UNIVERSITI PUTRA MALAYSIA

EFFECTS OF SELENIUM-ENRICHED BACTERIAL PROTEIN SUPPLEMENTATION ON EGG QUALITY, ANTIOXIDANT PROFILES, AND GENE EXPRESSION IN LAYING HENS

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By

IBRAHIM ALIYU MUHAMMAD

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

April 2021

DEDICATION

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

My Beloved Parents who stood firmly and always prays for my success
My Lovely Brothers and Sisters for their persistent encouragement and support
My Dearest Wife and Kids for being my inspiration
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 : Prof. Anjas Asmara @ Ab. Hadi Samsudin, PhD
Faculty : Agriculture

Micronutrients are paramount in animal nutrition for the normal biological, and physiological process of the living cells. Selenium (Se), known to play a vital role in human and animal biochemical functions. Se bioavailability depends on the form of dietary Se offered. Organic forms have been recognized to have superior absorption and efficacy compared to inorganic forms. Researchers are now developing an interest in exploring different forms of organic Se using strains of microorganisms, capable of bio-transforming inorganic Se to elemental Se and accumulate in their cells as Se-containing proteins. The use of Se-enriched bacterial proteins as 'dietary Se supplements' in animal nutrition would provide sufficient organic Se for humans and animals in the food chain, potentially addressing selenium deficiency. Thus, the current research is centered on investigating such an advantage and how it can successfully be transferred to the end product while reaping its benefits.

In the first experiment of this study, there was an optimization of culture medium and temperature for the production of organic selenium by the *Stenotrophomonas maltophilia* (ADS18) isolated from hot spring water. The results showed brain heart infusion supports higher cell biomass at 35 ^{0}C for an incubation time lesser than 48 h. The higher value of pellets totals Se yield was 45.10, 30.20, and 27.48 for nutrient broth, brain heart infusion, and tryptic soy media, respectively. However, after adding the same amount of Na_2SeO_3 to all tested media, organic Se of *S. maltophilia* was readily concentrated in both supernatant and cell biomass, with higher yields of cell biomass in brain heart media and organic Se nutrient broth media.

In the second experiment, the effects of sodium selenite, selenium yeast, and *Stenotrophomonas maltophilia* enriched bacterial protein (ADS18) on laying performance, egg quality traits, egg yolk, and tissue Se concentrations, and small intestine morphology under a normal environment was examined. A total of 144 Lohman

Brown-Classic laying hens, 18 weeks of age were randomly divided into four equal groups, each contained six replicates. A corn-soybean meal basal diet was supplemented with no Se supplementation (Con), basal diet plus 0.3 mg/kg feed inorganic sodium selenite (Na_2SeO_3) (SS), basal diet plus 0.3 mg/kg Se-yeast (SY), and basal diet plus 0.3 mg/kg *Stenotrophomonas maltophilia* ADS18 enriched bacterial protein. The results showed that different dietary Se sources affected laying rate, average egg weight, daily egg mass, FCR, live body weight (LBW) significantly ($p < 0.05$). However, average daily feed intake and shell-less and broken egg were unaffected ($p > 0.05$) among the treatment groups. Furthermore, the dietary Se supplemented group had higher ($p < 0.05$) Se concentrations in egg yolk and breast tissue compared to the control group. Hens fed ADS18 or Se-Yeast groups had significantly ($p < 0.05$) higher villi height in the duodenum and jejunum than those fed Na_2SeO_3 or a basal diet for the small intestine morphology. However, as compared to organic Se fed (ADS18 or Se-Yeast) hens, the basal diet group had the highest ($p < 0.05$) ileum villus height, while the SS group had the lowest among the treatment groups. In comparison to the Con group, dietary Se treatment decreased total serum cholesterol and serum triglycerides levels significantly ($p < 0.05$). Specifically, organic Se (Se-enriched bacteria and Se-yeast) sources show better results in some performance index, intestinal villus height, some serum biochemical parameters compared to the inorganic form (sodium selenite).

The third experiment examined the effect of sodium selenite, selenium yeast, and bacterial enriched protein on egg yolk and tissue antioxidant profiles and oxidative stability. The egg yolk and breast tissue samples were used for the analysis of antioxidant profile (total carotene, cholesterol, phenolic, and flavonoid content), oxidative stability-influencing status (antioxidant capacity, thiobarbituric acid reactive substances (TBARS)), and egg yolk color assay. Fresh eggs were sampled on the 16 weeks and stored at $4 \pm 2\ ^{0}C$ for 14 days for shelf-life determinations and subjected to the same analysis. The results showed that dietary Se supplementation significantly ($p < 0.05$) improved egg yolk color, the antioxidant profile of egg yolk, and breast meat (total carotenoid and phenol content). The oxidative stability parameters of the eggs, breast and thigh muscle, and plasma showed significantly ($p < 0.05$) decreased concentrations of primary oxidation products (MDA) formed in the egg yolk, tissue, and blood collected. However, the MDA content increased ($p < 0.05$) with an advanced storage period in Con and Na_2SeO_3 supplemented groups. Moreover, as compared to Na_2SeO_3-fed hen eggs, dietary organic Se (ADS18 or Se-Yeast) had the lowest MDA content after two weeks of storage. The source of organic Se supplemented is important for egg enrichment and antioxidant properties. Thus, possible to produce fortified eggs enriched with organic selenium.

The fourth experiment investigates the effects of organic and inorganic dietary selenium supplementation on gene expression profiles in oviduct tissue, antioxidant enzyme capacity, and the caecum microbiome of laying hens. A total of 24 hens were selected randomly from the four treatments and slaughter at 39 weeks old, and 16-weeks of the experiment. Uteri and liver tissue samples were collected from hens for quantitative polymerase chain reaction (qPCR). The hens ceacum digesta was collected for microbial population quantification. The basal diets supplemented with 0.3 mg/kg of different organic Se sources and sodium selenite improves the hen's caecum microbiota, upregulated uterine genes, and selenoproteins mRNA levels. This research reaps the advantage of tissue sampling from specialized segments of the oviduct that consecutively form different egg components. Both organic Se (bacterial Se proteins and Se-yeast) sources improve the expression of functional genes involved in the egg (eggshell biomineralization) formation and selenoproteins compared to inorganic and non-Se supplemented hens.

In conclusion, different dietary Se sources can improve the biological activities of the layers; however, Se from organic sources appears to be reliable and may be used to produce Se-enriched animal products. Eggs seem to be a valuable source of Se for humans, as the Se is distinctively incorporated into chemical compounds, and consequently, plays a vital role. Indeed, this will provide consumers with a range of animal-derived products that have been nutritionally enriched in a way that they can act as a vector for providing significant quantities of health-promoting nutrients to help achieve and maintain good health.

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

KESAN PEMBERIAN PROTEIN BAKTERIA YANG DIPERKAYA SELENIUM KEPADA PENAMBAHBAIKAN KUALITI TELUR, PROFIL ANTIOKSIDA DAN EKSPRESI GEN AYAM TELUR

Oleh

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Mikro-nutrien adalah yang terpenting dalam pemakanan haiwan untuk proses biologi normal, dan fisiologi sel hidup. Selenium (Se) terkenal dengan memainkan peranan yang penting dalam fungsi biokimia manusia dan haiwan. Biokeperolehan Se bergantung pada bentuk Se dalam makanan yang ditawarkan. Bentuk organik telah diiktiraf mempunyai penyerapan dan keberkesanan yang unggul berbanding dengan bentuk bukan organik. Para penyelidik kini berminat untuk meneroka pelbagai bentuk organik Se menggunakan mikroorganisma, yang mampu mengubah Se tidak organik secara bio menjadi Se unsur dan terkumpul di dalam sel mereka sebagai protin yang mengandungi Se. Penggunaan protin bakteria yang diperkaya dengan Se sebagai 'makanan tambahan Se dalam pemakanan haiwan akan menyediakan Se organik yang cukup untuk manusia dan haiwan melalui rantaian makanan dan dapat mengatasi masalah kekurangan selenium. Oleh itu, penyelidikan semasa bertujuan untuk menyelidik kelebihan yang dinyatakan dan bagaimana untuk memindahkan kebaikan tersebut ke produk akhir.

Dalam eksperimen pertama kajian ini, terdapat penambahbaikan medium dan suhu kultur untuk pembiakan selenium organik oleh *Stenotrophomonas maltophilia* (ADS18) yang diasingkan dari kolam air panas. Hasil kajian menunjukkan 'brain heart infusion' menghasilkan biojisim sel yang lebih tinggi pada suhu 35^0C untuk masa inkubasi kurang dari 48 jam. Nilai pelet yang lebih tinggi dari hasil Se masing-masing adalah 45.10, 30.20, dan 27.48 untuk kaldu nutrien, 'brain heart infusion', dan media 'tryptic soy'. Walaubagaimanapun, penambahan Na_2SeO_3 kepada semua media, Se organik iaitu *S. maltophilia* ADS18 terhasil pekat di dalam kedua-dua supernatan dan biojisim sel, dengan penghasilan biojisim sel yang lebih pekat di dalam media 'brain heart' dan media Se organik kaldu nutrien.

Dalam eksperimen kedua, kesan selenit natrium, yis selenium, dan protin bakteria diperkaya *Stenotrophomonas maltophilia* (ADS18) terhadap prestasi penelur, sifat kualiti telur, kuning telur, kepekatan Se di tisu dan morfologi usus kecil diuji di bawah persekitaran normal. Sebanyak 144 ekor ayam penelur 'Lohman Brown-Classic', berusia 18 minggu dibahagikan secara rawak kepada empat kumpulan yang sama, masing-masing mengandungi enam replika. Diet basal makanan jagung-kacang soya ditambah dengan tiada pemberian Se (diet kawalan), diet basal ditambah 0.3 mg / kg makanan selenit natrium tidak organik (Na_2SeO_3) (SS), diet basal ditambah 0.3 mg / kg Se-yis (SY), dan basal diet + 0.3 mg / kg *Stenotrophomonas maltophilia* ADS18 yang diperkaya protin bakteria. Hasil menunjukkan pemberian Se yang berbeza dalam makanan terkesan ke atas prestasi penelur, purata berat telur, jisim telur harian, nisbah penukaran makanan, berat hidup yang nyata ($p < 0.05$). Namun begitu, purata pengambilan makanan harian, penghasilan telur tiada kulit dan telur yang pecah tidak terkesan ($p > 0.05$) di antara kumpulan rawatan. Tambahan pula, kumpulan yang diberi Se mempunyai peningkatan ($p < 0.05$) kepekatan Se di dalam kuning telur dan tisu dada berbanding dengan kumpulan kawalan. Kumpulan ayam betina yang diberi ADS18 atau Se-yis mempunyai vilus yang nyata lebih tinggi ($p < 0.05$) dalam duodenum dan jejunum berbanding ayam yang diberi Na_2SeO_3 atau makanan kawalan bagi morfologi usus kecil. Walaubagaimanapun, untuk dibandingkan dengan ayam yang diberi Se organic (ADS18 atau Se-yis), kumpulan kawalan mempunyai vilus ileum yang paling tinggi ($p < 0.05$), dan kumpulan SS paling rendah di antara kumpulan rawatan. Jika dibandingkan dengan kumpulan kawalan, kumpulan yang diberi rawatan Se dengan nyata merendahkan ($p < 0.05$) jumlah kolesterol serum dan tahap trigliserida serum. Sumber Se organic (bakteria diperkaya Se dan Se-yis) khususnya menunjukkan hasil yang lebih memuaskan dalam beberapa indeks prestasi, ketinggian vilus usus, beberapa parameter biokimia serum berbanding dengan bentuk tidak organic (selenit natrium).

Eksperimen ketiga mengkaji kesan natrium selenit, selenium yis, dan protin yang diperkaya bakteria pada kuning telur dan profil antioksida tisu dan kestabilan oksidatif . Sampel kuning telur dan tisu dada digunakan untuk analisis profil antioksida (jumlah karotena, kolesterol, fenolik, dan kandungan flavonoid), status mempengaruhi kestabilan oksidatif (kapasiti antioksida, bahan reaktif asid thiobarbituric (TBARS)), dan ujian warna kuning telur. Telur baru diuji pada minggu ke-16 dan disimpan pada suhu 4 ± 2 ºC selama 14 hari untuk penentuan jangka hayat dan dijalankan analisis yang sama selepas tempoh penyimpanan. Hasil menunjukkan makanan tambahan Se dengan nyata ($p < 0.05$) meningkatkan warna kuning telur, profil antioksida kuning telur, dan tisu dada (jumlah karotena dan kandungan fenol). Parameter kestabilan oksidatif telur, otot dada dan peha, dan plasma menunjukkan penurunan yang nyata ($p < 0.05$) pada kepekatan produk utama oksidatif (MDA) yang terkumpul dalam kuning telur, tisu dan darah yang disimpan. Tetapi, kandungan MDA bertambah ($p < 0.05$) apabila disimpan dalam kumpulan yang diberi kawalan dan Na_2SeO_3. Tambahan pula, berbanding dengan ayam yang diberi Na_2SeO_3, Se organik (ADS18 atau Se-yis) mempunyai kandungan MDA paling rendah selepas disimpan selama dua minggu. Sumber makanan tambahan Se organik penting untuk pengkayaan telur dan sifat antioksida. Oleh itu, dapat menghasilkan telur yang diperkaya dengan selenium organik.

Eksperimen keempat mengkaji kesan makanan tambahan selenium organik dalam makanan dan bukan organik terhadap profil ekspresi gen pada tisu oviduktus, kapasiti antioksida, dan mikrobioma caecum pada ayam penelur. Sebanyak 24 ekor ayam dipilih secara rawak dari empat kumpulan dan disembelih pada umur 39 minggu dan 16 minggu eksperimen. Sampel tisu uteri dan hati dikumpulkan dari ayam bagi tindakbalas berangkai polimerasi (qPCR). Digesta dari caecum ayam dikumpulkan untuk penkuantitian populasi mikroba. Diet asas dilengkapi dengan 0.3mg / kg sumber Se organik yang berbeza dan selenit natrium meningkatkan mikrobiota caecum ayam, meningkatkan gen uteri dan tahap mRNA selenoprotin. Penyelidikan ini mengambil kira pengambilan sampel tisu dari segmen khas oviduktus yang membentuk komponen telur yang berbeza. Kedua-dua sumber Se organik (protin bakteria Se dan Se-yis) terbukti lebih baik dalam meningkatkan ekspresi fungsi gen yang terlibat dalam pembentukan telur (biomineralisasi kulit telur) dan selenoprotin berbanding dengan ayam yang yang diberi Se bukan organik dan tanpa Se.

Kesimpulannya, sumber makanan Se yang berbeza dapat meningkatkan aktiviti biologi ayam penelur, namun, Se dari sumber organik terbukti berkesan, dan mungkin dapat digunakan untuk menghasilkan produk haiwan yang diperkaya dengan Se. Telur merupakan sumber yang diperkaya dengan Se bagi manusia, kerana Se secara khusus dicampurkan ke dalam sebatian kimia, dan justeru, memainkan peranan penting. Malah, ini dapat memberi pengguna pelbagai produk haiwan yang diperkaya secara nutrisi yang berfungsi sebagai vektor penyampaian sejumlah besar nutrien yang dapat meningkatkan kesihatan untuk memperoleh dan menjaga kesihatan.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment 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 APPENDICES

LIST OF ABBREVIATIONS

μg	Microgram
μL	Microliter
μM	Micromole
a*	Yellowness
ADS18	*Stenotrophomonas maltophilia* bacterial strain
A/G	Albumin/globulin ratio
AL	Albumin
$AlCl_3$	Aluminum chloride
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
AST	Aspartate aminotransferase
ATS	Amino acid transport system
b*	Redness
BHI	Brain heart infusion
BUN	Blood urea nitrogen
BW	Body weight
BWG	Body weight gain
$(CH_3)_x$ SeH	Dimethylselenide or trimethylselenonium
C*	Chroma
$C_6N_6FeK_3$	Potassium ferricyanide
CAT	Catalase
cDNA	Complementary-DNA

CFU	Colony forming units
CH_3SeH	Methaneselenol
CIE	*Commission Internationale d'Eclairage*
Cm	Centimeter
Cr	Creatinine
CRD	Complete randomized design
CT	Cycle threshold
DIO1	Iodothyronine deiodinase 1
DIO2	Iodothyronine deiodinase 2
DMSeP	Dimethylselenonium propionate
DNA	Deoxyribonucleic acid
EAA	Equivalents of ascorbic acid
EAC	Ehrlich's ascites carcinoma
EDTA	Ethylenediaminetetraacetic acid
EGA	Equivalents gallic acid
EGCG	Epigallocatechin-3-gallate
FCR	Feed conversion ratio
FDA	Food and Drug Administration
$FeCl_3$	Ferric chloride
FI	Feed intake
FRAP	Ferric reducing antioxidant power
G	Gram
G	Globulin
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase

GGT	Gamma-glutamyl transferase
GIT	Gastro intestinal tract
GLM	General linear model
GNMT	Gene glycine N-methyltransferase
GSH-Px	Glutathione peroxidase
GS-Se-SG	Seleno-diglutathione
H*	Hue
H.S	Hot spring water
H:D	Height to crypt depth ratio
H_2O_2	Hydrogen peroxide
H_2Se	Hydrogen selenide
H_2SeO_3	Selenious
H_2SeO_4	Selenic
Hb	Hemoglobin
HDL-C	High density lipoprotein cholesterol
HMG-CoA	3-hydroxy 3-methylgluatryl coenzyme A
HMSeBA (OH-SeMet)	2-hydroxy- 4-melhylselenobutanoic acid
HNO_3	Nitric acid
HSe	Selenide
ICP.MS	Inductively coupled plasma mass spectrometer
ICP-OES	Inductively coupled plasma-optical emission spectrometry
K_2HPO_4	Di potassium hydrogen phosphate
kg	Kilogram
L	Liter

L^*	Lightness
LAB	Lactic acid bacteria
LDH	Lactate dehydrogenase
LDL-C	Low-density lipoprotein cholesterol
MDA	Malondialdehyde
MeSeCys	Se-methyl selenocysteine
mL	Milliliter
Mm	Millimeter
mM	Millimolar
mM	Millimolar
mRNA	Messenger ribonuclic acid
$NaC_{12}H_{25}SO_4$	Sodium dodecyl sulfate
NADPH	Nicotinamide adenine dinucleotide phosphate
$NaNO_2$	Sodium nitrite
NaOH	Sodium hydroxide
OC-116	Ovocleidin-116
OC-17	Ovocleidin-17
OCX32	Ovocalyxin-32
OCX-36	Ovocalyxin-36
OD	Optical density
PBS	Phosphate buffer saline
PCV	Packed cell volume
PM	Phosphomolybdenum
PPAR γ	Peroxisome proliferator-activated receptor-γ ligand

qPCR	real-time PCR
RBCs	Red blood cells
RCBD	Randomized completely design
RDA	Recommended dietary allowance
RNA	Ribonuclic acid
RPM	Rotation per minutes
RT-PCR	real-time PCR
RYCF	Yolk Colour Fan® scale
SAS	Statistical analysis system
Se	Selenium
Se-Cys	Selenocystein
SeHLan	Selenium-homolanthionine
SELW1	Selenoproteins w
SEM	Standard error mean
Se-Met	Selenomethionine
SeNPs	Seleno-nanoparticles
SeO_3^{-2}	Selenite
SeO_4^{-2}	Selenate
SeY	Se-enriched yeast
SOD	Superoxide dismutase
SREBP	Sterol regulatory element-binding protein
TAC	Total Antioxidant Capacity
TBARS	Thiobarbituric acid reactive substances
TBS	Tris-buffered saline

TCE	Trichloroethylene
TCHOL	Total cholesterol
TEP	1, 1, 3, 3-tetraethoxypropane
TG	Triglyceride
TP	Total protein
TSB	Tryptic soy broth
TXNDR1	Thyroxine reductase
VFA	Volatile fatty acid
VLDL-C	Very density lipoprotein cholesterol
WG	Weight gain
XO	Xanthine oxidase
Zn-SeMet	Zinc selenomithionine

CHAPTER 1

GENERAL INTRODUCTION

Micronutrients are paramount in animal nutrition for normal health, growth, and development, biochemical, and physiological process of the living cells (Scott et al., 1982). In particular, Selenium (Se), known to play a crucial role in human and animal biochemical functions such as improving antioxidant and immune response, living dell hormone (thyroid) metabolism (Surai, 2006). As an essential trace mineral, Selenium (Se) was verified by (NRC, 1994). The keen interest in studying the biological activity of Se (selenosis) begun somewhat in 1930 and culminate in the 1950s by studies of liver necrosis (vitamin E and cysteine) in rats (Klaus Schwarz & Foltz, 1957), preventive measures against exudative hemorrhagic diathesis, muscle dystrophy (Muth et al., 1958) and investigations of the synergy between Vit E and Se continues. This paves the way for a nutritionist to deep-rooting their investigation in metabolic function and documenting the findings in the food of both humans and animals. However, Shamberger (1983) profile many studies particularly with its deficiency causing diverse diseases like; liver necrosis, exudative diathesis, nutritional muscular dystrophy, poor feathering, placental retention, mastitis, cystic ovaries, cancer, cardiovascular ailments, immunodeficiencies, poor fertility, etc., among many in animal and humans.

An imbalance between free radicals produced within the system and their detoxification (oxidative stress) has a tremendous impact on immune and reproductive systems as well as major growth and development parameters and the wellbeing of the animals (Mavangira & Sordillo, 2018). Thus, it is necessary to preserve the redox balance in the cells and tissues to prevent the disadvantageous consequences of relevant stress. The role of trace elements in living cells is well established among which are endocrine regulation of energy metabolism and energy homeostasis, and oxidative balance which in turn is directly linked to normal growth of the living cells (Wiernsperger and Rapin, 2010). Specifically, Selenium (Se) is confirmed to be a trace element of high advantage due to its antioxidant, chemopreventive, and anti-inflammatory properties that influence positively the health of both mammals and avian species (Pappas et al., 2019). Selenium has been identified and categorized to be an integral part of more than 30 distinct selenoproteins, i.e., Se-containing proteins products of twenty-five genes and enzymes particularly glutathione peroxidases (Rotruck et al., 1973), a group of antioxidant enzymes that play key roles in the defense of body cells from harm due to formation of free-radicals (Sunde, 1997; Arthur, 2000). They were meticulously characterized based on their functions, for instance; glutathione peroxidases, the thioredoxin reductases, and the iodothyronine deiodinases (Pappas et al., 2019).

While trace minerals requirements are critical, Se has previously been identified as a toxic microelement in animals (Davidson, 1940; Muth & Binns, 1964), and humans (Watts, 1988; Yang et al., 1983). In the European Union, organic Se supplementation is limited to 0.2 mg Se per kg of complete feed, while total Se content is limited to 0.5 mg/kg of complete feed (EU, 2013). Similarly, the Food and Drug Administration (FDA) sets a limit of 0.3 mg/kg (as fed) for dietary Se supplementation (FDA, 2019). According to FDA guidelines, any concentration greater than 0.3 mg/kg but less than the maximum tolerable amount can be considered supranutritional. The chemical form of Se supplementation is thus a common practice in nutritional (poultry) diets. Se occurs principally in plants and animal meat in both inorganic forms such as; selenite, selenate, selenide, and the organic forms like selenomethionine, methylselenocystine, and selenocysteine (Rayman et al., 2008; Rayman, 2008; Dörr et al., 2013). Organic selenomethionine forms are present in plant sources, such as yeast (Schrauzer, 2000) whereas selenocysteine is from animal foods and meat (Petrovič et al., 2006; Mahima et al., 2012).

For the aforementioned reason, Surai & Fisinin (2016), Moreda-Piñeiro et al. (2017), and Juniper et al. (2019) entrenched with specific to animal species that Se bioavailability depends on the form of dietary Se offered. Having been previously mentioned, selenium compounds assimilation varies with the chemical form (> 90% for selenomethionine and < 80% selenite (Santhosh Kumar & Priyadarsini, 2014)) in which is administered and its quantity. Selenium absorption mainly occurred in the duodenum (Wright and Bell, 1966), by enterocytes via amino acid transport system (ATS), thereafter reduced to elemental selenium and fused into glutathione peroxidase (GPx) (Rayman, 2008; Navarro-alarcon and Cabrera-vique, 2008; Riaz and Mehmood, 2012). Organic forms have been recognized to have superior absorption and efficacy compared to inorganic forms (Surai et al., 2017). Examples, organic form are Se-enriched yeast, selenomethionine among others and in contrast to inorganic form such as the commonly used sodium selenite, were shown to be absorbed and fast by the small intestine, independently of its levels in the organism and defecated mainly via kidneys (Suchý et al., 2014; Zia et al., 2018). The Se (excess or unutilized) is expelled from the body mainly via urine, sweat, exhaled air (mammals), and feaces or droppings (avian *spp*) in excess of high intake in the diet. The transportation medium of absorbed Se is via the bloodstream, which is bound to plasmatic proteins and distributed to all tissues within the body (Cousins and Cairney, 1961). SeMet is, however, contemplate as a storage form of Se, starting from synthesized selenocysteine (SeCys) as the functional biological form of Se and incorporated into the active selenoproteins site in cellular units (Surai et al., 2019). SeMet reserves within the animal body system can have been suggested to be utilized when the Se requirements increase with a decrease in supply which may result from the decrease in feed intake or other related factors during stress conditions (Surai et al., 2017; Surai, 2018).

Se-yeast of various commercial types were found to populate markets and proved to be an effective source of Se for poultry and animal production (Surai, 2006; Fisinin et al., 2008; Surai et al., 2010; Surai & Fisinin, 2014; Surai and Fisinin, 2015). SeMet as a dietary supplement was recommended as an alternative to enhanced the Se status of poultry and other related farm animals (Schrauzer, 2000; Schrauzer and Surai, 2009). Therefore, after the FDA (2000) approval, many researchers developed an interest in exploring different forms of organic Se. Among which are Selenomethionine (SeM), Se-enriched yeast (SeY) (Rayman, 2004; Briens et al., 2013; Nyquist et al., 2013; Yuan et al., 2013), selenium-enriched algae *Scenedesmus quadricauda* (selenium-enriched *Scenedesmus* biomass) (Umysová et al., 2009; Skrivan et al., 2010), were few to mention. There were limited available findings on the prospect of transferring Se in stable organic and bioavailable form using Se-enriched bacterial proteins to animals. Conversely, a strain of bacteria identified from hot spring water was found capable of absorbing sodium selenite (inorganic form), and retained in its cells as Se-containing proteins otherwise known as enriched bacterial proteins (Dalia et al., 2017).

Despite the beneficial roles of microbes in micronutrients bioremediation, there is a paucity of information on optimizing the growth culture medium and temperature for the *S. maltophilia* ADS18 strain, which would produce the organic Se-enriched bacterial proteins, and the efficacy of transferring the Se in organic and bioavailable form to laying hens on laying performance, egg quality characteristics, intestinal morphology, Se concentration, egg yolk antioxidant profile and oxidative stability, selenoprotein and non-selenoprotein gene expression, antioxidant enzyme activity and caecum microbiome of laying hens. Thus, the current study was initiated to examine such effects with the following hypothesis and objectives.

Hypothesis statements

1. Optimizing the culture medium and temperature for the growth of the *S. maltophilia* ADS18 strain could result in high cell biomass with organic Se-bacterial selenoproteins.
2. Diet supplemented with selenium-enriched bacterial proteins would alter hens laying performance, egg quality characteristics, selenium status, egg yolk colour, antioxidant profiles, and oxidative stability.
3. Different Se sources would alter antioxidant enzyme activity and antioxidant status, caecum microbiome, and hepatic selenoprotein, and oviduct non-selenoprotein gene expression in laying hens.

Objectives

The main objective of this research was to optimize the culture medium and temperature for the growth of the *S. maltophilia* ADS18 strain and investigate its use as a feed supplement for laying hens as a source of organic selenium.

The specific objectives of this study were:

i. Optimization of culture medium and temperature for organic selenium production by *Stenotrophomonas maltophilia* strain (ADS18)
ii. Effect of selenium sources on laying performance, egg quality characteristics, Se concentrations, and intestinal morphology in laying hens
iii. Effect of sodium selenite, selenium yeast, and bacterial enriched protein on chicken egg yolk colour, antioxidant profiles, and oxidative stability
iv. Effects of organic and inorganic dietary selenium supplementation on gene expression profiles in oviduct tissue, antioxidant enzyme activity, caecum microbiome, and mRNA hepatic expression of laying hens.

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