

## BIOCHEMICAL AND HEMATOLOGICAL CHANGES DUE TO COPPER DEFICIENCY IN BROILER FARMS

By

M. M. Arafa; Magda Sh. Taha and Eman Sh. Laz

*Animal Health Research Institute*

### SUMMARY

*This study was conducted in a private broiler farm in Cairo Governorate. The flock was suffered from sever anemia and general weakness. Blood and serum samples were collected from affected broilers and were examined for detection of hematological and biochemical alteration. Poultry feed was chemically and mycologically analyzed. Results revealed that there was a significant decrease in copper concentration in serum as a result of copper deficiency in feed. Also, there was a significant decrease in Hb% and R.B.Cs. No significant change was recorded in the rest of biochemical and hematological parameters except slight significant decrease in M.C.V%, and the serum concentration of total protein, sodium, potassium and iron. Elevating copper level in poultry feed is recommended to enhance health status of broiler.*

### INTRODUCTION

Nutritional deficiency diseases of chicken may constitute the main factor of direct and indirect losses.

Broiler performance has been considerably improved during the past 30 years due to nutritional recommendations for chicken have been adjusted to theses changes. The modifications of nutritional recommendations have been made mainly taking growth into account. From a practical point of view, wide safety margins are often introduced, especially for minor nutrients such as trace elements to avoid problems caused by the heterogeneity of food staff.

Copper is essential trace element for all animals. It is vitally concerned in the process of pigmentation, growth and myelination of nerve fibers, (Underwood 1977).

Copper present in all body cells, being particularly concentrated in the liver which act as the main copper storage organ in the body (McDonald et al., 1995). It's an essential element which is involved in hemoglobin formation, disulphide cross, Linkage in elastin, collagen keratinization and oxidaze enzyme activity (Tanner et al 1988). Also it is associated with humoral and cell mediated immune mechanism as well as non specific immune ones. So its deficiency cause alteration in hummural response and killing ability of neutrophill (Radostits et al., 2000).

*William et al.*, (1983) thought that copper is involved in the absorption of iron from intestinal mucosa, its mobilization from the tissue and its utilization in hemoglobin synthesis. This function could be accomplished by ceruloplasmin (a copper containing  $\alpha_2$  globulin with ferrioxidase activity in the plasma) facilitating iron transport through formation of transferrin.

The molybdate restrict copper utilization by depressing copper solubility in the digestive tract through precipitation of insoluble copper salts as molybdate interact with copper to form a biologically unavailable copper - molybdenum complex called cupric - molybdate which appear to be not absorbed or transported from intestine and excreted as a unit to make both copper and molybdenum less available, (*Under wood 1977*).

*Hungerford*, (1990) approved that copper is an essential trace element for utilization of iron for formation of hemoglobin. With deficiency of copper, the iron is not utilized and anemia will occur.

This work aimed to study the biochemical and hematological changes accompanied with copper deficiency in broiler chickens.

### **MATERIAL AND METHODS**

In a private farm of broiler in Cairo Governorate, there was a flock of broiler suffered from anemia and general weakness. Two blood samples were collected from 50 anemic birds. The collected blood and serum samples were subjected to hematological and biochemical assays according to following procedures:

Red blood cell count, mean corpuscular volume (M.C.V), mean corpuscular hemoglobin (M.C.H) according to method described by *Schalm et al.*, (1975).

Serum concentration of total protein, iron, copper, calcium, inorganic phosphorus and magnesium were measured according to the methods described by *Hoffman & Richterrich (1979)*; *Ramsay (1958)*; *Khalifa et al.*, (1972); *Bett and Fraser (1959)*; *Klichling and Freiburg (1951)* and *Neil and Neely (1956)*, respectively. Serum sodium and potassium concentration were measured through flame photometry using corning 410 -C flame photometer as described by *Oser*, (1979). The obtained results were compared with serum samples collected from 50 apparently healthy broilers.

Four diet samples were collected from the available diets in front of birds and from stocks. They were subjected for chemical analysis according to the following methods:

Total ash content (*A.O.A.C, 1990*); humidity (*Kirmiz, 1962*); Crude protein (*David, 1976*); crude fiber (*Lees, 1975*); calcium (*Bett and Fraser, 1959*); Phosphorus (*Fiske and Subbarow, 1952*); Magnesium (*Neil and Neely, 1956*); copper (*Khalifa et al., 1972*); iron (*Ramsay, 1958*); sodium and potassium (*Oser, 1979*) concentration.

Statistical analysis of the obtained data was carried out according to *Petrie and Watson (1999)*. The ration samples were examined for presence of mycotoxin aflatoxin and ochratoxin as described by *A.O.A.C (1990)*.

### **RESULTS AND DISCUSSION**

The copper deficiency is either primary due to lack of copper in the diet or secondary when the dietary level is adequate but there is a failure in digestion, a absorption or metabolism of the copper.

Form diet analysis (table 3), it was obvious that copper is not sufficient for birds requirement. The low value of mean corpuscular hemoglobin (M.C.H), mean corpuscular volume (M.C.V.), hemoglobin concentration and red blood cell count among examined bird revealed that these birds have not utilized adequate iron for haemoglobin formation this results agreed with those recorded by *Magda, (1993) Aganbiade and Babatunda (1995)* and *Hassan et al., (2002)* who reported that during hypocuprosis, there was a decrease in hemoglobin level in sheep which might be due to the role of copper in the production of hemoglobin through utilization of iron liberated from normal break down of hemoglobin *Saleh et al., (1998)*.

The prolonged decrease in the available copper for the birds produces anemia syndrome among them as revealed in table (1). This agreed with *Brewer, (1987)*, who reported that prolonged copper deficiency generally resulted in anemia in mammals and is supported by the fact that copper is an essential element during iron transportation (*Frieden, 1983*) and heme synthesis in the mitochondria (*Porra and John, 1963*).

Table (2) showed that there were no significant difference in serum concentration of calcium, phosphorus, magnesium in diseased group compared with control birds. These results agreed with those recoded by *Ahmed et al., (1999)*.

The lowered serum protein may be a sign of dietary disorders as serum protein is sensitive to nutritional influences (*Kaneko et al., 1997*).

Low sodium and potassium serum concentration in the examined birds could be attributed to the low daily available sodium and potassium this in turn agreed with *Aoyagi and Baker, (1993)*.

It can be concluded that copper is very important nutrient for the health of broilers and its deficiency lead to anemia and general weakness cause delaying in marketing.

So, monitoring of the nutritional status of the broiler especially for copper is very important to avoid occurrence of anemia.

### **References**

- A.O.A.C (1990): Official Methods Analysis of the Association of Official analytical Chemists. – 15<sup>th</sup> Ed. Edited by Kennesh herlich, Arlington, Virginia, 2220, USA.*

- Aganbiade, J.A. and Babatunda, G.M. (1995):** Copper and iron supplementation in the tropical environment effect on hematological measurements, organ weight in layer, *Nigerian J. of Ani. Prod.*, 22, (1-2): 49 – 55.
- Ahmed, E. E. K. Haleem, H. H. and Aly, A. A. (1999):** Effects of copper and ascorbic acid in restriction of cadmium toxicity. *J. Egypt. Vet. Med. Ass* 59 (5): 1549 – 1572.
- Aoyagi, S. and Baker, D. H. (1993):** Biological efficiency of copper in chicken. *J. of Nutrition* 123 (5): 870 – 875.
- Bett, J.M. and Fraser; C.P. (1959):** Biochemical estimation of calcium. *Clin. Chem. Actas* 4: 346.
- Brewer, N. R. (1997):** *J. A. V. M. A.* igo: 645-658. Cited in Kaneko et al., 1997.
- David, P. (1976):** "The Chemical Analysis of Food". 7<sup>th</sup> Ed. Churchill Livingstone Press. Edinburgh, London and New York.
- Fiske, C.M. and Subbarow, Y. (1952):** The colorimetric determination of phosphorus. *J. Biol. Chem.* 66: 375.
- Frieden, E. (1983):** *Semin. Hematol.*, 20: 114-117. Cited in Kaneko et al., 1997.
- Hassan, Y.K.; Magda, S. Elsayed, Selim, H.M. amnd Hammoda, F.K. (2002):** Influence of hypocuprosis on the immune response of sheep vaccinated with inactivated foot and mouth disease (FMD) vaccine. *Munifia Vet. J.* 2 (1): 219-225.
- Hoffman, Von T.P. and Richterrich, R. (1979):** Die Eliminierung von trubungan bei der Bestimmung non plasama – proteinen mit dem biuret Reagenz. *Z. Klin. Chem. U. Klin. Biochem.*, 8: 505.
- Hungerford, T. G. (1990):** *Diseases of Livestock* 9<sup>th</sup> ed. Pp.1525 – 1526.
- Kanko, J. J.; Harvey, J. W. and Bruss, M. L. (1997):** *Clinical Biochemistry of Domestic Animals.* 5<sup>th</sup> Ed., Acad. Press, Harcourt Brace and Company, San Diego, Calif., U. S. A.
- Khalifa, H. ; Fouad, M.T. and Georgy, M.E. (1972):** Application of fast grays R. A. to the photometric determination of copper in serum of Egyptian camels. *Microchem. J.*, 17: 266 – 272.
- Kirmiz, J.P. (1962):** *Technique in Adaptation to Desert Environment.* Pp. 110, Butterworth, London.
- Klichling, H. and Freiburg, I.R.R. (1951):** *Klin Photometrie.* 3<sup>rd</sup> Ed. End. Wiss. Verl. Ges. MBH.
- Lees, R.(1975):** *Food Analysis and Quality Control Methods for the Food Manufacture.* Leonard Hill books. International textbook company Ltd. London.
- Mc Donald, P.; Edward, R.A. and Green balgh, J.F. (1995):** *Animal Nutrition.* 5<sup>th</sup> Ed. Pp. 103 – 106, English Language Book Society.
- Magda, S. Elsayed (1993):** Some biochemical studies on serum of copper deficient sheep. *Beni-Suef; Vet. Méd. Res.* 3 (2).
- Neil, D. W. and Neely, R.A. (1956):** Estimation of magnesium in serum using titan yellow. *J. Clin. Path.*, 9:162 – 163.
- Oser, B.L. (1979):** *Hawk's Physiological Chemistry.* 14<sup>th</sup> Ed. McGraw, Hill Book company, New Delhi.
- Petrie, A. and Watson, P. (1999):** "Statistics for veterinary and Animal science". 1<sup>st</sup> Ed. Pp. 90 – 99. the Black well Sci-Ltd. U.K.
- Porra, R. J. and John, O. T. (1963):** *Biochem. J.* 87: 181- 185. Cited in Kaneko et al., 1997.
- Ramsay, W.N.M. (1958):** *Advances in clinical Chemistry* Edit. Sobotha, H. and Stewart, C.P. Acad. Press. New York.
- Radostits, O. M.; Blood, D. C.; cay, L. C and Hincheliff, K. W. (2000):** *Veterinary Medicina Text Book of the Disease of Cattle, Pigs, Sheep, Goat and Horses.* 9<sup>th</sup> Ed.

- Saleh, I.A.; Elsamee, A.A. Rakha, G.M. (1998): Clinical studies on wool slip (alopecia) in sheep with reference to hematological and biochemical changes. Vet. Med. J. Giza, 46 (1): 57-66.
- Schalm, O.W.; Jain, N.C. and Carrol, E. J. (1975): Veterinary Hematology, 3<sup>rd</sup> Ed., Lea, Fabiger, Philadelphia.
- Tanner, D. Q; Stednick, J.D. and Leininger, W.C. (1988): Minimal herd sample size for determination of blood copper status of cattle. JAVMA, 192, (8): 1074 – 1076.
- Underwood, E.J. (1977): Trace elements in Human and Animal Nutrition 3<sup>rd</sup>. ed. Pp. 57 – 127. Acad. Press. N.Y.
- William, D.M.; Kenedy, F.S & Green B.G. (1983): Hepatic iron accumulation in copper difeent rats. Br. J. of Nut. 50, 653 – 660.

Table (1): Hematological picture of affected broiler chicken compared with normal healthy chicken.

Group	Parameter	R.B.Cs $10^6 \times \mu\text{g}$	M.C.V fl	M.C.H Pg	Hb gm/dL
Examined samples		$1.9 \pm 0.03^{**}$	$3.05 \pm 0.8^*$	$12.3 \pm 0.31^*$	$7.5 \pm 0.9^*$
Control		$3.6 \pm 0.07$	$5.53 \pm 0.3$	$15.9 \pm 0.44$	$11.3 \pm 0.7$

\* Significant at  $p \leq 0.05$

\*\* Significant at  $p \leq 0.01$

Table (2): Serum Biochemical Parameters of affected broiler chickens compared with normal healthy chicken.

Parameter	Total protein gm/dL	Calcium mg/dL	Phosphorus mg/dL	Magnesium mg/dL	Sodium meq	Potassium meq	Iron mg/dL	Copper mg/dL
Examined samples	$6.47^* \pm 0.21$	$10.31 \pm 0.17$	$7.4 \pm 0.41$	$1.71 \pm 0.8$	$133.81^* \pm 2.71$	$3.8 \pm 0.91$	$95.7^* \pm 4.31$	$35.7^{**} \pm 6.3$
Control	$8.41 \pm 0.35$	$11.7 \pm 0.18$	$7.9 \pm 0.18$	$1.95 \pm 0.77$	$145.71 \pm 4.29$	$4.7 \pm 0.85$	$101.3 \pm 3.37$	$65.3 \pm 8.41$

\* Significant at  $p \leq 0.05$

\*\* significant at  $p \leq 0.01$

Table (3): Chemical and mycological analysis of ration

Parameter	Value
Ash%	5 ± 0.7
Crude fiber %	8.7 ± 0.9
Crude protein %	19.5 ± 1.3
Humidity %	2.45 ± 0.7
Calcium %	1.5 ± 0.5
Phosphorus %	0.63 ± 0.08
Magnesium %	0.22 ± 0.03
Copper (ppm)	3.7 ± 0.7
Iron (ppm)	9.3 ± 0.45
Potassium (meq.)	33.7 ± 0.79
Sodium (meq.)	65.1 ± 3.71
Aflatoxin (ppm)	5 ppm
Ochratoxin (ppm)	- ve

## الملخص العربي

التغيرات البيوكيميائية والهيماتولوجية نتيجة نقص النحاس في مزارع الدواجن

محمود محمد عرفه، ماجدة شعبان طه، إيمان شوقي لافظ

قسم الكيمياء والسموم والنقص الغذائي - معهد بحوث صحة الحيوان - الدقى

أجريت هذه الدراسة في مزرعة دجاج تسمين حيث ظهرت حالات تعاني من أنيميا حادة وضعف عام. تم تحليل عينات من الدم ومصل الدم من الدجاج المصاب وتم مقارنته بدجاج آخر سليم لم تظهر عليه أعراض. تم تحليل العليقة المقدمة للدجاج كيميائياً وفطرياً. أظهرت النتائج أن هناك نقص في نسبة الهيموجلوبين وعدد كرات الدم الحمراء في الدم ويرجع هذا إلي النقص المعنوي في تركيز النحاس والحديد في مصل الدم. لم يحدث أى تغيير معنوي في بقية مكونات الدم الهيماتولوجية والبيوكيميائية فيما عدا انخفاض معنوي طفيف في تركيز البروتين الكلى والصوديوم والبوتاسيوم والحديد. ينصح بضبط تركيز نسبة النحاس في العليقة في الحدود المسموح بها لرفع كفاءة الحالة الصحية للدواجن.