Cairo University
Faculty of Veterinary Medicine
Dept. of Nutrition and Clinical Nutrition

Influence of Dietary Selenium Nanoparticles on Performance, Antioxidant Status and Gene Expression of Broiler Chickens in Comparison to Other Selenium Sources

Thesis Submitted by

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Supervision Sheet

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بسم الله الرحمن الرحيم

" قالوا سبحانك لا علم لنا الا ما علمتنا إنك أنت العليم الحكيم"

صدق الله العظيم

البقره (32)

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Abstract

A 38 days comparison study was carried out to investigate the impact of nano-selenium (the average particle size of NS was about 48.4 nm), that was physically prepared and characterized in the lab, to different commercial selenium sources (inorganic and organic) on the productive performance, immune status, seleno-proteins gene expression, mitochondrial activity, total antioxidant capacity, carcass traits, and selenium (Se) concentration in some tissues of broiler chickens. A total of 300 one-day old unsexed Hubbard broiler chicks was randomly assigned into five equal groups each with 3 replicates. The experimental groups were as the following: the first group (G1) served as the control group supplemented with sodium selenite (commercial inorganic Se source, SS), the second (G2) and the third groups (G3) were supplemented with commercially available organic selenium sources (Se- enriched yeast, SEY & seleno-methionine, SM, respectively), whereas, the fourth (G4) and the fifth groups (G5) were supplemented with the prepared NS. All the groups were supplemented with dietary Se at the level of 0.3 mg of Se/kg of diet except for the G5 that was supplemented with the half dose of NS (0.15 mg of Se/kg of diet). The results revealed significant ($P\leq 0.05$) positive effects of NS at the level of 0.3 mg Se/kg of diet (G4) on most of the productive performance parameters. Meanwhile, the results of antibody titers against Newcastle disease and Infectious Bronchitis viruses indicated that none of the dietary Se sources was able to boost significantly the humeral immune response. The gene expression of Sel-P and Sel-W, the mitochondrial oxygen consumption values, and the total antioxidant capacity indicators achieved the highest significant increase ($P\leq 0.05$) in NS full dose (0.3 mg/kg) supplemented group (G4) compared to all other groups. Moreover, the dressing and the breast muscle yields as well as the Se tissue concentration in pectoral muscles and liver tissues recorded significant increase ($P\leq 0.05$) in the birds supplemented with NS at full dose supplemented group (G4) in comparison to the rest of experimental treatments indicating better bioavailability for such source. It can be concluded that the use of Se in the nano form exhibits a better effects not only on productive performance, Se-dependant gene expression, tissue selenium concentration, and carcass traits but also, it helps to alleviate oxidative stress and to optimize mitochondrial oxygen consumption in broilers chickens compared to other traditional commercial Se sources.

Key words: Broiler, inorganic selenium, organic selenium, nano selenium, performance, gene expression, mitochondrial activity, enriched meat.

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Contents

List of tables

List of figures

Introduction

There is fast and rapid development in poultry production in Egypt to satisfy the increased population needs for meat and eggs. Proper nutrition is considered the foundation upon which the successful poultry production is built, as the cost of broiler diet represent about 65-70% of the total production costs at the farm level.

Since the target of broiler production is to achieve the maximum weight gain within a minimum period with the best cost. So research activities were focused to maximize feed efficiency which is considered a critical point in nutritional management practices of broiler.

It is a well-established fact that the tables of feeding standards as well as the breed manuals state the nutrient requirements of the birds at different stages of production only under normal condition and does not pay attention to the extra nutrient required in disease and/ or stress conditions. Recently, the use of surplus levels of certain vitamins, minerals, amino acids and/or their different combinations to chicken in excess of their supposed requirements enhances the growth performance as well as their disease resistance.

Minerals are naturally occurring inorganic nutrients that play a very important role as a structural constituent of different tissues and mediators and/ or regulatory constituents helping in regulation of acid-base balance, fluid distribution as well as maintaining normal neuro-muscular irritability (**McDonald *et al.*, 1988).**

Trace minerals can significantly impact animal performance and immunity. Furthermore, dietary trace elements, particularly Selenium (Se), has been recognized to play an important role in the regulation of various metabolic processes in the body, and is an integral part of at least 25 selenoproteins (**Pappas *et al.* 2008; Zhou *et al.* 2009).** However, the physiological role of Se in organisms is chiefly concentrated on the activity of glutathione peroxidases. It has been suggested that Se might enhance immunity, growth, reproductive performance, and the ability to resist disease **(Habibian *et al.*, 2015)**.

In chickens, it was reported that Se deficiency is linked to a number of diseases, which include exudative diathesis, pancreatic dystrophy, nutritional muscular dystrophy and suppression of immunity **(Arthur *et al.*, 1993; Habibian *et al.*, 2015). NRC (1994)** stated that the Se requirement for broilers throughout the growth period is 0.15 ppm, and **AAFCO (2003)** stated that the maximum allowable level of Se supplementation is 0.30 ppm**.**

The natural Se content of grain used in poultry feedstuffs is only 0.02 to 0.12 mg/kg, with values more commonly at the lower end of this range **(Zhou and Wang, 2011).** The intake of such feeds may result in a serious Se deficiency, with subsequently impaired poultry efficiency, health problems, or both. Thus, a selenium source must be added to poultry diet.

So, it is common practice to supplement broiler diets with Se. The traditional Se supplement that has been primarily used in animal and /or poultry diets, is the inorganic form sodium selenite,(SS), which has a very narrow margin between its nutritional dose and its toxic dose (**Wolffram *et al.*, 1986)** as well as it may act as a pro-oxidant in some conditions (**Spallholz, 1997).** However, recently the organic forms of Se are commercially available in Egyptian market, including seleno-methionine (SM), or Se-enriched yeast (SEY), as supplemental sources of Se. Usually, the organic forms of Se have higher bioavailability and antioxidant properties than inorganic forms (**Mahmoud and Edens, 2003)**. More recently, **Zhang *et al.* (2005)** synthesized nano-sized elemental Se (NS) of size 5 to 100 nm and observed that (NS) had a similar bioavailability in rat and much less acute toxicity in mice compared with (SS). **Wang *et al.* (2007)** Showed that NS (20 to 60 nm) possesses equal efficacy in increasing the activities of glutathione peroxidase in plasma and liver of male Kunming mice compared with seleno-methionine

(SM). Moreover, the toxicity of nano-selenium is 7 times lower than that of inorganic selenium and 3 times lower than that of organic selenium **(Peng *et al.*, 2007).**

On the basis of the above-mentioned data**, our research hypothesis was:**

The health status and the overall performance parameters of broiler chickens may be improved by using more safe sources of selenium having higher bioavailability and less toxicity than the traditional inorganic or organic sources. Moreover, the possibility to use such source to produce Se enriched meat a situation that of public health importance as it is considered as an effective tool to probably eliminate Se deficiency in human.

Overall goal:

To determine the most safe and economic selenium source that achieves the best broiler health (immune and antioxidant status) and productivity simultaneously with profitability.

Specific objectives of the experimental trial:

To investigate the impact of supplementing the broiler diet with different sources of selenium stated as follow: sodium selenite as an inorganic source; selenomethionine and selenium enriched yeast as two organic sources; and selenium nano-particles on:

1. Productive performance parameters:

a) Body weight development (g).

b) Weight gain (g)

c) Feed intake (g).

d) Feed conversion ratio (FCR).

e) Mortality rate (%).

2. Immune status (antibody titers against Newcastle Disease Virus and Infectious Bronchitis Virus).

3. Gene expression of seleno-protein P (Sel-P) in liver, and seleno-protein W (Sel-W) in breast muscle.

4. Mitochondrial activity indicators.

5. Oxidation stress bio-markers.

6. Carcass traits:

a) Dressing Wt. (g) and yield %.

b) Breast Wt. (g) and yield (%).

c) Maryland Wt. (g) and yield (%).

d) Liver Wt. (g) and index (%).

e) Heart Wt. (g) and index (%).

f) Spleen Wt. (g) and index (%).

7. Tissue selenium content (liver and pectoral muscle).

Review of literature

2.1. Historical background about Selenium

Selenium was discovered in (1817) by ***J.J. Berzelius*** and primary interest in the element was shown by various branches of industry (glassmaking, pottery, rubber, steel, and electronic industries). Since its discovery, Se has had an interesting history.

In the 1930's, several researchers identified Se toxicity to be a direct cause of alkali disease and blind staggers **(Franke, 1934a, b; Franke and Potter, 1935; Moxon, 1937).**

Around 1930 studies of selenosis evoked interest in biological activity of Se, culminating in 1950s by studies of the origin of liver necrosis in rats.

Nelson *et al.* (1943) classified Se as a carcinogen. While, **Schwarz and Foltz (1957)** reported that Se was an essential factor protecting rats against liver necrosis and degeneration.

Several reports showed that Se has a preventive action against exudative haemorrhagic diathesis and muscle dystrophy **(Swchwarz and Foltz 1957; Muth *et al.* 1958; Patterson and Zech, 1992).**

Muth *et al.* (1958) studied the relationships between vitamin E and Se in ewes and lambs.

Scott *et al.* (1967) observed that Se could prevent gizzard and cardiac myopathies in young turkey poults. The FDA in 1967 permitted the use of Se as a feed supplement for pigs and poultry at level of 0.1 ppm and 0.2 ppm for turkeys.

Despite the establishment of a dietary need for Se, it still is considered to be the most toxic dietary essential trace mineral. Therefore, the FDA regulates supplementation of Se into poultry diets **(FDA, 2000; AAFCO, 2003)**. The dietary requirement of poultry for Se often can be met by natural feedstuffs in the diet, but these feedstuffs vary widely in Se concentration depending on the region that they are grown. Therefore, nowadays it is common practice in poultry production to supplement diets with Se.

2.2. Selenium functions in broiler chickens

Selenium is an essential trace element that fulfills a number of significant biological functions in human and poultry organisms by means of specific selenium enzymes, including antioxidant protection of the organism against free radicals, maintenance and strengthening of natural immunity of the organism, support for correct function of the thyroid gland and reproductive organs **(Arthur, 2000).**

The most important and known action of Se is its antioxidant effect because it forms selenocysteine, part of the active center of glutathione peroxidase **(Avanzo *et al.*, 2002)**.

Glutathione peroxidase enzyme has antioxidant activity and contributes to the oxidant defense by catalyzing the reduction of hydrogen peroxide and lipid peroxides to less harmful hydroxides **(Arthur, 2000)**.

The activity level of this enzyme in the liver or plasma is indicative of the Se supply to the organism. In addition, dietary selenium is essential for the activity of virtually all arms of the immune system **(Spallholz, 1990).**

Previous studies have shown that in chickens, growth performance, survival, meat quality, and antioxidant protection level are affected by dietary Se status **(Avanzo *et al.* 2002; Pappas *et al.* 2005).**