Fe concentrations were observed to be unaffected by either added dietary Cu or Fe or by both. It can therefore be postulated that the lack of effect of Cu or Fe supplementation or of their interaction on heart Cu and Fe storage is an indication that the heart is not significantly involved in Cu and Fe storage or excretion in mature laying hens.

REFERENCES

- AGUNBLADE, J.A. and BABATUNDE, G.M. (1988). Copper and iron supplementation in the diets of laying hens in the tropical environment - effects on production traits. *Nig. J. Anim. Prod.* 13: 112-118.
- BANKOWSKI, R.A. (1942). Studies of the haemoglobin content of chicken blood and evaluation of methods for its determination. *Amer. J. Vet. Res.*, 3:373.

COPPER AND IRON SUPPLEMENTATION FOR LAYERS

- BECK, A.B. (1961). Observations on the copper metabolism of the domestic fowl and duck. *Aust. J. Agric. Res.* 12: 743-753.
- CHARLES, H.H. and GENNARD, M. (1961). Studies on copper and iron deficiencies in growing chickens. *J. Nutr.* 73:425-437.
- DUNCAN, D.B. (1955). Multiple range and multiple F-tests *Biometrics* 11: 1-42.
- GUBLER, C.J. (1956). Absorption and metabolism of iron. *Science* 123: 87-90.
- JACKSON, N. (1977). The effect of dietary copper sulphate on laying performance, nutrient intake and tissue copper and iron levels of the mature, laying, domestic fowl. *Br. J. Nutr.* 38: 93-100.
- JACKSON, A.N., STEVENSON, M.H. and KIRKPATRICK, G.M. (1979). Effects of the protracted feeding of copper sulphate supplemented diets to laying, domestic fowl on egg production and on specific tissues, with special reference to mineral content. *Br. J. Nutr.* 42:253-266.
- KING, J.O.L. (1972). The feeding of copper sulphate to growing fowls. *Br. Poult. Sci.* 13:61-65.
- KLINE, R.D., HAYS, V.W. and CROMWELL, G.L. (1972). Related effects of copper, zinc and iron on performance, haematology and copper stores of pigs. *J. Anim. Sci.* 34 (3) : 373 - 423.
- MAYNARD, L.A., LOOSLI, J.K., HINTZ, H.F. and WARNER, R.G. (1979). *Animal Nutrition*. 7th Edition Mc. Graw-Hill Book Company New Delhi. pp 244-250.
- MEHRING, A.L., BRUMBAUGH, J.H., SUTHERLAND, A.J. and TITUS, H.W. (1960). The tolerance of growing chickens for dietary copper. *Poult. Sci.* 39:1713.
- MERCEDES, H.K., MACIA, E., ZINNI, R.A., MERKELAND, J. and HALL, L. (1967). Effect of iron and copper intake and myoglobin levels in selected pig tissues. *J. Agric. Food Chem.* 15 (41): 721-725.
- MILNE, D.H. and WESWIG, P.H. (1968). Effect of supplementary copper containing fractions in rats. *J. Nutr.* 95:429.
- MITCHELL, H.S. and MILLER, L. (1931). Studies in nutritional anaemia: Qualitative variations in iron, copper and manganese supplements. *J. Biol. Chem.* 92:421.
- NORVELL, M.J., CALVERT, C.C., THOMAS, M.C. and GOATCHER, W.D. (1974). The effects of copper sulphate supplementation in broiler chickens diets on growth and selected toxicological parameters. *Poult. Sci.* 53: 1641 (Abstr).
- SOURKES, T.L., LLOYD, K. and BIRNBAUM, H. (1968). Factors affecting the concentration of copper in the liver of the rat. *Can. J. Biochem.* 46:267.
- STEEL, R.G.D. and TORRIE, J.H. (1960). *Principles and procedures of statistics*. McGraw-Hill Book Company, Inc. New York.
- STEVENSON, M.H. and JACKSON, N. (1980). Effects of level of dietary copper sulphate and period of feeding on the laying domestic fowl, with special reference to tissue mineral content. *Br. J. Nutr.* 43:205.