COMPARISON OF ORGANIC AND INORGANIC SELENIUM ON PERFORMANCE, SERUM ATTRIBUTES AND CELLULAR IMMUNITY IN BROILER CHICKEN

MD. TORIKUL ISLAM

DEPARTMENT OF POULTRY SCIENCE

SHER-E-BANGLA AGRICULTURAL UNIVERSITY

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BY

MD. TORIKUL ISLAM

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APPROVED BY

Prof. Dr. Md. Anwarul Haque Beg
Supervisor
Department of Poultry Science
Sher-e-Bangla Agricultural University

Hossan Md. Salim, PhD
Co-Supervisor
Assistant Director
Livestock Economic Section,
Department of Livestock Services

Prof. Dr. Md. Anwarul Haque Beg
Chairman
Department of Poultry Science
Sher-e-Bangla Agricultural University

Dr. Md. Anwarul Haque Beg
Professor
Department of Poultry Science
Sher-e-Bangla Agricultural University
Sher-e-Bangla Nagar, Dhaka-1207, Bangladesh
Mobile No: +8801718026200
E-mail: mahbegsau@yahoo.com

CERTIFICATE

*This is to certify that the thesis entitled "**COMPARISON OF ORGANIC AND INORGANIC SELENIUM ON PERFORMANCE, SERUM ATTRIBUTES AND CELLULAR IMMUNITY IN BROILER CHICKEN**" submitted to the Faculty of Animal Science & Veterinary Medicine, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of Master of Science in Poultry Science, embodies the result of a piece of bona fide research work carried out by **MD. TORIKUL ISLAM**, Registration No. **13-05734** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

I further certify that any help or source of information, received during the course of this investigation has been duly acknowledged.

Dated: June, 2020
Place: Dhaka, Bangladesh

Prof. Dr. Md. Anwarul Haque Beg
Supervisor
Department of Poultry Science
Sher-e-Bangla Agricultural University

DEDICATED TO

MY BELOVED PARENTS,

TEACHERS AND FRIENDS

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The Author

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ACRONYMS AND ABBREVIATIONS

Abbreviation	Full meaning
A.M	Ante meridiem
ANOVA	Analysis of Variance
BANSDOC	Bangladesh National Scientific and Technical Documentation Center.
BARC	Bangladesh agricultural research council
BBS	Bangladesh bureau of statistics
BLRI	Bangladesh livestock research institute
CBC	Complete blood count
CF	Crude fiber
Cm	Centimeter
cm^2	Square centimeter
CONTD.	Continued
CP	Crude protein
CRD	Complete randomized design
e.g.	For example
EDTA	Ethylene diamine tetraacetic acid
et al.	And others/Associates
FC	Feed consumption
FCR	Feed conversion ratio
GRA	Granulocyte
GSH-Px	Glutathione peroxidase

Abbreviation	**Full meaning**
Hb	Hemoglobin
I	Organic
i.e.	That is
IBD	Infectious bursal disease
In	Inorganic
kcal	Kilo –calorie
Kg	Kilogram
M.S.	Master of science
ml	Milliliter
mm	Millimeter
mmol	Millimoles
MT	Metric-ton
NS	Non-significant
P.M	Post meridiem
ppm	Parts per million
RBC	Red blood cell
SAU	Sher-e-Bangla Agricultural University
Se	Selenium
SE	Standard error
SPSS	Statistical package for social sciences
viz.	Such as
Vs	Versus
WBC	White blood cell
WHO	World health organization

LIST OF SYMBOLS

Symbols	Full meaning
@	At the rate of
+	Plus
<	Less than
>	Greater than
°C	Degree Celsius
%	Percentage
&	And
*	5% level of significance
/	Per

COMPARISON OF ORGANIC AND INORGANIC SELENIUM ON PERFORMANCE, SERUM ATTRIBUTES AND CELLULAR IMMUNITY IN BROILER CHICKEN

ABSTRACT

A feeding trial was conducted on 200 day-old Lohman meat broiler chicks for a period of 35 days in the poultry farm of Sher-e-Bangla Agricultural University, Dhaka. The aim of the study was to assess the efficiency of dietary organic Se, inorganic Se and their combined dose on the production index, blood parameters and immunity of commercial broiler chicken. The chicks were assigned to 4 treatment groups comprising of T_1 (control), T_2 (1.5ml/l organic Se), T_3 (0.75ml/l inorganic Se) and T_4 (combined Se) randomly. Treatments were replicated five times with 10 chicks per replication. The results showed that dietary supplementation of organic Se, inorganic Se and their Combined dose had significant ($P<0.05$) difference on feed consumption, body weight gain, final live weight, dressing percentage of broiler compared to control group. Higher feed consumption found in T_4 (1126.40±18.99g) group compared to other groups. However, superior final live weight (2257.66±27.95g) obtained in T_4 group where birds fed with combined dose of Se compared to those other treatment and control group. Improved FCR value (1.46±.00) found in T_3 group which is statistically significant ($P<0.05$) with the values of other groups. Dressing percentage is also higher in T_3 (75.10±0.90) group where birds fed with 0.75ml/l inorganic Se. Dietary supplementation of organic Se, inorganic Se and their combined dose had no significant ($P>0.05$) effect on the abdominal fat weight in different treatment groups. Birds supplemented with combined dose of Se (T_4) showed insignificantly ($P>0.05$) higher abdominal fat weight (31.90±1.603g).The glucose concentration had no significant ($P>0.05$) difference among all treatment groups but cholesterol concentration had significant ($P>0.05$) difference among all treatment groups and comparatively lower cholesterol level 143.60±15.79mg/dl was found in 0.75ml/l inorganic Se (T_3) supplemented group. In addition, the hematological parameters including white blood cell (WBC), lymphocyte and granulocyte which were significantly ($P<0.05$) lower in organic se, inorganic se and their combined dose groups compared to control group. Diets supplemented with organic se, inorganic se and their combined dose had no significant ($P>0.05$) effect on blood parameters compared to control group. Birds fed with 0.75ml/l inorganic se (T_3) supplemented diet achieved superior result.

CHAPTER 1

INTRODUCTION

The poultry industry has occupied a prominent position in the list of exports of products marketed in Bangladesh, producing broilers with high technology and very competitive costs. It has become a specialized and dynamic business sector at present time in all over the world. It is an integral part of farming system in Bangladesh and has created direct, indirect employment opportunity for the people. This sub-sector has proved as an attractive economic activity, thereby, indicating its importance for the entire economy. In Bangladesh malnutrition and unemployment problem are major of all problems. Poultry provides nutritious meats and eggs for human consumption within the shortest possible time which can efficiently and rapidly fulfill the shortage of protein requirements since it can be produced at shortest possible time as compared to meat of other meat producing animals. Commercial poultry could also serve as a tool for employment generation and poverty alleviation. Recently, broiler industry has become take the leading position among the other sector of poultry production.

The animal nutrition industry is searching for more suitable diet formulations and dietary supplements to provide better animal nutrition at lower production costs. Most nutrients required for normal metabolic functions are either not endogenously synthesized or their synthesis is insufficient, and therefore must be continuously supplied in the diets. Standard feed supplements have been used over the years, but they do not properly supply the requirements of many nutrients when immune competence and not only animal performance is considered (Karadas and Surai, 2004). Considering essential nutrients, trace minerals (particularly Se) are critical to maintain poultry health and performance. Selenium is essential for animals due to its roles as metabolism regulator, adequate body development and reproductive performance, and aiding the immune system to neutralize free radicals and protect the body against infections. Despite being essential for animal metabolism, Se levels in almost all feedstuffs are not sufficient to supply animal requirements. Se is usually supplemented in broiler diets in its inorganic form (sodium selenite).

Selenium concentration in the soil of Bangladesh is low (Oldfield, 2002). For this reason, plant cannot absorb the proper amount of Se from soil. As a result, natural feedstuffs such as cereal grains like maize, rice, wheat and soybean contain lower levels of Se .As a consequence, farmer supply the commercial organic Se (e.g., OSE-Vet) with natural feedstuffs to fulfill the bird's requirement. Approximately, 100% of the organic Se is imported from abroad that increase the feed cost and ultimately the production cost. Until today, no data on optimum level of se supplementation in the breeder ration at Bangladesh condition is available. As a result, farmers are using the OSE-Vet or other organic form of Se in poultry diets, particularly in breeder rations, depending upon their imagination only. Sometimes the trader's exaggerated advertisement in favor of organic Se misleads the farmers to use the organic Se at over doses that results poor performance of the birds and cause an unexpected loss in the business. However, the identification of appropriate level of organic Se supplementation in breeder diets that is mainly manufactured from locally available ingredients is still lacking.

The use of antioxidant vitamins and minerals such as Se and vitamin E alone or combined, in broiler diets, can minimize the effects of stress due to the effect of free-radical sequestration and consequently improve the growth performance of birds (Habibian *et al*., 2013).

In poultry production Se is added to food mainly for the purpose of prevention of certain diseases by its positive effect on immunological system and increase if production characteristics, primarily body mass and more efficient utilization of food (Jokić *et al*., 2005). Selenium has been defined as an essential element for growth (Wang and Xu, 2008), immune competence (Liao *et al*., 2012), antioxidant (Zhou and Wang, 2011) and reproductive functions, immune competence, and ageing (Leeson *et al*., 2008) of broilers. Selenium as an essential trace mineral is crucial in human health (Rayman, 2004), and improving performance and health of the birds (Yoon *et al*., 2007).

Commercial organic and inorganic forms of Se are available in the market. Organic forms (OSE Vet-Sk^{+}f) and inorganic forms (Sel-E Vet-ACI). The main used Se source in poultry diets is sodium selenite. However, research has shown other sources of Se, have been examined as alternatives to inorganic Se supplementation (Baylan *et al*., 2010). Se supplementation elevated the Se concentration in body tissues and breast muscles (Dong *et al*., 2011).

In another study, it was demonstrated that Se supplementation in broiler diets significantly improved weight gain, final body weight and meat quality without increase of feeding cost (Ibrahim *et al*., 2011). A number of research workers reported an optimistic correlation between organic Se and body weight in broilers (Upton *et al*., 2008). Payne and Southern (2005) in a study noticed that chick production was improved in organic Se treated birds. Yoon *et al*., 2007 conducted a research and reported that organic Se supplementation showed better growth than that of inorganic Se.

Organic Se has shown an enhancement in the tissue Se concentration, while has no other effects on plasma activity, carcass characteristics and growth performance compared to inorganic Se (Yoon *et al*.,2007). Use of Se yeast as an organic Se in poultry diets was authorized by Food and Drug Administration .Organic Se which is more efficiently absorbed and retained in tissues compared to inorganic Se salts such as sodium selenite (Yoon *et al*., 2007).

On the basis of this background, the experiment was planned to explore the possibilities of using organic Se, inorganic Se and their combined effect with the following objectives:

- To determine the effect of Se on growth performance of broiler Chicken.
- To determine the effect of Se on blood parameters of broiler Chicken.
- To determine the effect of Se on cellular immunity of broiler Chicken.

CHAPTER 2

REVIEW OF LITERATURE

Sources of literature

1. Book and journal in different libraries as mentioned below:
   - ✓ Sher-e-Bangla Agricultural University (SAU) Library, Dhaka
   - ✓ Bangladesh Agricultural Research Council (BARC) Library, Farmgate, Dhaka
   - ✓ Bangladesh National Scientific and Technical Documentation Centre (BANSDOC) Library, Agargaon, Dhaka.
   - ✓ Bangladesh Livestock Research Institute (BLRI) Library, Savar, Dhaka.

2. Internet browsing.

A total of about 68 literatures were reviewed to assimilate the background, drawbacks and prospects of research, to understand previous findings and to answer the research status of this field.

Among them 26 were full article and 31 abstracts, 11 were only titles and some were miscellaneous. A brief account is given below depending on 8 main headlines viz, Se in poultry production, Organic Se in immune response, Organic and Inorganic Se in poultry, Se effect on body weight and temperature, effect of different sources and levels of Se, se effect on meat quality, Se effect on serum attributes. The traditional system or sequences in stating the references is avoided. Crucial inspection was made of each article and significant information was collected and compiled in a systematic arrangement according to specific title. For further higher research review attempts, it is look forward to be a pathfinder effort in Bangladesh.

The poultry sub-sector is the most commercialized agricultural sub-sector all over the world. Poultry are raised for their meat and eggs, and are an important source of edible animal protein. Poultry meat accounts for 30% of global meat consumption (FAO Statistics Division, 2009). The poultry sector was the most dynamic meat sector during the last decade, showing the greatest growth of all meat sectors as reflected in world consumption. The dynamism of the global poultry sector has been supported by a strong growth in demand. To fulfill this demand appropriate nutritional supplement like Se should be used in poultry feeds to maximize the rate of production.

2.1 History of Se

The Swedish chemist, Jons Jakob Berzilius discovered Se in 1817 in the flue dust of iron pyrite burns. In the 1930's, several researchers identified Se toxicity to be a direct cause of alkali disease and blind staggers and then categorized Se as carcinogens. Selenium was essential for the proper function of the glutathione peroxidase enzyme, further establishing Se as nutritionally essential. Selenium can be found in all cells and tissues of the body but its highest concentration is in kidneys, followed by testes, liver, adrenals, erythrocytes, plasma, spleen, pancreas, lungs, heart, thymus, gastrointestinal tract, skeleton, brain and muscles. Selenium exists in inorganic and organic form and usually it was supplemented in poultry feeds via inorganic sources, such sodium selenate . However, the organic source of Se was also approved as a feed supplement in poultry rations. Organic sources of Se are in the form of organic Se compounds, such as SY, Se-enriched alga, and SM (Payne *et al*., 2005).

2.2 Distribution

Selenium can be found in all cells and tissues of the body, but the concentration of Se will depend on the chemical form and amount of Se in the diet. The highest concentration of Se is in the kidneys, followed by the testes, liver, adrenals, erythrocytes, plasma, spleen, pancreas, lungs, heart, thymus, gastrointestinal tract, skeleton, brain, and muscle. Se levels of the blood, muscle, liver, kidneys, and skin increased linearly in chicks fed up to 0.30 ppm Se from an inorganic Se source. It is reported that increasing Se to 0.80 ppm only resulted in higher levels of Se in the liver and kidneys with no significant increase in blood or muscle Se concentration.

2.3 Selenium sources and their efficiency

Sources of Se can be divided into several groups according to their efficiency:

Elementary Se. Elementary Se is stable and exists in modifications. It is virtually biologically inactive, especially for its poor resorption.

Inorganic Se compounds. Inorganic Se (sodium selenite) is not too biologically active. It accelerates oxidization processes in organism and may cause health problems. Most inorganic Se is excreted from the body. Higher doses are toxic.

Organic Se compounds.

Organic Se compounds perform a key role in biological processes. They are more active than inorganic salts. They are part of proteins and include Se-Met and selenocysteine (Se- Cys). Se- Met exists in two isomer forms, d and l, and was identified in plant proteins. Only the l-form occurs naturally, d-form may only be prepared synthetically. Se-Cys is the only Se compound forming part of effective Se enzymes. It is mainly found in food of animal origin and in plants able to accumulate high levels of Se (Hartikainen 2005). Se -Met is quickly absorbed with the consequence of higher blood levels in comparison to inorganic Se. Bioavailability of Se depends on the chemical compound it is part of. Organically bound Se is mostly used in the form of Se-enriched yeast or other preparations. Se -enriched yeast contains Se in the form of Se-Met. This form is also contained in most plants and cereals.

2.4 Symptoms of Se deficiency

The disease has observed in regions with soil low in Se. In human symptoms of the disease include joint swelling, pain, general malaise, short status (due to the effect of the disease on the growth plate of tubular bone), and secondary osteoarthritis.The sign of Se deficiency have also been reported in quail and chicken. It includes decrease of body weight, poor feathering, impaired reproduction, reduced hatchability, and reduced viability. In ducklings, Se deficiency reduced plasma glutathione peroxidase activity and body weight gain, increased mortality.

2.5 Metabolism of Se

The metabolism of Se is dynamic. Animals synthesize many different intermediary metabolites in the cause of converting inorganic Se to organic forms, which can be enzymatically catalyzed. Hydrogen selenite is a key metabolite, formed from inorganic sodium selenite (oxidation state +4) via Selenodiglutathione through reduction by thiols and NADPH-dependent reductases. Methylation is a major pathway for Se metabolism in microbes, plants, and animals. The hydrogen selenite is generally regarded both as substrate for biosynthesis of selenocysteine by cysteine synthases and as molecule for the transformation into selenophosphate by selenophosphatesynthetase, and both are required for synthesis of selenoproteins (Birringer *et al.,* 2002).

2.6 Selenium in Broiler production

In poultry production Se is added to food mainly for the purpose of prevention of certain diseases by its positive effect on immunological system and increase if production characteristics, primarily body mass and more efficient utilization of food (Jokić *et al*., 2005).Se has been defined as an essential element for growth (Wang and Xu, 2008), immune competence (Liao *et al*., 2012), antioxidant (Zhou and Wang, 2011) and reproductive functions, immune competence, and ageing (Leeson *et al.*, 2008) of broilers. Se as an essential trace mineral is crucial in improving performance and health of the birds (Yoon *et al.*, 2007).

2.6.1 Effect of Selenium on growth performance

While comparing the influence of Se sources, the attempts have been made to study the impact of Se in poultry birds and concluded that the Se supplementation positively affected the length of the body as well as the width of the chest (Zia *et al*., 2016b). The Selenium status of progeny chicks was improved at hatching by supplementing breeder hen diet with Se-yeast (Macalintal *et al*., 2011). Similarly, Sel-Plex (organic Se) in turkey showed significantly higher body weight and length compared to the birds in the control group (Zia *et al*., 2016b). Se supplementation elevated the Se concentration in body tissues and breast muscles (Dong *et al*., 2011). In another study, it was demonstrated that Se supplementation in broiler diets significantly improved weight gain, final body weight and meat quality without increase of feeding cost (Ibrahim *et al*., 2011).

The results of a study showed that organic Se treated birds presented better growth performance than the birds treated with SS (Anthony, 2012). Organic Se exhibited the most striking response in breast muscles and had significantly higher Se levels in heart, lungs and gizzard tissue (Leng *et al*., 2013). A number of research workers reported an optimistic correlation between organic Se and body weight in broilers (Upton *et al*., 2008). Payne and Southern (2005) in a study noticed that chick production was improved in organic Se treated birds. Yoon *et al*., (2007) conducted a research and reported that organic Se supplementation showed better growth than that of inorganic Se.

2.6.2 Effect of Selenium on meat quality

Antioxidant effects of Se are manifested in meat quality by reduced oxidization of lipids (De Almeida *et al*., 2012), as well as by better color stability of hem pigments (Yang *et al*., 2012).Se also positively affects reduction of weight loss of meat, expressed by loss of water by dripping (De Medeiros *et al*. 2012) and improvement of certain organoleptic properties of broiler chicken meat. Selenium rich meat is more juicy, crispy, and better looking. For animal fodder enrichment, Se is used in combination with other antioxidants, such as tocopherol (vitamin E).Positive effects of Se on quality and stability of broiler chicken meat have been confirmed by a number of authors (Yang *et al*., 2012). Further studies focus on the effects of Se on egg quality (Skrivan *et al*., 2013).

2.6.3 Effect of Selenium on quality and stability of Chicken meat.

Values of Se levels in meat and other animal products show seasonal fluctuations and significant changes related to ration composition. Selenium shows a clearly positive effect on the quality or stability of poultry meat. Oxidative stability in broilers under heat stress is improved by supplemental vitamin E and Se (Harsini *et al.*, 2012).Compared with the control (Na_2SeO_3), organic Se (Se-enriched yeast) increased meat red color degree of chest and thigh muscles by 13.98 and 20.83%, respectively; the drip losses of chest and thigh muscles were decreased by 13.57 and 24.92%, respectively (Yang *et al.*, 2012). Selenium in the feed improved meat quality by reducing the lipid oxidation and cooking loss (De Almeida *et al.*, 2012).

2.6.4 Effect of Selenium on antioxidant stability of chicken meat

The antioxidant effect of Se on the stability of broiler chicken meat has been documented by a number of authors (Rama Rao *et al*., 2013).The inclusion of Se-Chlorella in the diet enhanced the oxidative stability of meat in broilers expressed as reduced malondialdehyde values in breast meat after a 0-, 3-, and 5-day refrigeration at 3–5°C (Dlouha *et al*,. 2008). Skrivan *et al*., (2008) confirmed these findings in a similar study.Comparisons of effects of various forms of Se in the diet on growth, meat quality, Se storage, and antioxidant properties in broilers were performed by Wang *et al.* (2011b). Yang *et al.* (2012) confirmed that the effects of organic Se on enhancing body oxidation resistance were superior to those of inorganic Se. The supplementation with Se produced a linear reduction on the abdominal fat of the carcasses assessed.

Regarding meat quality, the supplementation with organic Se linearly increased pH levels at the breast. Besides, it linearly reduced the loss of water by pressure and the shear force, which in turn improved the final quality of meat (De Medeiros *et al.*, 2012). Skrivan *et al*., (2012) studied oxidative stability of meat of broilers fed diets enriched with vitamin C (280 and 560 mg/kg) and Se (sodium selenite or selenized yeast, 0.3 mg/kg). Both Se sources in-creased the activity of GSH-Px and the oxidative stability of meat. Diets supplemented with vita-min C and Se increased protein concentrations in meat. Vitamin C reduced lipid oxidation in meat stored for 5 days.

2.6.5 Effect of Se in immune system

Selenium is essential for the activity of multiple components of the human and animal immune system. Se deficit damages both cellular and humoral immunity .Se stimulates the immune system, strengthening proliferation of activated T lymphocytes. Daily intake of 200 µg of Se causes increased reaction of lymphocytes to antigenic stimulation and increase of their ability to mature to cytotoxic lymphocytes destroying tumour cells. The activity of natural killers increases, too. This mechanism is closely connected with increased numbers of receptors for interleukin-2 on the surface of the activated lymphocytes and natural killers. These interactions are critical for clonal expansion and differentiation to cytotoxic T cells. Se insufficiency also affects humoral immunity resulting in reduced levels of IgG and IgM antibodies.

Effects of supplemented organic Se on immune response in broiler chickens were studied by Rama Rao *et al*., (2013). The cell-mediated immunity (lymphocyte proliferation ratio) increased linearly with dietary Se concentration. Another study (Funari *et al.*, 2012) was conducted to evaluate the effect of different levels and sources of Se on humoral immunity of broilers.

2.6.6 Effect of Se on hematological and immunological parameters

It is founded that the Se singly or combining with vitamin-E supplementation to the broiler chickens and Japanese quail diets caused a significant ($p<0.05$) rise in WBC's or RBC's counts. It is also reported that Se supplementation enhanced the immune system and increased the natural resistant of animals by increasing response of the organism to antigenic stimuli. Arshad *et al*., (2005) conducted an experiment Results indicate that Se supplementation may help to increase post vaccination humoral immune response against IBD in broiler chicks.

2.6.7 Effects of Se sources on body maintenance and survivability

Wang YanBo (2009) observed the improved survival rate in commercial broiler fed on both the sodium selenite and nano-Se supplemented diet, whereas Korosi *et al.,* (2005) had reported the lower mortality on male and female on Sel-Plex^{TM} supplemented broiler parent stock.

2.6.8 Effect of Selenium on Slaughter traits and tissue Se deposition

Sevikova *et al.,* (2006) conducted an experiment to estimate the Se effects on the broiler birds and reported that organic Se increased the tissue Se levels of broiler birds more than birds offered inorganic Se supplemented diet, the higher live weight of broiler chickens was recorded in the treated groups. Se contents in breast and thigh muscles were more in Se treated birds as compared to the birds in the control group (Zia *et al*., 2016d). Zhao and Xu (2009) at the end of his research project demonstrated that the supplementation of SM in maternal diet can increase Se deposition in muscles of the progeny and lead to more effective protection against lipid oxidation in progeny's thighs. The organic Se significantly increased the meat red coloration and drip loss was noticed comparatively low. The growth performance, meat quality and antioxidant status of meat were also found better (Jiang *et al*., 2009). Yang *et al*., (2012) reported that organic Se supplementation significantly increased the meat red color, and decreased the cooking loss. In contrast, no significant ($P>0.05$) impact of Se was found on final body weight of broilers (Yoon *et al*., 2007.

2.6.9 Se and activity of glutathione-peroxidase

Glutathione peroxidase is an enzyme transforming the toxic and carcinogenic hydrogen peroxide to harmless water and oxygen. Its activation requires small amounts of Se (selenocysteine), probably substituting sulphur in the glutathione molecule and causing development of modified enzyme GPx4. The basic function of GSH-Px is elimination of excessive peroxide and hydrogen peroxides of fatty acids resulting from oxidative elimination of lipids (De Almeina *et al*., 2012). In this it acts in synergy with vitamin E. Lipid peroxidation in plasma decreased, while activities of GPx and glutathione reductase in plasma increased linearly with Se concentration in a broiler chicken diet (Rama Rao *et al*., 2013). The Se source (Se-enriched yeast and Se-enriched alga Chlorella) level, including sodium selenite, significantly influenced the GSH-Px activity in breast and thigh meat (Heindl *et al*., 2010).

Some researchers showed that the addition of organic Se source in diet of broilers significantly elevated plasma GPx activity and hence improved antioxidant activity (Khajali *et al*., 2010. The effect of organic and inorganic Se on growth, meat quality, and antioxidant properties of broiler meat was studied by Yang *et al*., (2012). These results indicate that the effects of organic Se on enhancing body oxidation resistance were superior to those of inorganic Se.

2.6.10 Toxicity of Se

In livestock, interest in the toxic effect of Se was obtained after the discovery in the early 1930's by scientists from the U.S. Department of Agriculture and from South Dakota and Wyoming State Agricultural Experiment Stations that Se was the toxic substance in forages and grains responsible for "blind staggers" and "alkali disease" which sometimes occurred in livestock in the certain areas of the American western plains. Chronic Se toxicity in livestock occurs when animals consume seleniferous plants containing 3-20 ppm of Se over a prolonged period. When it occurs in cattle and horses, it is often called alkali disease. Symtoms include lameness, loss of vitality, hoof malformations, loss of hair in the mane and tail, atrophy cirrhosis of the liver and chronic nephritis. Se toxicity had been reported to be a cause of death and deformities of embryos and chicks in aquatic birds within Kesterson area of California.

2.6.11 Intoxication with Se

Generally speaking, inorganic compounds are more toxic than organic ones. In the order of decreasing toxicity the compounds may be sorted as follows: the most toxic selenite >selenate>selenocysteine> methylated Se compounds. Se acid is the most toxic form of Se defined three types of intoxication with Se: acute, sub-acute, and chronic poisoning (alkali disease). Acute intoxication is manifested with respiratory disorders, ataxia, diarrhoea or death. The signs include garlic odour of the breath caused by the presence of methyl selenide. The chronic form of intoxication caused by long-term supply of high Se levels in the diet causes reduced feed intake, slowed down growth, hair loss, liver cirrhosis or anaemia. Chronic poisoning, called selenosis, most often occurs in regions with high Se levels in soil and drinking water.

2.7 Effect Se in poultry yield

Effects of various sources and levels of Se in the diet on poultry yield have been subject of a number of studies (Rama Rao *et al.*, 2013). The achieved results are not uniform, both negative and positive responses being reported.

2.7.1 Negative response to the application of inorganic and organic sources of Se

Rama Rao *et al.*,(2013) studied various levels (0, 100, 200, 300, or 400 μg/kg diet) of organic Se in broiler chickens in tropical conditions. The results of the study indicate that the supplementation of Se did not influence body weight and feed efficiency. Similar findings have been reported by Chen *et al.*, (2013), who fed the chickens with different levels of Se yeast. The results showed that effects of different levels of Se on growth performance, slaughter performance, the immune status, drip loss, and flesh did not significantly differ. Organic Se was also fed to broiler chicks by De Medeiros et al., (2012).The results revealed that the supplementation with organic Se did not affect productive characteristics of the broilers.

The effects of dietary vitamin E (0, 125, and 250 mg/kg), Se (0, 0.5, and 1 mg/kg), or their different combinations under either thermos neutral or heat stress conditions were studied by Habibian *et al.*, (2013). Body weight and feed intake were not influenced significantly by dietary vitamin E and Se, whereas feed conversion was improved significantly by 125 mg/kg vitamin E. The different levels of Se and vitamin E applied in the feed mixtures were found not to affect the final body weight of the chickens (Zdunczyk *et al.*, 2011).

2.7.2 Positive response to the application of inorganic and organic sources of Se

In contrast to the above-mentioned reports, Attia *et al.*, (2010) stated that addition of organic and inorganic Se improved the productive and reproductive performance of Gimmizah breeding hens. Effect of organic and inorganic Se supplementation on growth performance, meat quality, and antioxidant status of broilers was also studied by Yang *et al.*, (2012). In the control group, 0.3 mg/kg inorganic Se (Na_2SeO_3) was added to the diets while in the experimental group, 0.3 mg/kg organic Se (Se-enriched yeast) was added to the same basal diets. The results show that organic Se could increase daily weight gain and feed intake by 8.92 and 3.99%, and decrease survival rate and feed conversion by 0.93 and 4.84%, respectively, indicating that the effects of organic Se on broiler growth performance were better than those of inorganic Se.

Dlouha *et al*., (2008) studied the effects of supplementation of dietary sodium selenite and sodium-enriched alga Chlorella on the growth performance of sexed broiler cockerels Ross 308. The basal diet was supplemented with 0 (control) or 0.3 mg/kg Se from sodium selenite or Se-Chlorella (Se-CH). Dietary supplementation with Se-CH increased body weight. Also Heindl *et al*., 2010 confirmed that Se addition influenced body weight in 21- and 35-day-old broiler chickens. Feeding of selenized yeast increased the live body weight of chickens compared with the controls (Rozbicka-Wieczorek *et al*., 2012.

2.8 Research gap and scope of present investigation

From the above literatures, it is clear that the supplementation of Se to poultry with appropriate doses is always favorable for better growth, reproduction and survivability, and the organic form was found to be superior to inorganic one in most of the cases. The organic form of Se is available in natural feed staffs including the cereal grains like maize, wheat, sorghum etc. However, Se content in feed ingredients depends upon the Se concentration in soil. The Se concentration in Bangladeshi soil has been reported to be lower (Jason *et al*., 2004) than the standard. So, it is obvious that the feed grains grown on Bangladeshi soil will be deficient of Se. Therefore, there is a scope of investigating the necessity of Se supplementation in poultry as well as in other animals. But, until recently no work has been done to study the effects of Se supplementation with the appropriate form and levels in poultry rations formulated from locally available ingredients.

CHAPTER 3

MATERIALS AND METHODS

3.1 Statement of the experiment

The research work was conducted at Sher-e-Bangla Agricultural university poultry farm, Dhaka, with 200 day old straight run(Lohman Meat) commercial broilers for a period of 35 days from 02 february to 8 march, 2020 to assess the feasibility of using selenium in commercial broiler diet on growth performance, hematological and immune status of broilers. This research helps to make a conclusion about Selenium as an nutrient and growth supplement.

3.2 Collection of experimental broilers

A total of 200 day old Lohman meat broiler chicks were collected from Kazi hatchery, Gazipur, Dhaka.

3.3 Experimental materials

The collected chicks were carried to the university poultry farm early in the morning. They were kept in electric brooders equality for 7 days by maintaining brooding protocol. During brooding time only basal diet was given .After seven days 200 chicks were distributed randomly in four(4) dietary treatment .Each treatment had five (5)replication with 10 birds per replication.

3.4 Experimental treatments

T_1 : Basal diet / Control

T_2: Organic Se(1.5ml/L)

T_3 : Inorganic Se (0.75 ml/L)

T_4 : Combined (O+In Se)(1.5ml+0.75ml / L)

3.5 Preparation of the experimental house

The experimental room was cleaned and washed by using detergent mix water. Ceiling walls and floors were thoroughly cleaned and disinfected . After proper drying ,the house was divided into 20 pens of equal size using wood materials and wire net. The height of wire net was 36 cm. A group of 10 birds were allocated to each pen (replication) of the four dietary treatments. The stocking density was 1 m^2/ 10 birds.

Table 1. Layout of the experiment

Treatments with Replication (10 birds / replication)					No. of birds
T_1R_1(n=10)	T_1R_2(n=10)	T_1R_3(n=10)	T_1R_4(n=10)	T_1R_5(n=10)	50
T_2R_1(n=10)	T_2R_2(n=10)	T_2R_3(n=10)	T_2R_4(n=10)	T_2R_5(n=10)	50
T_3R_1(n=10)	T_3R_2(n=10)	T_3R_3(n=10)	T_3R_4(n=10)	T_3R_5(n=10)	50
T_4R_1(n=10)	T_4R_2(n=10)	T_4R_3(n=10)	T_4R_4(n=10)	T_4R_5(n=10)	50
Total					200

3.6 Experimental diets

The composition of starter and grower diets are shown in the table 2

Table 2. **Name of composition present in Starter and Grower ration.**

Name of the Ingredient	Minimum percentage present in Starter diet	Minimum percentage present in Grower diet
ME	3000 kcal / kg	3050 kcal / kg
Crude protein	21.0%	19.0%
Crude Fat	6.0%	6.0%
Fiber	5.0%	5.0%
Ash	8.0%	8.0%
Lysine	1.20%	1.10%
Methionine	0.49%	0.47%

Feed were supplied 3 times daily by following Lohman meat Manual and ad libitum drinking water 2 times daily.

3.6.1 Collection of Se

Organic (Sk+f) & Inorganic Se (ACI) was purchased from Market.

Plate 1: Organic and Inorganic Se

Table 3. Composition of Se

Organic Se(100ml solution)	Inorganic Se(100ml oil emulsion)
Vitamin E Acetate (10gm)	Vitamin E (BP 100mg)
Se (as selisseo) (20mg)	Sodium selenite (BP 0.5 mg)

3.7 Management procedures

Body weight and feed intake were recorded every week and survivability was recorded for each replication up to 35 days of age.

The following management procedures were followed during the whole experiment period.

3.7.1 Brooding of baby chicks

The experiment was conducted during 2 February to 8 March, 2020. The average temperature was 27.9^{0}C and the RH was 50% in the poultry house. Common brooding was done for one week. After one week the brooder size was increased. After seven days chicks were distributed in the pen randomly. There were 10 chicks in each pen and the pen space was 1m^2. Brooding temperature was adjusted with birds behavior. At day time only an electric bulb was used to stimulate the chicks to eat and drink.

3.7.2 Room temperature and relative humidity

Daily room temperature (°C) and humidity were recorded every six hours with a thermometer and a wet and dry bulb thermometer respectively. Then the Averages of room temperature and percent relative humidity for the experimental period were calculated **(Appendix 1 & 2).**

3.7.3 Litter management

Rice husk was used as litter at a depth of 4cm. At the end of each day, litter was stirred to prevent accumulation of harmful gases and to reduce parasite infestation. At 10 days of age & 3 weeks of age, droppings on the upper layer of the litter were cleaned and for necessity fresh litter was added. Regular supervision of the litter material to observe any bad smell or abnormality.

3.7.4 Feeding and watering

The birds were offered with ad libitum feed and clean fresh water. One large feeder and one big round drinker were provided in each pen for 10 birds. Feeders were cleaned at the end of each week and drinkers were washed daily in the morning before supplying water. Feces and dirt contamination in the feeder and drinker were avoided by raising the feeder and drinker at a manageable height by using brick.

3.7.5 Lighting

There was provision of light in the broiler farm to stimulate feed intake and body growth at night. For first 2 weeks 24 hours lighting schedule was used. Thereafter 1 hours dark period was scheduled up to 35 days.

3.7.6 Bio security measures

Biosecurity components were properly maintained during the experimental period. Entry of wild birds and animals were prohibited. Foot bath (PPM) was used in front of farm gate to avoid the risk of pathogen transmission. Proper hygienic and sanitation program was undertaken in the farm and its premises. Strict sanitary measures were taken during the experimental period. Disinfectant (Virkon) was used to disinfect the feeders and waterers and the house also. Regular cleansing of the farm was done. Some vitamins like Vitamin B-Complex, Vitamin-AD$_3$K, Vitamin-C, Calcium and electrolytes were supplied to the birds.

3.7.7 Vaccination

To prevent diseases in the farm, chicks were vaccinated as per standard vaccination schedule. The vaccines collected from medicine shop (Hipraviar & Ceva Company) and applied to the experimental birds according to the vaccination schedule.

Table 4. Vaccination schedule

Age of birds	Name of Disease	Name of vaccine	Route of administration
3 days	ND + IB	Hipraviar(live)	One drop in each eye
9 days	Gumboro	G-228E (inactivated)	Drinking Water
18days	Gumboro	G-228E (inactivated) booster dose	Drinking Water
22 days	ND + IB	Hipraviar (live)	Drinking Water

3.7.8 Ventilation

The broiler shed was south facing and open-sided. Due to wire-net cross ventilation it was easy to remove polluted gases from the farm. Besides ventilation was regulated as per requirement by folding polythene screen.

3.8 Study Parameters

3.8.1 Recorded parameters

Weekly live weight, weekly feed consumption and death of chicks to calculate mortality percent. FCR was calculated from final live weight and total feed consumption per bird in each replication. After slaughter dressing weight, Abdominal fat, were measured from each broiler chicken. 60 broiler Dressing yield was calculated for each replication to find out dressing percentage. Cholesterol and Glucose level was analysis from each replication.

3.9 Data collection

3.9.1 Live weight:

The initial day-old live weight and weekly live weight of each replication was kept to get final live weight record per bird.

3.9.2 Dressing yield:

Live weight- (blood + feathers + head + shank+ digestive system + Liver+ Heart)

3.9.3 Feed consumption:

Daily feed consumption record of each replication was kept to get weekly and total feed consumption record per bird.

3.9.4 Mortality of chicks:

Daily death record for each replication was counted up to 35 days of age to calculate mortality.

3.9.5 Dressing procedures of broiler chicken:

Three birds were picked up at random from each replication at the 35 day of age and sacrificed to estimate dressing percent of broiler chicken. All birds to be slaughtered were weighed and fasted by overnight (12 hours) but drinking water was provided ad-libitum during fasting to facilitate proper bleeding. All the live birds were weighed again prior to slaughter. Birds were slaughtered by severing jugular vein, carotid artery and the trachea by a single incision with a sharp knife and allowed to complete bleed out at least for 2 minutes. Defeathering was done by defeathering machine. Then the carcasses were washed manually to remove loose singed feathers and other foreign materials from the surface of the carcass. Afterward the carcasses were eviscerated and dissected according to the methods by Jones (1982). Abdominal fat removed and weight. Dressing yield was found by subtracting blood, feathers, head, shank, liver, heart and digestive system from live weight.

3.9.6 Blood sample analysis

Blood samples (1 ml/bird) were collected into ethylene diethyltetraacetic acid (EDTA) tubes from the wing veins. Samples was calculated by Easy Touch meter for glucose & cholesterol.

3.10 Calculations

3.10.1 Live weight gain

The average body weight gain of each replication was calculated by deducting initial body weight from the final body weight of the birds. Body weight gain = Final weight – Initial weight

3.10.2 Feed intake

Feed intake was calculated as the total feed consumption in a replication divided by number of birds in each replication.

Feed intake (g/bird) = No. of birds in a replication / Feed intake in a replication

3.10.3 Feed conversion ratio

Feed conversion ratio (FCR) was calculated as the total feed consumption divided by weight gain in each replication.

FCR= Weight gain (kg) / Feed intake (kg)

3.11 Statistical analysis

The data was subjected to statistical analysis by applying one way ANOVA using statistical package for social sciences (SPSS) version 16. Differences between means were tested using Duncan‟s Multiple Comparison Test and significance was set at $P<0.05$.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Production index of broiler chicken

Calculation of Production Index (PI) is one the major parameter to assess the successfulness of broiler chicken production which compare broiler results from different flocks, region and treatment groups. The performance of broiler chickens is measured through five factors. These factors are:

- ✓ The level of feed consumption
- ✓ The achievement of body weight
- ✓ Feed Conversion Ratio
- ✓ Dressing Percentage
- ✓ Survivability rate

Measurement and assessment of the five factors reflect the quality of maintenance and performance maintenance of broiler chickens.

4.1.1 Feed Consumption (FC)

The mean weekly feed consumption (g) of broiler chicks at the end of 5^{th} week in the dietary group T_1, T_2, T_3, T_4 were 1063.75±20.92, 987.58±27.08, 1028.98±35.48, 1126.40±18.99 accordingly. The overall mean feed consumption of different groups showed that there was significant difference ($P<0.05$) among control (T_1), 1.5ml/L organic Se (T_2), 0.75ml/L, inorganic Se (T_3) and their combined effect (Table 5 and Figure 1).

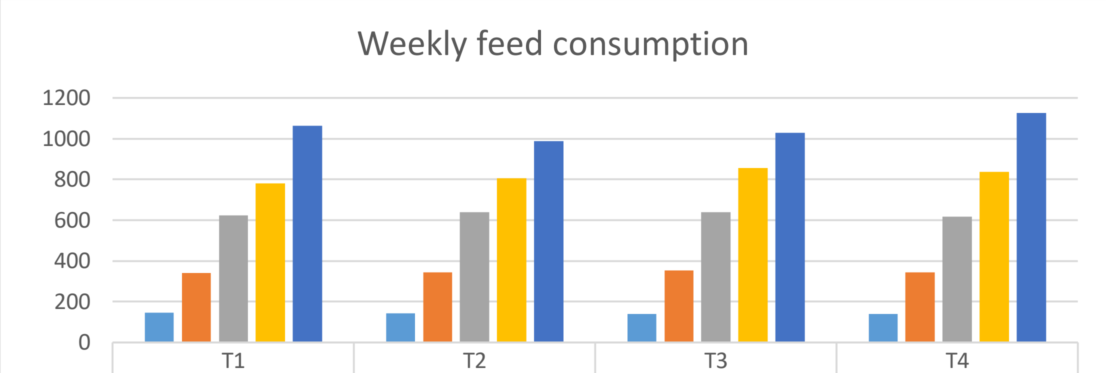
These results are in agreement with the findings of Yang *et al*. (2012) reported that dietary organic Se and inorganic Se had significant effect on weekly feed consumption in broiler chickens at different inclusion level compared to control group. Zelenka and Fajmonova (2005) who reported that sodium selenite increased feed intake in chicks. These results are contradictory with the findings of Habibian *et al*., (2014) mentioned that feed intake were not influenced significantly by dietary vitamin E and Se.

Table 5. Effects of feeding different level of organic Se, inorganic Se and their combined dose on feed consumption (g/bird) of broiler chickens at different week.

Treatment	1st wk FC	2nd wk FC	3rd wk FC	4th wk FC	5th wk FC
T1	$145.29^{c}\pm0.33$	$341.43^{a}\pm0.36$	622.82 ± 19.78	$781.94^{a}\pm25.25$	$1063.75^{ab}\pm20.92$
T2	$142.89^{b}\pm0.34$	$342.44^{ab}\pm0.55$	640.32 ± 12.32	$804.88^{ab}\pm18.87$	$987.58^{a}\pm27.08$
T3	$138.70^{a}\pm0.34$	$354.58^{b}\pm0.40$	638.74 ± 12.22	$856.28^{b}\pm13.96$	$1028.98^{a}\pm35.48$
T4	$138.17^{a}\pm0.33$	$344.71^{ab}\pm8.03$	616.08 ± 18.65	$838.62^{ab}\pm24.12$	$1126.40^{b}\pm18.99$
Mean ± SE	$141.26^{s}\pm0.69$	$345.79^{s}\pm2.20$	629.49 ± 7.77	$820.43^{s}\pm11.71$	$1051.68^{s}\pm16.82$
	*	*	NS	*	*

Here, T_1=(control), T_2=(organic Se, 1.5ml/L), T_3=(0.75ml/L, inorganic Se), T_4=(organic + inorganic Se).Values are Mean ± S.E (n=20) one way ANOVA (SPSS, Duncan method).

- ✓ Mean with different superscripts are significantly different ($P<0.05$)
- ✓ Mean within same superscripts do not differ ($P>0.05$) significantly
- ✓ SE= Standard Error
- ✓ * = Significant
- ✓ NS=Non-significant



	T1	T2	T3	T4
W1	145.29	142.89	138.7	138.17
W2	341.43	342.44	354.58	344.71
W3	622.82	640.32	638.74	616.08
W4	781.94	804.88	856.28	838.62
W5	1063.75	987.58	1028.98	1126.4

Figure1. Effects of feeding different level of organic Se, inorganic Se and their combined dose on feed consumption (g/bird) of broiler chickens at different week.

4.1.2 Body Weight Gain

The mean body weight gains (g) of broiler chicks at the end of 5th week in different groups were T_1, T_2, T_3, T_4 $654.72^a \pm 14.43$, $667.04^a \pm 13.18$, $701.98^b \pm 22.04$ and $755.70^b \pm 12.87$ respectively (Table 6 and Figure 2).The overall mean body weight gain of different groups showed that there was significant ($P<0.05$) difference in groups compared to control group. These result are in agreement with Yang *et* al.,(2012) and Ibrahim *et* al.,(2011). Rama Rao *et* al.,(2013) and Chen *et* al.,(2013) result is contradictory to this result.

Table 6. Effects of feeding different level of organic Se, inorganic Se and their combined dose on body weight gain (BWG) (g/bird) of broiler chickens at different week.

Treatment	1st wk wt.gn	2nd wk wt.gn	3rd wk wt.gn	4th wk wt.gn	5th wk wt gn
T_1	120.02±0.44	291.18^a±2.78	458.36^a±18.84	501.30^a±20.77	654.72^a±14.43
T_2	119.82±0.37	320.30^b±5.27	469.20^a±14.86	484.00^a±14.54	667.04^a±13.18
T_3	120.62±0.51	312.78^b±5.87	478.24^a±10.39	558.16^b±11.03	701.98^a±22.04
T_4	120.42±0.51	307.72^{ab}±9.90	521.76^b±8.02	549.34^b±12.78	755.70^b±12.87
Mean ± SE	120.22^s±.22	308.00^s±3.85	481.89^s±8.36	523.20^s±10.02	694.86^s±11.62
	NS	*	*	*	*

Here, T_1=(control), T_2=(organic Se, 1.5ml/L), T_3=(0.75ml/L, inorganic Se), T_4=(organic + inorganic Se).Values are Mean ± S.E (n=20) one way ANOVA (SPSS, Duncan method).

- ✓ Mean with different superscripts are significantly different (P<0.05)
- ✓ Mean within same superscripts do not differ (P>0.05) significantly
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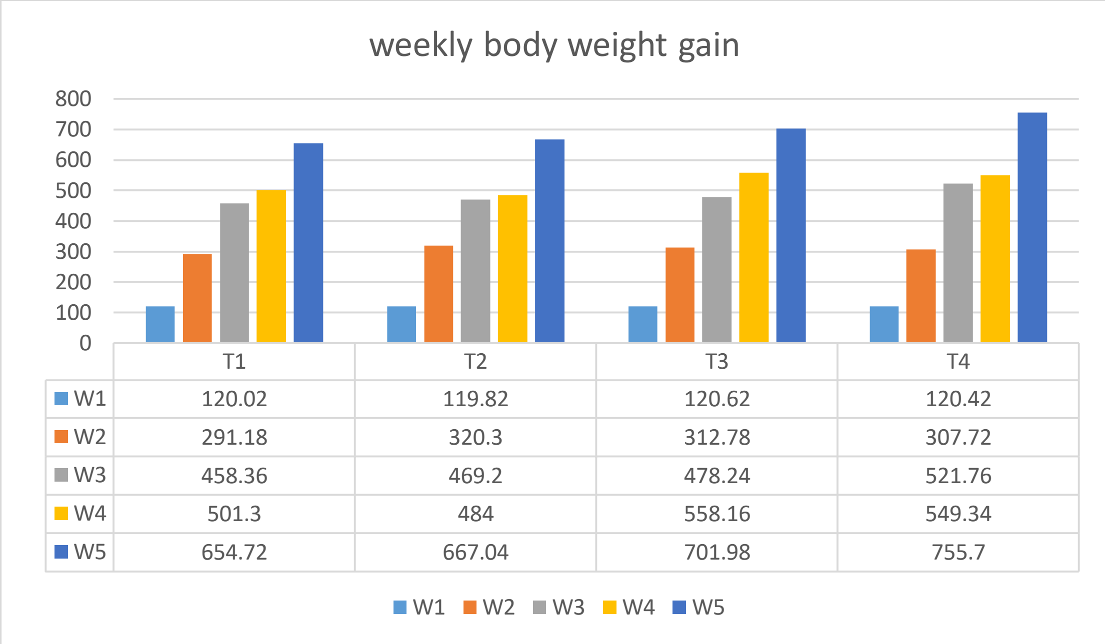

Figure2. Effects of feeding different level of organic Se, inorganic Se and their combined dose on body weight gain (BWG) (g/bird) of broiler chickens at different week.

4.1.3 Feed Conversion Ratio (FCR)

The FCR of broiler chicks at the end of 5th week in different groups T_1, T_2, T_3, T_4 were 1.62±0.01, 1.47±0.01, 1.46±0.00, 1.49±0.01 respectively. The overall mean FCR of different groups showed that there was significant ($P<0.05$) increase in groups T_2, T_3 and T_4 compared to control (T_1) (Table 7 and Figure 3).

These results are coincided with the findings of previous researchers (Habibian *et al.*, 2013) who concluded that dietary Se supplementation respectively had significant effect on weekly feed conversion ratio (FCR).On the other hand, other results revealed that the supplementation with organic Se did not affect productive characteristics like FCR of the broilers (de Almeida *et al.*, 2012).

Table 7. Effects of feeding different level of organic, inorganic and their combined dose of Se on FCR of broiler chickens at different week.

Treatment	1st week FCR	2nd week FCR	3rd week FCR	4th week FCR	5th week FCR
T_1	1.21^c±0.00	1.17^b±0.01	1.36^b±0.01	1.56^a±0.02	1.62^b±0.01
T_2	1.19^b±0.00	1.07^a±0.01	1.36^b±0.01	1.66^b±0.02	1.47^a±0.01
T_3	1.15^a±0.00	1.13^b±0.02	1.33^b±0.01	1.53^a±0.02	1.46^a±0.00
T_4	1.14^a±0.00	1.12^b±0.01	1.22^a±0.02	1.52^a±0.01	1.49^a±0.01
Mean ± SE	1.17±0.00	1.12±0.01	1.32±0.01	1.57±0.01	1.51±0.01
	*	*	*	*	*

Here, T_1=(control), T_2=(organic Se, 1.5ml/L), T_3=(0.75ml/L, inorganic Se), T_4=(organic + inorganic Se).Values are Mean ± S.E (n=20) one way ANOVA (SPSS, Duncan method).

- ✓ Mean with different superscripts are significantly different ($P<0.05$)
- ✓ Mean within same superscripts do not differ ($P>0.05$) significantly
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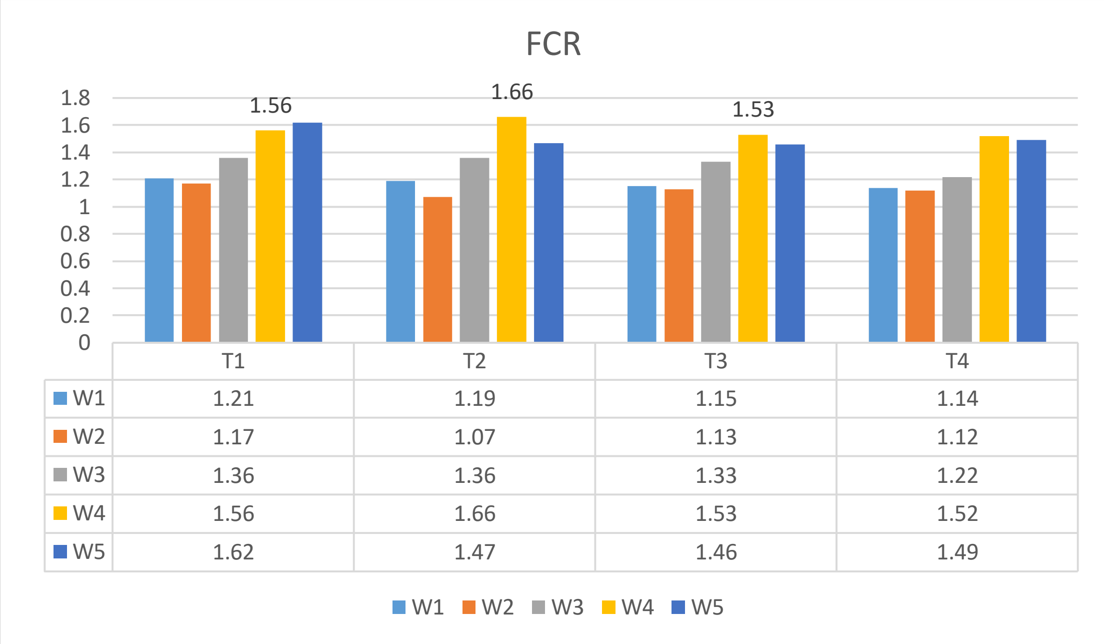

Figure3. Effects of feeding different level of organic, inorganic and their combined dose on FCR of broiler chickens at different week.

Table 8. Production index of broiler chicken supplemented with organic Se, inorganic Se and their combined dose.

Treatment	**T_1**	**T_2**	**T_3**	**T_4**	**Mean ± SE**
Final Lv. wt.(g/Broiler)	2019.00^{a}±40.14	2062.30^{a}±21.39	2195.50^{b}±37.49	2257.66^{b}±27.98	*
FC (g)	1063.75^{ab}±20.92	987.58^{a}±27.08	1028.98^{a}±35.48	1126.40^{b}±18.99	*
FCR	1.62^{b}±.01	1.47^{a}±.01	1.46^{a}±.00	1.49^{a}±.01	*
DP% with Skin	73.40±1.25	74.82±.69	75.10±.90	74.21±1.20	NS
Survivability(%)	96.00±2.44	100±0.00	100±0.00	98.00±2.00	NS

Here, T_1=(control), T_2=(organic Se, 1.5ml/L), T_3=(0.75ml/L, inorganic Se), T_4=(organic + inorganic Se).Values are Mean ± S.E (n=20) one way ANOVA (SPSS, Duncan method).

- ✓ Mean with different superscripts are significantly different (P<0.05)
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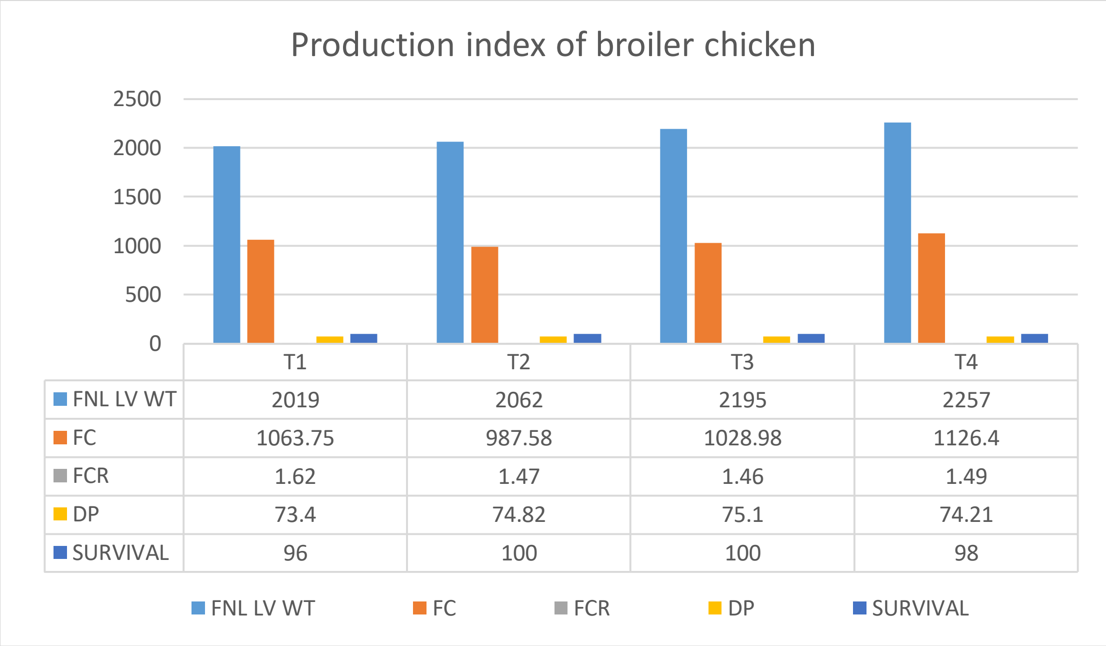


Figure4. Production index of broiler chicken supplemented with organic se, inorganic se and their combined dose.

4.1.4 Final Body Weight

Data presented in (Table 8 and Figure4) showed that the effect of treatments on final live weight (gram per broiler chicken) was significant (P>0.05). The relative final live weight (g) of broiler chickens in the dietary groupT_1, T_2, T_3, T_4 were $2019.00^{a}\pm40.14$, $2062.30^{a}\pm21.39$,$2195.50^{b}\pm37.49$, $2257.66^{b}\pm27.98$ respectively. The highest result was found in T_4 (2257.66±27.98) and lowest result was in T_1 (2019.00±40.14) group. However, Final live weight of broiler fed with organic and inorganic Se (combined) diet increased and that was significant (P<0.05) compared to that of the control and other groups. The final live weight of T_2 and T_3 group was also higher than the control group (T_1) (Table 8 and Figure 4).

Our study is in agreement with the finding of Krstic *et al*. (2012). Heindl *et al*., (2010) confirmed that Se addition influenced body weight in 21- and 35-day-old broiler chickens. In another study, it was demonstrated that Se supplementation in broiler diets significantly improved final body weight and without increase of feeding cost (Ibrahim *et al*., 2011).

In contrast, no significant (P>0.05) impact of Se was found on final body weight of broilers (Yoon *et al.*, 2007). Payne & Southern (2005) noticed that organic and inorganic sources of Se did not have any effects on performance in broilers.

4.1.5 Dressing Percentage (DP)

The dressing percentage of broiler chicks at 35^{th} days presented in (Table 8 and Figure 4) were not significantly (P>0.05) differ in T_1 (control), T_2 (organic Se), T_3 and T_4 group. Broiler supplemented with 0.75ml/L Inorganic Se (T_3) had a greater (P>0.05) dressing percentage (75.10±.905) compared with the organic Se ($T_{2)}$ (74.82±.691) and control (73.40±1.251) group. Dressing percentage of combined group T_4 was 74.215±1.207.

Generally dressing percentage will increase by increasing live body weight and by advancing age of broiler (Naji *et al*., 2007). Agreement with this finding of Sevcikova *et al*., (2006). The result was in contrast with (El-Sheikh *et al*., 2006) who noted that the effect of Se on meat yield could be due to changes in thyroid hormone metabolism or a result of changes in broiler feathering. The Se and vitamin E significantly increased the carcass weight, dressing percentage and carcass parts percentages at 6 weeks of age Tayeb and Qader (2012).

On the other hand Chen *et al*., (2013), stated that who fed the chickens with different levels of Se showed that effects of different levels of Se on slaughter performance, cooking loss, and flesh did not significantly differ. Payne & Southern (2005) noticed that organic and inorganic sources of Se did not have any effects on carcass traits and in broilers.

4.1.6 Abdominal fat weight

The abdominal fat weight range was 25.70±3.48 to 31.90±3.33 (Table 9). The highest abdominal fat was found (31.90±3.33) in combined se (T_4) group and lowest abdominal fat was found 25.70±3.48 in control ($T_{1)}$ group. In inorganic Se group (T_3) abdominal fat (26.40±3.20) was comparative lower than organic Se ($T_{2)}$ treatment group (29.50±3.02). However, there was no significant (P>0.05) difference among the treatment group statistically (ANOVA).

Se also positively affects the improvement of certain organoleptic properties of broiler chicken meat by increasing abdominal fat percentage. Se-rich meat is more juicy, crispy, and better looking. Another researcher stated that the supplementation with Se produced a linear reduction on the abdominal fat of the carcasses assessed. (De Almeida *et al*., 2012).

Table 9: Abdominal fat weight of broiler chicken supplemented with organic se, inorganic se and their combined dose.

Treatments	T_1	T_2	T_3	T_4	MEAN±SE
Abdominal fat weight(g)	25.70±3.4	29.50±3.02	26.40±3.20	31.90±3.33	28.98±1.60
NS					

Here, T_1=(control), T_2=(organic Se, 1.5ml/L), T_3=(0.75ml/L, inorganic Se), T_4=(organic + inorganic Se).Values are Mean ± S.E (n=20) one way ANOVA (SPSS, Duncan method).

- ✓ Mean with different superscripts are significantly different (P<0.05)
- ✓ Mean within same superscripts do not differ (P>0.05) significantly
- ✓ SE= Standard Error
- ✓ NS=Non-significant

4.1.7 Survivability

Data presented in Table (8 and Figure 4) showed that dietary supplementation of Se had no significant effect on the survivability rate. There are less mortality of broiler chicken. 1.5% mortality was seen (3 among 200) during the time of research period.

The role of Se which was decrease mortality (Hoffmann, 2007) where the non - significant differences in mortality rate between experimented groups may be due Se enhances immune responses**.**

Wang YanBo (2009) observed improved survival rate in commercial broiler fed on both the sodium selenite and nano-Se supplemented diet, whereas Korosi *et al.,* (2005) had reported the lower mortality on male and female on Sel-Plex^{TM} supplemented broiler parent stock**.** However, lower mortality rate in Se supplemented Japanese quail compared to control were also reported by Elaroussi *et al.,* (2002).

4.2 Serum Biochemical Parameters

4.2.1 Glucose

Effects of dietary supplementation of Se on concentration of glucose of broiler chickens are presented in (Table 10 and Figure 5). Dietary incorporation of organic and inorganic Se had no significant ($P>0.05$) effect on blood glucose level. The lowest level (263.60±8.04) of plasma glucose found in T_4 (combined Se) and supplementation of 1.5ml/L organic Se (T_2) showed higher glucose level (271.20±6.304) compared to that of inorganic Se and control group

No effect of ingredients on blood glucose is already described by others (Collin *et al*., 2003). The blood glucose levels retain constant, even when broilers are submitted to fasting (Swennen *et al.*, 2007).In contrast to the results of this study, it is reported that the addition of Se as nanoparticles at 0.3 mg/kg level in chicks significantly elevated serum glucose levels when compared to that of control treatment (Mohapatra *et al.,* 2014)

Table 10. Effects of feeding different level of organic, inorganic and their combined dose on serum biochemical level of different broiler chicken under different treatment.

Parameters	T_1	T_2	T_3	T_4	Mean ± SE
Glucose mg/dl	265.20±7.82	271.20±6.30	267.80±6.39	263.60±8.04	266.95^{NS}±3.36
Cholesterol mg/dl	165.00^{ab}±9.60	150.40^{a}±8.54	143.60^{a}±15.7	186.60^{b}±6.28	161.40^{S}±6.18

Here, T_1=(control), T_2=(organic Se, 1.5ml/L), T_3=(0.75ml/L, inorganic Se), T_4=(organic + inorganic Se).Values are Mean ± S.E (n=20) one way ANOVA (SPSS, Duncan method).

- ✓ Mean with different superscripts are significantly different (P<0.05)
- ✓ Mean within same superscripts do not differ (P>0.05) significantly
- ✓ SE= Standard Error
- ✓ * = Significant
- ✓ NS=Non-significant

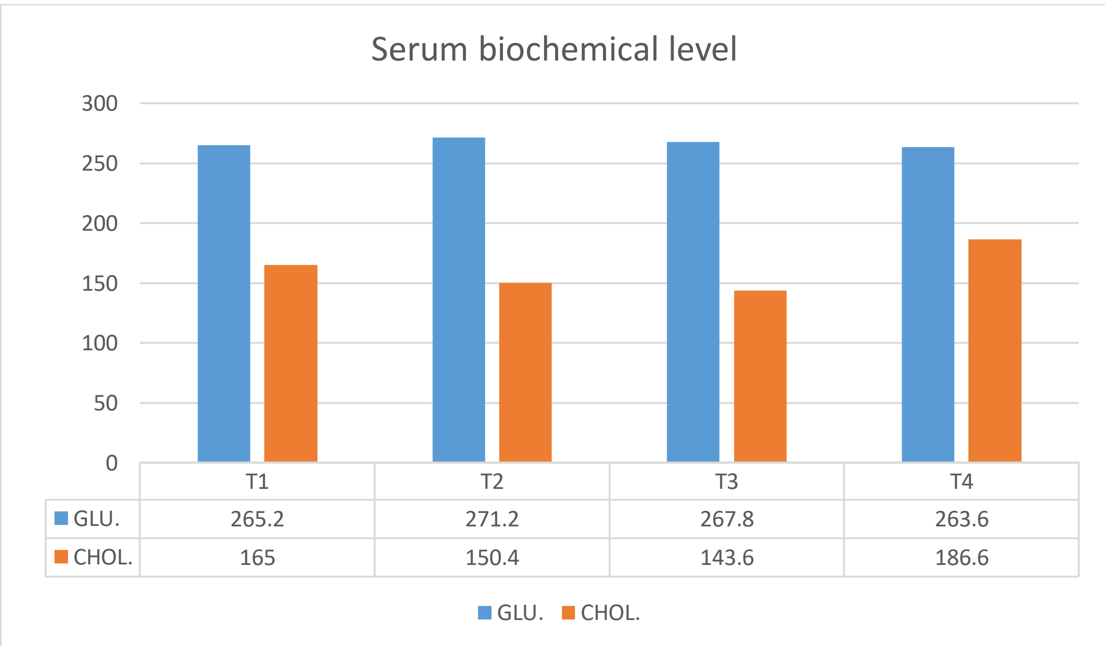


Figure 5. Effects of feeding different level of organic, inorganic and their combined dose on serum biochemical level of different broiler chicken under different treatment.

4.2.2 Cholesterol

Total cholesterol concentration (mg/dl) in the serum of different groups ranged from 143.60±15.79 to 186.60±6.28. Statistical analysis revealed that significant ($P>0.05$) difference among the groups (Table 10 and Figure 5). The lower amount (143.60±15.79) of cholesterol found in 0.75ml/L, Inorganic Se supplementation group (T_3) comparable to organic Se and control group but there was statistical difference. It is reported that the addition of Se as nanoparticles at 0.3 mg/kg level in chicks significantly decreased cholesterol levels when compared to that of control treatment (Mohapatra *et al*., 2014).

Yang *et al*. (2012) showed that chickens fed diet containing 0.3 mg/kg organic Se had no significant effect on their total cholesterol when the serum samples were compared to those fed diet without Se supplementation. Se added to the diet of broilers showed no significant effect on serum total cholesterol levels when compared to that of control group. (Collin *et al*., 2003)

4.3 Hematological parameters

Data presented in (Table 11 and Figure 6) showed the effect of dietary supplementation of organic Se, inorganic Se and their combined dose on some blood parameters of broiler chicken. Considering the treatment impact on blood components, the results indicated significant ($P<0.05$) differences due to supplementation of organic Se, inorganic Se and their combined dose, except HB, RBC, which were not significantly affected ($P>0.05$). The values of WBC, Lymphocyte and Granulocyte had higher in T_1 (control), T_3(0.75ml/L inorganic Se) had lower amount of WBC, Lymphocyte and Granulocyte. The highest value of WBC (70.20±.3.56), Lymphocyte (60.90±2.60) and Granulocyte (9.22±.63) were found in T_1 group.

El-Sebai, (2000) founded that the Se singly or combining with vitamin-E supplementation to the broiler chickens diets caused a significant ($p<0.05$) rise in RBC's counts. This result was in agreement with (Choct *et al*., 2004**)**.

Arshad *et al*., (2005) conducted an experiment Results indicate that Se supplementation may help to increase post vaccination humoral immune response against IBD in broiler chicks. Se deficit damages both cellular and humoral immunity (Artur *et al.*, 2003). Rama Rao *et al*., (2013). The cell-mediated immunity (lymphocyte proliferation ratio) increased linearly with dietary Se concentration.

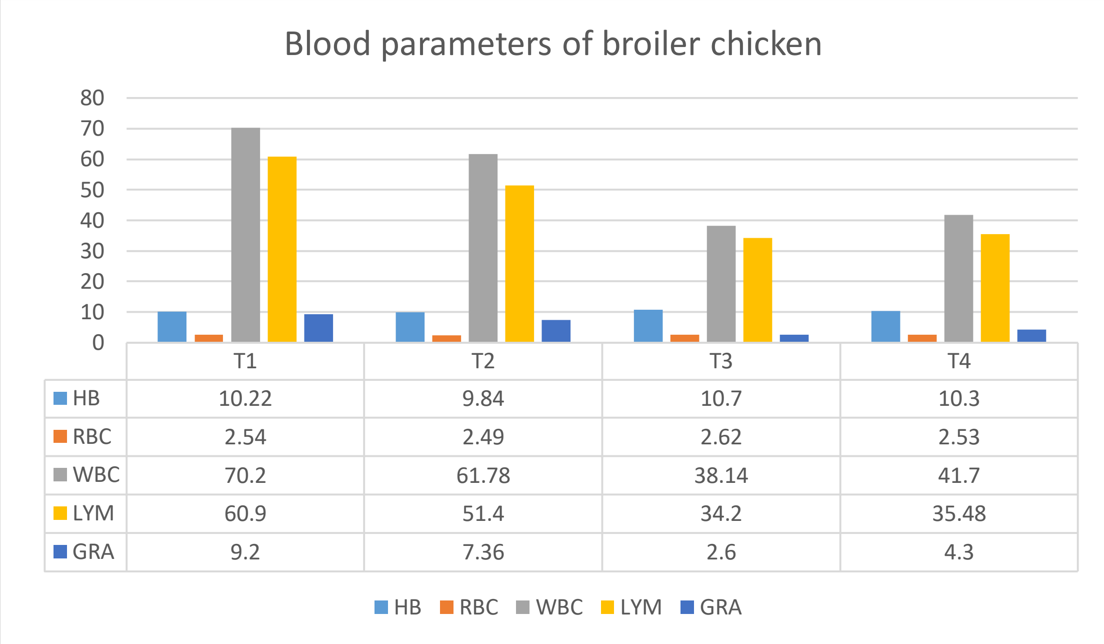
Tayeb and Qader (2012) showed that addition of 0.45mg Se along with 100mg vitamin E/kg diet of broiler significantly increased lymphocytes, when compared to that of control group received no Se and vitamin E. This result was in agreement with finding of Shlig (2009).

Table 11. Effects of feeding different level of organic, inorganic and their combined dose on blood parameters of broiler chickens.

Parameters	**T_1**	**T_2**	**T_3**	**T_4**	**Mean ± SE**	
Hemoglobin (g/dl)	10.22±.45	9.84±.32	10.70±.58	10.30±.53	10.26±.23	NS
RBC (($*10^{12}$/L)	2.54±.10	2.49±.06	2.62±.12	2.53±.10	2.55±.04	NS
WBC ($*10^{9}$/L)	70.20^{b}±3.56	61.78^{b}±5.76	38.14^{a}±3.55	41.70^{a}±1.85	52.95±3.57	*
Lymphocyte ($*10^{9}$/L)	60.90^{c}±2.60	51.40^{b}±3.89	34.20^{a}±3.11	35.48^{a}±1.20	45.49±2.88	*
Granulocyte (($*10^{9}$/L)	9.2^{b}±.63	7.36^{b}±1.43	2.60^{a}±.36	4.30^{a}±.51	5.87±.71	*

Here, T_1=(control), T_2=(organic Se, 1.5ml/L), T_3=(0.75ml/L, inorganic Se), T_4=(organic + inorganic Se).Values are Mean ± S.E (n=20) one way ANOVA (SPSS, Duncan method).

- ✓ Mean with different superscripts are significantly different (P<0.05)
- ✓ Mean within same superscripts do not differ (P>0.05) significantly
- ✓ SE= Standard Error
- ✓ * = Significant
- ✓ NS=Non-significant



	T1	T2	T3	T4
HB	10.22	9.84	10.7	10.3
RBC	2.54	2.49	2.62	2.53
WBC	70.2	61.78	38.14	41.7
LYM	60.9	51.4	34.2	35.48
GRA	9.2	7.36	2.6	4.3

Figure 6. Effects of feeding different level of organic, inorganic and their combined dose on blood parameters of broiler chickens.

CHAPTER 5

SUMMARY AND CONCLUSION

A feeding trial was conducted on 200 day-old Lohman meat broiler chicks for a period of 35 days in the poultry farm of Sher-e-Bangla Agricultural University, Dhaka. The chicks were assigned to 4 treatment groups comprising of T_1 (control), T_2 (1.5ml/L organic Se), T_3 (0.75ml/L inorganic Se) and T_4 (combined Se) randomly. Treatments were replicated five times with 10 chicks per replication. At 35 days of age, 60 broilers were sacrificed in halal method to evaluate the efficacy of dietary organic se, inorganic se and their combined diet supplementation. The production indexes viz. feed consumption, body weight, body weight gain, FCR, dressed weight, dressing percentage, abdominal fat percentage, hematological and blood biochemical parameters and survivability of broiler on different replication of different treatments was recorded and compared.

All collected data were subjected to one-way analysis of variance using Statistical Package for Social Science (SPSS) version 16 and differences in compare means using Duncan method. There was significant difference ($P<0.05$) on the feed intake, final and live body weight, body weight gain, FCR, cholesterol among different treatment groups. The higher body weight found in combined se (T_4) treated group compared to other groups and values were followed in an ascending order in T_3, T_2, and T_1 group. The dressing percentage, abdominal fat percentage, glucose level showed no significant ($P>0.05$) difference among all treatment groups. The better FCR (1.46±.00) found in birds fed diets with 0.75ml/L inorganic Se supplementation compared to other treatment and control group. The dressing percentage in T_3 (75.10±.90) and T_2 (74.825±.69) group was non-significantly ($P>0.05$) higher than control group. The relative weight of abdominal fat percentage did not show any significant difference among the treatment groups.

The serum biochemical parameters viz. glucose and cholesterol concentration was measured. The result showed that level of glucose decreased non-significantly ($P>0.05$) and cholesterol decreased significantly ($P<0.05$) in the Organic Se and Inorganic Se supplemented groups compared to combined and control group. The result indicated that effects of supplementation of organic Se and inorganic Se in blood constituents had significant effect , except Red blood cell (RBC) and Hemoglobin (HB) concentration which were non-significant ($P>0.05$).Although highest amount of HB and RBC was found in Inorganic Se supplementation group compared to other and control group. The control group (T_1) showed higher values of White blood cell (WBC), lymphocytes and Granulocyte than T_2, T_3 and T_4 treated group.

On the basis of analysis of the above mentioned research findings, it can be concluded that organic Se, inorganic Se and their combined dose had very effective impact on production performance, serum biochemical and hematological parameters and immune stimulation state of broiler chicken. Birds fed with 0.75ml/L inorganic Se supplemented diet achieved superior result. So, organic Se, inorganic Se could be used as safe growth stimulant in broiler production. Therefore, the present study recommends that implementation of these formulations in the field aspect for commercial broiler production which is safe, sound, and economically viable and environmentally suitable for our country. However, further more experimental trials are required to assess the impact of these material on the better quality of broiler meat production to ensure the safety of human consumption.

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APPENDICES

Appendix 1. Recorded temperature (^{0}C) during experiment

Weeks	Date	Period					Average
		7 A.M	**11 A.M**	**3P.M**	**7P.M**	**11P.M**	
1st	03.02.2020-09.02.2020	25.3	28.4	30.6	32.8	28.3	29.08
2nd	10.02.2020-16.02.2020	25.7	27.8	29.1	31.7	27.8	28.42
3rd	17.02.2020-23.02.2020	22.6	26.2	29.5	32.8	26.4	27.5
4th	24.02.2020-01.03.2020	24.3	26.6	28.8	31.2	26.7	27.52
5th	02.03.2020-08.03.2020	23.3	26.2	29.1	31.6	26.8	27.4

Appendix 2. Recorded relative humidity (%) during experiment

Weeks	Date	Period					Average
		7 A.M	**11 A.M**	**3P.M**	**7P.M**	**11P.M**	
1st	03.02.2020-09.02.2020	43	32	25	44	62	41.2
2nd	10.02.2020-16.02.2020	38	31	27	47	58	40.2
3rd	17.02.2020-23.02.2020	71	55	42	56	62	57.2
4th	24.02.2020-01.03.2020	72	53	41	52	66	56.8
5th	02.03.2020-08.03.2020	72	54	40	51	65	56.4

Appendix 3. Feed consumption (g/bird) of 1st, 2nd, 3rd, 4th and 5th week under different treatment groups.

Treatment	Replication	1st wk(g/bird)	2nd wk(g/bird)	3rd wk(g/bird)	4th wk(g/bird)	5th wk(g/bird)	Cumulative FC/bird (g)
T1	R1	144.21	340.2	586.8	734.6	1132.11	2937.92
	R2	145.1	341.42	600	799.3	1043.33	2929.15
	R3	145.68	342.21	617.4	791.6	1012.7	2909.59
	R4	146.21	341.22	699.2	862.5	1088.3	3137.43
	R5	145.23	342.1	610.7	721.7	1042.32	2862.05
T2	R1	142.56	340.8	676.1	845.6	963.5	2968.56
	R2	141.82	341.64	632.6	806.4	923.5	2845.96
	R3	143.76	342.56	661.3	798.1	946.8	2892.52
	R4	142.87	343.43	621	738.2	1054	2899.5
	R5	143.45	343.76	610.6	836.1	1050.1	2984.01
T3	R1	137.85	353.65	623.6	870.5	1083.8	3069.4
	R2	138.65	354.34	679.5	902.5	1087.2	3162.19
	R3	139.95	355.34	649.6	841	894.88	2880.77
	R4	138.62	353.87	633	821.3	1022.4	2969.19
	R5	138.43	355.7	608	846.1	1056.6	3004.83
T4	R1	137.56	351.56	652.8	849.2	1090	3081.12
	R2	138.54	352.21	650.5	865.7	1140.7	3147.65
	R3	139.2	353.76	627.3	889.4	1115.1	3124.76
	R4	137.32	353.43	596.2	840.8	1093.1	3020.85
	R5	138.22	312.6	553.6	748	1193.1	2945.52

Appendix 4. Body weight (g/bird) of 1st, 2nd, 3rd, 4th and 5th week under different treatment groups.

Treatment	Replication	Weight of DOC	1st wk(g/bird)	2nd wk(g/bird)	3rd wk(g/bird)	4th wk(g/bird)	5th wk(g/bird)
T1	R1	35.98	155	475.4	893	1378	1988.8
	R2	35.98	157	442.9	876	1378.9	1945.2
	R3	35.98	156	451.9	922.1	1340.1	1949
	R4	35.98	155	454	1033	1526.2	2160
	R5	35.98	157	417.7	863.2	1305.2	2052
T2	R1	35.98	156	483	996.3	1507	2004.4
	R2	35.98	155	456.3	924.1	1431	2073.2
	R3	35.98	157	485.7	972.7	1455.7	2024
	R4	35.98	156	484.3	936	1365.6	2121.9
	R5	35.98	155	471.2	897.4	1387.2	2088
T3	R1	35.98	158	461.4	942.7	1536.3	2284
	R2	35.98	156	478.5	972.4	1528	2258
	R3	35.98	157	474	973.3	1516.6	2076
	R4	35.98	155	450	926.7	1456	2151.5
	R5	35.98	157	483	923	1492	2208
T4	R1	35.98	156	483	996.3	1550.3	2278.1
	R2	35.98	158	471.2	976.5	1550.4	2290.9
	R3	35.98	155	463.6	1012.2	1586.2	2329
	R4	35.98	157	476.7	864	1403.5	2170.3
	R5	35.98	156	426.1	957.5	1419.4	2220

Appendix 5. Abdominal fat weight in different treatment group.

Treatment	Replication	**Abdominal fat Weight (g)**
T1	R1	28
	R2	27.5
	R3	30.5
	R4	12
	R5	30.5
T2	R1	20
	R2	28
	R3	28
	R4	38
	R5	33.5
T3	R1	19.5
	R2	28.5
	R3	35
	R4	30.5
	R5	18.5
T4	R1	39
	R2	22
	R3	35.5
	R4	26
	R5	37

Appendix 6. Serum biochemical data in different treatment groups.

Treatment	Replication	Glucose (mg/dl)	Cholesterol (mg/dl)
T_1	T_1R_1(1)	241	142
	T_1R_1(2)	291	127
	T_1R_1(3)	216	132
	T_1R_2(1)	243	140
	T_1R_2(2)	257	142
	T_1R_2(3)	280	130
	T_1R_3(1)	282	195
	T_1R_3(2)	275	180
	T_1R_3(3)	286	185
	T_1R_4(1)	236	181
	T_1R_4(2)	252	182
	T_1R_4(3)	277	183
	T_1R_5(1)	257	176
	T_1R_5(2)	265	173
	T_1R_5(3)	230	172

Appendix 6 (**Cont'nd**)

Treatment	Replication	Glucose (mg/dl)	Cholesterol (mg/dl)
T_2	T_2R_1(1)	305	120
	T_2R_1(2)	292	183
	T_2R_1(3)	295	170
	T_2R_2(1)	281	101
	T_2R_2(2)	248	149
	T_2R_2(3)	255	135
	T_2R_3(1)	276	146
	T_2R_3(2)	274	132
	T_2R_3(3)	272	150
	T_2R_4(1)	250	151
	T_2R_4(2)	267	149
	T_2R_4(3)	262	145
	T_2R_5(1)	256	137
	T_2R_5(2)	295	141
	T_2R_5(3)	275	139

Appendix 6 (Cont'nd)

Treatment	Replication	Glucose (mg/dl)	Cholesterol (mg/dl)
T3	T3R1(1)	256	193
	T3R1(2)	286	203
	T3R1(3)	244	190
	T3R2(1)	219	179
	T3R2(2)	263	108
	T3R2(3)	245	153
	T3R3(1)	258	140
	T3R3(2)	265	109
	T3R3(3)	270	135
	T3R4(1)	260	132
	T3R4(2)	293	148
	T3R4(3)	273	137
	T3R5(1)	232	115
	T3R5(2)	247	135
	T3R5(3)	242	132

Appendix 6 (Cont'nd)

Treatment	Replication	Glucose (mg/dl)	Cholesterol (mg/dl)
T4	T4R1(1)	245	140
	T4R1(2)	257	189
	T4R1(3)	246	163
	T4R2(1)	178	233
	T4R2(2)	249	190
	T4R2(3)	266	195
	T4R3(1)	289	200
	T4R3(2)	266	155
	T4R3(3)	275	185
	T4R4(1)	261	154
	T4R4(2)	254	197
	T4R4(3)	245	192
	T4R5(1)	286	198
	T4R5(2)	174	230
	T4R5(3)	309	207

Appendix 7. Average Live Weight, Eviscerated Weight and Dressing Percentage of broiler chicken of different replication under different treatment groups.

Treatment	Replication	**Live Weight (g)**	**Eviscerated Weight (g)**	**Dressing Percentage (%)**
T1	R1	1843	1327	72.002
	R2	1807	1405	77.753
	R3	1956	1428	73.006
	R4	1785	1254	70.252
	R5	1951	1444	74.013
T2	R1	1997	1481	74.161
	R2	2065	1584	76.707
	R3	1956	1479	75.613
	R4	2127	1596	75.035
	R5	2165	1572	72.609
T3	R1	2163	1656	76.560
	R2	2215	1653	74.627
	R3	2333	1693	72.567
	R4	2203	1711	77.666
	R5	2145	1589	74.079
T4	R1	2432	1826	75.082
	R2	2135	1628	76.252
	R3	2246	1676	74.621
	R4	1960	1482	75.612
	R5	2217	1541	69.508

Appendix 8. Data of Complete Blood Count (CBC) under different treatment groups.

Treatment	Replication	WBC *10^9	Lymphocyte *10^9	Granulocyte *10^9	RBC *10^12	HB(g/dl)
T_1	T_1R_1(1)	75.1	65.8	8.2	2.61	9.5
	T_1R_1(2)	75.2	64.5	8.1	2.63	9.7
	T_1R_1(3)	73.6	63.7	8.3	2.62	9.8
	T_1R_2(1)	79.1	67.2	8.3	2.22	9.1
	T_1R_2(2)	78.2	66.5	8.4	2.19	9.4
	T_1R_2(3)	78.6	65.4	8.2	2.21	9.6
	T_1R_3(1)	70.9	59.7	11.6	2.42	9.9
	T_1R_3(2)	69.6	58.6	10.8	2.41	9.3
	T_1R_3(3)	68.4	58.5	10.2	2.40	9.5
	T_1R_4(1)	58.2	52.7	9.5	2.75	11.2
	T_1R_4(2)	57.5	53.2	9.4	2.74	11.3
	T_1R_4(3)	56.3	54.7	8.9	2.73	11.6
	T_1R_5(1)	67.7	59.1	8.5	2.74	11.4
	T_1R_5(2)	66.5	58.3	8.2	2.75	11.6
	T_1R_5(3)	65.4	56.5	8.6	2.73	11.3

Appendix 8 (**Cont'nd**)

Treatment	Replication	WBC *10^9	Lymphocyte *10^9	Granulocyte *10^9	RBC *10^12	HB(g/dl)
T_2	T_2R_1(1)	69.1	57.6	8.3	2.7	10.6
	T_2R_1(2)	68.5	54.6	8.1	2.69	10.5
	T_2R_1(3)	67.8	52.4	8.2	2.74	10.2
	T_2R_2(1)	76.3	61.4	10.9	2.51	9.8
	T_2R_2(2)	75.4	58.3	9.8	2.53	9.7
	T_2R_2(3)	74.8	54.8	10.2	2.59	9.5
	T_2R_3(1)	65.3	52.6	9.6	2.3	8.7
	T_2R_3(2)	65.8	48.5	9.4	2.37	8.3
	T_2R_3(3)	64.2	46.5	9.1	2.35	8.4
	T_2R_4(1)	43.3	40.3	4.2	2.43	9.8
	T_2R_4(2)	43.1	38.6	4.1	2.41	9.6
	T_2R_4(3)	42.5	37.6	4.4	2.45	9.5
	T_2R_5(1)	54.9	45.1	3.8	2.55	10.3
	T_2R_5(2)	53.6	43.2	3.4	2.54	10.7
	T_2R_5(3)	54.8	42.4	3.2	2.51	10.1

Appendix 8 (**Cont'nd**)

Treatment	Replication	WBC *10^9	Lymphocyte *10^9	Granulocyte *10^9	RBC *10^12	HB(g/dl)
T3	T_3R_1(1)	51.3	45.7	3.6	2.74	11.6
	T_3R_1 (2)	50.7	43.5	3.4	2.73	11.2
	T_3R_1 (3)	52.6	41.8	3.5	2.71	10.8
	T_3R_2(1)	36.6	32	3.3	2.37	9.8
	T_3R_2 (2)	35.5	30	3.2	2.36	9.6
	T_3R_2 (3)	34.2	29	3.1	2.35	8.9
	T_3R_3(1)	35	33	1.8	3.02	12.3
	T_3R_3 (2)	36	28	1.6	3	12.1
	T_3R_3 (3)	37	31	1.7	3.05	11.9
	T_3R_4(1)	37.1	33.5	2.4	2.67	10.8
	T_3R_4 (2)	36.7	32.6	2.3	2.63	10.7
	T_3R_4 (3)	35.8	31.5	2.1	2.61	10.5
	T_3R_5(1)	29.7	26.8	1.9	2.32	9.2
	T_3R_5 (2)	28.5	25.4	1.6	2.34	9.5
	T_3R_5 (3)	29.3	24.8	1.8	2.36	9.4

Appendix 8 (**Cont'nd**)

Treatment	Replication	WBC *10^9	Lymphocyte *10^9	Granulocyte *10^9	RBC *10^12	HB(g/dl)
T4	T_4R_1 (1)	34.5	31.5	2	2.71	11.4
	T_4R_1 (2)	34.8	32.5	2.1	2.70	10.9
	T_4R_1 (3)	33.6	30.3	2.5	2.72	11.2
	T_4R_2(1)	43	33.9	6.5	2.11	8.4
	T_4R_2 (2)	42	32.5	6.1	2.13	8.3
	T_4R_2 (3)	41	29.7	5.9	2.10	8.6
	T_4R_3(1)	44.2	37.9	4.6	2.64	11
	T_4R_3((2)	43.6	38.4	4.1	2.66	11.4
	T_4R_3((3)	42.9	39.6	3.8	2.63	10.6
	T_4R_4(1)	43.3	36.8	4.3	2.56	10.8
	T_4R_4 (2)	44.2	35.5	4.6	2.54	10.7
	T_4R_4 (3)	43.6	33.4	4.1	2.53	10.5
	T_4R_5(1)	44.5	37.3	4.1	2.63	9.9
	T_4R_5 (2)	43.6	36.5	4	2.66	9.7
	T_4R_5 (3)	42.8	33.6	4.3	2.67	9.8

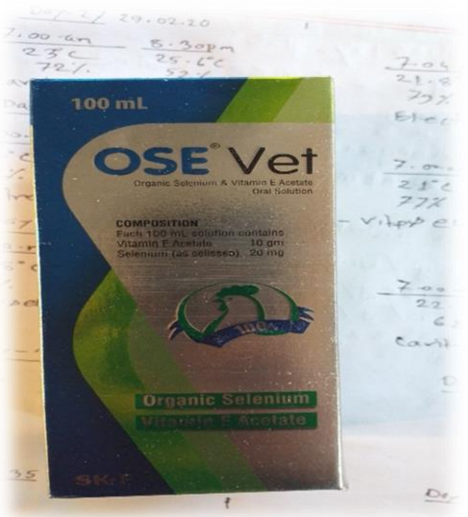
Appendix 9. Some photographs during the period of experiment conducted at SAU poultry farm.

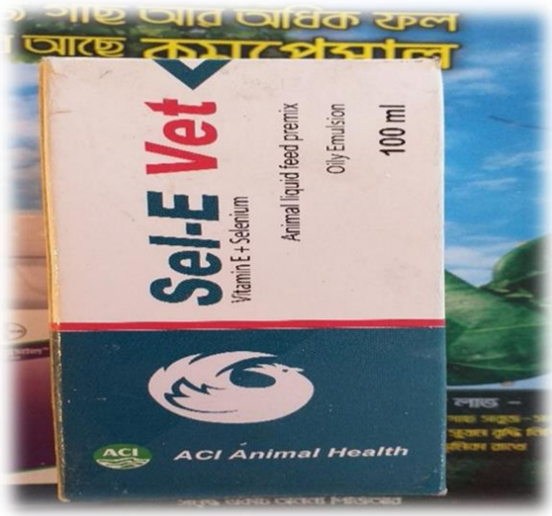
Some activities performed before and after arriving of DOC

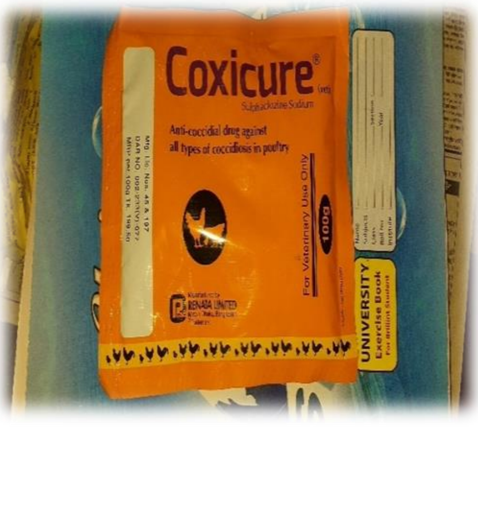
Appendix 9. Cont'd

Monitoring of research activities by the honorable supervisor

Appendix 9. Cont'nd







Different types of medication and vaccine used in the experiment

Appendix 9. Cont'd

Feeding and watering management of broiler chicken

Appendix 9. Cont'd

Collection of blood sample at the age of 35 days

Appendix 9. Cont'd

Postmortem of dead broiler

Appendix 9. Cont'd

Appendix 9. Cont'd

Weight measurement of broiler after dressing