The Effects of Selenium and Vitamin E Deficiency and Subsequent Selenium Supplementation on Immune Response in Chicks

A Thesis

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By

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THESIS ABSTRACT

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TITLE OF THESIS

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This experiment was conducted in an effort to track the healing process in chicks resupplemented with selenium following a combined selenium and vitamin E deficiency. Chicks were fed a low selenium diet with no added vitamin E or synthetic antioxidant. A control group was fed the same diet with 0.1 mg selenite selenium added per kilogram. Selenium-deficient chicks first displayed visible symptoms of exudative diathesis at 10 days of age. Trios of birds were matched for severity. Two birds were treated with an injection of 0.15 μg selenite selenium in 1 mL distilled water. The remaining bird was given an injection of 1 mL distilled water. The process was repeated for the five remaining trios of chicks.

Immune responses were observed among each group. In selenium-deficient chicks, leukocyte counts more than doubled. Heterophil counts for this group were also increased. Upon reconstitution of selenium, leukocyte, heterophil, monocyte, and basophil counts approached normal values. Eosinophils, however, increased in number in the selenium corrected-group.

The selenium-deficient birds displayed evidence of cellular damage, as indicated by the results of the blood chemistries. An increase in immature erythroid cellular elements resulted in a myeloid:erythroid (M:E) ratio of 0.45. The deficient group had the highest heterophil and lowest lymphocyte counts. Necrosis in the pectoral muscle and varying degrees of pancreatic fibrosis were also observed. In birds resupplemented with selenium, fibrosis was extensive, and necrosis was uncommon. The (M:E) ratio was 1, as was the case with the selenium-adequate birds. Leukocyte and heterophil counts in selenium-corrected birds were similar to those observed in the selenium-adequate birds. However, the selenium-corrected birds had the lowest body weights over a 27-day period. Resupplementation of selenium in the diet decreased the severity of exudative diathesis, myocyte necrosis and began to restore immunocompetence in chicks. Immunological criteria in selenium-adequate birds was normal.

Introduction

The ability to provide a proper selenium concentration to poultry prevents muscular dystrophy, exudative diathesis, and other forms of muscular degeneration. Selenium, which is closely associated with vitamin E and other antioxidants in practical feed formulation, may prevent the aforementioned degenerative diseases by functioning as a cofactor for glutathione peroxidase (1). Although selenium and vitamin E are very dissimilar chemically, they spare the requirement for one another in the prevention of these diseases (1). Selenium and vitamin E function as key elements of a multi-component system of antioxidant protection within cells (2).

Together, vitamin E and glutathione peroxidase reduce and terminate free oxygen radicals generated within the cell, thus preventing the initiation of oxidative reactions that are known to impair normal cellular function (3). Vitamin E, which is fat soluble, resides in membranes and protects tissue against oxidative damage by acting as a free radical scavenger in the lipid portion of biological membranes (2). Glutathione peroxidase is present in the aqueous portions of the cell, such as the cytoplasm and mitochondrial matrix of the cell. Glutathione peroxidase catalyzes the conversion of hydrogen peroxide and other peroxides to water and alcohols, thereby decreasing the amounts of peroxides available for reaction with the unsaturated fatty acids of biomembranes.

In selenium-deficient birds, the activity of plasma creatine phosphokinase (CPK) has been found to be 20,000 times greater than normal (4). Creatine phosphokinase is an enzyme that can be found in high activities in skeletal muscle and is necessary for the breakdown of phosphate compounds for energy. A possible explanation for the high CPK activity recorded for selenium-deficient birds may be due to leakage of CPK out of the cell. This leakage would be caused by the destruction of cell membranes due to the peroxides whose accumulation could have been prevented by adequate selenium supplementation (5).

Deficiencies of selenium and/or vitamin E can affect several organ systems of poultry. Exudative diathesis in chicks is characterized by severe subcutaneous edema, particularly in the abdomen, feet, and ventral aspect of the neck and wings. The edema results from abnormally increased capillary permeability (1). Breakage of blood vessels results in a green-blue discoloration of the breast tissue. The affected chicks are also anemic, having smaller than normal red blood cells (RBC). In this anemia, there is no condensation of the RBC nucleus.

Dietary supplementation of selenium is effective in reducing the incidence of nutritional muscular dystrophy in the chick. This disease involves degeneration of the skeletal muscles, and affected chicks show generalized muscular weakness and marked decreases in activity (1). The metabolic conditions leading to muscular dystrophy may relate to oxidative stresses associated with either reduced utilization of, or increased need for, the glutathione peroxidase system (1).

Chicks deprived of selenium have shown an impaired development of immunocompetence (6). Chicks made deficient with respect to either selenium or vitamin E within the first two weeks after hatching showed impaired humoral, or antibody-mediated, responses. Subsequent studies have shown lesions of the epithelial tissue of the bursa of Fabricius, which is responsible for immunological responses in poultry, in selenium and vitamin E deficient chicks. These lesions appear to be associated with the depletion of lymphoid cells in that organ and may explain the diminished antibody function observed in chicks with the combined deficiency. This suggests that selenium and/or vitamin E deficiency may affect disease resistance in young chicks. The purpose of this research was to study the effect of an induced selenium deficiency and subsequent selenium supplementation in chicks in the immune system, red blood cells, and muscle tissues.

Materials and Methods

Commercial broiler breeders were fed a diet with no supplemental selenium or vitamin E for one month prior to collection of eggs for this study. Chicks that hatched from these eggs showed no signs of a deficiency, but stores of selenium and vitamin E were low. Twenty-six chicks were fed a low selenium diet (Table 1) with no added selenium, vitamin E, or synthetic antioxidant. Ten chicks were fed the same diet that had 0.1 mg selenite selenium added per kilogram.

Several chicks first showed signs of exudative diathesis at 10 days of age. A trio of chicks was matched for severity. One was injected subcutaneously with 1 ml of distilled water. The other two were injected subcutaneously with 0.15 micrograms selenite selenium in 1 mL of distilled water. A chick that was fed the selenium supplemented diet was also injected subcutaneously with 1 mL of distilled water. Two days later, blood was collected from the heart of 3 of the chicks. An aliquot of the blood from each chick was delivered to a tube with EDTA (1.5 mg per mL blood). This blood was used for manual counting of white blood cells and hemoglobin determination. The remainder of the blood was delivered to a tube with heparin (30 units per mL blood). One mL was withdrawn for determination of selenium content (7). Plasma from this remaining blood was used to determine blood chemistry and glutathione peroxidase activity (8). After blood collection, the chicks were euthanized with carbon dioxide. Tissues were collected from the chicks and placed in formalin. The remaining chick from the original 4 recovered for 6 days after the selenium injection. An identical procedure was then followed with that chick.

The process was repeated several times over an 16-day period. When a matched trio of selenium deficient chicks was identified, procedures were initiated. Statistical procedures using analysis of variance (ANOVA) were used to compare blood chemistry and blood differential results among groups.

TABLE 1: Low Selenium Basal Diet

Ingredient	% of Diet
Corn (Low Selenium)	11.48
Soybean Meal (48%) (Low Selenium)	15.00
Torula Yeast	20.00
Glucose	22.50
Corn Starch	22.50
Dicalcium Phoshpate	0.87
Limestone	1.56
Iodized Salt	0.40
Animal Fat	3.00
Soybean Oil	2.00
D,L-Methionine	0.32
Arginine (Free Base)	0.12
Trace Mineral[1]	0.05
Vitamin Mix[2]	0.20

1. The following was supplied in mg per kg of diet: Mn, 50; Fe, 50; Cu, 5.0; Co, 0.5; and Zn, 50.

2. Supplied per kg of diet: vitamin A, 1500 I.U.; cholecalciferol, 800 I.C.U.; (in mg) riboflavin, 3.6; pantothenic acid, 10; vitamin B12, .01; choline chloride, 650;vitamin K, 0.5; niacin, 17.0.

Results

Blood Chemistries:

The results of the blood chemistries for the selenium-adequate (A), selenium-deficient (D), and selenium-corrected (C) groups can be found in Table 2. The amount of CO_2 in the blood of the D group is significantly lower ($p<0.05$) than that of the A group. The deficient birds had significantly lower blood glucose levels than either the A or C groups. S and C chicks had significantly more creatine phosphokinase (CPK) activity than A chicks. Glutathione peroxidase activity highest in selenium-adequate birds and lowest in the selenium-deficient birds.

Blood Differentials:

The results of the blood differentials for the A, D, and C groups are displayed in Table 3. Hemoglobin level was lowest in the D group. The D chicks had significantly higher leukocyte, heterocyte, and monocyte counts than A or C chicks. Lymphocyte count was highest in the A chicks and lowest in the D chicks. Basophil count in the A group was significantly higher than the D and C groups.

Muscle Tissue:

Necrosis was observed in the muscle of the D chicks. The extent of myocyte necrosis varied from rare-focal to severe-diffuse. Varying degrees of fibrosis also occurred. Acute muscular degeneration occurred in the D chicks. Muscle tissue from C chicks showed fibrosis and proliferation of myocytes. In most 6-day selenium-corrected birds, fibrosis was extensive and necrosis was uncommon.

Bone Marrow:

Selenium-adequate chicks had a myeloid:erythroid (M:E) ratio of 1. The bone marrow cellularity was increased in the D chicks. The M:E ratio in D chicks was 0.45 because of

increased immature erythroid elements. In addition, there was mild lymphocyte depletion. On days 2 and 6 of selenium reconstitution, the M:E ratios were 0.75 and 1, respectively.

TABLE 2: The Effect of Selenium Status on the Blood Chemistry of Chicks.

		Selenium Status		
Criterion	Units	Adequate	Deficient	Corrected
		(n =7)	(n =6)	(n = 5)
Total CO_2	mEq/L	23.4^{a}	19.8^{ab}	18.2^{b}
Glucose	mg/dL	278^{a}	215^{b}	292^{a}
Na	mEq/L	151^{b}	149^{b}	157^{a}
Alanine transaminase	iu/L	0.14^{b}	2.00^{a}	0.00^{b}
Aspartic transaminase	log iu/L	5.19^{b}	6.54^{a}	5.92^{ab}
Creatine phosphokinase	log iu/L	7.35^{b}	10.2^{a}	9.05^{a}
Glutathione peroxidase	iu/L	$.369^{a}$	$.117^{c}$	$.235^{b}$
Blood Selenium	µg/L	$.08^{a}$	$.01^{c}$	$.04^{b}$

a, b, c, Groups with different superscripts denote significant statistical difference ($p < .05$).

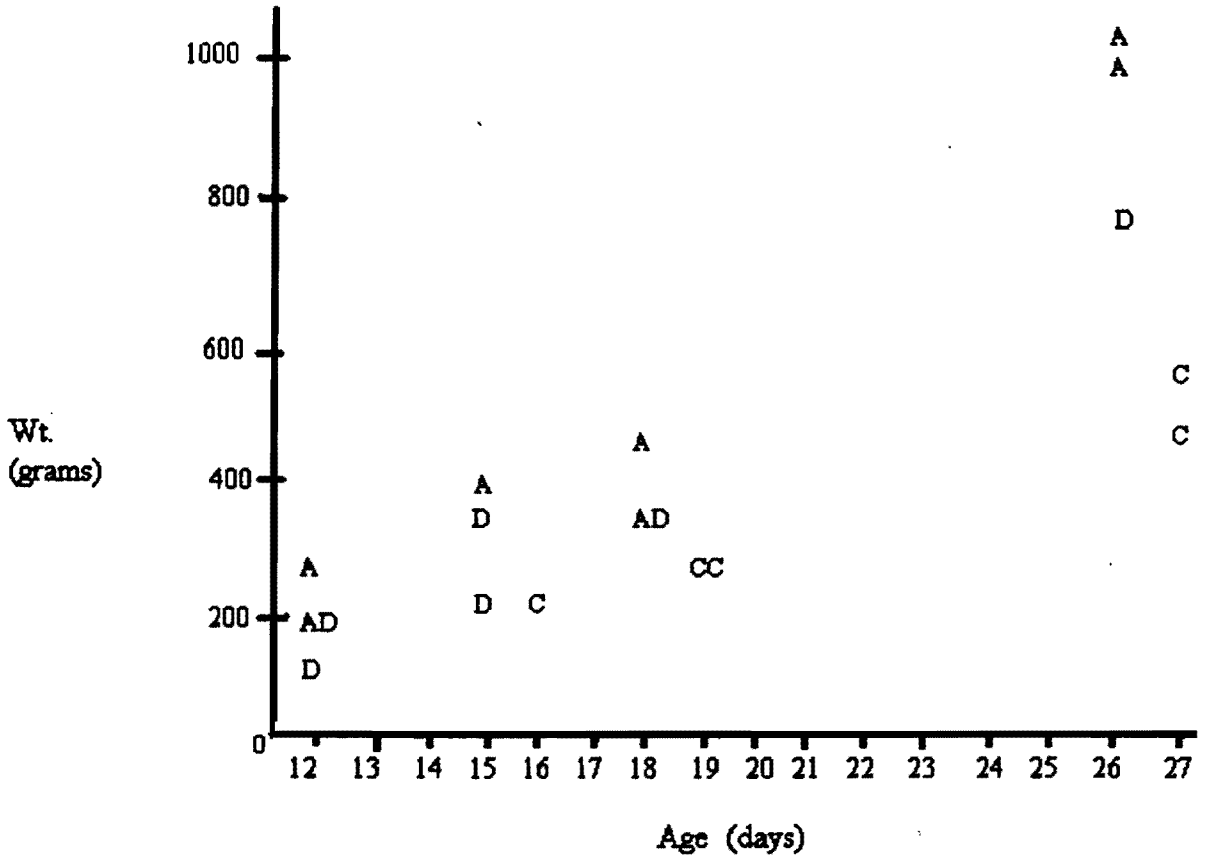
TABLE 3: The Effect of the Selenium Status on the Blood Differentials of Chicks

		Selenium Status		
Criterion	Units	Adequate (n =7)	Deficient (n =6)	Corrected (n =5)
◊Hemoglobin	gm/dL	8.05^{ab}	6.93^{b}	8.72^{a}
Leukocytes	x 10^9/L	25.2^{b}	53.7^{a}	29.6^{b}
Heterocytes	x 10^9/L	7.18^{b}	40.0^{a}	13.0^{b}
Lymphocytes	x 10^9L	13.9^{a}	6.20^{b}	9.36^{ab}
Monocytes	x 10^9/L	1.94^{b}	6.76^{a}	1.82^{b}
Eosinophils	x 10^9/L	$.643^{b}$	$.600^{b}$	4.62^{a}
Basophils	x 10^9/L	1.56^{a}	$.117^{b}$	$.880^{ab}$
Heterophils	% of leukocytes	27.4^{c}	74.5^{a}	45.4^{b}
Lymphocytes	% of leukocytes	55.3^{a}	11.0^{c}	32.0^{b}
Eosinophils	% of leukocytes	2.71^{b}	1.00^{b}	13.4^{a}
Basophils	% of leukocytes	7.00^{a}	$.333^{b}$	3.20^{b}

a, b, c, Groups with different superscripts denote significant statistical difference ($p < .05$).

◊ Denotes statistical significance $p < .06$.

FIGURE 1: Age at Which Chicks Were Sampled and Weight at that Time.



Discussion

The purpose of this experiment was to examine how selenium status affects immunological responses in chickens. The birds were fed a selenium-deficient diet to develop desired deficiency symptoms. These symptoms include, but are not limited to, exudative diathesis (as evidenced by color of tissue), myocyte necrosis, muscular fibrosis, and loss of muscle striations.

The results of blood chemistry sampling (Table 2) indicate that there is leakage of CPK out of the cells in the selenium-deficient birds. This leakage of CPK into the blood plasma may be the result of cell membrane damage by peroxides. Selenium, as an antioxidant, may have prevented this cellular damage by reducing free oxygen radicals. Glutathione peroxidase activity was found to be the lowest in selenium-deficient birds. This enzyme converts peroxides to water and alcohol. Cellular functions may be impaired in the deficient chicks as hydrogen peroxide accumulates.

The low levels of CO_2 and glucose in the deficient chicks may attributed to the mechanisms of glycolysis. Glucose is necessary in the initiation of glycolysis. CO_2 and adenosine triphosphate (ATP), which is a source of energy for cells, are end products of glycolysis. When blood glucose is decreased, the amounts of the products derived from glycolysis will decrease also. The decrease in ATP in deficient chicks results in a decease in body growth (see Figure 1), as available energy is used for body maintenance. The corrected chicks had the highest blood glucose levels, however, body growth was low. Available energy in selenium-corrected birds is utilized for repairing cells damaged by the implications of selenium deficiency. Because the selenium-adequate chicks did not have severe cellular damage to contend with, excess energy was available for body growth, as evidenced in Figure 1.

The blood chemistry results revealed that the selenium-deficient birds had significantly

higher monocyte, leukocyte, and heterophil counts. Basophils, which are originate in the bone marrow, were lowest in the deficient chicks. Basophils play a role in inflammatory response by preventing blood clotting. When basophils degrade, histamine releases--causing eosinophils to increase in number. Histamine, a vasodilator, increases the permeability of capillaries allowing plasma protein and fluid to leak into the tissue. The increased vascular permeability allows plasma and plasma proteins to traverse the endothelial lining. This leaking of fluid causes swelling associated with inflammatory response. The increased permeability may affect the characteristic edema of exudative diathesis.

Leukocytes, which are macrophages, were found in the highest concentrations in the deficient chicks. These antibodies phagocytize and destroy foreign proteins and cellular debris, such as blood clots. A decrease in basophils, which results in an increase in this cellular debris, may contribute to the increased need for leukocyte activity. Additionally, plasma proteins leaking from cells are not recognized by leukocytes and are labeled as foreign proteins. The macrophages digest these proteins and enhance inflammatory response.

Hemoglobin, which is required for oxygen transport in the blood, is lowest in selenium-deficient birds and highest in selenium-corrected birds. Hemoglobin stimulates increased production of erythrocytes in the bone marrow. The higher level recorded in the corrected group may be the result of a rebound effect. Red blood cells, maturing at the same time, reach a peak level before plateauing.

When necrosis is present, damaged cells are in need of repair. Heterophils are the first cells to remove the dead, damaged tissue. Secondly, monocytes respond for the same purpose. The M:E ratio of 0.45 for deficient birds indicates that there are more white blood cells present than normal in this group. Erythrocytes increase because of the decrease in hemoglobin.

Adequate selenium supplementation in poultry is essential for the prevention of the previously mentioned conditions. Selenium is an integral part of glutathione peroxidase, which destroys peroxides in the plasma and cytosol of cells and organelles. Glutathione peroxidase decreases the amounts of peroxides available to react with the unsaturated fatty acids of biomembranes. When the integrity of the biomembranes maintained, leakage of proteins form the cell may be stopped, thereby decreasing the incidence of disease and decreased immunological function.

Conclusions

1. Providing chicks with an adequate amount of dietary selenium may prevent exudative diathesis, fibrosis, and other forms of muscular degeneration.

2. Destruction of cells initiates an inflammatory response. Leukocytes increase to remove foreign protein from the blood and/or remove debris of dying cells.

3. A decrease in myeloid cells in bone marrow suggests slowed cell division or an increased rate of release. An inreased number of erythroid cells suggests a slower release or an increaased production due to loss.

4. Selenium supplementation to deficient chicks rapidly initiates repair of muscle tissue. Changes in cell proliferation in bone marrow also occur quickly.

References

1. M. L. Scott, M. C. Neisheim, and R. J. Young, Nutrition of the Chicken. M. L. Scott & Associates, pp.344-348 (1976).

2. G. F. Combs, The role of selenium in nutrition and health; implications in poultry nutrition. Poult. Processing Proc. 9:21 (1994).

3. E. J. Underwood, Trace Elements in Human and Animal Nutrition. Academic Press, Inc.; New York. pp. 307 (1977).

4. A. C. Guyton, The Textbook of Medical Physiology. W. B. Saunders Company, Ch. 79, pp. 943-944 (1986).

5. M. E. Putnam, Vitamin E for pigs, Veterinary Society Proceedings. 9:178 (1982).

6. J. A. Marsh, R. R. Dietert, and G. F. Combs, Influence of dietary selenium and vitamin E on the humoral immune response of the chick. Proc. Soc. Exp. Biol. Med. 166:128 (1981).

7. T. S. Koh, and T. H. Benson, Critical Re-appraisal of Fluorometric Method for Determination of Selenium in Biological Materials. J. Assoc. Off. Anal. Chem., Vol. 66 (1983).

8. R. P. Burk, and R. A. Lawrence, Glutathione Peroxidase Activity in Selenium Deficient Rat liver. Biochem. Biophys. Res. Commun., Vol 71 (1976).