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SELENIUM-ENRICHED BACTERIAL PROTEIN AS A SOURCE OF ORGANIC SELENIUM IN BROILER CHICKENS

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By

DALIA ABD ALLA MOHAMED ABD ALLA

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

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DEDICATION

This work is dedicated to those who motivated and helped me throughout my study

My father and mother who always prays for my success

My brothers and sisters for their constant encouragement and support

My husband (Mohammed) and sons (Moayed, Eyad, Kareem) for being my inspiration

My sister (Kaltham), without her support I could not have accomplished this work

My friends and colleagues

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

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Chairman : Associate Professor Anjas Asmara @ Ab. Hadi Samsudin, PhD
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Selenium (Se) is an essential dietary trace mineral associated with several important roles in the biological processes. Livestock are routinely supplemented with various Se sources and many studies have indicated that organic Se is more efficient and bioavailable than inorganic sodium selenite (SS). Some bacterial strains have the ability to produce organic selenium biologically through selenite microbial reduction and accumulate it in their cells as Se-containing proteins. The possibility of using bacterial organic Se as a feed supplement may provide an interesting source of organic Se for animals and also humans through the food chain. Hence, this study aimed to identify a potential new product of bacterial organic Se and show the possibility of using it in animals' feed to improve animals' Se status and antioxidant status.

In the first experiment of this study, four isolates, identified as *Enterobacter cloacae* (ADS1, ADS7, ADS11), and *Klebsiella pneumoniae* (ADS2) from rumen fluid origin, and one isolate from hot spring water (*Stenotrophomonas maltophilia* (ADS18), were able to transform 47.17- 62.52% of the absorbed inorganic Se into organic form (Se-containing proteins). Organic Se-containing proteins in all the selected strains showed *in vitro* antioxidant properties, therefore, these strains may offer a potential source of organic Se due to their Se-tolerant nature and higher biomass organic to inorganic Se ratio.

The second experiment examined the influence of bacterial organic Se on growth performance, meat quality and meat antioxidant, immunity and selenoproteins gene expression in broiler chickens. A total of 180, day-old, broiler chicks were randomly assigned to 5 groups: T1, basal diet; T2; basal diet + 0.3 mg/kg SS; T3, basal diet + 0.3 mg/kg ADS1-Se; T4, basal diet + 0.3 mg/kg ADS2-Se; T5; basal diet + 0.3 mg/kg

ADS18-Se. Different sources of bacterial Se or SS, increased the birds' survival rate, serum and tissues antioxidants and Se accumulation, improved meat's water holding capacity, and up-regulated some selenoproteins mRNA. Birds fed bacterial Se-protein showed high intestinal villus height, better Se retention with higher tissues Se deposition, better anti-oxidative capacity and meat tenderness with lower serum and tissues TBARS level more than SS. Selenium of ADS18 had a superior action in improving Se retention, antioxidant system and expression of selenoproteins compared to ADS1 and ADS2 bacterial Se.

The third experiment examined the effects of the combination of Vit E with bacterial organic Se and inorganic Se on growth performance, meat quality, immunity and antioxidant status of broiler chickens. A total of 216, day-old chicks were randomly assigned to six dietary treatments: T1, basal diet; T2, basal diet + 100 mg/kg α-tocopherol acetate; T3, Basal diet + 0.3 mg/Kg SS; T4, Basal diet + 0.3 mg /kg ADS18-Se; T5, Basal diet + 0.3 mg /kg SS+ 100 mg/kg α-tocopherol acetate; T6, 0.3 mg/kg ADS18-Se + 100 mg/kg α-tocopherol acetate. Supplementation of ADS18-Se in broiler chickens improved the performance, antioxidant system, meat quality, ceacum microbiota, and immunity response more than SS. Moreover, adding of 100 mg/kg of Vit E to the diet resulted in better performance, meat quality, and meat fatty acid modulation, with no effect on ceacum microbial population. The synergistic effect of Se and Vit E appear clearly in the performance, antioxidant system, and immunity response. The supplementation of Vit E alone had a better effect on most of the antioxidant and immunity parameters than the combination with Se, while the combination with bacterial organic Se had better effect than the combination with SS. Moreover, the inclusion of 100 mg/kg Vit E with 0.3 mg/kg ADS18-Se, effectively could support performance, antioxidant system, and immunity more than using ADS18-Se alone, while no difference between using SS alone or combined with Vit E.

In conclusion, sodium selenite and bacterial organic Se of ADS1, ADS2, and ADS18 could enhance broiler biological activities, however, bacterial organic sources were more efficient, and can be used to produce Se-enriched meat. Moreover, inclusion of Vit E with bacterial organic Se could improve it's efficiency.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PROTEIN BAKTERIA YANG DIPERKAYAKAN DENGAN SELENIUM SEBAGAI SUMBER ORGANIK SELENIUM UNTUK AYAM PEDAGING

Oleh

DALIA ABD ALLA MOHAMED ABD ALLA

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Selenium (Se) merupakan mineral surih pemakanan penting yang berkaitan dengan beberapa peranan penting dalam proses biologi. Secara rutin, ternakan diberi suplemen daripada pelbagai sumber Se, dan banyak kajian menunjukkan bahawa Se organik lebih efisien dan tersedia daripada segi biologi berbanding sodium selenite (SS) yang tidak organik. Sesetengah strain bakteria mempunyai keupayaan untuk menghasilkan selenium organik secara biologi melalui pengurangan mikroba selenit dan mengumpulnya dalam sel mereka sebagai protein yang mengandungi Se. Kebarangkalian menggunakan Se organik bakteria sebagai suplemen makanan dapat memberikan sumber organik Se yang menarik untuk haiwan dan manusia melalui rantai makanan. Maka, kajian ini bertujuan untuk mengenal pasti potensi satu produk baru Se organik bakteria dan menunjukkan kemungkinan untuk menggunakannya dalam makanan haiwan untuk menambah baik status Se haiwan.

Dalam eksperimen pertama kajian ini, empat asingan, yang dikenali sebagai *Enterobacter cloacae* (ADS1, ADS7, ADS11), dan *Klebsiella pneumoniae* (ADS2) daripada bendalir rumen, dan satu asingan daripada air mata air panas (*Stenotrophomonas maltophilia* (ADS18) untuk mengubah 47.17- 62.52% daripada Se tidak organik yang diserap, kepada organik (protein yang mengandungi Se). Protein organik yang mengandungi Se dalam semua strain terpilih menunjukkan sifat antioksidan *in vitro*. Maka, strain tersebut berpotensi menjadi sumber Se organik kerana mampu menahan Se, dan mempunyai nisbah biojisim Se organik yang tinggi berbanding Se tidak organik.

Eksperimen kedua meneliti pengaruh bakteria organik Se pada prestasi pertumbuhan, kualiti dan antioksidan daging, imuniti, dan selenoprotein ekspresi gen dalam ayam pedaging. Sebanyak 180 anak ayam pedaging berusia 1 hari, dibahagikan kepada 5 kumpulan secara rawak: T1, diet asas; T2; diet asas + 0.3 mg / kg SS; T3, diet asas + 0.3 mg / kg ADS1-Se; T4, diet asas + 0.3 mg / kg ADS2-Se; T5; diet asas + 0.3 mg / kg ADS18-Se. Sumber yang berbeza bagi Se dan SS bakteria meningkatkan kadar kelangsungan hidup burung, serum dan tisu antioksidan, dan pengumpulan Se meningkatkan kapasiti pengekalan air daging tersebut, serta “upregulated” sesetengah selenoprotein mRNA. Burung yang diberi makan bakteria organik Se menunjukkan vilus usus yang lebih tinggi, pengekalan Se yang lebih baik dengan tisu yang lebih tinggi, pengendapan Se, kapasiti anti-oksidatif yang lebih baik dan kelembutan daging dengan serum yang lebih rendah dan tahap tisu TBARS yang tinggi berbanding SS. Selenium ADS18 mempunyai hasil yang lebih baik dalam meningkatkan pengekalan Se, sistem antioksidan dan ekspresi selenoprotein, berbanding dengan ADS1 dan ADS2 Se bakteria.

Eksperimen ketiga mengkaji kesan-kesan gabungan vitamin E dengan bakteria Se organik dan Se tidak organik dalam prestasi pertumbuhan, kualiti daging, imuniti dan status antioksidan ayam pedaging. Sejumlah 216 anak ayam berusia 1 hari dibahagikan secara rawak kepada 6 rawatan pemakanan: T1, diet asas; T2, diet asas + 100 mg / kg α-tokoferol asetat; T3, diet asas + 0.3 mg / kg SS; T4, diet asas + 0.3 mg / kg ADS18-Se; T5, diet asas + 0.3 mg / kg SS + 100 mg / kg α-tocopherol asetat; T6, 0.3 mg / kg ADS18-Se + 100 mg / kg α-tokoferol asetat. Suplemen ADS18-Se bagi ayam pedaging meningkatkan prestasi, sistem antioksidan, kualiti daging, mikrobiota ceacum, dan tindak balas imuniti lebih daripada SS. Selain itu, penambahan 100 mg / kg vit E ke atas diet meningkatkan prestasi, kualiti daging dan modulasi asid lemak daging tanpa sebarang kesan pada populasi mikrob ceacum. Kesan bersinergi Se dan vit E jelas kelihatan dalam prestasi, sistem antioksidan, dan tindak balas imuniti. Suplemen vit E dengan sendiri mempunyai kesan yang lebih baik pada kebanyakan parameter antioksidan dan imuniti berbanding dengan gabungan bersama Se, sementara kombinasi dengan Se bakteria mempunyai kesan yang lebih baik berbanding kombinasi dengan SS. Selain itu, kemasukan 100 mg / kg vit E dengan 0.3 mg / kg ADS18-Se, dapat menyokong prestasi, sistem antioksidan, dan imuniti lebih daripada menggunakan ADS18-Se semata-mata, sementara SS sahaja atau gabungan dengan vit E, tidak membawa apa-apa perbezaaan.

Kesimpulannya, natrium selenit dan bakteria Se dari ADS1, ADS2 dan ADS18 boleh meningkatkan aktiviti biologi pedaging, namun, sumber bakteria organik jauh lebih cekap, dan boleh digunakan untuk menghasilkan daging diperkaya. Selain itu, kemasukan vit E dengan bakteria Se boleh juga meningkatkan kecekapan bakteria Se.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fullfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

µg	Microgram
µL	Microliter
µM	Micromole
a*	Yelloness
ADG	Average daily gain
ADS1	*Enterobacter cloacae* bacterial strain
ADS2	*Klebsiella pneumonia* bacterial strain
ADS18	*Stenotrophomonas maltophilia* bacterial strain
A/G:	Albumin/ globulin ratio
AL	Albumin
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
b*	Redness
BUN	Blood urea nitrogen
BW	Body weight
BWG	Body weight gain
CAT	Catalase
cDNA	Complementary-DNA
CFU	Colony forming units
Cm	Centimeter
CP	Crude protein
Cr	Creatinine

CRD	Complete randomized design
DIO1	Iodothyronine deiodinase 1
DIO2	Iodothyronine deiodinase 2
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
FA	Fatty acids
FCR	Feed conversion ratio
FI	Feed intake
G	Gram
G	Globulin
GAPDH	Glyceraldehyde-3-phosphate Dehydrogenase
GLM	General linear model
GSH-Px	Glutathione peroxidase
HB	Hemoglobin
HDL-C	High density lipoprotein cholesterol
HNO_3	Nitric acid
H_2O_2	Hydrogen peroxide
H.S	Hot spring water
HSe	Selenide
ICP.MS	Inductively coupled plasma mass spectrometer
IFN-γ	Interferon gamma
IgA	Immunoglobulin A
IgG	Immunoglobulin G

IgM	Immunoglobulin M
IL	Interleukin
kg	Kilogram
L*	Lightness
L	Liter
LDH	Lactate dehydrogenase
LDL-C	Low-density lipoprotein cholesterol
MDA	Malondialdehyde
mL	Milliliter
Mm	Millimeter
mM	Millimolar
MUSFA	Mono-unsaturated fatty acids
mRNA	Messenger ribonuclic acid
NADPH	Nicotinamide adenine dinucleotide phosphate
OD	Optical density
PBS	Phosphate buffer saline
PCV	Packed cell volume
PUSFA	Poly-unsaturated fatty acids
R.B	Rumen bacteria
RBCs	Red blood cells
RNA	Ribonuclic acid
SAS	Statistical analysis system
Se	Selenium
Se-Cys	Selenocystein

SELW1	Selenoproteins w
Se-Met	Selenomethionine
SFA	Saturated fatty acids
SOD	Superoxide dismutase
TAC	Total antioxidant
TBARS	Thiobarbituric acid reactive substances
TBS	Tris-buffered saline
TCOL	Total cholestrol
TG	Triglyceride
TNF-α	Tumor necrosis factor alpha
TP	Total protein
TCHOL	Total cholesterol
TXNDR1	Thirodxine reductase
USFA	Un-saturated fatty acids
VLDL-C	Very density lipoprotein cholesterol
WBCs	White blood cells
WG	Weight gain

CHAPTER 1

GENERAL INTRODUCTION

Selenium (Se) is an essential trace mineral, which is a natural constituent of the earth's crust and available in living organisms in small quantities. In 1817, Se was recognised by Jacob Berzelius Jöns as a toxic compound that causes major health problems in humans and animals. However, the outcome of researches over the years demonstrated that Se can be absorbed by the intestinal tract of animals and humans, and then play a vital role in their body functions and metabolic pathways. Selenium has several important roles: For example, it is a significant structural part of at least 25 proteins, such as glutathione peroxidase (GSH-Px) and thioredoxin reductase (Zhang, 2009). These catalysts have important roles in the body, and the discovery of their structural Se inclusion illustrated the biochemical role of this mineral. Selenium is involved in the body for reproduction and fertility, thyroid hormone metabolism, redox control of enzymes and proteins, immune system function, DNA synthesis, and in the reduction of the aging process (Suttle, 2010; Méplan, 2011). Moreover, dietary Se affects the oxidative status in the host by altering seleno-proteins expression (Ferguson & Karunasinghe, 2011). Therefore, an adequate supply of Se element in the diet of animals is essential for maintenance of their health, growth, and biochemical-physiological functions. The Se status must be controlled in humans and animals and the daily recommended intake level should be adequate to perform its functions (Mehdi *et al.*, 2013).

To ensure optimal level of Se in livestock, inorganic Se is usually added to the diet in the form of sodium selenite or sodium selenate. However, most of the researches showed that inorganic Se sources are poorly utilised and poorly absorbed by livestock (Suttle, 2010), while organic Se sources such as seleno-methionine (SeMet) and seleno-cysteine (SeCys) resulted in high bioavailability of Se in the body compared to inorganic sources.

Concerning the improvement in Se status of livestock, most recent studies have tended to investigate the effective organic sources of Se to be used in animal diets. The results of these previous studies showed different sources of organic Se, but the most popular and commercially available one is Se-yeast, which, showed good results in improving performance, production, fertility and physiological aspect in poultry (Surai *et al.*, 2010). This is because yeast has the ability to produce SeMet in the presence of Se. The SeMet can be used by the host immediately for protein synthesis, or be deposited in the tissues and animal products, and in turn benefit the human population via the food chain (Kuricová *et al.*, 2003).

Many studies showed that organic Se also can be produced by bacterial species during respiration or detoxification processes (Stolz *et al.*, 2002). Several bacterial strains isolated from different environmental sources were found to be capable of converting inorganic Se to organic forms. However, limited data are available on using bacterial

Se in animal feed as a source of Se, but some studies indicated that various biological compounds can be produced by microorganisms, such as Se-enriched exopolysaccharide, which is produced by *Enterobacter cloacae* Z0206 (Zeqing *et al.*, 2013). The product of bacterial strain *Enterobacter cloacae* Z0206 showed significant improvement in the innate and humoral immunity in mice (Xu *et al.,* 2009). Moreover supplementation of Se-enriched exopolysaccharides product to broiler chickens improved the antioxidant system and enhanced the immunity response (Zeqing *et al.*, 2013).

Poultry production is associated with various stresses, and Se as an important trace mineral in poultry nutrition is the main structural component in antioxidant enzymes to overcome oxidative stresses (Fairweather-Tait *et al.*, 2010). All recent studies demonstrated that the organic Se is more bioavailable than inorganic forms in poultry nutrition (Stępińska *et al.*, 2012; Chen *et al.*, 2014), which may be due to the differences in the absorption and metabolism of organic and inorganic Se. Based on different poultry studies, it appears that excess Se supplementation of different sources could increase the concentration of the Se mineral in plasma (Kuricová *et al.*, 2003), and higher Se concentration in serum, kidney, liver, and breast muscle were observed (Wang *et al.*, 2011b). Some studies indicated that organic Se in broiler and layer nutrition affects the antioxidant status and the total antioxidant capacity in the blood and tissues of the birds; the inhibition of hydroxyl radical was also elevated when Se-yeast was used rather than sodium selenite (Chen *et al.*,2014).

With regard to poultry performance and production, some researchers demonstrated that there is no relation between Se sources and poultry performance (Chantiratikul *et al.*, 2008a; Maysa *et al.*, 2009; and Chen *et al.*, 2014). However, some studies revealed that in comparison with inorganic Se, organic sources significantly increased live body weight in poultry (Maysa *et al.*, 2009), increased the birth rate and feed consumption and decreased mortality in the progeny of the layer (Wang *et al.*, 2011b). Although most of the reviewed studies reported better effects for organic Se on poultry performance in comparison with the inorganic Se, there are still some conflicting results between the studies.

In recent years, there has been interest in dietary incorporation of some minerals and vitamins as an economical way to enhance the body antioxidant status and increase the disease resistance in animals, as well as, enhance the delivery of essential nutrients to humans via animal products. The relation between dietary Se and Vit E had been well illustrated. Researchers suggested that supplementation of Se or Vit E has a beneficial effect on the antioxidant system and immune response (Guo *et al.*,2003), however, their dietary combination may be more effective in improving growth performance, serum and meat antioxidant status and enhance innate and humoral immunity (Habibian *et al.,* 2015).

The possibility of delivering Se in a highly bioavailable form using Se-enriched bacterial protein has not been deeply investigated. However, according to recently data, there are some strains of bacteria capable of up-taking inorganic Se and accumulate it in their cells as Se-containing proteins. Thus, the current study was initiated to examine such effects with the following hypothesis and objectives.

Hypothesis statements

1. Se- enriched bacteria could be isolated from rumen fluid and hotspring water, and their Se- protein extract would identify as organic Se source.
2. Se-enriched bacterial protein would alter growth performance, Se- status meat quality, antioxidant status and immunity response in broiler chickens.
3. Combination of Vit E with Se-enriched bacterial protein would alter growth performance, antioxidant status, ceacum microbial profile and cytokines gene expression in broiler chickens.

The general objective

To identify a potential source of bacterial organic Se from rumen fluid and hotspring water and examine the possibility of using it as a feed supplement to improve animals' Se status.

The specific objectives:

1. To isolate and identify potential selenium-enriched bacteria of high Se-containing protein, and extraction of their biomass Se-protein as a source of organic selenium.
2. To examine the effects of dietary supplementation of inorganic and bacterial organic selenium on growth performance, meat quality, selenium status, immunity, and hepatic selenoproteins gene expression in broiler chickens.
3. To investigate the effects of dietary supplementation of inorganic and bacterial organic Se sources and their Vit E combination on growth performance, antioxidant status, immunity response, cytokine gene expression in broiler chickens.

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