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STUDYING BIOAVAILABILITY OF RED AND GREY NANO-SELENIUM IN ANIMAL MODELS

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Written by **Aya Ferroudj** certified **Applied Biochemistry**

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Table of contents

ABBREVIATIONS AND ACRONYMS

2θ	2-Theta
AMPk	Adenosine monophosphate kinase
BW	Body weight
cm^{-1}	Per centimetre
DNA	Deoxyribonucleic Acid
EDS	Energy-dispersive X-ray spectroscopy
FCR	Feed conversion rate
FI	Feed intake
GPx	Glutathione peroxidase
GSH	Reduced glutathione
GSSG	Oxidized Glutathione
H_2O_2	Hydrogen Peroxide
H_2Se	Hydrogen selenide
HCl	Hydrochloric Acid
HNO3	Nitric Acid
HSPs	Heat shock proteins
KSCs	Keratinocyte Stem Cells
MDA	Malondialdehyde
mg/kg	Milligram per kilogram
mg/L	Milligram per Liter
MsrB1	Methionine Sulfoxide Reductase B1
NAD^+	Nicotinamide Adenine Dinucleotide
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
Nano-Se	Nano-Selenium

NPs	Nanoparticles
RBF	Red blood fraction
Se	Selenium
SeCys	Selenocysteine
SelenoP	Selenoprotein P
SelP	Selenoprotein
SEM	Scanning electron microscopy
SeMet	Selenomethionine
SeNPs	Selenium nanoparticles
SeO_3^{2-}	Selenite
SeO_4^{2}-	Selenate
SOD	Superoxide dismutase
TAC	Total antioxidant capacity
TrxR	Thioredoxin reductase
TXNRD1	Thioredoxin Reductase 1
UV	Ultra-violet
v/v	Volume per volume
XRD	X-ray diffraction spectroscopy
MIC	Minimum Inhibitory Concentration
PVP-SeNPs	PVP-stabilized SeNPs
MRC-5	Normal Human Lung Fibroblast Cell Line

1. INTRODUCTION

Nanotechnology has emerged as a promising tool in animal production systems, offering innovative solutions to improve both the quantity and quality of animal-derived products (Huang et al., 2015a). In poultry production, recent advances in nanotechnology have demonstrated improvements in growth performance, feed efficiency, health status, and product quality (Panea et al., 2014). Nanoparticles (NPs) possess unique physicochemical properties compared to their bulk counterparts, including a high surface-to-volume ratio, enhanced surface reactivity, improved stability, increased bioactivity and bioavailability, controlled particle size, and the ability for targeted and controlled delivery (Youssef et al., 2019).

Nanoparticles have also shown strong antimicrobial properties and the potential to reduce microbial load in poultry products, thereby offering alternatives to antibiotics and contributing to the control of antibiotic-resistant pathogens of relevance to human health (Hassanen & Ragab, 2021; Verma et al., 2012). For instance, silver nanoparticles exhibit antibacterial activity through direct interaction with bacterial cell membranes, generation of reactive oxygen species (ROS), release of Ag^{+} ions, penetration into bacterial cells, and subsequent interaction with DNA (Durán et al., 2016). Similarly, zinc oxide nanoparticles (ZnONPs) exert antimicrobial effects by inhibiting bacterial glycolysis, disrupting transmembrane proton transport, interfering with DNA replication, and releasing Zn^{2+} ions and ROS (Sirelkhatim et al., 2015). In poultry nutrition, dietary inclusion of nanoparticles has yielded promising outcomes. Supplementation with selenium and silver nanoparticles has been shown to enhance antioxidant status and reduce oxidative stress in broilers (F. Ahmadi & Kurdestany, 2010; Aparna & Karunakaran, 2016). Copper nanoparticles improve immune responses and growth performance (Y. Wang et al., 2011), while nano-iron has been associated with improved hatchability and productive performance (Saki et al., 2014; Sizova et al., 2015). Among trace elements, selenium is of particular importance in poultry nutrition due to its essential role in antioxidant defense, immune regulation, thyroid hormone metabolism, and reproductive function(Marković et al., 2018; Verma et al., 2012). Selenium exerts its biological effects primarily through its incorporation into selenoproteins, including glutathione peroxidases and thioredoxin reductases, which protect cells and tissues from oxidative damage (Pelyhe & Mezes, 2013; Skalickova et al., 2017). Adequate selenium intake is therefore

critical for maintaining physiological homeostasis and productive performance in poultry. However, the biological effectiveness of selenium depends not only on its dietary concentration but also on its chemical form and bioavailability. Traditional inorganic selenium sources, such as sodium selenite, are characterized by limited bioavailability and a narrow margin between nutritional requirement and toxicity, prompting the search for safer and more efficient selenium supplements (Bhattacharjee et al., 2019; Hadrup & Ravn-Haren, 2023).

Selenium nanoparticles (SeNPs) have emerged as promising alternative selenium sources due to their improved bioavailability, reduced toxicity, and enhanced biological activity compared with inorganic selenium forms (Fairweather-Tait et al., 2010). At the nanoscale, selenium exhibits increased surface reactivity and improved interaction with biological membranes, facilitating absorption and utilization. Numerous studies have reported that dietary nano-selenium supplementation can enhance antioxidant status, immune responses, growth performance, and selenium enrichment of animal-derived products, particularly in poultry (T.-T. Meng et al., 2021). These findings highlight nano selenium as a nutritionally effective and biologically relevant form of selenium.

Importantly, elemental selenium exists in multiple allotropes, primarily amorphous red selenium and crystalline grey selenium, which differ fundamentally in atomic arrangement, crystallinity, stability, and reactivity(Xiong et al., 2006). Red selenium is generally characterized by an amorphous structure with higher chemical reactivity, whereas grey selenium possesses a crystalline trigonal structure associated with greater thermodynamic stability. These structural differences are known to influence the physicochemical behaviour of selenium; however, their implications for biological performance, particularly at the nanoscale, remain insufficiently understood.

In nutritional and toxicological literature, red selenium is often considered more bioavailable, while grey selenium is frequently described as biologically inert or poorly absorbed. This assumption, however, is largely based on studies of bulk selenium rather than nanoscale materials. At the nanoscale, particle size, surface properties, and crystallinity may substantially alter dissolution behaviour, cellular uptake, tissue distribution, and metabolic fate. Consequently, it remains unclear whether the traditional view of black selenium as biologically inactive holds true for selenium nanoparticles.

Japanese quail (Coturnix japonica) represents an important avian species in poultry production and an excellent experimental model for studying selenium bioavailability and metabolism. Approximately 20 species of wild quails have been identified worldwide, whereas domesticated quails comprise more than 70 recognized lines and strains (Northcutt et al., 2022). Japanese quails are particularly popular due to their rapid growth, efficient muscle accretion, and high egg-laying capacity, making them valuable meat- and egg-producing birds (Lukanov, 2019; Northcutt et al., 2022). They represent an excellent alternative to chickens owing to their high metabolic rate, early sexual maturity, and high egg production (approximately 300–320 eggs per laying cycle), in addition to their lower space requirements (200–250 cm^2 for growing birds and 150–200 cm^2 for laying birds), reduced susceptibility to common poultry diseases, and similar feed efficiency despite lower daily feed intake (20–25 g/bird/day) (Aygun & Sert, 2013; Jatoi et al., 2013; Northcutt et al., 2022). Quail production offers economic advantages compared to conventional chicken farming, as it requires lower initial investment, reduced housing space, and fewer management resources (Bakoji et al., 2013; Nasar et al., 2016; Northcutt et al., 2022; Redoy et al., 2017). Correspondingly, consumption of quail eggs has increased steadily in recent years, driven by their nutritional value and consumer acceptance (Fernandez et al., 2011; Lukanov, 2019; Northcutt et al., 2022). Despite these advantages, both quails and their eggs remain sensitive to environmental fluctuations, particularly temperature variations. Improper thermal conditions during egg storage negatively affect offspring growth, health, and productivity and increase microbial contamination, including Gram-negative bacteria and Molds, while reducing albumen quality, eggshell integrity, and shelf life (Nepomuceno et al., 2014; Northcutt et al., 2022; Surai & Fisinin, 2014, 2016b, 2016c; Surai & Kochish, 2019; Surai & Fisinin, 2016c).

In poultry nutrition, systematic comparative studies evaluating the bioavailability and biological effects of red and grey selenium nanoparticles are indetermined. Available data are fragmented and often focus on a single selenium form, dose, or biological endpoint, making it difficult to draw definitive conclusions regarding allotrope-dependent differences in selenium metabolism. Moreover, limited information is available on how selenium nanoparticle allotropy influences tissue-specific selenium distribution, antioxidant enzyme activity, and selenium retention. Addressing these gaps is essential for optimizing nano-selenium supplementation strategies and for understanding the structure–function relationships that govern selenium metabolism in vivo.

The primary aim of this study was to determine whether selenium nanoparticles allotropes are bioavailable and biologically active in vivo; red amorphous and grey crystalline selenium nanoparticles. Despite the well-established assumption that bulk (black) selenium is biologically inert. In addition, the study aimed to clarify whether the transformation of red selenium nanoparticles into the more stable grey form represents a loss of biological function or a structural stabilization that preserves bioavailability at the nanoscale. To achieve this overarching aim, the specific objectives were to:

- Synthesize red (amorphous) and grey (crystalline) selenium nanoparticles and confirm their structural distinction at the nanoscale, using complementary physicochemical techniques (SEM–EDS, XRD, Raman spectroscopy, and fluorescence analysis), to verify that the materials differ in allotropy while retaining identical elemental composition.
- Investigate the influence of selenium nanoparticle allotropy on in vivo selenium metabolism, by comparing red and grey selenium nanoparticles with respect to bioavailability, tissue distribution, antioxidant activity, and post-withdrawal patterns in adult male Japanese quails.
- Examine dose-dependent selenium retention and depletion after dietary withdrawal, in order to determine how selenium nanoparticle allotropy and the transformation of red selenium into the more stable grey form influence selenium bioavailability, biological efficacy, and the practical stability (shelf life) of nano-selenium.

2. LITERATURE REVIEW

2.1. Nano-selenium and its characterization

Selenium was discovered in 1817 by J. J. Berzelius, and the first detailed description of the major selenium-containing enzyme, glutathione peroxidase, was reported in 1973 (Suchý et al., 2014; Surai & Kochish, 2019). Selenium is an essential trace element for living organisms and is widely distributed in various tissues, including the liver, heart, kidneys, and skeletal muscle (He et al., 2020; Y. Huang et al., 2016; D. Sun et al., 2015; Xia et al., 2022; Q. Zhang et al., 2019; X. Zhao et al., 2014). Selenium plays distinct physiological roles in plants and animals, contributing to improved growth, productivity, and product quality, particularly under stress conditions, as documented in studies on selenium nutrition (El-Ramady et al., 2022).

The bioavailability of selenium is influenced by its chemical formula, size, and physicochemical properties, and gastrointestinal absorption remains a major determinant of its biological efficacy. Selenium compounds may transform into insoluble forms as a result of pH changes and microbial activity in the gastrointestinal tract, particularly in ruminants (Hosnedlova et al., 2018). Elemental selenium nanoparticles (SeNPs) are characterized by low toxicity and zero oxidation state (Se^0), with particle diameters typically ranging from 100 to 500 nm (**Fig. 1)**. Despite being poorly soluble, their nanoscale size enhances bioavailability across microorganisms, plants, and animals (Santhappan & Kumar, 2016). The advantages of selenium nanoparticles arise from their small and uniform size, high permeability, increased stability, and resistance to oxidative and enzymatic degradation, resulting in prolonged residence time and improved biological efficacy (Hosnedlova et al., 2018). Consequently, the physical form of selenium strongly determines its bioavailability and physiological impact (Cai et al., 2012).

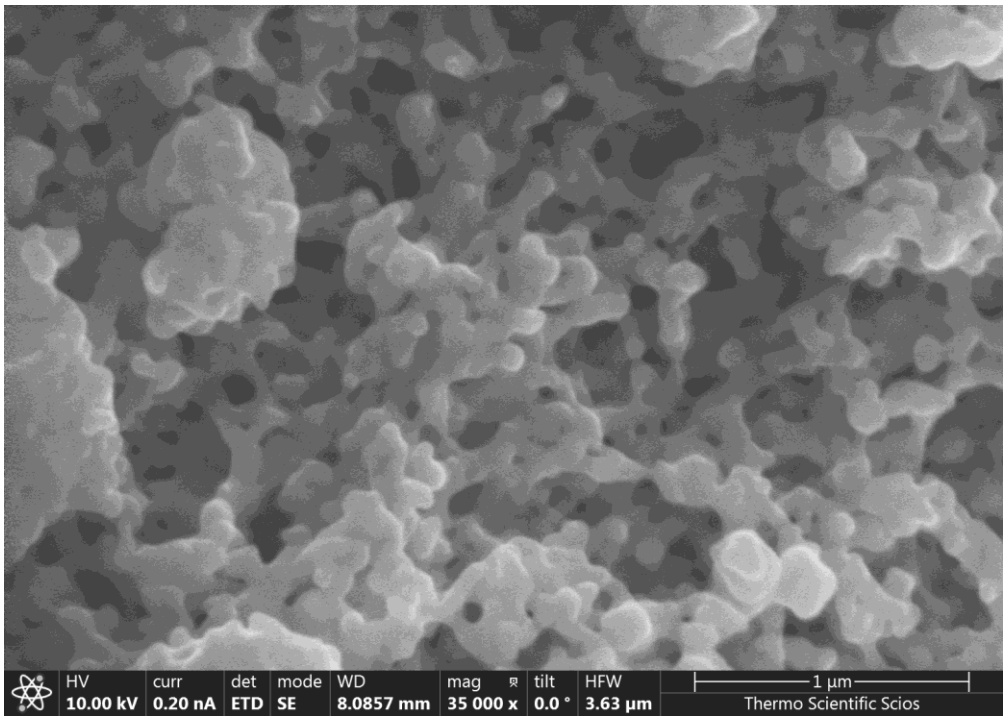


Figure 1: Aggregation of purified nano-selenium (Se^0) spherical particles observed using scanning electron microscopy (SEM) (created by the author)

The chemical form of selenium governs both its bioavailability and toxicity (Prokisch et al., 2011). Inorganic selenium exists primarily as selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}), with selenate generally exhibiting higher toxicity (Santhappan & Kumar, 2016). Regardless of their initial form—selenate, selenite, selenomethionine (SeMet), and selenocysteine (SeCys)—organic selenium compounds are ultimately converted to hydrogen selenide (H_2Se), which serves as a central intermediate for selenoprotein synthesis or is excreted in urine as selenosugars or via exhalation after methylation (Fairweather-Tait et al., 2010).

Dietary organic selenium enhances antioxidant capacity by increasing the activity of selenoenzymes that limit peroxide and free radical formation in serum, liver, and peripheral tissues (Cai et al., 2012; Zhou & Wang, 2011). Selenocysteine, the principal biologically active form of selenium, plays a pivotal role in regulating antioxidant defence systems (Surai & Kochish, 2019). Common organic selenium sources include selenium yeast, selenomethionine (SeMet), and the hydroxy-analogue of selenomethionine (OH-SeMet), a more stable and highly concentrated source (Surai et al., 2018).

Elemental selenium provides additional physiological benefits through its conversion to hydrogen selenide, an endogenous gasotransmitter involved in immune, endocrine,

cardiovascular, and metabolic regulation. These effects are mediated through the incorporation of selenium into redox-active enzymes (selenoproteins) and the maintenance of cellular redox homeostasis (**Fig.2)** (Kuganesan et al., 2019; Sztrik, 2016).

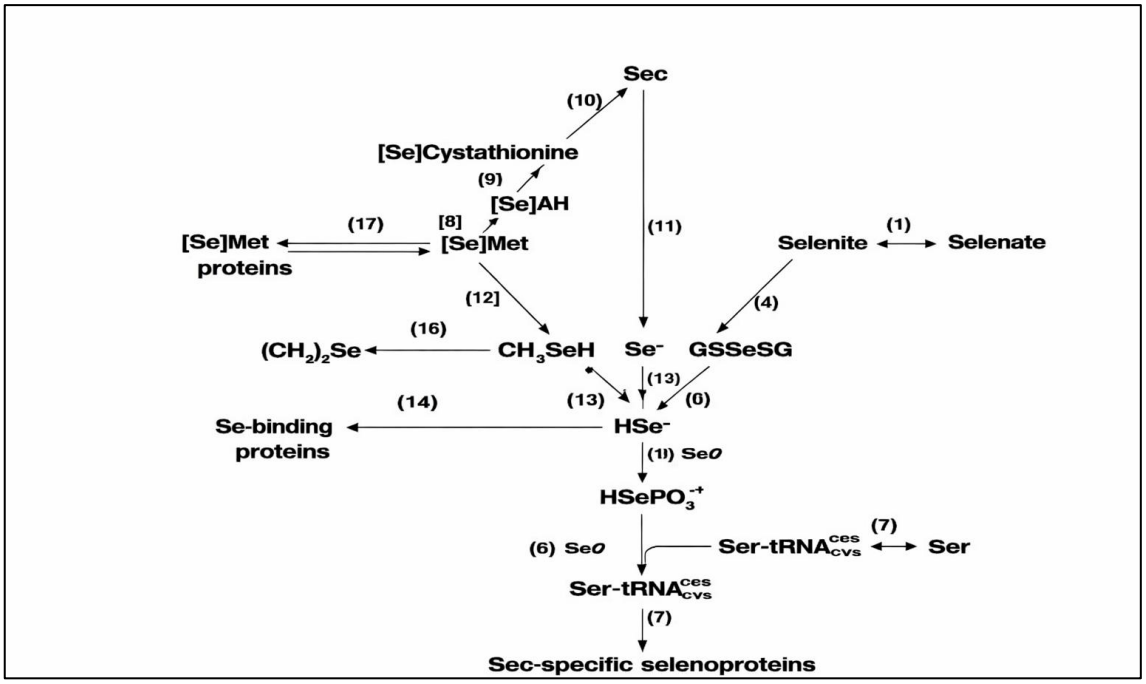


Figure 2: Chemical and biochemical conversion of selenium species from elemental Se to H_2Se and subsequent incorporation into selenoproteins (created by Sztrik, 2016)

Selenium nanoparticles possess distinctive physicochemical characteristics, including a large surface area, enhanced catalytic efficiency, high adsorption capacity, and reduced toxicity (Youssef et al., 2019). Both nano-selenium and organic selenium sources exert beneficial effects on poultry health by upregulating selenoprotein expression and activity (**Fig. 3)** (Surai & Kochish, 2020). Their high bioavailability, combined with relatively low toxicity, makes SeNPs promising alternatives to conventional selenium supplements; however, exceeding physiological selenium requirements remains hazardous and may induce toxicity (Hosnedlova et al., 2018; Marković et al., 2018; Surai & Kochish, 2020)**.**

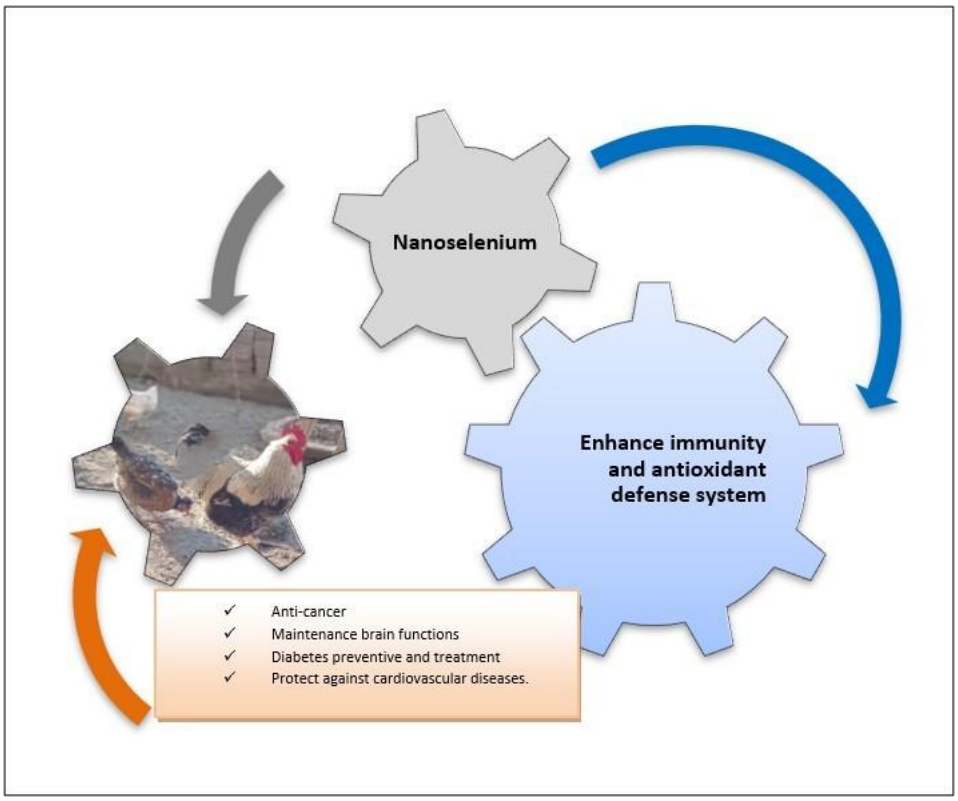


Figure 3: Preventive potential of Nano-Selenium in poultry against several diseases (created by the author)

Selenium nanoparticles can be synthesized using chemical, physical, or biological methods. Chemical synthesis commonly involves the reduction of selenious acid (H_2SeO_3) or selenite ions using reducing agents such as ascorbic acid in aqueous solutions, with the characteristic colour change to red indicating nanoparticle formation (Hosnedlova et al., 2018). Physical methods include laser ablation and ultrasonic fragmentation using selenium-containing substrates (Fardsadegh et al., 2019; Hosnedlova et al., 2018). Biological synthesis exploits the ability of plants and microorganisms to convert selenium ions into elemental nanoparticles, yielding uniformly spherical and biocompatible SeNPs with minimal toxicity (Hosnedlova et al., 2018; Prokisch et al., 2011)**.** Prokisch et al. (2011) demonstrated that probiotic bacteria present in yoghurt can produce elemental SeNPs (50–500 nm) through intracellular detoxification of high selenite concentrations, reducing selenium ions to elemental nanospheres. Such biological methods are considered environmentally friendly and suitable for nutritional applications(Hosnedlova et al., 2018; Prokisch et al., 2011).

Approximately 80% of dietary selenium absorption occurs in the duodenum, with organic forms generally exhibiting higher absorption efficiency than inorganic forms.

Selenium enters enterocytes through distinct mechanisms: selenite diffuses passively across cell membranes, selenate is absorbed via sodium-dependent transporters, and selenomethionine utilizes amino acid transport pathways similar to methionine (Hosnedlova et al., 2018; Surai & Kochish, 2019). Once absorbed, selenium is transported in the bloodstream bound to plasma proteins, mainly albumin, and delivered to the liver, where selenium metabolism occurs. It is subsequently converted to hydrogen selenide and redistributed to peripheral tissues as selenocysteine or excreted following methylation (Hosnedlova et al., 2018; P. F. Surai & Kochish, 2019).

2.2. Main application of nano-Se in poultry

Adequate selenium (Se) nutrition is essential in poultry to ensure optimal immune function and overall physiological performance **(Fig.4)**. Appropriate dietary selenium levels reduce the risk of several health disorders, including oxidative stress, muscular dystrophy, cardiovascular dysfunction, cystic fibrosis, and inflammatory joint conditions (Weekley & Harris, 2013). Cellular structures and macromolecules are highly sensitive to oxidative stress, which disrupts cellular homeostasis. Oxidative stress leads to the excessive generation of reactive oxygen species (ROS), including hydroxyl radicals, superoxide anions, and hydrogen peroxide, thereby promoting apoptotic pathways at the cellular level (Sarkar et al., 2015).

Major physiological and metabolic sources of oxidative stress include mitochondrial respiration, phagocytic and inflammatory responses of the innate immune system, xenobiotic metabolism, detoxification processes, prostanoid synthesis, redox-active transition metals (iron and copper), and exposure to high concentrations of oxygen and polyunsaturated fatty acids (P. F. Surai, 2018; P. F. Surai & Fisinin, 2016b a). The expression of selenoproteins is primarily regulated by dietary selenium availability and cellular redox status (Gladyshev, 2016). During mitochondrial respiration, molecular oxygen is partially reduced to reactive oxygen species, while phagocytes deliberately produce peroxides as antimicrobial agents. However, excessive peroxide generation may cause collateral damage to host tissues. Moreover, detoxification by superoxide dismutase (SOD) is incomplete, as it results in hydrogen peroxide formation, which must be further reduced to water by catalase or glutathione peroxidase (GPx) (Surai, 2018). Reactive oxygen species target critical biological macromolecules, including proteins, lipids, and DNA, thereby impairing essential cellular and tissue functions (Surai et al., 2018). In poultry production, oxidative stress is exacerbated by environmental stressors such as

temperature fluctuations, immunosuppression, and mycotoxin exposure, negatively affecting meat and egg quality. Selenium supplementation in poultry diets mitigates the deleterious effects of ROS by enhancing antioxidant defence systems (Habibian et al., 2015; Liu et al., 2020a; Xia et al., 2022). Conversely, selenium deficiency compromises productivity, weakens immune responses, and increases chick mortality (Emamverdi et al., 2019; Xia et al., 2022; Z. Yang et al., 2016). Malondialdehyde (MDA), the terminal product of polyunsaturated fatty acid peroxidation, is widely used as a biomarker of oxidative stress and lipid peroxidation in poultry tissues (Cai et al., 2012; R. Yang & Liu, 2017; Zhang et al., 2014).

Figure 4: Selenium nutritional advantages in poultry and poultry production (created by the author)

2.2.1. Selenium and Nano Selenium Mediated Immune Enhancement in Poultry

The immunomodulatory effects of selenium reported in **Table 1** are primarily mediated through selenium-dependent proteins and antioxidant regulation. Selenium is incorporated into key selenoproteins, including glutathione peroxidases, thioredoxin reductases, and selenoprotein P, which maintain intracellular redox balance and support both innate and adaptive immune responses (Dalgaard et al., 2018; P. F. Surai et al., 2018). By strengthening enzymatic and non-enzymatic antioxidant defences, selenium protects

immune cells from oxidative damage, reduces lipid peroxidation, and enhances immune resilience under normal and stress conditions (Habibian et al., 2015).

In addition to its antioxidant role, selenium contributes directly to antiviral and cell-mediated immunity by upregulating interferon-related genes, enhancing lymphocyte proliferation, and improving antibody production following vaccination (Azab et al., 2019; Salah et al., 2024; Shojadoost et al., 2020). Selenium supplementation has also been shown to modulate cytokine responses under stress, reducing pro-inflammatory signalling and supporting immune stability during heat stress (Habibian et al., 2015; Salah et al., 2024; Shojadoost et al., 2020). Collectively, these mechanisms explain the improved immune performance and disease resistance observed in selenium-supplemented poultry.

Importantly, the magnitude of these effects is strongly dependent on selenium source and dosage. Organic selenium and nano-selenium consistently outperform inorganic sodium selenite, with optimal responses generally reported at dietary levels of 0.15–0.5 mg/kg (Hu et al., 2012; Rana, 2021). However, the available evidence is derived almost exclusively from studies using red, amorphous selenium nanoparticles, leaving unresolved whether grey selenium nanoparticles exhibit comparable bioavailability and immunological activity.

Table 1: Biological Immuno-Antioxidant Effects of Selenium Forms in Poultry

Study	Se Form	Species / Model	Dose (mg/kg diet)	Antioxidant Effects	Immune Effects
(Dalgaard et al., 2018)	Various Se forms (review)	Poultry & mammals	Not specified	Selenoprotein-mediated redox regulation	Increased resistance to Eimeria, Clostridium, E. coli; modulation of innate and adaptive immunity
(Saad et al., 2009)	Various Se forms (review)	Poultry	Not specified	Enhanced antioxidant defense under stress conditions	Improved immune resilience during infection and environmental stress
(Rao et al., 2013)	Organic Se	Broilers	0–0.3	Increased GPx activity and reduced lipid peroxidation	Stimulated lymphocyte proliferation in a dose-dependent manner
(Elnaggar et al., 2020)	Organic Se (yeast enriched) vs. Na_2SeO_3	Broilers	0.3	improved antioxidant indices compared with inorganic selenium	Increased IgG, IgM, IgA and globulin
(Azab et al., 2019)	Nano-Se	Broilers	0.5	Enhanced antioxidant enzyme activities and reduced oxidative stress	Increased antibody production
(Alagawany et al., 2021)	Nano-Se (red)	Broilers	0.2–0.6	Reduced MDA concentrations and increased GSH and GPx levels	Increased IgG, IgM, IgA; improved gut microbiota composition
(Hu et al., 2012)	Nano-Se vs. Na_2SeO_3	Broilers	0.15–1.2	Increased GPx concentrations than sodium selenite	Not reported
(Kazaz et al., 2020)	Nano-Se vs. Na_2SeO_3	Japanese quail	0.1–0.2	Increased GPx activity and reduced lipid peroxidation	Not reported
(Cai et al., 2012)	Nano-Se	Broilers	~0.3	Increased GPx and SOD activities and reduced lipid peroxidation	Increased serum IgG and IgM levels at 42 days
(Gangadoo et al., 2020)	Nano-Se	Broilers	~0.9	Improved overall oxidative status	Accumulation in spleen indicating targeted immune tissue uptake

Study	Se Form	Species / Model	Dose (mg/kg diet)	Antioxidant Effects	Immune Effects
(Bami et al., 2022)	Green Nano-Se vs. Na_2SeO_3	Broilers	~0.3	Reduced oxidative damage and increased antioxidant capacity	enhanced total antibody response to SRBC and elevated IgG levels at 42 days
(Rana, 2021)	Nano-Se (review)	Poultry	Not specified	Improved oxidative balance	increased IgG, IgM, boosted IL-2 and IFN-γ and the bursa and thymus
(Galić et al., 2020)	Functionalized SeNPs	In vitro	Not specified	Oxidative stress modulation dependent on nanoparticle surface chemistry	Not reported
(Shojadoost et al., 2020)	Organic & inorganic Se	Chickens	Nutritional	Not reported	Increased HI antibody titers; increased IgM and IgY levels; reduced viral shedding
(Salah et al., 2024)	PVP-SeNPs	In vitro bacteria	MIC ≈ 0.3 µg/mL	Not reported	Antibacterial activity against *Staphylococcus aureus, Bacillus cereus, Klebsiella pneumoniae , Escherichia coli and Pseudomonas aeruginosa* and antitumor activity against MRC-5 carcinoma cell line
(Yuan et al., 2023)	SeNPs	In vitro pathogens	0.5-100µg/mL	Not reported	Antibacterial activity against *Listeria monocytogenes, Staphylococcus aureus, Staphylococcus epidermidis, Vibrio alginolyticus, Salmonella enterica*, and antifungal activity against *Candida albicans*
(Surai & Kochish, 2020)	Various Se forms (review)	Poultry	Various	Stronger antioxidant effects for nano and organic Se compared with inorganic Se	Enhanced immune responses for nano and organic Se compared with inorganic Se

2.2.2. Growth-promoting effects

Japanese quails are generally more robust and tolerant to poultry diseases and environmental fluctuations compared with other avian species; however, environmental variations can still negatively influence their behaviour, productivity, and egg-laying performance (El-Tarabany, 2016; Nguyen et al., 2021). In poultry production systems, stressors are commonly classified as environmental or technological, nutritional, and biological. In recent years, excessive production of free radicals has been recognized as a primary underlying mechanism linking these stressors to impaired physiological performance (Surai, 2016; Surai & Fisinin, 2016a, 2016b, 2016c; Surai & Kochish, 2019). The thermoneutral zone for Japanese quail ranges between 25 and 36 °C, while the optimal temperature for egg production is approximately 23.8 °C (El-Tarabany, 2016; Sousa et al., 2014). Ambient temperature directly affects feed intake, water consumption, growth rate, body weight gain, egg production, and the biological and microbiological quality of poultry products (El-Tarabany, 2016; Nguyen et al., 2021; Santos et al., 2012). Under moderate temperatures (around 25 °C), quails exhibit increased egg production, higher activity levels, and reduced water intake, whereas exposure to severe heat stress (≈38 °C) results in increased water consumption, reduced feed intake, and suppressed productivity (Nguyen et al., 2021). Selenium nanoparticles (SeNPs) have been widely investigated in poultry nutrition and have demonstrated improvements in growth performance and muscle deposition **Table 2.** Furthermore, nano-selenium supplementation has been shown to influence poultry growth and production performance under different thermal stress conditions, highlighting its role in supporting physiological stability during environmental challenges **Table 3** (Kumaran et al., 2015).

In addition, SeNPs mitigate several adverse effects associated with inorganic selenium, suppress pathogenic microorganisms, and reduce the incidence of fatty liver syndrome in broilers (Y. Wang et al., 2013). Dietary inclusion of SeNPs at levels ranging from 0.15 to 1.20 ppm significantly increased body weight gain compared with birds receiving 0.30 ppm inorganic selenium (Hu et al., 2012). These findings indicate that SeNPs exert beneficial effects at lower dietary concentrations while exhibiting reduced toxicity relative to sodium selenite. Supplementation of broiler diets with 0.3 mg/kg SeNPs significantly improved feed conversion ratio (FCR), growth performance, meat quality, and antioxidant status in broilers and Guangxi yellow chickens (Zhou & Wang, 2011). Similarly, dietary SeNPs supplementation at doses of 0.2, 0.3, 0.4, and 0.5 mg/kg

enhanced growth performance and carcass characteristics without inducing adverse effects on internal organs (Ahmadi et al., 2018). More recently, SeNPs have been reported to improve the quality of frozen broiler meat more effectively than inorganic or organic selenium sources (Ibrahim et al., 2019). According to Surai & Fisinin (2014), recommended dietary selenium supplementation levels are approximately 0.06–0.2 mg/kg for laying hens, turkeys, and ducks. Cai et al. (2012) demonstrated that supplementation with 0.3 mg/kg SeNPs enhanced immune responses, improved meat quality, and increased resistance to oxidative stress in broilers by elevating glutathione peroxidase activity in serum and liver tissues. Enhanced humoral immunity was reflected by increased immunoglobulin M (IgM) levels. Conversely, supplementation with 2 mg/kg SeNPs resulted in minimal additional benefits, indicating the importance of optimal dosing (Cai et al., 2012). Regulatory authorities such as the Association of American Feed Control Officials (AAFCO) (2023) and; Ministry of Agriculture of the People's Republic of China (2010) recommend limiting dietary selenium supplementation for poultry to a range of 0.5–2.0 mg/kg feed to avoid toxicity (AAFCO, 2023; Ministry of Agriculture of China, 2010). The optimal SeNPs dose for Guangxi yellow broilers has been identified as 0.3 mg/kg (Zhou & Wang, 2011).

Nano-selenium also plays a crucial role in egg production and quality. SeNPs enhance eggshell membrane synthesis by stimulating epithelial secretion on the eggshell surface, resulting in improved eggshell integrity and reduced shell defects Boostani et al., 2015). During embryonic development, the egg yolk contains high concentrations of fatty acids susceptible to β-oxidation and peroxide formation, which may cause embryonic malformations if antioxidant protection is insufficient. Selenium deficiency in maternal diets has been associated with skeletal muscle abnormalities in offspring (Gao et al., 2018; Xia et al., 2022). Selenium is efficiently transferred from the maternal diet into egg components and subsequently to developing embryos, where antioxidant defence systems operate within the yolk, albumen, and embryonic tissues (Karadas et al., 2011; Surai, 2015a, 2016, 2017; Surai & Fisinin, 2014, 2016d; Surai & Fisinin, 2016c).

Dietary supplementation with SeNPs enhances egg-laying rate, egg mass, albumen quality, eggshell thickness, and tissue mineral content (Rana, 2021); Improvements in ovulation rate, hatchability, egg weight, Haugh unit, eggshell quality, and reduced mortality have also been reported (Nabi et al., 2020). These benefits are partly attributed to selenium's antimicrobial properties, which reduce bacterial penetration through the

eggshell and mitigate nutritional, environmental, metabolic, and genetic factors influencing egg quality (Qu et al., 2017).

Despite these advantages, quail eggs remain sensitive to environmental conditions, particularly storage temperature. Elevated storage temperatures promote microbial growth, including Gram-negative bacteria and Molds, resulting in reduced albumen thickness and shortened shelf life (Nepomuceno et al., 2014; Xia et al., 2022). Storage of quail eggs at 4 °C for up to 120 days significantly reduces total aerobic microbial counts and eliminates yeast contamination, thereby preserving egg quality and safety (Northcutt et al., 2022).

Table 2: Effect of Nano-Selenium in poultry growth

Bird	Reference	Dose (mg/kg)	Conditions/ Age	Body weight (g)	Food conversion ratio(g/g)	Feed intake(g/d)
Arbor broiler	(Hassan et al., 2020)	0	Heat stress 40C°, 6–8h/day, 0 to 38 days old	368.30	1.54	557
		0.5		387.40	1.48	580.9
Ross broiler		0		364.20	1.76	643.2
		0.5		412.80	1.40	543
Broiler Japanese quail	(Khazraei et al., 2022)	0	Controlled T°/ 20 days old	4.43	6.37	28.18
		0.2		3.91	6.78	26.46
		0.5		3.95	6.77	26.71
Japanese quail	(Alagawany et al., 2021)	0	Controlled T°/1 to 5 weeks old	5.41	3.49	18.9
		0.4		6.1	2.81	17.1
Broiler Arbor Acres	(Cai et al., 2012)	0	Controlled T°/0 to 42 days old	47.30	1.64	77.3
		0.3		48.10	1.61	77.2
		0.5		48.20	1.62	77.9
Hen	(Meng et al., 2021)	0	Controlled T°/29 week old	NA	NA	130.77
		0.3		NA	NA	129.1

Table 3: Impact of nano-Selenium in poultry growth and production performance under different thermal conditions.

Birds	Impact of nano-Selenium under different temperatures	Reference
Broiler	Enhances birds' growth progression, increasing body weight, feed intake, and conversion under ambient temperature.	(Ahmadi et al., 2018)
	Improvement of antioxidant and antibacterial defence systems and reduction of lipid accumulation contributing to enhanced meat quality, with rearing temperature maintained at 34°C at hatch and progressively decreased by 3.5°C per week until 25 °C at the end of the third week	(Ali et al., 2020)
	Decreasing mortality ratio, ameliorating antioxidant and immune systems under heat stress 35°C, and they are accustomed to warm conditions and increasing their production and growth	(Hassan et al., 2020)
	Effective doses of Se-NPs ranged between 0.3 to 0.5 mg/kg, and the overdose is toxic and causes alterations in liver and macromolecule metabolisms, and it may promote bird death in thermoneutral condition.	(Cai et al., 2012)
	Gives an antioxidant impact at 21°C by improving animal health, reducing cholesterol levels in plasma, and augmenting HDL concentrations.	(Safdari-Rostamabad et al., 2017)
	Broiler chicks under 33-35°C and Nano-Se addition gave an anti-apoptotic effect.	(Xueting et al., 2018)
Hens	Improve digestive tube functions by reaching a beneficial microbial repertoire in controlled temperature.	(Nabi et al., 2020)
	Se-NPs support hen's laying and reproduction performance with a low supplementation and controlled temperature.	(T. Meng et al., 2019)
	Se-NPs enhance laying hens' production and the quality of their eggs under heat stress.	(Salaheldin, 2015)
Japanese quails	Enhanced its feed intake in 21 days, the birds gained weight, MDA levels were lower, GPx and TxR activities were at the highest levels, and the mortality rate was diminished compared with control samples at 24°C.	(Khazraei et al., 2022)
	In controlled conditions, quails supplied with Che-SeNPs gained weight, and Antioxidant potential was increased by SOD, GPx actions, and a remarkable increase in the levels of reduced glutathione GSH. At the same time, MDA concentrations measured and harmed microbial	(Alagawany et al., 2021)

Birds	Impact of nano-Selenium under different temperatures	Reference
	count in the birds were low, which supported immune functions and raised immunoglobulin production.	

2.2.3. Selenium as an Anti-Stress and Antioxidant Agent

Antioxidant protection is achieved through the neutralization and removal of reactive oxygen species (ROS) via several complementary mechanisms. Antioxidants can be broadly classified into fat-soluble components, such as vitamin E, carotenoids, and coenzyme Q, and water-soluble components, including vitamin C, glutathione, thioredoxin, carnitine, and taurine. In addition, antioxidant defence relies on a complex enzymatic system that includes glutathione peroxidases (GPx1–4, 6), multiple selenoproteins (I, M, K, H, N, O, P, V, R, S, and T), glutathione transferase, glutathione reductase, superoxide dismutase (SOD), and thioredoxin reductases (Surai, 2016; Surai & Kochish, 2019; Yang & Liu, 2017). Glutathione represents a major non-enzymatic intracellular antioxidant component and plays a central role in maintaining redox homeostasis (Cai et al., 2012)**.** The primary enzymatic defence against oxidative stress is mainly mediated by GPx and SOD, whose activities are functionally interconnected (Cai et al., 2012;Surai & Kochish, 2020). Glutathione peroxidase (GPx) is a selenium-dependent enzyme that is activated under oxidative stress conditions arising from β-oxidation and peroxide generation, which can disrupt normal cellular functions. GPx protects cells by reducing peroxides and limiting the activity of enzymes responsible for the generation of toxic free radicals **Table 4** (Hassnin et al., 2013)**.** GPx activity is reported to be highest in the liver and kidneys, lower in plasma and red blood cells, and lowest in thigh and pectoral muscles (Surai & Kochish, 2019; Zhou & Wang, 2011)**.**

Selenoproteins are classified into two major groups: those that maintain essential biological functions under normal conditions and stress-responsive selenoproteins that are induced during oxidative stress or selenium deficiency (Surai & Kochish, 2019)**.** In chickens, 25 genes encoding selenoproteins have been identified (Lei, 2017; Li et al., 2018; Zhao et al., 2017)**.** The synthesis and activity of these selenoproteins strongly depend on selenium availability and physiological stress status. While some selenoproteins show constitutive low-level expression to support basal cellular functions, the majority exhibit selenium-dependent regulation linked to its biological activity (Surai, 2018; Surai et al., 2018; Surai & Fisinin, 2014; Surai & Kochish, 2019)**.**

Dietary supplementation with selenium nanoparticles (SeNPs) has been shown to enhance both the expression and activity of selenoenzymes in living organisms (Cai et al., 2012; Zhou & Wang, 2011)**.** The active sites of selenoproteins are selenium-specific, enabling selenium to function as an essential cofactor responsible for enzyme activation

and biological function (Fardsadegh et al., 2019). Optimal nano-selenium doses required for the activation of GPx1, GPx4, and TxR have been demonstrated previously (Zhou & Wang, 2011). Furthermore, SeNPs supplementation enhances GPx and SOD activities, supports glutathione redox cycling, and prevents excessive accumulation of malondialdehyde (MDA), a key marker of lipid peroxidation (Hassnin et al., 2013; Surai & Kochish, 2020).

As a preventive strategy, cells store selenium intracellularly in the form of selenoproteins to ensure availability during periods of selenium deficiency or physiological stress (Hosnedlova et al., 2018; Kojouri et al., 2012; Surai & Kochish, 2019). Skeletal muscle serves as a major selenium reservoir, which can be mobilized under stress to maintain the basal expression of essential selenoproteins (Surai, 2018; Surai et al., 2018; Surai & Fisinin, 2014; Surai & Kochish, 2019).

Biological systems operate through three distinct levels of antioxidant protection. The first level involves TxR, selenoproteins R and W, and cellular detoxification by SOD, which converts superoxide radicals into hydrogen peroxide (H_2O_2). Although H_2O_2 is potentially harmful, it is subsequently reduced to water by catalase and glutathione reductase (Surai, 2016, 2017). This level also includes the sequestration of free transition metals by specific binding proteins to reduce oxidative reactions (Surai & Kochish, 2019). Additionally, carnitine, taurine, and coenzyme Q play crucial roles in maintaining mitochondrial functional integrity(Surai, 2015 b, 2017).

The second defence level relies primarily on the glutathione system, comprising reduced glutathione (GSH), GPx, glutathione reductase, and glutathione disulfide reductase, alongside ascorbic acid, vitamin E, carotenoids, thioredoxin, thioredoxin reductase, and peroxiredoxins (Surai, 2016). Within this system, GPx catalyses the reduction of hydrogen peroxide and lipid hydroperoxides using GSH as an electron donor, thereby converting GSH to oxidized glutathione (GSSG) and preventing oxidative damage to cellular lipids, proteins, and membranes. The oxidized glutathione is subsequently regenerated to its reduced form by glutathione reductase using NADPH, maintaining the intracellular redox balance. In addition, oxidized vitamin E is regenerated to its reduced, biologically active form through reactions involving vitamin C and the thioredoxin system, with NADPH supplied by the pentose phosphate pathway (Surai, 2016).

The third and final level consists of cellular repair mechanisms, including heat shock proteins (HSPs), methionine sulfoxide reductase, phospholipases, and DNA repair enzymes, which collectively restore macromolecular integrity following oxidative damage (Surai & Fisinin, 2016c; 2016d).

Table 4: Effects of Nano-Selenium on Antioxidant Capacity and Oxidative Stress Markers in Serum of Poultry Species.

Species	GPx (mg/dL)	MDA (nmol/ml)	SOD (U/ml)	SeNPs (mg/kg)	Conditions/ Age	Reference
Arbor broiler	NA	7.4	251.4	0	Heat stress 40C°, 6–8h/day, 0 to 38 days old	(Hassan et al., 2020)
	NA	27.4	273.8	0.5		
Ross broiler	NA	8.2	256.3	0		
	NA	30.3	275.1	0.5		
Broiler Japanese quail	74.60	3.86	NA	0	Controlled T°/ 20 days old	(Khazraei et al., 2022)
	60.90	4.88	NA	0.2		
	66.60	4.54	NA	0.5		
Japanese quail 0.30	0.13	0.33	0.12	0	Controlled T°/1 to 5 weeks old	(Alagawany et al., 2021)
	0.30	0.22	0.29	0.4		
Broiler Arbor Acres	1.18	3.90	146	0	Controlled T°/0 to 42 days old	(Cai et al., 2012)
	1.41	3.20	171	0.3		
	1.39	3.30	156	0.5		
Hen	2817.49	12.86	67.39	0	Controlled T°/29 week old	(Meng et al., 2021)
	3437.77	9.8	55.02	0.3		

2.3. Health-related and medical applications

Selenium plays a crucial role in numerous aspects of human and animal health, including inflammation, cancer, immune function, and diabetes **Figure 5** (Prabhu & Lei, 2016). It exhibits a dual role in glucose metabolism by improving insulin synthesis and secretion and by exerting insulin-mimetic effects that enhance intracellular glucose uptake (Prabhu & Lei, 2016). High doses of inorganic and organic selenium have been administered to alleviate symptoms in experimental models of both type 1 and type 2 diabetes. Furthermore, improvements in metabolic status have been reported in patients with type 2 diabetes (T2D) following dietary selenium supplementation. Comparable antioxidant regulation in poultry contributes to reduced oxidative stress, improved feed efficiency, and enhanced energy metabolism, particularly under heat or nutritional stress conditions. However, several livestock studies indicate that excessive selenium intake may exert diabetogenic effects, highlighting the narrow therapeutic window of selenium supplementation (Prabhu & Lei, 2016).

Selenium nanoparticles (SeNPs) have demonstrated efficacy in inhibiting cell proliferation in high-grade serous ovarian cancer cells through multiple biological mechanisms. Previous studies have shown that selenium can suppress cancer cell growth via autophagy in colorectal cancer, apoptosis in skin, breast, and liver cancers, and ROS-mediated necrosis in prostate cancer (Toubhans et al., 2020). Selenium enhances cellular detoxification against reactive oxygen species (ROS) in most tissues; excessive ROS production induces oxidative stress, leading to DNA damage and apoptosis. Selenium deficiency may prolong oxidative stress and increase cancer risk. In several lung cancer cell lines, certain inorganic selenium compounds are more effective than conventional cytotoxic drugs through modulation of the thioredoxin system. Another key mediator of selenium activity is selenoprotein P (SelP), which serves a dual function as both an antioxidant and a selenium transport protein with anticarcinogenic properties. Downregulation of SelP expression commonly leads to increased oxidative stress and altered glutathione peroxidase (GPx) activity in tumour tissues (Yang & Liu, 2017).

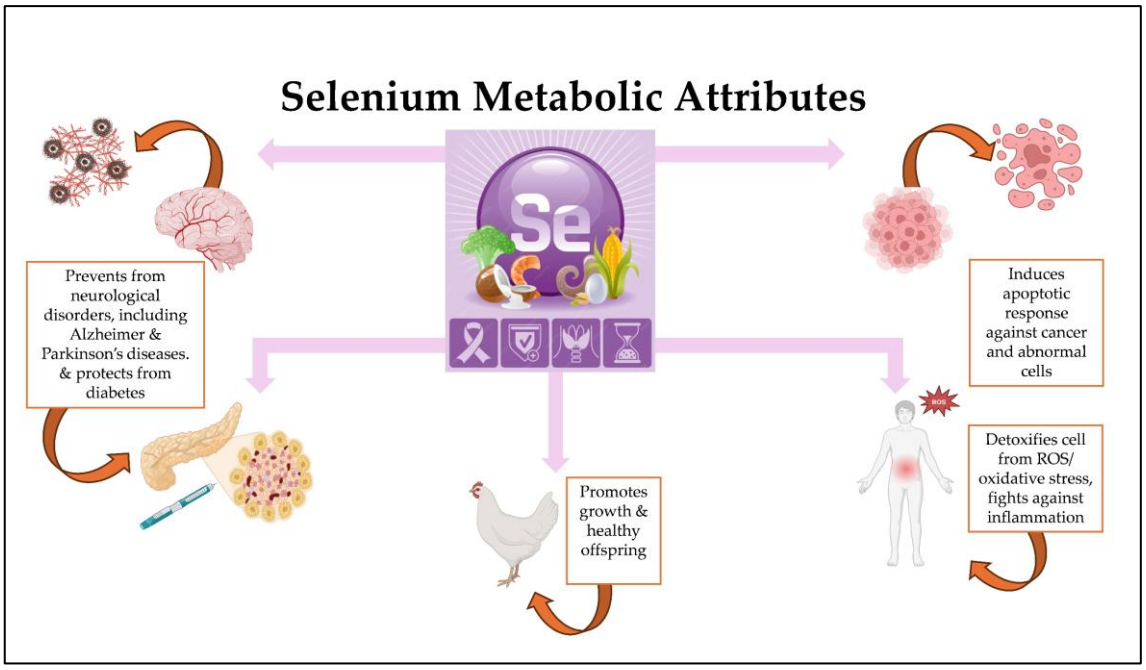


Figure 5: Selenium metabolic and medical attributions (created by the author)

2.3.1. Selenium and Cardiovascular Health

Cardiovascular diseases are associated with hyperlipidaemia, increased blood viscosity, atherosclerosis, hypertension, and elevated mortality rates, particularly among elderly populations. In poultry, dietary selenium reduces lipid peroxidation in cardiac and muscular tissues, maintains membrane integrity, and supports erythrocyte stability under heat or oxidative stress (Ji et al., 2024). The beneficial effects of selenium on cardiovascular health have been well documented by Rayman (2012). Selenoenzymes such as thioredoxin reductase (TrxR) and GPx play a protective role in the vascular endothelium by reducing oxidative damage, inhibiting platelet aggregation, and attenuating inflammation. GPx4 contributes to the reduction of phospholipid hydroperoxides and cholesterol ester hydroperoxides in lipoproteins, thereby limiting oxidized low-density lipoprotein (LDL) accumulation in vascular walls and reducing thrombotic risk. Selenium deficiency leads to reduced GPx activity and elevated lipid hydroperoxide levels, which inhibit prostacyclin synthase, an enzyme essential for prostacyclin production. A decline in prostacyclin—a potent vasodilator and inhibitor of platelet aggregation—favours increased thromboxane synthesis, promoting platelet aggregation and increasing cardiovascular risk (R. Yang & Liu, 2017).

2.3.2. Selenium and Neurodegenerative Disorders

Low selenium (Se) levels have been associated with several neurological disorders on humans and animals, including Alzheimer's disease, Parkinson's disease, epilepsy, and schizophrenia. Deficiency of selenoprotein P reduces glutathione peroxidase (GPx) and thioredoxin reductase (TrxR) activities and decreases selenium availability in the brain, contributing to oxidative damage and motor dysfunction (Yang & Liu, 2017). Evidence indicates a protective role of selenium against Alzheimer's disease. Selenium supplementation has been shown to prevent and attenuate Alzheimer's pathology by enhancing antioxidant defences and reducing oxidative stress (Zhang & Song, 2021). Consistently, decreased selenium levels have been linked to the onset and progression of Alzheimer's disease (Zhou et al., 2023). The relationship between selenium status and Parkinson's disease appears dose-dependent. While excessive selenium exposure may increase Parkinson's disease risk (Chen et al., 2024), higher physiological blood selenium levels have been associated with reduced disease prevalence (Zeng et al., 2023). This duality highlights the narrow optimal range of selenium for neuroprotection. Adequate glutathione levels play a key role in mitigating oxidative stress and supporting neuronal recovery in Parkinson's disease (Yıldızhan & Nazıroğlu, 2020), underscoring the importance of selenium-dependent antioxidant systems.

2.3.3. Selenium and Cancer

Selenium (Se) is an essential cofactor incorporated into a family of selenoproteins, such as glutathione peroxidase (GSH-Px), Thioredoxin reductase (TrxR) and selenoprotein P1 (Misra et al., 2015); those are the most well-known antioxidant enzymes in living systems (Djebara et al., 2025; Rayman, 2005). These selenoproteins play an essential role in cellular antioxidant defence by detoxifying reactive oxygen species (ROS) and maintaining intracellular redox balance. Because oxidative stress contributes to DNA damage, mutagenesis, and abnormal cell proliferation, selenium-dependent antioxidant enzymes are frequently discussed in relation to cancer-related cellular processes. Although cancer incidence is rare in poultry, similar antioxidant pathways determine cellular repair, apoptosis regulation, and tissue renewal, especially in the liver, reproductive, and muscle systems. All selenium compounds might contribute to selenoenzymes biosynthesis, and several studies indicate that the bioavailability of Se increases Selenoproteins (SelP) expression in vivo and in vitro (Minich, 2022; Misra et

al., 2015; Moreda-Piñeiro et al., 2017). Different metabolism pathways are involved to produce a useful intermediate form of Se, like Selenide, and it will insert as a Se-amino acid (SeCys) in the peptide chain during translation of mRNA due to its unique codon (**Fig.6)** (Misra et al., 2015; Rayman, 2005).

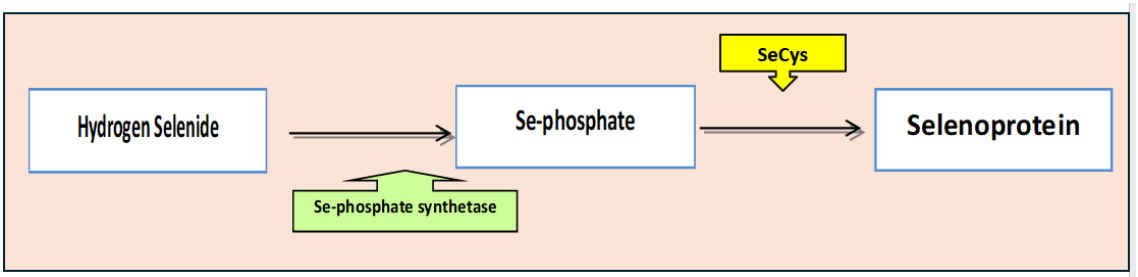


Figure 6: Selenium metabolism pathway (created by the author)

Selenoproteins catalyse disulfide bonds reduction between protein macromolecules to maintain their functional structures (Yim et al., 2018; Z. Zhao et al., 2022). Selenium potentiates to diminish oxidative cell status by integrating into selenoproteins is the key behind its anticancer and antioxidant impacts(Steinbrenner et al., 2013). Through these mechanisms, selenoproteins contribute to the protection of cellular DNA, lipids, and proteins from oxidative damage, which is one of the major factors involved in carcinogenesis. Methyl-Selenol (CH_3SeH) is one of the intermediate metabolites of selenium that has a powerful anticancer effect in case of over-nourishment of that element, by detoxifying cells from carcinogens and inducing caspases and limits tumour cell proliferation (Rayman, 2005). On the other hand, inorganic forms such as Selenite enhance immune system response at low doses by favouring natural killers (NK) and lymphocytes production(Rayman, 2005). Selenoenzymes exhibit distinct activities in carcinoma cases; thus, the molecular expression of these proteins is altered. Such an example, GPx4, was shown to have a therapeutic and biomarking behaviour in lymphatic system cancer (Chen et al., 2024). Their high expression in sane tissues provides cancer protection, or, in case of TrxR, can lead to tumour cells 'apoptosis (Lin et al., 2024; Scalcon et al., 2018). Selenium has 2 opposite effects; an anticancer by preventing against malignancy in several organs, and it may also induce tumour development also. Its different compounds have a remarkable impact to protect from hepatic, colorectal, and breast carcinoma by favouring (SeP.) expression and protecting DNA from damage, destroying the abnormal cells (Maiyo & Singh, 2017). In addition, Se deficiency or over-exposure can produce harmful responses. In case of high uptake, the epidemiologic cancer

risk is significantly increased and results a keratinocyte carcinoma, squamous cell cancer, and hyperglycaemia Type 2 diabetes (T2D) (Steinbrenner et al., 2013; Vinceti et al., 2018). Selenium lack promotes cardiovascular disorders and neuropathy because of the oxidative stress and low rate of selenoenzymes, and might influence the reproductive capacity for both genders and general immune system weakness (Maiyo & Singh, 2017; Shreenath et al., 2024). Redox-active selenium species conversion to elemental (Se^0) is due to their pro-oxidant properties can generate more free radicals that promote mitochondrial apoptosis or cell necrosis in very high amounts(Abozaid et al., 2023; Misra et al., 2015). As a positive side, these pro-oxidant compounds can be used as a chemotherapeutic cure as the cancer cells have a high affinity to them; several studies reported the positive impact of Se to prevent from prostate cancer and reduce side effects of conventional radio-chemotherapies. Typically, tumour cells produce high amounts of reactive oxygen species (ROS) but it has low resilience with them, so the active Se-compounds can induce an oxidative stress which lead to the apoptosis of these abnormal cells (Djebara et al., 2025; Estevez et al., 2023). Selenium acts as a chemoprotection in the early stage of tumour progression by inhibiting fat peroxidation and preventing DNA adducts produced by carcinogens such as (7,12-dimethylbenz(a) anthracene) "DMBA"(Djebara et al., 2025). The chemical forms of Selenium might also interact differently with the tumour suppressor gene P53 and induce different responses (F. Sun et al., 2019). So, they may stimulate DNA repair or cell apoptosis. Even serum content of Se can lead to a modulation of chemotherapy and radiotherapy drugs (Smith et al., 2004; Yildiz et al., 2019). A recent study demonstrates that in vitro SeNPs have the aptitude to distinguish between normal and cancer cells and promote cell death via high cytotoxicity in tumours(Martínez-Esquivias et al., 2022). The key behind the impressive effect of nano-sized selenium to fight against cancer development is the multiple mechanisms that can induce, as the shortage of energy in the abnormal cells, like reduction of ATP, ADP, and NAD^+ and blocking the cellular respiratory pathways(L. Chen et al., 2017; Estevez et al., 2023). Supplementation of chemically produced SeNPs leads to elevate the cysteine and fructose levels in cancer cells to imitate the effect of hypoxia and oxidative stress and suppressed its uncontrolled multiplication and arrests the cellular cycles(Estevez et al., 2023; Rani et al., 2025). As well as the nanoparticles of selenium restrict the essential amino acids and fatty acid necessary for the cancer progression(L. Chen et al., 2017; Estevez et al., 2023).

2.3.4. Selenium and Diabetes

Selenium is an indispensable element for the well-functioning selenoproteins in several organs and tissues of the living body. The rate of alteration of selenium in a population can induce numerous medical issues. In poultry, selenium supplementation influences glucose metabolism and lipid mobilization, contributing to better feed conversion, growth, and reproductive efficiency under metabolic stress (W. Liu & Zhao, 2014). Diabetes is one of the most common illnesses nowadays. The relation between diabetes and Se abundance is a bit critical multifaced relationship; depending on several conditions such as gender, age, and nutritional status (Kohler et al., 2018; Misu et al., 2010; Rayman & Stranges, 2013). Many studies demonstrate that the high uptake levels of selenium can induce to the high expression of selenoprotein P (Sep P) and serum Se content which may cause the insulin resistance and diabetes types 2 (T2D); the mechanism by which Sep (P) influences glucose metabolism involves the inhibition of adenosine monophosphate-activated protein kinase (AMPK), an enzyme responsible for maintaining insulin sensitivity and regulating fatty acid oxidation. At the same time, Sep (P) may stimulate the expression of gluconeogenic enzymes, which can increase hepatic glucose production and contribute to the development of hyperglycaemia (Misu et al., 2010; Rayman & Stranges, 2013).In contrast, a new study revealed that no correlation between Se concentrations in serum and glycemia parameters (Kurniati et al., 2024). Additionally, Se is known as a protective factor against hypertensive nephropathy and decreases the risk of diabetes related to it (Shi et al., 2024). Ogawa-Wong et al. (2016) reported that high exposure to Se increase T2D risk in males, whereas females are not affected, which poses the hypothesis that selenium-glucose homeostasis is sex-dependent regulation. The high Se uptake increases the expression of several selenoproteins as Sep P, GPx1, and Sep S and over-scavenges intercellular ROS and impairs insulin receptors and downstream signalling this leading to hyperinsulinemia and glucose intolerance (Chung et al., 2009; Vinceti et al., 2018; S. J. Yang et al., 2011; J. Zhou et al., 2013). In different side Al-Quraishy et al. (2015), Rayman & Stranges (2013) and Steinbrenner et al. (2010, 2022) suggested that large quantities of antioxidant biomarkers could be a consequence rather than be cause of T2D, and it may signify a hyperglycaemia that proposes an insulin mimetic effect for selenium and SeNPs and be an antidiabetic element in certain levels. The crystalline SeNPs biosynthesize can prevent tissues damages induced by diabetes via the enhancement of the antioxidant activities in these organs, such

testis(Fan et al., 2020). Y. Liu et al. (2018) demonstrated that nanoparticles of selenium stabilized with polysaccharides had antidiabetic potential in diabetic mice. Additionally, these NPs of selenium could prevent the offspring of gestational diabetic mothers from diabetes(Hassan et al., 2020). Moreover, the combination of SeNPs with plasma-rich platelets can reduce the complications associated with diabetes (Karas et al., 2024). As well as the green synthesised SeNPs with *Moringa oleifera* have the same impact as insulin by reducing hyperglycaemia and deactivate α-amylase and α-glucosidase(Olaoye et al., 2024).

2.3.5. Selenium and Dermatological Conditions

Stress severely compromises skin health by increasing ROS (Reactive Oxygen Species), which drives inflammation, collagen breakdown, and accelerated ageing (Tobin, 2017). Selenium counters this by boosting antioxidant capacity and maintaining thyroid homeostasis. In avian species, Same antioxidant pathways promote feather integrity, skin health, and improved plumage colouration, which are vital for birds thermoregulation (Leeson & Walsh, 2004). This systemic support facilitates the optimal growth and differentiation of keratinocytes, thereby improving the health and elasticity of the skin, hair, and nails(Winther et al., 2020). Selenium, as a mineral cofactor, protects the external layer of the skin from various dermatological disorders like eczema, acne and psoriasis (Iv et al., 2020); due to the antimicrobial and anti-inflammatory potential that could promote a well wound healing and prevent it from infections(Mao et al., 2021). In addition, selenium has anti-infection activity that protects against several germs from gram-positive, like *Staphylococcus aureus* and *Bacillus cereus* and gram-negative, such; *Listeria monocytogene* and yeast *Saccharomyces cerevisiae* (Galić et al., 2020). Besides recovering sores, SeNPs regulate the pro-inflammatory cytokines IL-6 and TNF-α by reducing their levels to accelerate the repairing(El-Sayed et al., 2023). Keshta A. T. et al. (2020) demonstrated that SeNPs at 0.5 ($mg.kg^{-1}$) can accelerate the healing by decreasing nitric oxide amounts and protect from the oxidative stress and apoptosis of the injured tissues and the cicatrization factors elevated its activities such vascular endothelial growth factor (VEGF) and collagenase I. additionally; selenium had different forms and ionic states and each could have its own impact on the skin health, for example selenium sulphide (SeS_2) reduces the formation of dandruff and other forehead eczema forms (Turner et al., 1994). Hon et al. (2010) has shown that zinc and copper are effective in cases of eczema severities (vitiligo and alopecia), but selenium has not clearly revealed

an impact as the previous elements mentioned. (Iv et al., 2020) exhibited that selenium deficiency can lead to other specific dermatological illnesses like acne vulgaris, chloric acne, and psoriasis. Although (Xu & Li, 2024) exposed that the prevalence of childhood eczema does not direct clear link with Se intake rate. Kaur & Rath, (2019) showed the powerful effect of sunburn skin issues, where selenium is accompanied by probiotics. So, we may say that the correlation selenium-skin wellness is such a complicated relationship. Se confers significant anti-ageing and photoprotective properties to the skin, primarily by acting as a powerful antioxidant and supporting genomic stability. Selenium is incorporated into selenoproteins (including GPx1, TXNRD1, MsrB1, and SelenoP), which are crucial for detoxifying reactive oxygen species (ROS) and facilitating cellular DNA repair(Z. Cai et al., 2019; Wu et al., 2014). This action is vital for maintaining the stability of the entire genome and preventing DNA damage, mutation, and age-related decline. Furthermore, selenium intake, specifically as Sodium Selenite, has been linked to the superior maintenance of telomere length, a key factor in slowing cellular senescence(Z. Cai et al., 2019). At the cellular level, selenium supports epidermal integrity by preserving the longevity of Keratinocyte Stem Cells (KSCs) and enhancing keratinocyte adhesion to the basement membrane via increased Collagen and Integrin expression. By controlling cell cycle progression through the activation of tumour suppressor pathways (e.g., p53, 21, 16), selenium prevents premature senescence and supports continuous epidermal renewal (Jobeili et al., 2017). Collectively, these actions underscore selenium's role in the skin's self-renewal pathway, its capacity to mitigate the damaging effects of chronic UV radiation exposure, and its overall support for long-term skin health and elasticity(Michalak et al., 2021).

2.4. Nano selenium Biofortification in Poultry Products for Human Consumption

Poultry products, particularly meat and eggs, represent an efficient and sustainable vehicle for selenium biofortification, offering a practical strategy to enhance human dietary selenium intake. Selenium deficiency remains a global nutritional concern, affecting immune competence, thyroid function, and antioxidant defence in many populations(Shreenath et al., 2024). The introduction of nano-selenium into poultry diets offers a viable solution to this problem via feed-to-food nutrient transfer (El-Ramady et al., 2020; Minich, 2022). SeNPs are more bioavailable, less toxic, and better retained in avian tissues than inorganic forms like sodium selenite (Bhattacharjee et al., 2019). This leads to elevated selenium accumulation in muscle, liver, and egg yolk (Ferroudj et al., 2025a; X. Zhou & Wang, 2011). These biofortified products can provide selenium in organic and nano-forms, which are better absorbed by humans than conventional sources (El-Ramady et al., 2020). Feed-to-food nutrient transfer approach is the potential solution to the deficiency issue by supplementation of nano-selenium to poultry diets. In addition to boost selenium content, nano-selenium supplementation improves poultry products' antioxidant defence and shelf life by reducing lipid peroxidation (Karadas et al., 2011; T. Meng et al., 2019). Nano-selenium-enriched eggs have increased albumen quality, yolk integrity, and antioxidant potential, whereas selenium-fortified meat retains great colour, tenderness, and nutritional value (S. J. Cai et al., 2012; Ferroudj et al., 2025a; Ferroudj et al., 2025b). Importantly, the utilisation of SeNPs has dual benefits, optimizing poultry productivity and contributing to public health nutrition, without altering the organoleptic qualities of the final products. However, obtaining safe and effective nano-selenium biofortification necessitates careful regulation of dose, nanoparticle size, and physicochemical form, as excessive accumulation may pose toxic to both animals and consumers. Future research should focus on consistent safety assessments, kinetic analysis of selenium transfer, and determining human bio-accessibility from enhanced poultry products. Integrating nanotechnology-based micronutrient fortification into poultry production provides an innovative, long-term solution for addressing shortages of selenium while linking the feeding of animals with human health interests(Abd El-Ghany et al., 2021).

3. MATERIALS AND METHODS

3.1. Production of Selenium nanoparticles

Sodium selenite, Vitamin C, nitric acid 65% (AR grade), hydrogen peroxide, and hydrochloric acid 37% (AR grade) were obtained from VWR, International Ltd. (Lutterworth, Leics, UK). Sodium borohydride 98% (AR grade) was purchased from Acros Organics (Geel, Belgium).

Selenium nanoparticles (SeNPs) were synthesized in aqueous solution by chemical reduction of sodium selenite using ascorbic acid. Sodium selenite was dissolved in deionized water to prepare stock solutions with selenium concentrations of 1000, 700, 500 mg/L (for physicochemical analysis), 5, 0.5, 0.05 mg/L (for animal testing). Reduction was initiated by the gradual addition of a 1% (w/v) ascorbic acid solution (corresponding to 1 g of ascorbic acid powder dissolved in 100 mL of distilled water) under continuous magnetic stirring at room temperature. For each synthesis, the sodium selenite and ascorbic acid solutions were mixed in equal volumes and allowed to react for 30 minutes at ambient temperature. The formation of red selenium nanoparticles was confirmed visually by the appearance of a characteristic red colouration. For analyses requiring solid material, including scanning electron microscopy coupled with energy-dispersive X-ray spectroscopy (SEM–EDS), X-ray diffraction (XRD), and Raman spectroscopy, the red SeNPs suspensions were purified by centrifugation at 6000 rpm for 10 min. This step was repeated three times, with washing using distilled water after each cycle to remove residual reactants. The purified nanoparticles were subsequently freeze-dried to obtain dry red SeNPs powder. Grey selenium nanoparticles were obtained by thermal transformation of red SeNPs in aqueous suspension. Red SeNPs suspensions were heated at 85 °C for 10 min, following the reported protocol (Khandsuren & Prokisch, 2021a), resulting in a visible colour change indicative of conversion to the grey selenium allotrope. Both liquid suspensions and freeze-dried powders were used for animal experiments and subsequent analyses, respectively.

3.2. Characterization of Selenium nanoparticles allotropes

3.2.1. Scanning Electron Microscopy (SEM) and Energy-Dispersive X-ray Spectroscopy (EDS)

The morphology and surface structure of red and grey selenium nanoparticles were examined using scanning electron microscopy (SEM). Measurements were performed at the Institute for Nuclear Research (ATOMKI) using a dual-beam focused ion beam–scanning electron microscope (FIB–SEM, Thermo Fisher Scientific, Model: Scios 2, Waltham, MA, USA). Freeze-dried SeNPs powders (1000mg/L) were mounted on carbon adhesive tape prior to imaging. Particle size analysis was conducted by measuring particle dimensions directly from SEM micrographs using image analysis software. Elemental composition was analyzed using an energy-dispersive X-ray spectroscopy (EDS) system (Bruker Quantax) integrated with the SEM. EDS spectra were collected to confirm the elemental composition of the selenium nanoparticles and to assess the presence of potential impurities.

3.2.2. X-ray Diffraction (XRD) Analysis

The crystalline structure of red and grey selenium nanoparticles was investigated by X-ray diffraction (XRD) using a Rigaku SmartLab diffractometer equipped with Cu Kα radiation ($\lambda = 0.154$ nm). Measurements were carried out in θ–2θ scanning geometry, with the X-ray tube operated at 45 kV and 200 mA. Freeze-dried SeNPs samples (1000mg/L) were gently ground to a fine powder using a mortar and pestle and then pressed into the sample holder. Diffraction patterns were recorded over a 2θ range of 15–75° to identify the structural phase and degree of crystallinity of the samples. Phase identification was performed by comparison with standard reference patterns from the ICDD database.

3.2.3. Raman Spectroscopy

Raman spectroscopy was used to characterize the vibrational properties and allotrope-dependent structural features of selenium nanoparticles. Raman spectra were recorded using a LabRAM HR Evolution Confocal Raman Microscope (Horiba Ltd., Kyoto, Japan). Measurements were performed on 1000mg/L freeze-dried red and grey SeNPs powders using a 532 nm excitation laser. Spectra were collected over the relevant Raman

shift range to identify characteristic selenium vibrational modes associated with amorphous and crystalline structures.

3.2.4. Fluorescence Spectroscopy

The optical properties of selenium nanoparticles were evaluated using fluorescence spectroscopy. Measurements were carried out on SeNPs aqueous suspensions with selenium concentrations of 500, 700, and 1000 mg/L. Fluorescence spectra were recorded using a Jasco FP-8500 spectrofluorometer (Jasco, Oklahoma, USA). Excitation–emission matrix (EEM) measurements were performed with excitation wavelengths scanned over 320-480 nm, and corresponding emission spectra were collected to evaluate excitation-dependent fluorescence behaviour. Fluorescence intensity data were analyzed as a function of selenium concentration, and the resulting relationships were fitted using polynomial models.

3.2.5. Atomic Fluorescence Spectrometry

Selenium concentrations were determined using an atomic fluorescence spectrometer (Millennium Excalibur, PSA, England) following the procedures recommended and validated by the instrument manufacturer. Selenium hydride was generated by a hydride formation reaction using two reagent solutions delivered at a flow rate of 1.5 mL/min: 3 M hydrochloric acid (HCl) and 1.4% (w/v) sodium borohydride ($NaBH_4$) prepared in 0.1 M sodium hydroxide (NaOH). The resulting hydrogen selenide (H_2Se) gas was transported by an argon stream at 15 L/min through a double-membrane separator (PermaPure). The diffused gas was introduced into a flame, where selenium atoms were excited by monochromatic radiation from a hollow cathode lamp and subsequently emitted fluorescence detected at right angles to the incident beam.

Each analysis consisted of a 30 second sample aspiration followed by a 30-second rinse for background correction. Measurements were performed in triplicate, and quantification was achieved using calibration with a Charlau selenite standard solution. For sample preparation, 1 mL of the selenium-containing solution was digested with 5 mL of concentrated nitric acid (HNO_3, 65% w/w) at 60 °C for 60 min. Subsequently, 3 mL of hydrogen peroxide (H_2O_2, 30% w/w) was added, and the mixture was further digested for 4 h at 120 °C. After cooling, the digests were diluted to a final volume of 15 mL with 3 M hydrochloric acid and filtered through filter paper before analysis.

3.3. Animal experiments

This study was conducted at the University of Debrecen, Faculty of Agricultural and Food Sciences and Environmental Management, Institute of Animal Science, Biotechnology and Nature Conservation, Department of Animal Husbandry, Nanofood laboratory, Hungary. It was approved by the institutional Ethics Committee of the University of Debrecen (ethical permission number: 4/2021/DEMÁB). All methods were performed following the relevant guidelines and regulations.

3.3.1. General Husbandry and Dietary Management

Both animal trials were conducted on adult male Japanese quails (Coturnix japonica) at the experimental facilities of the University of Debrecen, Hungary. Birds were individually housed in wire cages under standardized environmental conditions (temperature: 25 ± 2 °C; photoperiod: 16 h light/8 h dark) to allow precise monitoring of feed intake and health status. Throughout the experimental periods, quails had ad libitum access to drinking water, and approximately 18 g of feed per bird per day was offered. The basal diet **Table 5** was formulated using soybean, corn, wheat and sunflower oil taking into account the nutrient requirements of breeder quails according to (National Research Council, 1994). The premix included in the basal diet provided a background selenium content of 0.21 mg/kg. Selenium nanoparticle (SeNP) supplements (red and grey allotropes) were thoroughly homogenized into the basal diet to achieve the target selenium concentrations for each treatment group **Figure 7**.

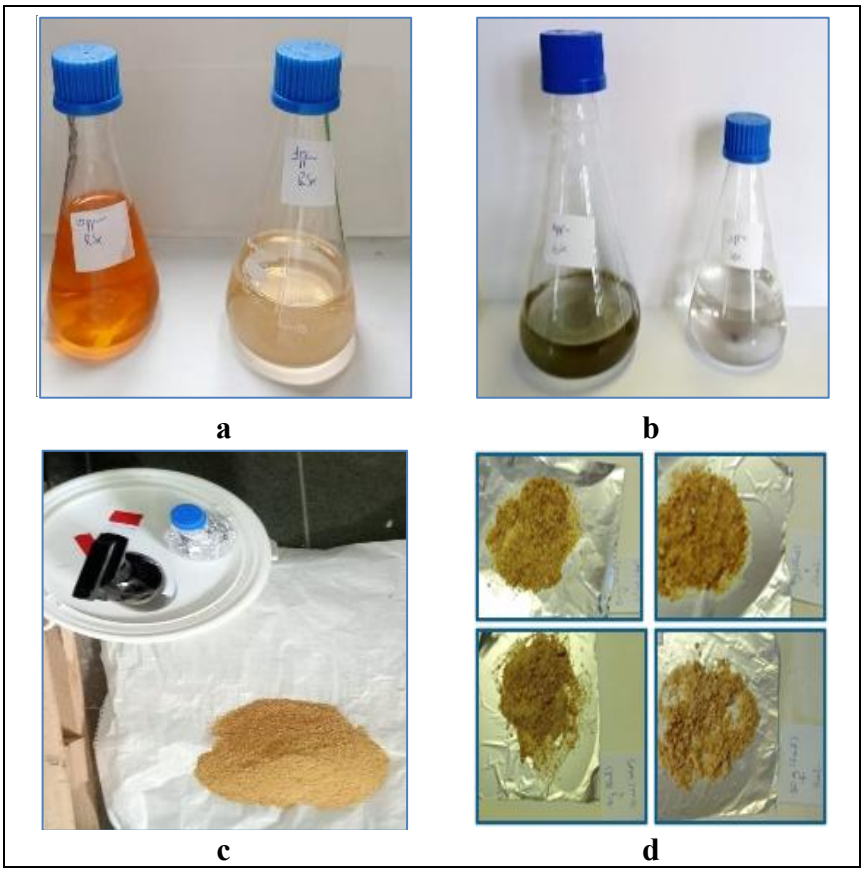


Figure 7: Preparation and Incorporation of Selenium Nanoparticles in Experimental Diets. (**a**) synthesis of red selenium nanoparticles; (**b**) synthesis of grey selenium nanoparticles; (**c**) preparation of selenium-supplemented feed; (**d**) homogenization of selenium nanoparticles in feed samples

Birds were randomly allocated to dietary treatments based on initial body weight to ensure uniformity among groups. All experimental procedures were carried out in accordance with institutional ethical guidelines for animal experimentation.

Table 5: Ingredients and nutrient composition of the diet

Feed Ingredients	Inclusion Rate, %
Soybean meal (46% CP)	34.88
Corn	30.37
Wheat	20.00
Sunflower oil	6.79
Limestone	5.64
MCP	1.29
Salt	0.38
DL-Methionine	0.15
Vitamin and mineral premix [a]	0.50
Nutrient content, %	
Metabolisable energy MJ/kg	12.13
Crude protein	20.0
Calcium	2.50
Available Phosphorus	0.35
Sodium	0.15
Methionine	0.45
Methionine + cysteine	0.75
Lysine	1.08
Threonine	0.74
Leucine	1.59
Isoleucine	0.86
Arginine	1.33
Tryptophan	0.25

[a] 1 kg premix provided: 1,000,000 NE vitamin A, 200,000 NE vitamin D3, 4900 mg/kg vitamin E, 200 mg vitamin K3, 150 mg vitamin B1, 500 mg vitamin B2, 1200 mg Ca-d-Pantothetane, 400 mg vitamin B6, 2 mg vitamin B12, 11 mg biotin, 2502 mg niacin, 60 mg folic acid, 300,000 mg choline chloride, 13,200 mg Zn, 1920 mg Cu, 9612 mg Fe, 13,200 mg Mn, 180 mg I, 42 mg Se, 12 mg Co.

3.3.2. First Animal Experiment: Growth Performance and Tissue Selenium Distribution

3.3.2.1. Experimental Design

The first animal experiment was conducted in May as a 28-day feeding trial to evaluate the effects of dietary red and grey SeNPs supplementation on growth performance and organ-specific selenium accumulation. A total of 20 adult male Japanese quails (11 weeks of age) were used. Birds were randomly assigned to five dietary treatment groups (n = 4 per group):

C0 (Control): basal diet without SeNPs supplementation

T1: basal diet + 0.05 mg/kg red SeNPs

T2: basal diet + 0.5 mg/kg red SeNPs

T3: basal diet + 0.05 mg/kg grey SeNPs

T4: basal diet + 0.5 mg/kg grey SeNPs

Considering the background selenium in the premix, total dietary selenium concentrations were approximately 0.21 mg/kg (C0), 0.26 mg/kg (T1 and T3), and 0.71 mg/kg (T2 and T4).

3.3.2.2. Growth Performance

Body weight (BW) and feed intake (FI) were recorded daily for each bird throughout the 28-day experimental period. Daily monitoring allowed precise tracking of growth dynamics and feeding behaviour and facilitated the early identification of any potential physiological or behavioural changes associated with selenium nanoparticle supplementation. Averages of BW and FI were subsequently calculated and used to evaluate overall growth performance **Figure 8**.

Figure 8: Daily measurements of body weight and feed intake

3.3.2.3. Tissue Sampling and Selenium Analysis

At the end of the feeding period, all birds were euthanized. Liver and spleen were excised and weighed immediately to determine organ indices. Tissue samples were collected from the liver, spleen, kidney, blood, testis, breast muscle, and eyes **Figure 9**. Blood samples were collected into appropriate tubes. All tissues were washed with phosphate-buffered saline (PBS) and stored at −80 °C until analysis.

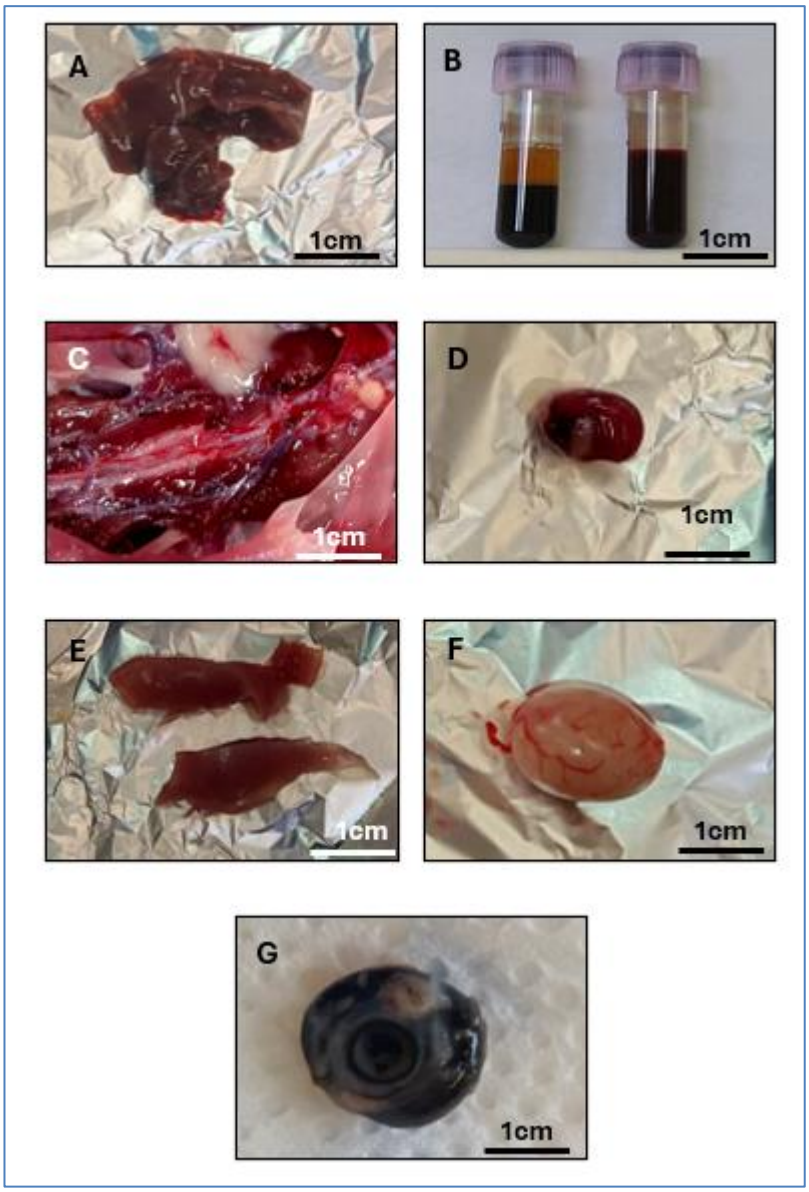


Figure 9: Japanese quails 'Tissues collection; **A:**Liver, **B:** Blood samples, **C:** Kidneys, **D:** Spleen, **E:** Breast Meat, **F:** Testis, **G:** Eye.

3.3.3. Second Animal Experiment: Antioxidant Status and Selenium Retention/Depletion

3.3.3.1. Experimental Design

The second animal experiment was conducted in July to investigate antioxidant responses and selenium retention and depletion following dietary SeNPs supplementation and a withdrawal phase. A total of 60 adult male Japanese quails (12 weeks of age) were used. Birds were randomly assigned to five dietary treatment groups (n = 12 per group):

C0 (Control): basal diet without SeNPs supplementation

T1: basal diet + 0.5 mg/kg red SeNPs

T2: basal diet + 5 mg/kg red SeNPs

T3: basal diet + 0.5 mg/kg grey SeNPs

T4: basal diet + 5 mg/kg grey SeNPs

The estimated total dietary selenium contents were 0.21 mg/kg (C0), 0.71 mg/kg (T1 and T3), and 5.21 mg/kg (T2 and T4).

Birds were fed the experimental diets for 28 days. Thereafter, half of the birds from each group (n = 6) were euthanized for sampling. The remaining birds continued for an additional 7 days on the unsupplemented basal diet (Nano-selenium-free withdrawal phase) and were euthanized on day 35 to evaluate residual selenium levels. Feed intake was recorded daily and expressed as g/bird/day.

3.3.3.2. Tissue Sampling and Selenium Analysis

At day 28, birds (n = 6 per treatment) were randomly selected and euthanized. Tissue samples were collected from the liver, kidney, spleen, testis, breast muscle, eyes, and blood. Blood samples were centrifuged in anticoagulant tubes at 3000 rpm for 15 min to separate the red blood fraction (RBF) and serum. All samples were washed with PBS and stored at −80 °C until analysis.

After the 7-day withdrawal period (day 35), the remaining birds in each treatment group were euthanized and tissue samples were collected from the liver, kidney, spleen, and blood, following the same sampling procedure.

Tissue digestion and selenium determination were performed as described in **Section 3.2.5**, Briefly, tissue samples were digested using nitric acid and hydrochloric acid, followed by selenium quantification using atomic fluorescence spectrometry (Millennium Excalibur 10.055, PSA, UK).

Selenium retention and depletion after the withdrawal period were calculated only for the liver, kidney, spleen, and RBF, as these organs represent the principal metabolic compartments and by using the following equations:

$$Total\ Se\ retention\ \% = \left(\frac{\sum(\text{Se in organ after withdrawal (35d)})}{\sum(Se\ in\ organ\ before\ withdrawal\ (28d))}\right) \times 100$$

$$Se\ depletion\ \% = \left(1 - \frac{\sum(\text{Se in organ after withdrawal})}{\sum(Se\ in\ organ\ before\ withdrawal)}\right) \times 100$$

3.3.3.3. Antioxidant Biomarker Analysis

Antioxidant capacities were assessed by measuring glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), and total antioxidant capacity (T-AOC). Liver homogenates were used for GSH-Px determination, while both liver and serum samples were analyzed for SOD and T-AOC. Commercial assay kits were employed: Invitrogen™ Glutathione Peroxidase (GSH-Px) Activity Kit (Cat. No. EEA010, Thermo Fisher Scientific, Waltham, MA, USA), Invitrogen™ Superoxide Dismutase (SOD) Colorimetric Activity Kit (Cat. No. EIASODC, Thermo Fisher Scientific, USA), and Antioxidant Assay Kit (Cat. No. KA1622, Abnova, Taipei, Taiwan). Absorbance was measured using a SPECTROstar Nano microplate reader (BMG LABTECH GmbH, Ortenberg, Germany), following the manufacturer's instructions.

3.3.4. Statistical analysis

All statistical analyses for both animal experiments were performed using appropriate software packages. Data from the first experiment were analyzed using MINITAB software (version 19.1, Minitab LLC, State College, PA, USA), while data from the second experiment were analyzed using GraphPad Prism (version 9.5.0, GraphPad Software, San Diego, CA, USA). Results are presented as mean values ± standard error of the mean (SEM). Statistical significance was accepted at $p < 0.05$.

For the first experiment, differences among dietary treatment groups were evaluated using one-way analysis of variance (ANOVA). When a significant treatment effect was detected, Fisher's pairwise comparison test was applied to identify differences between means.

For the second experiment, tissue selenium concentrations were analyzed using two-way ANOVA with selenium nanoparticle form (red vs. grey) and dietary dose (0, 0.5, and 5 mg/kg) as fixed factors, and analyses were conducted separately for each organ.

Feed intake, antioxidant parameters (GSH-Px, SOD, and T-AOC), and average selenium content were analyzed by one-way ANOVA with dietary treatment as the independent factor. Selenium distribution among organs within each treatment group was analyzed using one-way ANOVA with organ as the factor. When significant effects were observed, Tukey's honestly significant difference (HSD) post hoc test was used for multiple comparisons.

4. RESULTS

4.1. Selenium nanoparticles production

The chemical reduction of sodium selenite with ascorbic acid resulted in the successful formation of selenium nanoparticles, as evidenced by the development of a distinct reddish colouration in the reaction mixtures. The appearance of this colour indicated the formation of colloidal red selenium nanoparticles and was observed consistently for all prepared concentrations. The red SeNPs suspensions remained visually stable during the reaction period, with no apparent precipitation at low concentrations of 0.05, 0.5, and 5 mg/L. Upon thermal treatment of the red SeNPs suspensions at 85 °C for 10 min, a clear colour change from red to grey was observed, confirming the transformation of amorphous red selenium into the grey selenium allotrope. This visual transition was reproducible across all batches, indicating effective allotrope conversion under the applied conditions. After purification and freeze-drying, the red SeNPs were obtained as reddish solid powders, whereas the grey SeNPs formed dark grey to black powders, demonstrating that the characteristic colour differences were preserved in the solid state. Representative images showing the visual appearance of selenium nanoparticles in suspension and after freeze-drying are presented in the **Figure 10**. The marked differences between the red and grey forms provide qualitative confirmation of successful synthesis and thermal transformation prior to further physicochemical characterization.

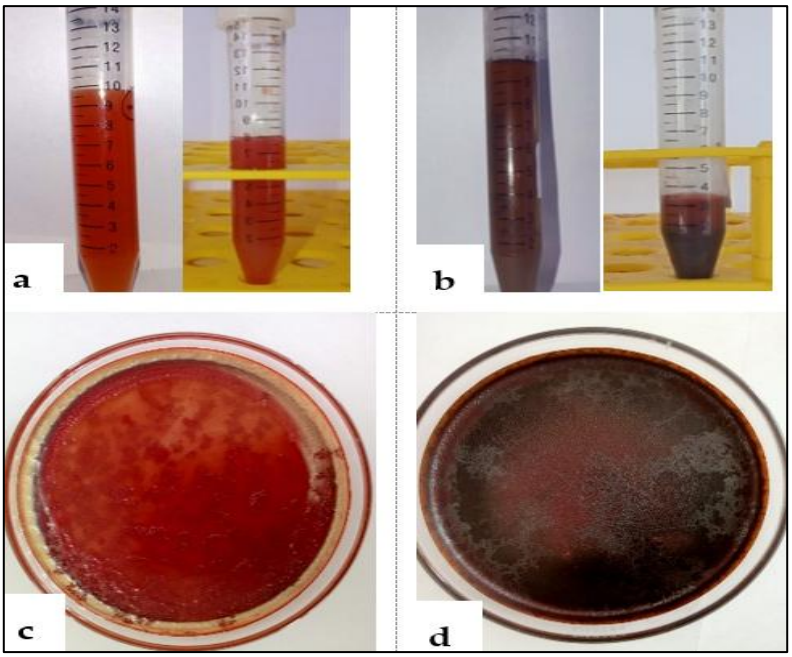


Figure 10: Visual appearance of selenium nanoparticles in two allotropes at 1000mg/L. (a) Red SeNPs suspension, (b) grey SeNPs suspension, (c) red SeNPs solid after freeze-drying, and (d) grey SeNPs solid after freeze-drying. Suspensions were used for fluorescence measurements, while solid forms were analysed by SEM-EDS, XRD and Raman spectroscopy

4.2. Characterization of Selenium nanoparticles allotropes

The physicochemical and optical properties of red and grey selenium nanoparticles (SeNPs) were systematically investigated using a combination of scanning electron microscopy (SEM), particle size analysis, energy-dispersive X-ray spectroscopy (EDS), X-ray diffraction (XRD), Raman spectroscopy, and fluorescence spectroscopy **Figure 11**. This multi-technique approach was employed to elucidate allotrope-dependent differences in morphology, structure, elemental composition, and optical behaviour.

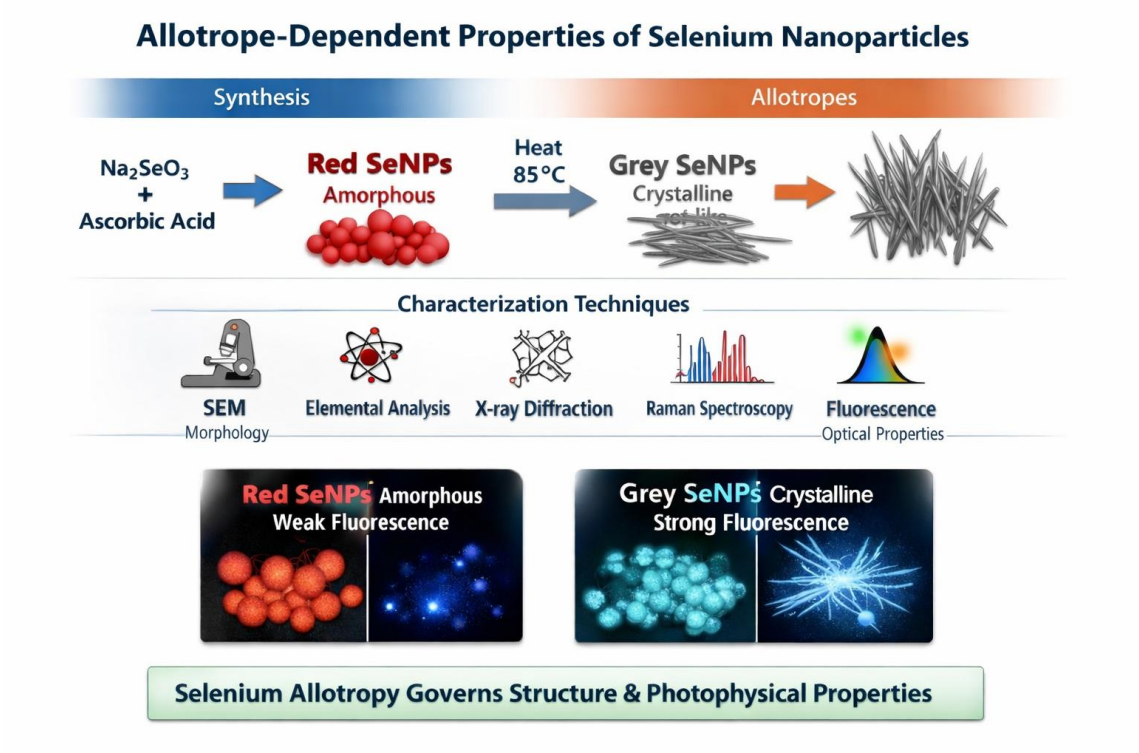


Figure 11: Graphical overview of selenium nanoparticle synthesis and characterization (created by the author)

4.2.1. SEM-EDS analysis

To examine allotrope-dependent differences in morphology and particle dimensions, scanning electron microscopy (SEM) was employed to characterize the surface structure and size distribution of red and grey selenium nanoparticles **Figures 12-13.**

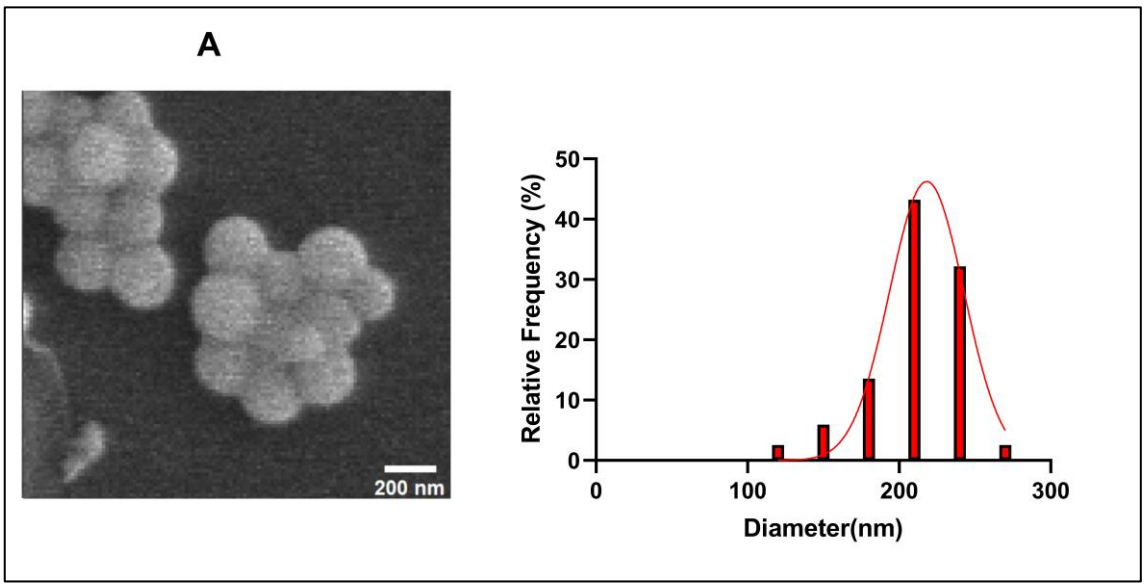


Figure 12: SEM Images of: (A) red SeNPs and diameter distribution histogram

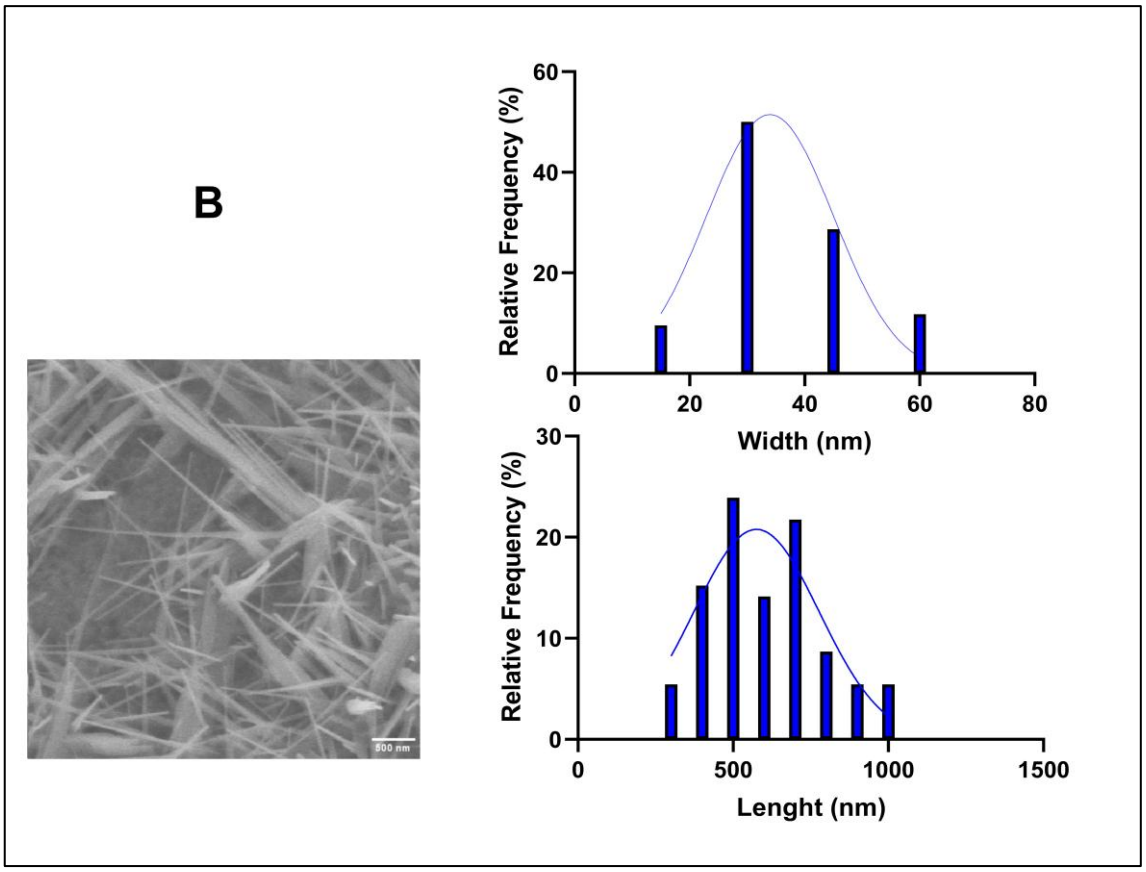


Figure 13: SEM Images of: (B) grey SeNPs and length and width distribution histograms

SEM analysis revealed pronounced morphological differences between the two selenium allotropes. Red SeNPs exhibited a quasi-spherical to spherical morphology, forming compact nanoaggregates composed of smaller primary particles with smooth, non-faceted surfaces **(Fig.12)**. Particle size analysis based on SEM images indicated a narrow and unimodal size distribution, with apparent particle diameters predominantly in the range of 200–220 nm, with a mean diameter of 218±24 nm. These dimensions represent aggregated nanoparticle domains typical of dried amorphous systems.

Such spherical morphology is commonly associated with amorphous selenium nanoparticles synthesized under kinetically controlled reduction conditions, where isotropic nucleation dominates due to the absence of long-range atomic order (Khandsuren & Prokisch, 2021a, 2021b; K. Li, Zhu, et al., 2024; Tendenedzai et al., 2022). The morphology observed in the present study, therefore, supports the amorphous nature of the red SeNPs.

In contrast, grey SeNPs displayed a highly anisotropic, needle-like morphology, forming interconnected networks of elongated nanostructures **(Fig.13)**. Due to their non-spherical

shape, size analysis was performed by separately evaluating length and width. The nanoneedles exhibited lengths mainly between 400 and 900 nm, with a mean value of 575±202 nm, while widths were significantly smaller, predominantly 20–50 nm and an average of 33.9±11. This pronounced aspect ratio reflects directional crystal growth, which is characteristic of crystalline selenium. This anisotropic morphology is consistent with preferential growth along the helical Se–Se chains that define the trigonal selenium lattice (Chellapa et al., 2020; Khandsuren & Prokisch, 2021a; L. Ren et al., 2004), indicating successful thermal transformation of amorphous red selenium into the crystalline grey allotrope.

Following morphological characterization, energy-dispersive X-ray spectroscopy (EDS) was used to verify the elemental composition and assess the purity of both red and grey selenium nanoparticles **Figure 14.**

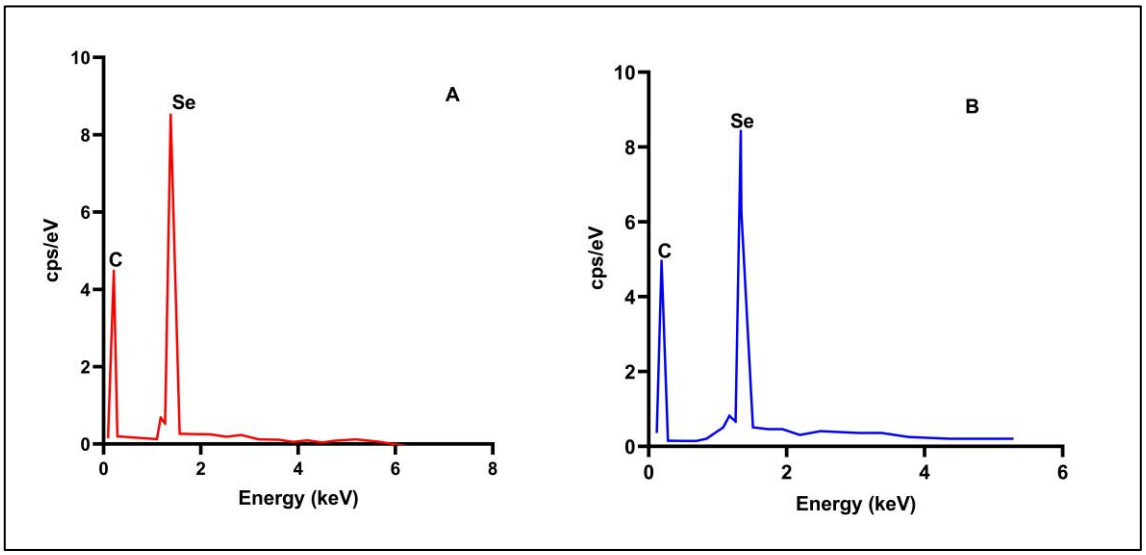
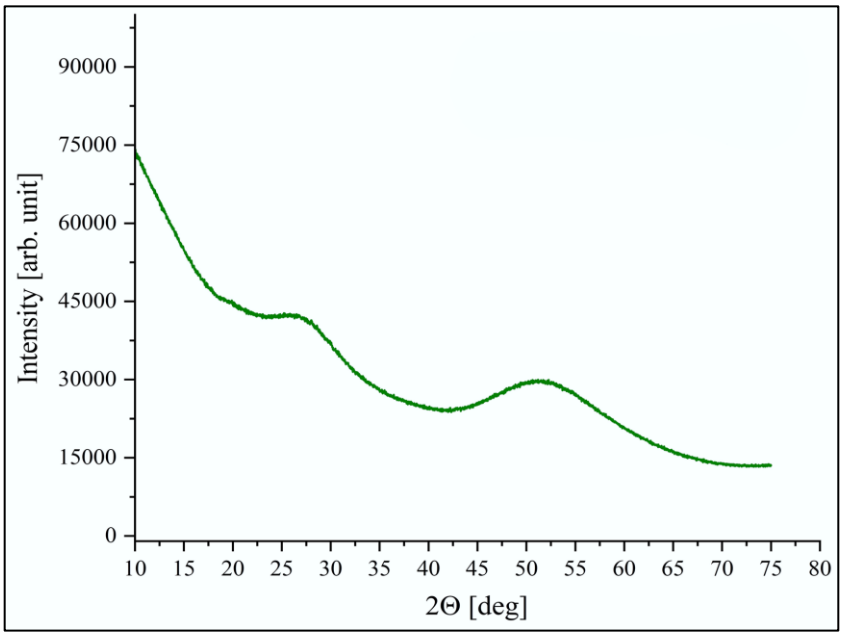


Figure 14: EDS spectra of (A) red SeNPs and (B) grey SeNPs

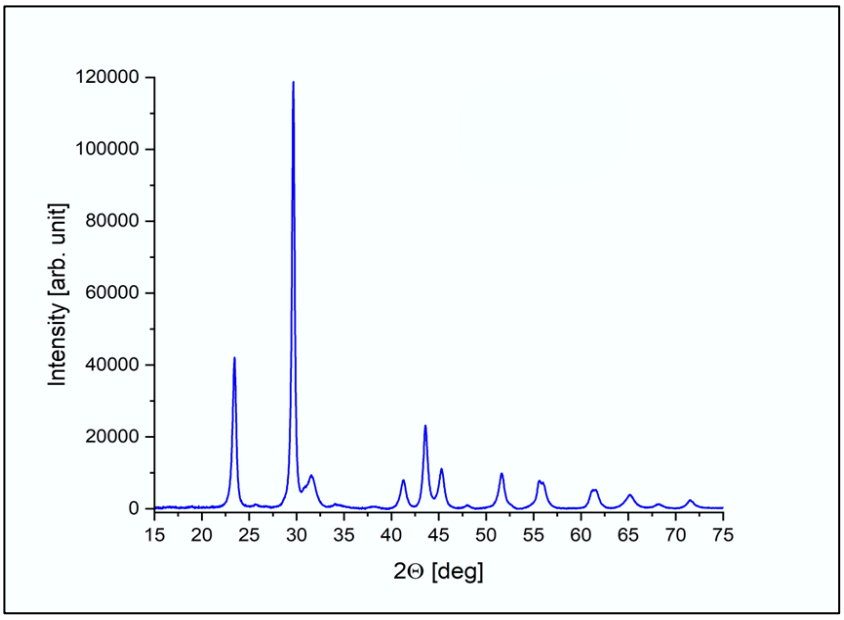
EDS analysis confirmed selenium as the dominant elemental constituent in both red and grey SeNPs, with a characteristic Se Lα peak at ~1.37 keV observed in all spectra. A carbon signal was also detected, which can be attributed to the carbon substrate used during SEM–EDS analysis. No additional elemental impurities were detected within the sensitivity limits of the technique, indicating comparable elemental purity for both allotropes. Importantly, EDS confirmed that the observed differences between red and grey SeNPs arise from structural and morphological variations rather than elemental composition.

4.2.2. XRD analysis

To determine the structural nature and crystallinity of the synthesized selenium nanoparticles, X-ray diffraction (XRD) analysis was performed on both allotropes **Figure 15.**



A



B

Figure 15: XRD patterns of (A) red SeNPs and (B) grey SeNPs

XRD analysis revealed a fundamental structural distinction between the two selenium allotropes. The diffraction pattern of red SeNPs was characterized by broad, diffuse scattering features and the absence of sharp Bragg reflections, indicating an

amorphous structure with no long-range atomic order. A weak hump in the 20–30° 2θ range was attributed to short-range atomic correlations typical of amorphous selenium.

In contrast, grey SeNPs exhibited multiple sharp and intense diffraction peaks across the scanned 2θ range, corresponding to crystalline trigonal selenium (t-Se). The narrow peak widths and high intensities confirmed a high degree of crystallinity and long-range periodicity. No amorphous halos or secondary phases were observed, indicating a complete transformation to the crystalline grey allotrope. These findings are in excellent agreement with previous reports describing amorphous red selenium and crystalline trigonal grey selenium nanostructures(Alex et al., 2024; Fardsadegh et al., 2019; Y. Wang et al., 2012; Xi et al., 2006; Xiong et al., 2006). Together with SEM results, the XRD patterns demonstrate that the two SeNPs systems differ fundamentally in atomic ordering, which underlies their distinct morphologies.

4.2.3. Raman spectroscopy

Raman spectra of selenium are presented in the **Figure 16,** confirming structural differences between red and grey SeNPs. The red SeNPs exhibited characteristic and weak peaks in the range of 250–255 cm^{-1}, which are typically associated with the amorphous state of selenium, where structural disorder leads to peak broadening and poorly resolved vibrational modes. In contrast, the grey SeNPs showed distinct peaks between 230–235 cm^{-1}, corresponding to the A_1 vibrational mode of trigonal selenium associated with Se–Se stretching along helical chains. The narrow linewidth and high intensity of this peak indicate well-ordered atomic arrangements, in excellent agreement with the crystalline structure identified by XRD.

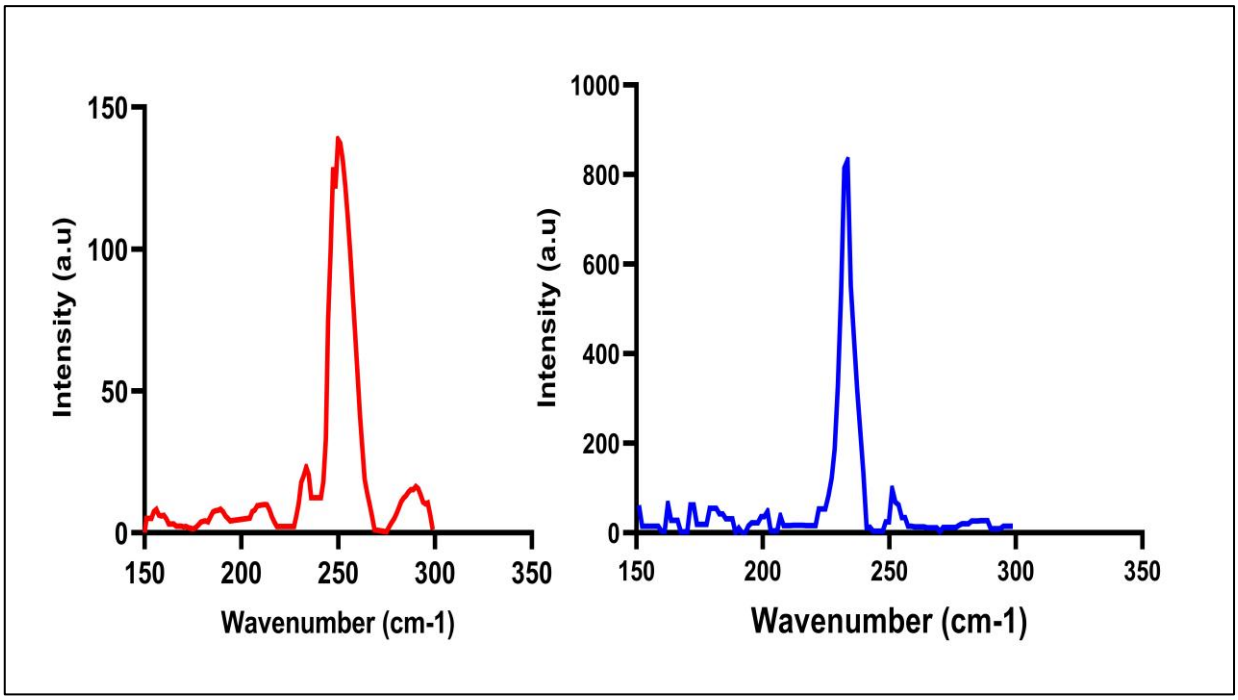


Figure 16: Raman spectra of Selenium (redline) Red Se. (blueline) Grey Se

Similar Raman features for amorphous and crystalline selenium have been reported previously (Anupama et al., 2021; Baganich et al., 1991; Goldan et al., 2016; Kizovský et al., 2021; Tugarova et al., 2018); the amorphous phase typically shows Raman bands around 250 cm^{-1}, while crystalline selenium occurs in two polymorphic forms: pure trigonal structure, characterized by the ~233-237 cm^{-1} band observed in this study, and the monoclinic crystals, which appear near 251 cm^{-1}. Raman spectroscopy, therefore, provides strong complementary evidence for the allotrope-dependent structural states of the synthesized SeNPs. The sensitivity of selenium Raman bands to environmental and oxidative conditions further reflects the redox-active nature of selenium (Lopez et al., 1981), emphasizing the usefulness of Raman spectroscopy in distinguishing selenium phases at the nanoscale.

4.2.4. Fluorescence analysis

The optical properties of the amorphous red selenium nanoparticles and crystalline grey selenium nanoparticles were further investigated using excitation–emission fluorescence spectroscopy to evaluate their electronic and surface-related states **Figure 17-18.**

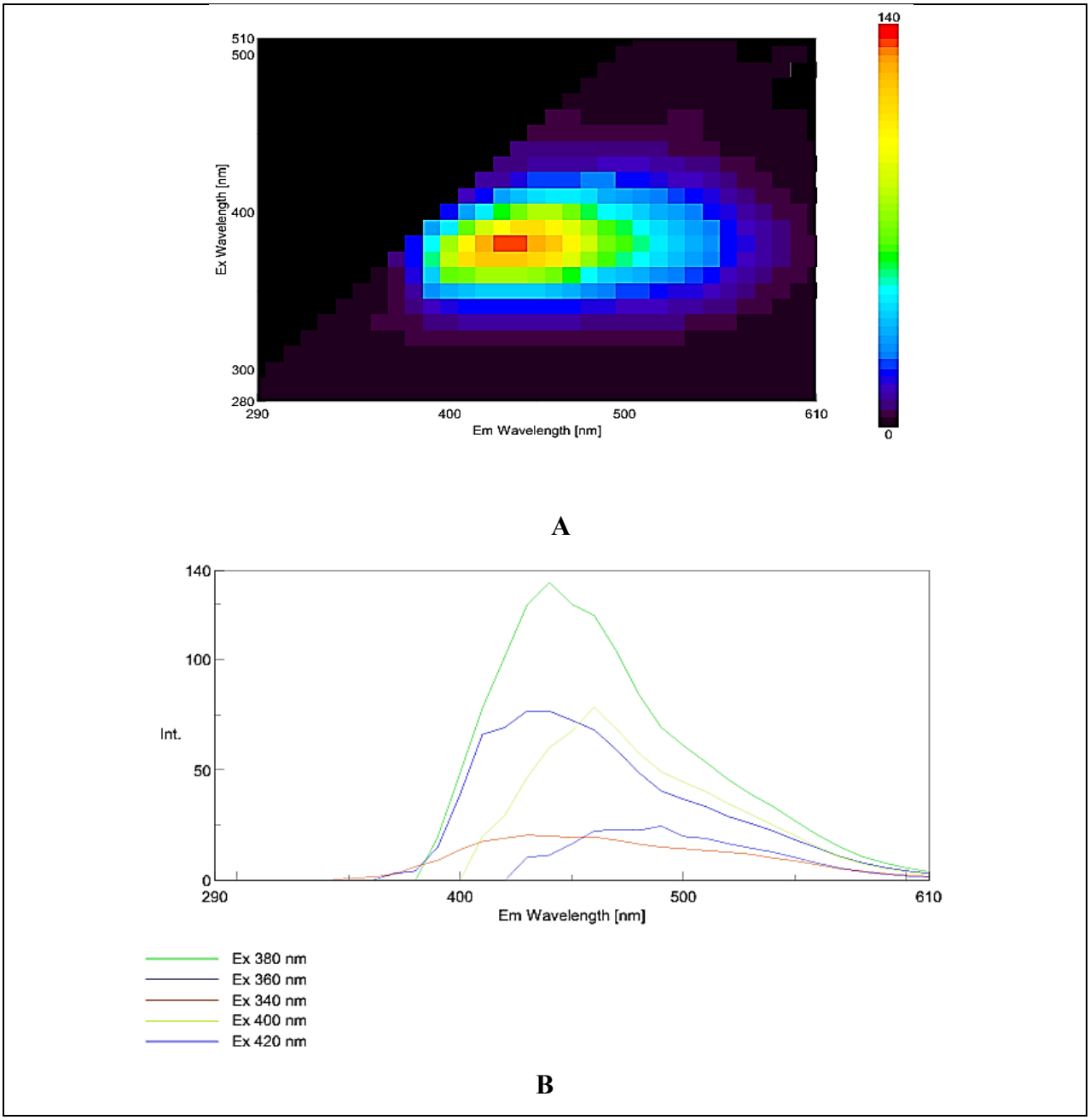


Figure 17: Fluorescence characteristics of red SeNPs at 1000 mg/L: (A) 3D excitation–emission fluorescence spectrum of red SeNPs, and (B) 2D emission spectra recorded at different excitation wavelengths

The fluorescence characteristics of red SeNPs at 1000 mg/L were evaluated using full scan fluorescence analysis **Fig. 17-A**. A weak fluorescence region was observed with the maximum emission around 430–450 nm when excited at ~380 nm. The emission spectra recorded at selected excitation wavelengths **Fig. 17-B** confirmed this behaviour, with the highest fluorescence intensity obtained at 380 nm excitation, followed by moderate signals at 360 nm and 400 nm, while 340 nm and 420 nm resulted in comparatively weaker emissions. The overall low intensity highlights the limited optical

response of red SeNPs, distinguishing them from grey SeNPs, which displayed stronger fluorescence under similar conditions.

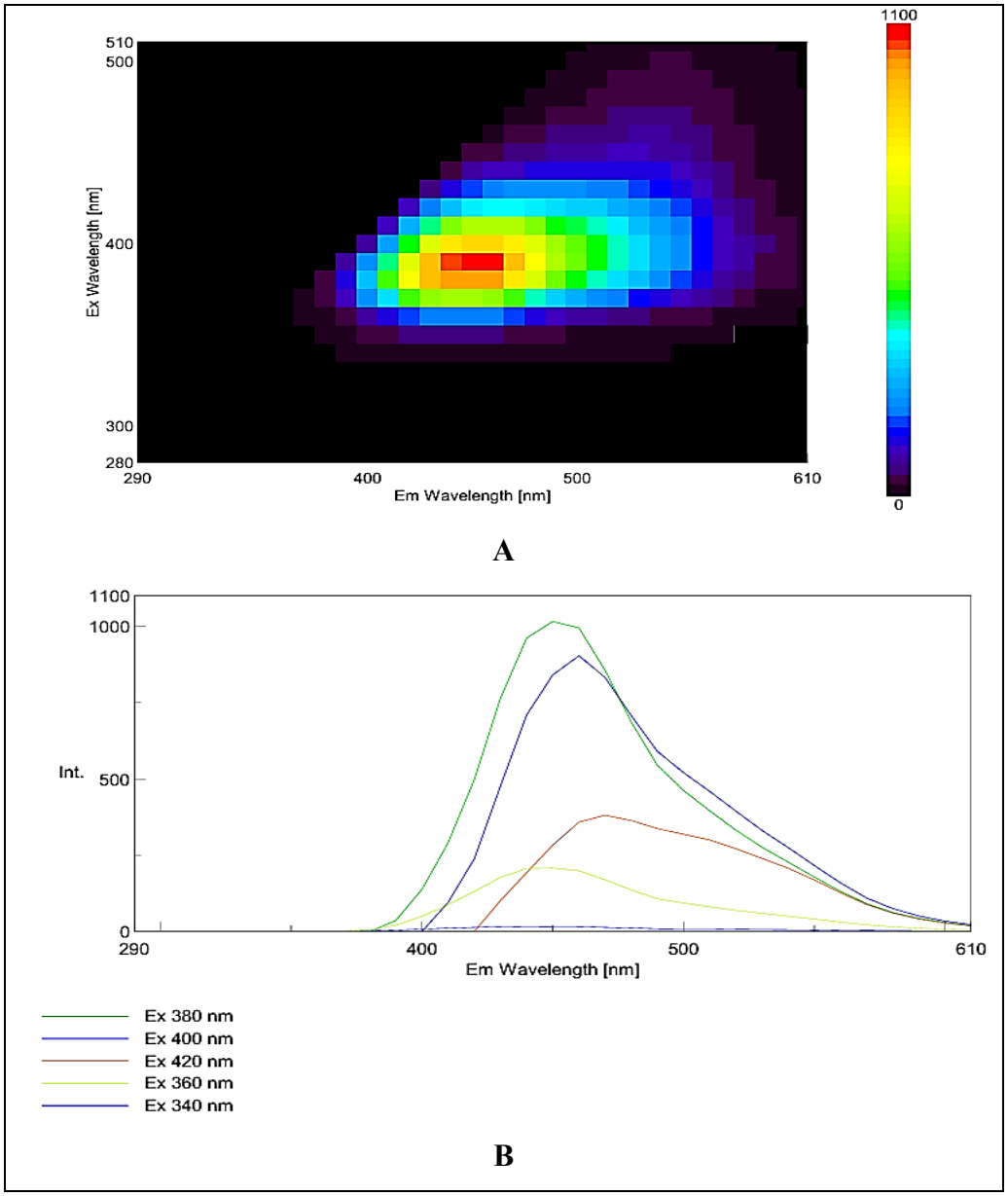


Figure 18: Fluorescence characteristics of grey SeNPs at 1000 mg/L: (a) 3D excitation–emission fluorescence spectrum of grey SeNPs, and (b) 2D emission spectra recorded at different excitation wavelengths

The fluorescence characteristics of grey SeNPs at 1000 mg/L were evaluated using full scan fluorescence analysis **Fig. 18-A**. A well-defined fluorescence region was observed with the maximum emission centred around 430–450 nm when excited at ~380–400 nm, indicating strong optical activity in the blue region. The emission spectra recorded at selected excitation wavelengths **Fig. 18-B** confirmed this behaviour, with the highest fluorescence intensity obtained at 380 nm excitation, followed closely by 400 nm,

while 420 nm, 360 nm, and 340 nm resulted in comparatively weaker emissions. The variation in intensity across excitation wavelengths highlights the excitation-dependent optical response of grey SeNPs. This behaviour is consistent with the strong optical activity of grey SeNPs, distinguishing them from red SeNPs, which exhibit lower fluorescence intensity under similar conditions. The optical properties of the SeNPs further support their nanoscale character. Biosynthesized SeNPs showed strong fluorescence activity in the 398–420 nm range, with excitation at 398 nm and emission intensities exceeding 800 a.u. (Tripathi et al., 2020). Red SeNPs additionally exhibited an absorption band in the UV–Vis spectrum at ~260–270 nm, confirming their distinct optical activity (Shahzamani et al., 2022). Such photoluminescence behaviour is strongly size- and surface-dependent, and selenium species are known to emit across the visible to near-infrared range (J. Wang et al., 2023). Notably, red SeNPs have been reported to fluoresce near the NIR region, making them suitable for biomedical applications such as imaging, diagnostics, and intracellular tracking (Khalid et al., 2016). For comparison, selenite itself typically emits at ~475 nm (Song et al., 2013), highlighting the altered optical behaviour of nano selenium. However, the literatures indicate that crystalline selenium nanostructures can indeed display significant fluorescence. For example, Gates et al. (2002) observed optical activity in trigonal selenium nanowires, and a more recent work demonstrated that selenium quantum dots with a trigonal structure exhibit strong solid-state fluorescence, with emission peaks varying between 418–449 nm depending on excitation wavelength (Anupama et al., 2021).

4.2.5. Concentration-Dependent Fluorescence Response

Fluorescence measurements were performed on red and grey selenium nanoparticles (SeNPs). Fluorescence intensity was measured across a concentration range of Se (0–1000 mg/L) in the sample solution **Figure 19**.

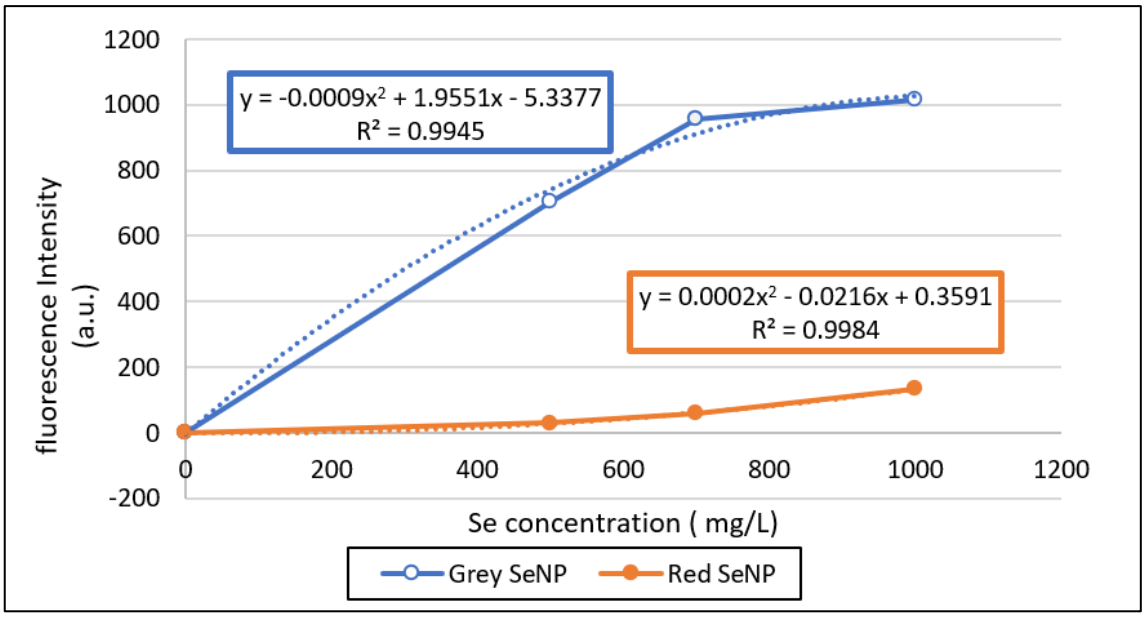


Figure 19: Fluorescence Intensity of Grey and Red Selenium Nanoparticles at Different Concentrations (mg/L)

Grey SeNPs exhibited a concentration-dependent increase in fluorescence intensity, reaching a maximum near 1000 mg/kg, with the relationship well described by a quadratic fit ($R^2 = 0.9945$). In contrast, red SeNPs showed only a marginal increase in fluorescence intensity across the tested concentrations, with considerably lower emission compared to grey SeNPs ($R^2 = 0.9984$). These findings highlight the stronger optical activity and concentration sensitivity of grey SeNPs relative to red SeNPs, suggesting distinct structural or surface-state contributions to their fluorescence behaviour (Anupama et al., 2021; Gates et al., 2002).

4.3. First Animal Experiment: Growth Performance and Tissue Selenium Distribution

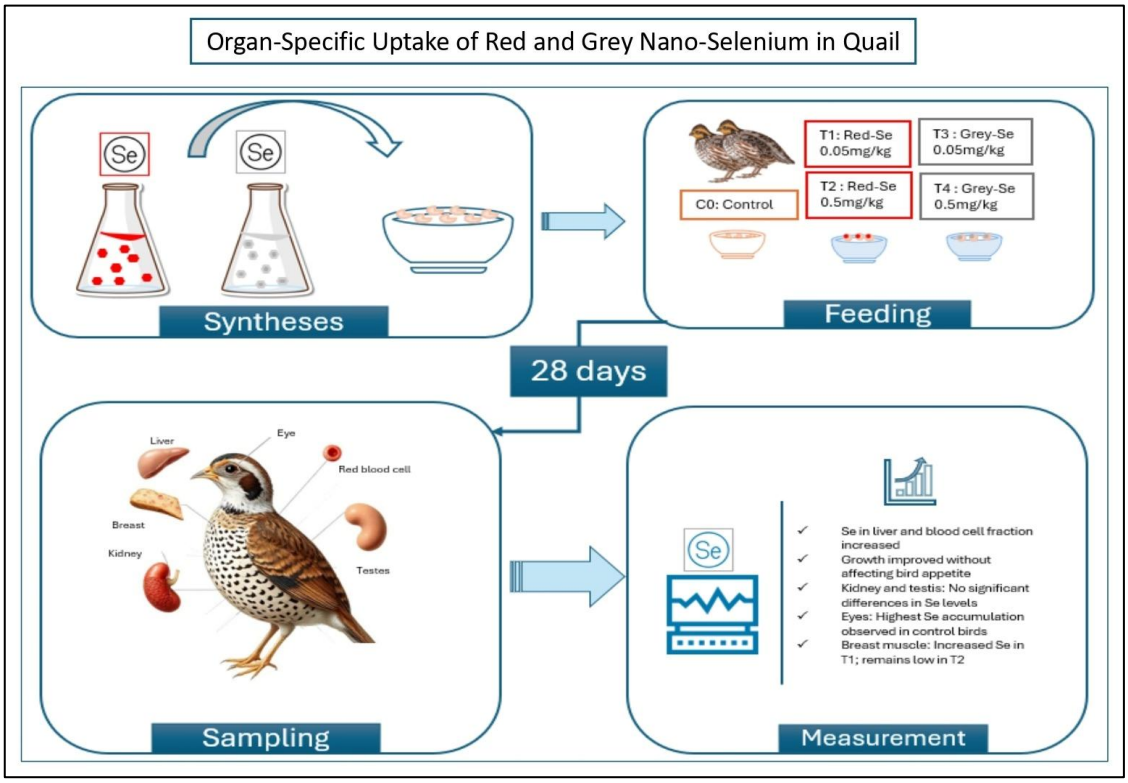


Figure 20: Graphical overview of the animal experimental design and main outcomes

4.3.1. Growth performance

Figure 21 presents the final live body weight of Japanese quails fed diets supplemented with different concentrations and forms of selenium nanoparticles. Body weight differed significantly among treatments ($p < 0.05$). The highest average body weight was observed in the T2 group (0.5 mg/kg red SeNPs). The control group (C0) showed an intermediate value and did not differ significantly from T2 or T4, as indicated by the statistical overlapping. The T4 group (0.5 mg/kg grey SeNPs) showed lower body weight than T2 but remained higher than T3 and T1. The T3 group (0.05 mg/kg grey SeNPs) exhibited a further reduction in body weight, while the lowest body weight was recorded in the T1 group (0.05 mg/kg red SeNPs). These results indicate that selenium nanoparticle supplementation influenced body weight in a dose- and form-dependent manner rather than consistently improving growth performance across all treatments.

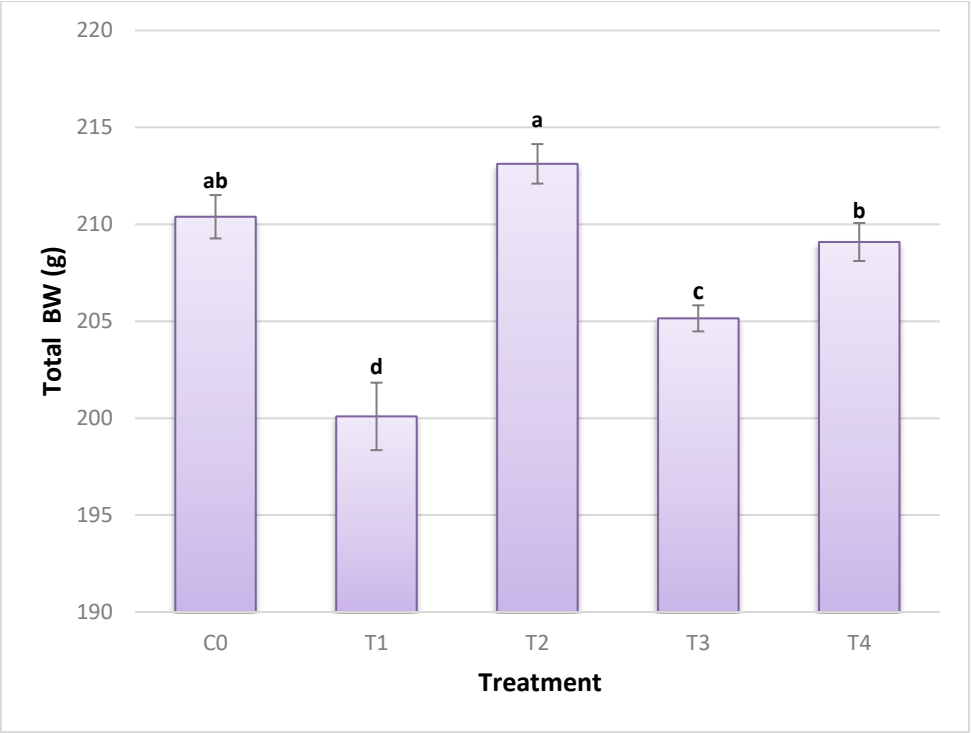


Figure 21: Influence of Different Levels of Selenium Nanoparticles (SeNPs) Dietary Supplementation on the Live Body Weight (g) ± SEM of Adult Japanese Quails. Means with the differing letters are Significantly Different ($p < 0.05$). C0: control; T1: 0.05 mg/kg Red SeNP; T2: 0.5 mg/kg Red SeNP; T3: 0.05 mg/kg Grey SeNP; T4: 0.5 mg/kg Grey SeNP

The feed intake was measured daily during the 28-day trial for the five groups treated with SeNPs supplements. **Figure 22** of average feed intake showed that the feed intake is not significantly affected by the various selenium doses and forms. All groups consumed comparable amounts of food; that indicates that Se did not influence the birds' appetite and feed consumption patterns. I Although differences in body weight were observed among treatments (Figure 21), these changes occurred without corresponding differences in feed intake, suggesting that the observed body weight responses were not related to variations in feed consumption. SeNPs are known to improve antioxidant defence, support thyroid hormone metabolism, and enhance selenoprotein synthesis—all of which are critical for maintaining physiological homeostasis and metabolic regulation in animals. Previous studies have shown that nano-selenium supplementation can improve antioxidant status and enzyme activity in poultry (Abdel-Moneim et al., 2020; Hosnedlova et al., 2018), who reported that nano-Se supplementation enhances antioxidant status, enzyme activity, and feed conversion efficiency. However, in the

present study, although feed intake remained unchanged among treatments, moderate selenium nanoparticle supplementation did not produce a consistent improvement in body weight compared with the control group.

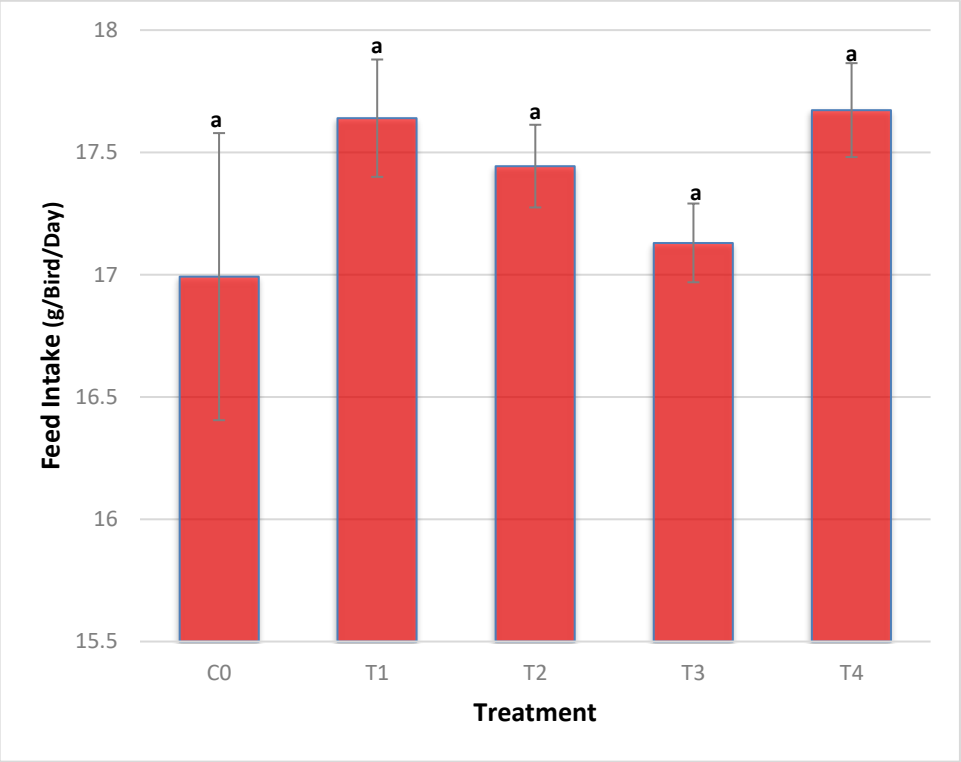


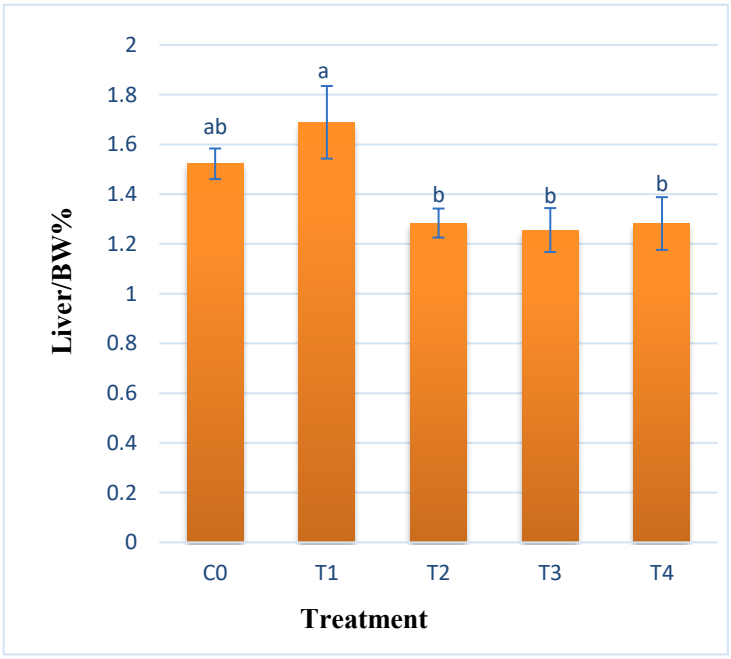
Figure 22: Impact of Different Doses of Selenium Nanoparticles (SeNPs) Dietary Supplementation on the average feed intake (g) ± SEM of Adult Japanese Quails. Means with the Same letter Are Not Significantly Different ($p > 0.05$). C0: control; T1: 0.05 mg/kg Red SeNP; T2: 0.5 mg/kg Red SeNP; T3: 0.05 mg/kg Grey SeNP; T4: 0.5 mg/kg Grey SeNP

Nano selenium supplementation of Japanese quails (Coturnix japonica) with 0.5 mg/kg in red and grey forms and with 0.05 mg/kg Grey Se produced treatment-dependent differences in body weight; however, these differences did not consistently result in higher body weight compared with the control group. Previous studies have reported improved growth performance in poultry supplemented with nano-selenium within the range of 0.2–0.6 mg/kg (Elkhateeb et al., 2024; Kaewsatuan et al., 2024; Marković et al., 2018; Reda et al., 2024; Tsekhmistrenko et al., 2020). Most of these studies, however, were conducted in rapidly growing chicks or broilers, where selenium supplementation may influence growth rate more markedly. In contrast, the birds used in the present study were adult Japanese quails, in which growth potential is already largely stabilized. Consequently, the physiological effects of selenium supplementation in adult birds are

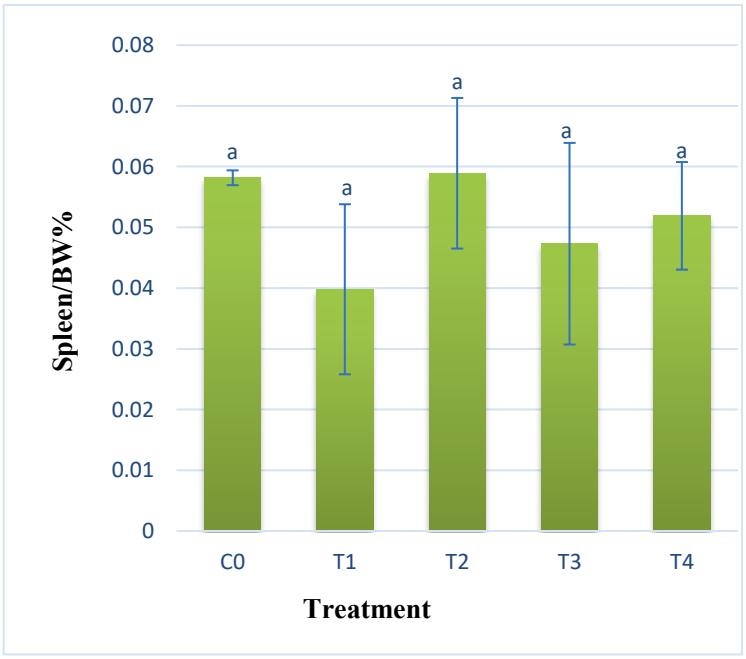
more likely related to metabolic regulation and antioxidant processes rather than measurable increases in body weight. Meanwhile, the recommended level of Se is 0.15 mg/kg in poultry feeding (National Research Council, 1994). In the present experiment, the lower body weight observed in the T1 and T3 groups (0.05 mg/kg red and grey SeNPs) may indicate that this supplementation level was insufficient to support optimal selenium status under the experimental conditions. It has been suggested that inorganic selenium forms such as selenite and elemental nano-selenium may share similar intestinal absorption pathways (Sindireva et al., 2023). This overlap could lead to competition during intestinal uptake, which may influence selenium utilization efficiency and contribute to variable physiological responses even under supplementation (Bakke et al., 2010; Schiavon & Pilon-Smits, 2017). Comparable observations have been reported in layer chicks where the growth performance was impaired at a level of 0.3 mg/kg (Mohapatra et al., 2014). Similar trend has been reported by Biswas et al. (2006) and Kaewsatuan et al. (2024) where selenium supplementation did not significantly affect feed intake, while growth responses varied depending on selenium dose, chemical form, and physiological stage of the birds.

4.3.2. Organ Indices

The liver index presented in **Fig. 23-A** had a notable variation among the groups treated with SeNPs ($p < 0.05$). The T1 group (0.05 mg/kg red SeNPs) exhibited the highest liver index and differed significantly from T2, T3, and T4, which shared the same statistical grouping. The control group (C0) showed an intermediate value and did not differ significantly from any of the treatments. **Fig. 23-B** showed the spleen relative weights, where there are no significant differences across all treatments ($p > 0.05$).



A



B

Figure 23: Effects of Dietary Selenium Nanoparticle Supplementation on Liver (A) and Spleen (B) weights (Relative to Body Weight) in Adult Japanese Quails ± SEM. Means with the Same superscript are Not Significantly Different ($p > 0.05$) while means with different letters are Significantly Different ($p < 0.05$). C0: control; T1: 0.05 mg/kg Red SeNP; T2: 0.5 mg/kg Red SeNP; T3:0.05 mg/kg Grey SeNP; T4: 0.5 mg/kg Grey SeNP

The liver or spleen index is the relative organ weight to the bird's body weight; they are markers that reflect the morphological and functional changes in organs and is used to evaluate the toxicity of the supplements (Long et al., 2021; Z. Ren et al., 2022). In a similar study, the evaluation of the glycine nano selenium effect on the immunity of the mice where the supplement indicated no significant difference in the liver, spleen and lung indices, which demonstrates that these nanoparticles had no poisoning effect (Z. Ren et al., 2022). According to (Khazraei et al., 2022), the heart, liver and digestive organs were not impacted by nanoSe and inorganic selenium in broiler quails. However, the younger quails in the fattening period can be affected by dietary chemical nano selenium, which increases the liver index (Alagawany et al., 2021). The spleen index remained within the normal range [0.04–0.06]%, indicating that SeNPs did not cause marked damage or affect the immune response (Abdel-Moneim et al., 2020). Se additive does not affect the spleen relative weight of growing Japanese quails but shows a great impact on the immune system (Biswas et al., 2006). In contrast, the Japanese quails' chicks demonstrated noticeable elevation of lymphoid organs' weights [1.8–3]% due to their enhanced absorption and targeted tissues delivery (Khazraei et al., 2022).

4.3.3. Organ-Specific Selenium Uptake

Selenium (Se) distribution varied across tissues depending on the treatment group and tissue type (**Fig.24 A–F**).

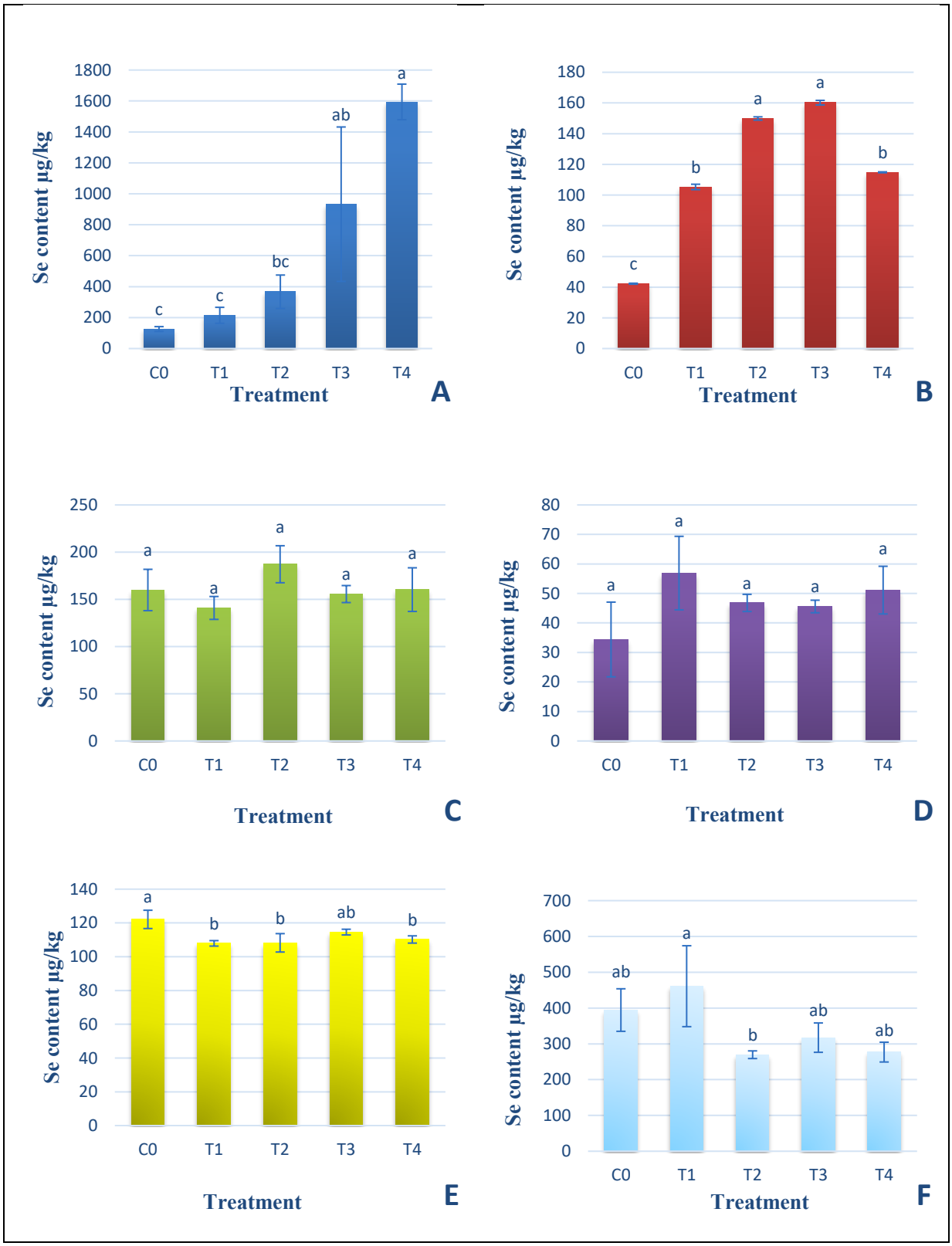


Figure 24: Nanoparticle of Selenium distribution on Liver (A), red Blood cellular fraction (B), Kidneys (C), Testis (D) and Eyes (E), and Breast (F). Means ± SEM with the Same Superscript Are Not Significantly Different ($p > 0.05$), while means with different letters are Significantly Different ($p < 0.05$). C0: control; T1: 0.05 mg/kg Red SeNP; T2: 0.5 mg/kg Red SeNP; T3: 0.05 mg/kg Grey SeNP; T4: 0.5 mg/kg Grey SeNP

In the liver **Fig.24-A**, selenium content was highest in T4 and lowest in the control group (C0). Significant differences were observed among groups, with the Se level in T4 significantly higher than all others ($p < 0.05$). The liver Se concentrations followed the pattern: control (C0), red Se 0.05 mg/kg (T1), red Se 0.5 mg/kg (T2), grey Se 0.05 mg/kg (T3), and grey Se 0.5 mg/kg (T4), indicating a dose-dependent increase, particularly with grey SeNPs supplementation. In the red blood cellular fraction (**Fig. 24-B**), the highest Se concentrations were observed in T2 and T3, both significantly higher than the control (C0), T1, and T4. The control group exhibited the lowest Se level among all groups. This suggests enhanced selenium uptake in red SeNPs-treated groups at high doses and grey SeNPs-treated groups at low doses. In the kidney **Fig.24-C** and testis **Fig.24-D**, selenium content showed no statistically significant differences among the treatment groups. This indicates stable selenium deposition in these organs, regardless of SeNPs form or concentration. In the eye tissue **Fig.24-E**, the control group had the highest selenium concentration, which was significantly different from all Se-supplemented groups. The lowest Se levels were observed in T1, T2, and T4, while T3 showed intermediate levels, suggesting a potential decrease in ocular selenium levels following SeNPs supplementation. Selenium levels in breast muscle **Fig.24-F** were highest in T1, followed by the control group, while T2 showed the lowest levels ($p < 0.05$), indicating a tissue-specific sensitivity to the form and dose of SeNPs.

The results of our study indicate that the selenium levels administered were well within the safe range for poultry. The toxicity threshold varies depending on the chemical form and species, with poultry generally exhibiting toxic effects at dietary selenium levels exceeding 0.15–0.50 mg/kg, as reported by the (Commission Implementing Regulation (EU) 2022/1459 of 2 September 2022 Amending Implementing Regulation (EU) 2019/804 as Regards the Terms of Authorisation of the Organic Form of Selenium Produced by Saccharomyces Cerevisiae CNCM I-3060 as Feed Additive for All Animal Species (Text with EEA Relevance), 2022; National Research Council, 1994). Organic selenium sources, such as selenomethionine and selenium nanoparticles, are typically less toxic than inorganic forms like sodium selenite and selenate (Bhattacharjee et al., 2019). Given that the selenium concentrations in our experimental groups remained significantly below the toxic range, it is unlikely that any adverse effects observed were due to selenium toxicity. Instead, the differences in physiological responses can be attributed to variations in selenium bioavailability and metabolism. The findings in **Fig.24-A** reveal

that selenium levels in the liver increase in a dose-dependent effect, with the highest accumulation observed in the grey selenium groups (T3, T4), followed by red selenium (T2). This pattern suggests that the liver, a primary organ for selenium storage and metabolism (Marković et al., 2018; Reda et al., 2024), effectively retained selenium from both Grey and Red selenium nanoparticles, particularly at higher doses. The lower selenium levels in the Control and Red selenium (T1) groups indicate minimal selenium availability for hepatic accumulation due to insufficient supplementation. The pronounced retention in Grey treated groups could be attributed to higher bioavailability or efficient uptake of Grey selenium nanoparticles compared to Red selenium, possibly due to differences in surface chemistry and particle stability (Filipović et al., 2021). Additionally, The liver plays a key role in selenium metabolism, serving as a major site for selenium storage and selenoprotein synthesis, particularly glutathione peroxidases (GPx); This metabolic function may explain the higher selenium deposition observed in the high-dose treatments, while the absence of toxicity suggests that the absorbed selenium was effectively incorporated into physiological pathways. (Elkhateeb et al., 2024; Mohapatra et al., 2014; X. Zhou & Wang, 2011). The selenium nanoparticles supplementation considerably boosted their incorporation into the red blood cellular fraction, which supports earlier research showing selenium's function in regulating oxidative stress and erythropoiesis (Reda et al., 2024). The highest levels were observed in the grey and red selenium-supplemented groups. This suggests that both Grey and Red selenium nanoparticles delivered substantially absorbed selenium to enhance uptake into Red blood cells (RBCs), likely due to their role in synthesizing selenoproteins like glutathione peroxidase (GPx) (Dehkordi et al., 2017; Reda et al., 2024; Sadeghian et al., 2012). The control group exhibited the lowest selenium content, confirming the limited selenium availability in a non-supplemented diet. Selenium deposition varied significantly among the treatment groups, where red selenium at a high dose and grey selenium at a low dose exhibited similar selenium incorporation, suggesting that Grey SeNPs might have a higher bioavailability, allowing lower doses to achieve comparable uptake. Conversely, low-dose red selenium and high-dose grey selenium also showed similar deposition levels, indicating a potential saturation threshold where excess dietary selenium, particularly in the more bioavailable Grey SeNPs form, did not further enhance tissue accumulation. These results highlight that selenium homeostasis plays a crucial role in regulating absorption and storage, preventing excessive accumulation beyond optimal physiological levels. The observed differences between selenium forms may be

attributed to variations in structural stability, absorption efficiency, and metabolic pathways, where Grey SeNPs exhibit enhanced bioavailability compared to Red SeNPs. While this study offers valuable insights into selenium deposition patterns and relative bioavailability of red and grey SeNPs, it did not include measurements of key selenoenzyme activities such as glutathione peroxidase (GPx), superoxide dismutase (SOD), or catalase (CAT), which play critical roles in oxidative stress regulation. Due to resource constraints, these functional biomarkers could not be assessed. Nevertheless, future research should prioritize the evaluation of these enzymes to better understand the physiological impacts of SeNPs uptake and their potential antioxidative benefits. However, the lack of substantial differences in selenium content in the kidneys and testis suggests a uniform distribution and systemic controls which prevent excessive deposits in these organs. The kidneys are key organs for selenium homeostasis, balancing absorption and excretion to prevent toxicity (Hadrup & Ravn-Haren, 2023).The large nanostructures can occur renal damage because of the prolonged keeping in the kidneys (Abdelnour et al., 2021); As a preventive measure, selenium content remains stable within the tolerable range in the kidney due to its immediate breakdown, as reported in (Kaewsatuan et al., 2024). Selenium concentrations in the testis remained relatively constant across treatments, suggesting that selenium accumulation in this organ is tightly regulated and may not increase proportionally with dietary selenium intake. In contrast, Abdelnour et al. (2021) reported increased selenium deposition in the testes of ruminants following nano-selenium supplementation, accompanied by improvements in semen quality and fertility. Similarly, Kazaz et al. (2020) observed increased selenium concentrations in the testes and ovaries of Japanese quail chicks during the early growth stage (14 days old). These discrepancies may be attributed to differences in species, physiological stage, selenium dose, nanoparticle form, and experimental conditions. In the present study, the birds were adult male quails, in which reproductive tissues may exhibit tighter regulation of selenium accumulation, possibly reflecting protective mechanisms that prevent excessive selenium deposition in sensitive organs. The selenium content in the eyes exhibited a regulated pattern **Figure 24-E**, with the control group showing the highest levels, significantly differing from most supplemented groups. Both Red selenium at low and high doses, as well as grey selenium at high doses, had similarly lower selenium levels, suggesting a saturation mechanism that limits excessive accumulation in ocular tissues. The low-dose grey selenium group showed an intermediate position, not significantly different from either the control or supplemented

groups, indicating potential variations in selenium uptake efficiency. These findings suggest that selenium homeostasis in the eye is tightly controlled, preventing excessive deposition regardless of dietary supplementation, possibly through endogenous regulatory mechanisms. In our findings the selenium levels in quails 'eyes remained constant within the different forms and dosage that consist with (McFarland et al., 1970) study, which reported a low and similar Se accumulation in the ocular tissue of other avian species such as chicken, turkey, and quails. This aligns with the human studies showing no significant impact of selenium on cataract prevention (Christen et al., 2015). Signifying that the eyes in poultry as like as in human tightly regulates selenium to avoid unnecessary deposits, while Se may help to enhance the antioxidant defences (Chandrinos et al., 2023). **Figure 24-F** presents selenium content in the breast muscle that exhibited a non-uniform distribution among treatment groups, indicating moderate variation in selenium deposition. The Red selenium at a low dose showing the highest deposition, significantly differing from the lowest selenium level observed in the high dose red selenium group. The control and both grey selenium groups (T3, T4) exhibited intermediate values without significant differences among them. These results suggest that a lower dose of red selenium may be more efficiently incorporated into muscle tissue, while higher doses could trigger regulatory mechanisms that limit excessive selenium accumulation. This may be due to the muscle's capacity to store selenium reaching a saturation point, beyond which excess selenium is either excreted or redistributed (Gawor et al., 2020; X. Zhou & Wang, 2011). Additionally, previous studies have demonstrated that muscle selenium content is relatively less sensitive to dietary supplementation, as observed in carp (Ashouri et al., 2015). However, nano-selenium has been reported to exhibit higher bioavailability than selenium methionine in several poultry species, including broilers, growing quails, and geese under different dietary and environmental conditions, similar observations have also been reported in certain fish species, such as crucian carp (Dlouhá et al., 2008; Kralik et al., 2012; Mahmoud et al., 2024; Mohammadi et al., 2020; Wan et al., 2020; X. Zhou et al., 2009). These findings are consistent with research indicating that selenium accumulation in muscle is regulated to prevent toxicity while ensuring adequate antioxidant defence (Zoidis et al., 2014). The similarity between the control and grey selenium groups may indicate a more balanced uptake and distribution of selenium in the muscle, maintaining tolerance levels.

4.4. Second Animal Experiment

The effects of dietary selenium nanoparticles on selenium distribution, growth, antioxidant activity, and retention dynamics were evaluated in adult male Japanese quails. The results are organized to first describe tissue selenium deposition across treatments, followed by changes in antioxidant biomarkers, and finally, the patterns of selenium retention and depletion after the withdrawal period.

4.4.1. Growth Performance

Dietary supplementation with red or grey SeNPs did not significantly affect feed intake and body weight progress throughout the 28-day feeding period ($p > 0.05$) **(Fig.25-26**). The mean FI presented in **Figure 25** remained comparable between the control and all supplemented groups, including the high-dose treatments (5 mg/kg), indicating normal feeding behaviour and the absence of overt selenium-induced anorexia.

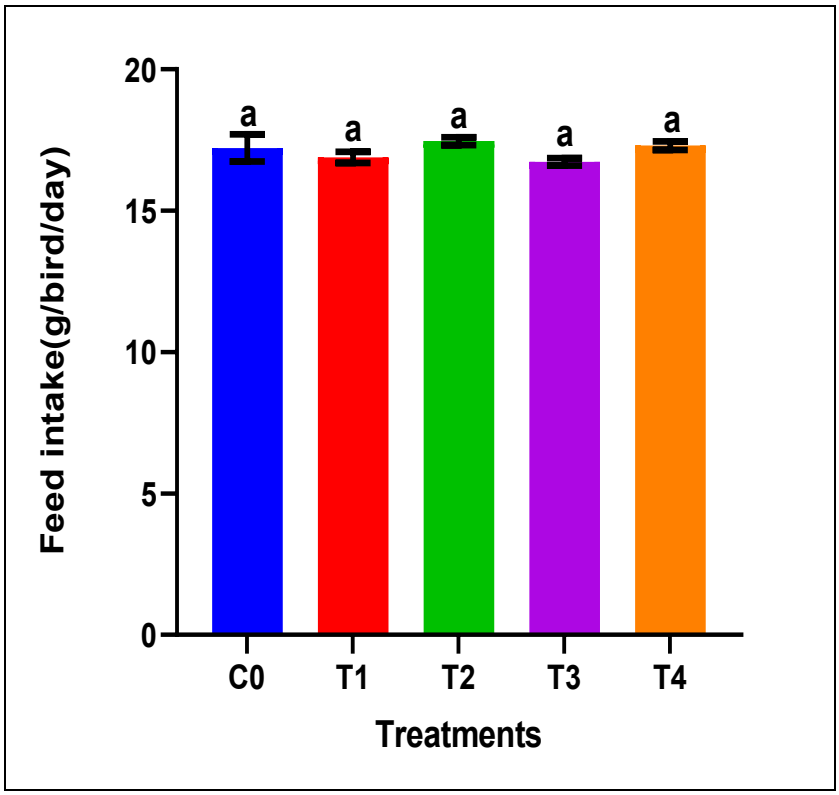


Figure 25: Feed intake of adult male Japanese quails during 28 days of dietary supplementation with selenium nanoparticles (n=6). Values are presented as mean ± SEM. No significant differences were observed among treatments ($p > 0.05$). C0: control

diet without SeNPs; T1: 0.5 mg/kg red SeNPs; T2: 5 mg/kg red SeNPs; T3: 0.5 mg/kg grey SeNPs; T4: 5 mg/kg grey SeNPs.

The body weight of Japanese quails at Week 1 and Week 4 in the second animal experiment is presented in **Fig.26.** At the beginning of the supplementation period (Week 1), no significant differences in body weight were observed among the experimental groups, indicating a homogeneous initial distribution of birds. After four weeks of feeding with diets supplemented with red or grey selenium nanoparticles at 0.5 and 5 mg/kg, body weight had no statistically significant differences been detected between the control and any of the SeNPs-treated groups ($p > 0.05$).

These results demonstrate that dietary supplementation with selenium nanoparticles, irrespective of allotrope form or dose, did not significantly influence body weight during the experimental period. This suggests that the applied SeNPs levels were physiologically safe and did not exert growth-promoting or growth-depressing effects under the present conditions. Similar observations have been reported in previous studies, where selenium supplementation primarily affected antioxidant status and selenium deposition in tissues rather than overall growth performance when basal diets already met selenium requirements(Mahmoud et al., 2024; Mohapatra et al., 2014).

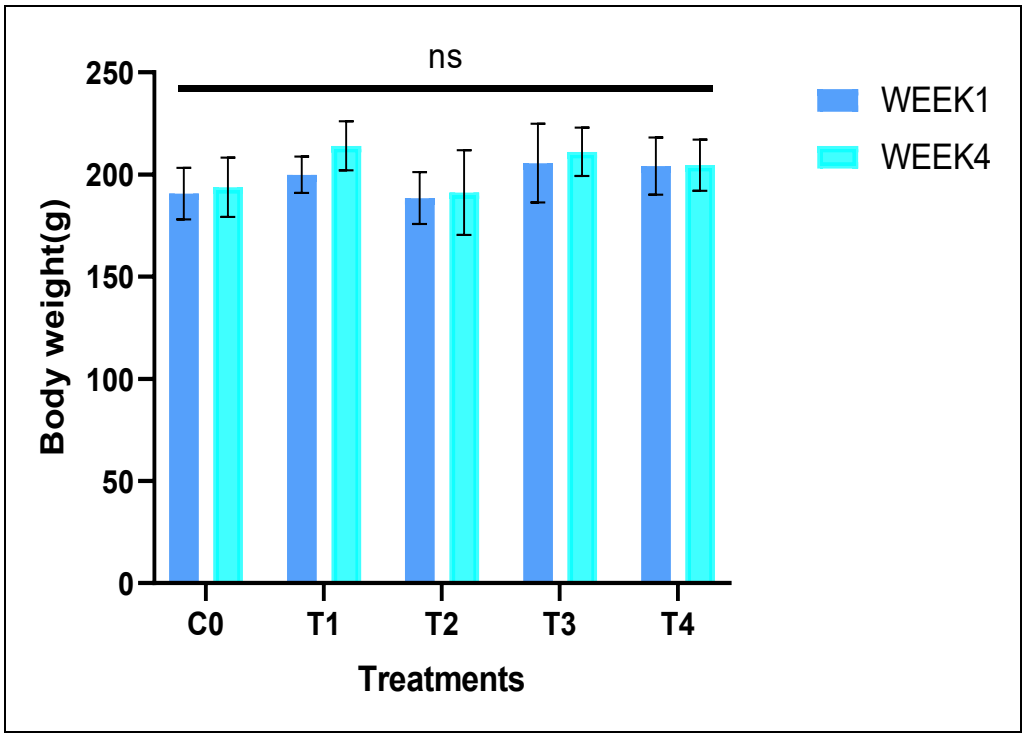


Figure 26: Live Body weight of Japanese quails in the second animal experiment at Week 1 and Week 4 under different dietary selenium nanoparticle treatments. C0: control diet without SeNPs; T1: 0.5 mg/kg red SeNPs; T2: 5 mg/kg red SeNPs; T3: 0.5 mg/kg grey SeNPs; T4: 5 mg/kg grey SeNPs. Bars represent mean ± SEM. No significant differences among treatments were detected (ns, $p > 0.05$)

4.4.2. Selenium distribution among Tissues

Table 6 shows the selenium distribution in organs of Japanese quails and average selenium accumulation after 28 days for both allotropes of SeNPs supplementation. Selenium concentrations varied significantly among treatments and organs ($p < 0.0001$). The red SeNPs treatments (T1 and T2) resulted in higher selenium concentrations in several metabolically active tissues, with the highest values observed in the T2 group. In this group, selenium levels reached 263.18 µg/kg ± 26 in red blood fractions, 128.86 µg/kg ± 1.6 in the liver, and 196.93 µg/kg ± 6.2 in breast muscle, indicating a dose-dependent increase for red SeNPs. The lower red SeNPs dose (T1) also increased selenium concentrations in the spleen, RBFs and breast compared with the control. Grey SeNPs supplementation showed a distinct accumulation pattern. The low dose (T3) resulted in lower selenium concentrations in the spleen and testis while maintaining comparable breast muscle selenium levels to the red SeNPs groups. At the higher dose (T4), grey SeNPs produced marked increases specifically in the spleen and testis, whereas

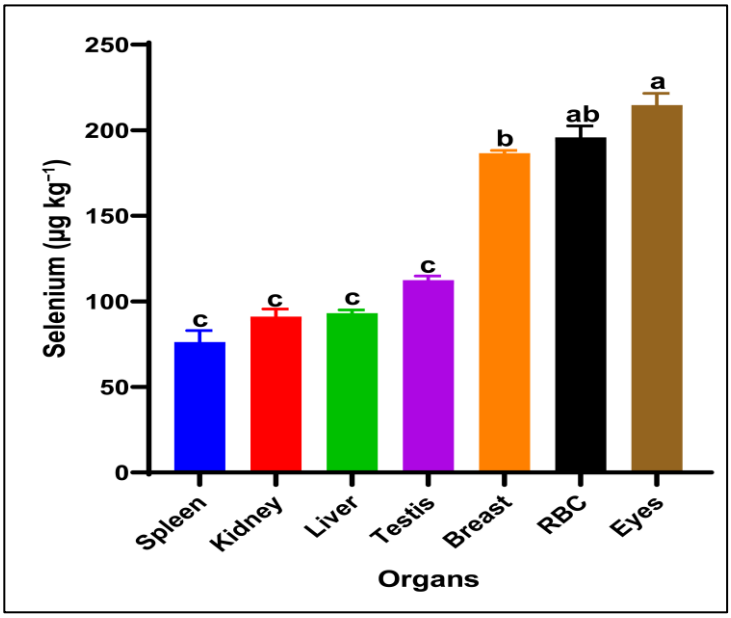
liver selenium levels were comparable to those observed in T2. Kidney selenium concentrations varied moderately among treatments, with a significant elevation only observed in the T2 group relative to the control. Selenium concentrations in the eyes remained relatively stable across all treatments. Average Se represents the mean selenium concentration calculated from the analysed tissues for each treatment group. Moreover, the high-dose groups T2 and T4 showed higher mean of Se levels compared with the control. Overall, the groups supplemented with nanoparticles of selenium produced the highest selenium accumulation and distribution in different organs; grey SeNPs demonstrated a strong dose dependence, with substantial increases observed only at higher concentrations in the spleen and testis.

Table 6: Selenium distribution in organs of Japanese quails and average selenium content after 28 days of control and SeNPs supplementation (n=6)

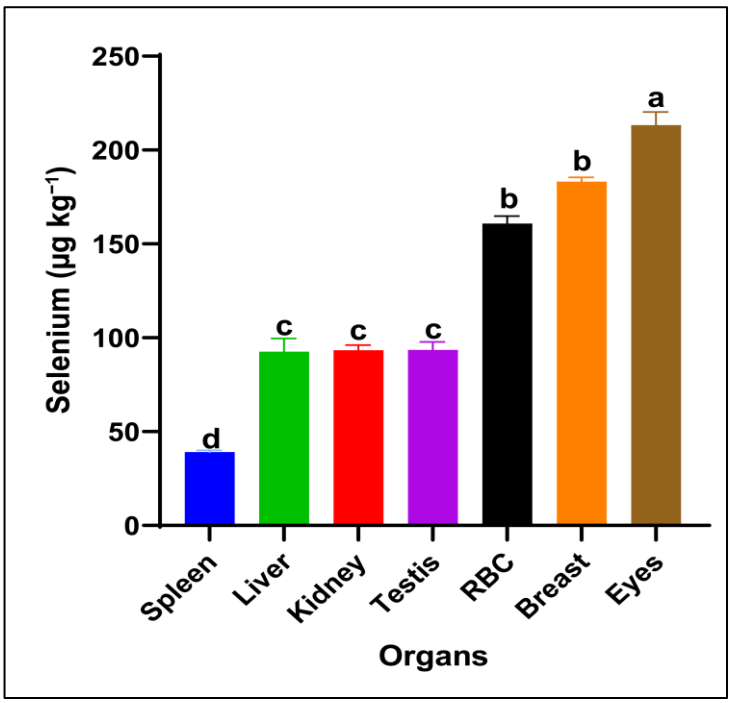
Se Content ($\mu g.kg^{-1}$)	C	T1	T2	T3	T4	*p* Value
Spleen	49.3±1.6 ^{c}	76.3±6.6 ^{b}	67.7±4.6 ^{b}	39.1±1.02 ^{d}	135.2±3.8 ^{a}	<0.0001
Kidney	101.3±3.7 ^{b}	91.2±4.4 ^{b}	114.8±1.5 ^{a}	93.4±2.8 ^{b}	89.6±4.4 ^{b}	<0.0001
Testis	119±5.3 ^{b}	112.4±2.5 ^{b}	124.2±3.1 ^{b}	93.5±4.3 ^{c}	151±7.2 ^{a}	<0.0001
Eyes	220.4±7.1 ^{a}	214.7±6.9 ^{a}	240.8±7.7 ^{a}	213.4±6.9 ^{a}	222.1±4.5 ^{a}	<0.0001
RBF (red blood fraction)	166.5± 4.9^{c}	195.9±6.7 ^{b}	263.2±27 ^{a}	160.8± 4 ^{c}	162.4±3.8 ^{c}	<0.0001
Breast muscle	178.8±1.5 ^{b}	186.6±1.6 ^{ab}	196.9±6.2 ^{a}	183.2±2.3 ^{ab}	192.1±9.7 ^{ab}	<0.0001
Liver	96.4±2.3 ^{b}	93.1±1.9 ^{b}	128.9±1.6 ^{a}	92.6±7 ^{b}	110.7±7.5 ^{ab}	<0.0001
Average Se content	133.1± 3.8^{b}	138.6±4.3 ^{b}	162.3±6.9 ^{a}	125.1±3.9 ^{b}	151.9±5.7 ^{a}	0.0007

Data are presented as mean ± SEM. Different superscript letters within each row indicate statistically significant differences among dietary treatments ($p < 0.05$).

The selenium distribution notably differed between the two nanoparticle forms at the similar dietary dose (0.5 mg/kg) in **Fig.27.** In the red SeNPs group (T1), **Fig.27-a,** selenium concentrations varied significantly among organs ($p < 0.05$). Red blood fraction selenium concentrations did not differ significantly from those in the eyes or breast muscle, whereas a significant difference was observed between the eyes and breast muscle, with the overall pattern being eyes ≥ RBF ≥ breast muscle > testis = liver = kidney = spleen. The highest levels were observed in the eyes, followed by the red blood fraction (RBF) and breast muscle, indicating relatively strong systemic availability and consistent deposition across tissues. The remaining visceral organs and testis showed lower but similar selenium concentrations (93–112 µg/kg), suggesting a homogeneous distribution pattern at this dose. In contrast, birds receiving the similar dose of grey SeNPs (T3) in **Fig.27-b** displayed a distinct deposition hierarchy of eyes > breast muscle = RBF > liver = kidney = testis > spleen ($p < 0.05$). While selenium levels in the eyes and breast muscle were comparable to those in T1, RBF selenium was notably lower, and spleen selenium accumulation was the lowest among all measured tissues (39.10 µg/kg ± 1). Overall, red SeNPs (T1) produced a slightly higher and more evenly distributed selenium profile across organs, whereas grey SeNPs (T3) resulted in reduced accumulation in circulating and immune tissues despite maintaining similar levels in muscle and ocular tissues.



(a)



(b)

Figure 27: Selenium distribution in spleen, kidney, liver, testis, breast muscle, red blood cells (RBCs), and eyes of Japanese quails supplemented with red SeNPs ((**a**): T1, 0.5 mg/kg) and grey SeNPs ((**b**): T3, 0.5 mg/kg). Bars represent mean ± SEM. Different superscript letters within each treatment indicate significant differences in selenium concentration among organs ($p < 0.05$)

4.4.3. Antioxidant Biomarkers

Figure 28 shows the glutathione peroxidase (GPx) activity, superoxide dismutase (SOD) levels, and total antioxidant capacity (TAC) in liver and serum samples after 28 days of treatment. Hepatic GPx activity responded strongly to dietary selenium supplementation. The high-dose SeNPs-treated groups showed significantly higher GPx activity compared with the control and low grey Se treatment T3, while T1 showed an intermediate value ($p < 0.05$), with the greatest increases observed in T2 (red SeNPs, 5 ppm) and T4 (grey SeNPs, 5 ppm). These findings indicate a clear dose-dependent enhancement of GPx-mediated antioxidant capacity in the liver, irrespective of nanoparticle form. In contrast, hepatic SOD activity remained statistically unchanged among treatments, suggesting that liver superoxide dismutase activity is relatively stable and not markedly influenced by selenium level or nanoparticle type. Serum SOD activity exhibited an opposite trend: all SeNPs-supplemented birds showed significantly lower serum SOD activity than the control group ($p < 0.05$); the activity levels were still maintained within the normal reference range, suggesting that the improvement in selenium status reduced the systemic demand for circulating SOD(Alagawany et al., 2021). Liver TAC (Total antioxidant activity) values did not differ significantly among treatments, indicating that hepatic non-enzymatic antioxidant capacity was maintained across dietary groups. However, serum total antioxidant capacity differed significantly among treatments ($p < 0.05$). The highest serum TAC was observed in T2, which was significantly higher than the control, T1, and T4, but did not differ significantly from T3. In addition, the T3 group exhibited significantly higher serum TAC compared with the control, whereas no significant differences were detected among the control, T1, and T4 groups. Taken together, these results demonstrate that selenium nanoparticles enhanced antioxidant status primarily through GPx upregulation and improved systemic TAC, with red SeNPs at 5 ppm exerting the strongest overall effect. Grey SeNPs also increased GPx activity, but their impact on serum antioxidant capacity was less pronounced with high supplementation, indicating comparatively lower bioefficacy at equivalent high doses.

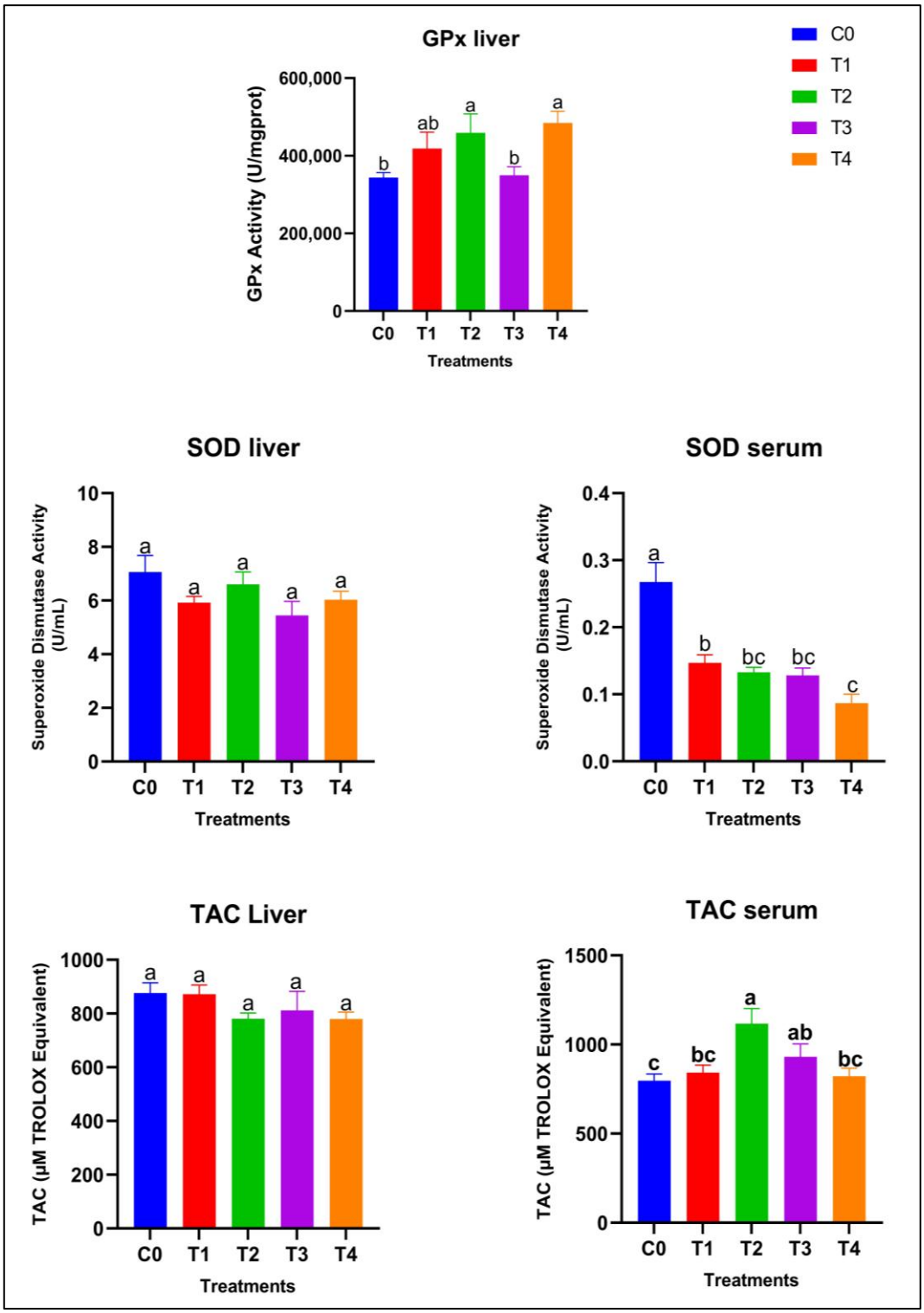


Figure 28: Antioxidant biomarkers in liver and serum of Japanese quails fed diets supplemented with selenium nanoparticles (SeNPs). Data are presented as mean ± SEM. Different superscript letters indicate statistically significant differences among dietary treatments ($p < 0.05$)

4.4.4. Selenium Retention after Withdrawal & Selenium Depletion Patterns

Table 7 shows the total retention and depletion rate (in liver, kidney, spleen and red blood fraction RBF). The total selenium retention and depletion percentages revealed clear treatment-dependent patterns. Control birds exhibited the most stable selenium balance (96% retention, 3.9% depletion), reflecting normal homeostatic regulation. Among the supplemented groups, the low-dose treatments (T1 and T3) achieved the highest overall retention (91% and 88%, respectively), indicating efficient incorporation and stable maintenance of selenium during the withdrawal period. In contrast, higher doses (T2 and T4) showed markedly lower retention (78% and 57%). This drop is a direct consequence of the body reaching tissue saturation, which subsequently triggers enhanced excretion and biological regulatory mechanisms to rapidly clear the excess selenium once supplementation is withdrawn. Red SeNPs displayed higher initial bioavailability and retention overall (T1 was 91%), while grey SeNPs followed closely in second place, confirming that both nanoparticle forms lead to efficient selenium incorporation, but the clearance rate was primarily governed by the dose.

Table 7: Total selenium retention and depletion rates in Japanese quails following SeNPs supplementation and a 7-day withdrawal period

	C	*T1*	*T2*	*T3*	*T4*
Total Se Retention %	96%	91%	78%	88%	57%
Total Se Depletion %	4%	9%	22%	12%	43%

This study demonstrated that selenium nanoparticles exerted clear form- and dose-dependent effects on selenium deposition, post-withdrawal retention, and antioxidant responses in adult male Japanese quails (Ferroudj et al., 2025c; Malik et al., 2025; Olaoye et al., 2024; Urbankova et al., 2021). Across all evaluated parameters, red and grey SeNPs behaved differently, reflecting their contrasting dissolution rates, bioavailability, and tissue affinities (Filipović et al., 2021; Hageman et al., 2017; Lesnichaya et al., 2020; J. Zhang et al., 2018). Red SeNPs, particularly at 5 mg/kg (T2), consistently produced the highest selenium levels in metabolic and circulating compartments, including the liver,

red blood fraction (RBF), and breast muscle (Debata et al., 2023; Ferro et al., 2021; K. Li, Li, et al., 2024; Zambonino et al., 2021). This pattern aligns with the known higher reactivity and faster dissolution of amorphous red selenium, enabling efficient intestinal absorption and rapid incorporation into selenoproteins (Ashraf. S.S., 2021). The pronounced increase in hepatic GPx activity and elevated serum TAC observed in T2 further supports the notion that red SeNPs provide readily available selenium for antioxidant enzyme synthesis and systemic redox balance (Guleria et al., 2020). Despite the supranutritional selenium level used in the 5 mg/kg treatments, no clinical signs of selenium toxicity were observed during the 28-day feeding period as the birds maintained normal feed intake and showed no abnormal behaviour or signs of distress during routine daily observations. However, the low-dose Red SeNPs (T1) demonstrated high initial bioavailability and also the highest overall retention (91%) among all supplemented groups following dietary withdrawal, indicating highly efficient incorporation and maintenance (Loeschner et al., 2014). In contrast, grey SeNPs exhibited slower and more selective accumulation patterns (K. Li, Li, et al., 2024; Ruiz Fresneda et al., 2018). At the low dose (T3), grey SeNPs resulted in generally lower organ selenium concentrations compared with the equivalent red SeNPs dose, confirming reduced bioavailability at small doses (K. Li, Li, et al., 2024). Nonetheless, T3 birds retained selenium more consistently during the withdrawal phase than T2 and T4, suggesting slower release and more prolonged tissue retention, but low-dose Red SeNPs-treated T1 birds showed superior retention compared to T3 and showed the most stable maintenance overall during the withdrawal phase (Ho, 2022). At the high dose (T4), grey SeNPs produced strong accumulation in specific organs, most notably in the spleen and testis, while showing a marked decrease in total retention after withdrawal, reflecting saturation of the storage capacity followed by compensatory mobilization or excretion of excess selenium after supplementation ceased. These findings indicate that higher selenium doses do not necessarily improve long-term retention and may reduce supplementation efficiency, highlighting the importance of moderate dosing to achieve sustained selenium status while maintaining safety (Burk & Hill, 2015; Ye et al., 2022). Across all treatments, the eyes consistently displayed the highest selenium concentrations, independent of SeNPs form or dose. This stability suggests a strong physiological requirement for selenium in ocular antioxidant defences, likely driven by high GPx expression and the sensitivity of retinal tissues to oxidative stress (Christen et al., 2015; H. Wang et al., 2007). Meanwhile, the spleen and visceral organs showed greater variability among treatments, indicating

dynamic selenium redistribution associated with immune and metabolic processes (Amini & Mahabadi, 2018; Niu et al., 2022). The retention–depletion analysis provided further insight into the kinetic differences between nanoparticle types. Low doses (T1 and T3) resulted in the highest overall retention, whereas high doses (T2 and T4) were associated with accelerated clearance following the withdrawal period (Burk & Hill, 2015; H. Wang et al., 2007; X. Zhang et al., 2019). This dose-dependent clearance pattern suggests that moderate selenium supplementation supports stable tissue incorporation, while higher doses trigger homeostatic regulation and enhanced excretory responses to maintain physiological selenium balance (Burk & Hill, 2015; Ye et al., 2022; X. Zhang et al., 2019). Red SeNPs supported greater overall bioavailability and maintenance, with the low dose (T1) achieving the highest total retention among all supplemented groups. Grey SeNPs—particularly at the low dose—provided a sustained release profile that ensures longer-term availability due to their structure. Collectively, these findings highlight that selenium form and dose strongly influence the balance between absorption, utilization, tissue storage, and clearance in quails. Red SeNPs excelled in rapid antioxidant enhancement and systemic distribution and had superior overall retention. In contrast, grey SeNPs showed distinct advantages in slower, targeted accumulation (spleen/testis) and a release mechanism designed for prolonged selenium provision (Selmani et al., 2024; Tışlı et al., 2024).

5. CONCLUSIONS, RECOMMENDATIONS

This work investigated how the allotropy of selenium nanoparticles influences their physicochemical properties and biological performance in adult male Japanese quails. By combining controlled synthesis of red (amorphous) and grey (crystalline) selenium nanoparticles with detailed structural characterization and two in vivo feeding experiments, clear structure–function relationships were established between nanoparticle form and selenium bioavailability, tissue distribution, antioxidant activity, and selenium retention following dietary withdrawal.

A major conclusion is that selenium nanoparticle allotropy fundamentally determines both material characteristics and biological behaviour. Although red and grey nanoparticles consist of the same elemental selenium, they exhibited markedly different morphology, crystallinity, and optical properties, which translated directly into distinct in vivo responses. These findings demonstrate that nanoscale structure, rather than elemental composition alone, influences selenium metabolism in poultry.

The physicochemical characterization provided strong and complementary evidence for the successful preparation of two structurally distinct selenium allotropes. Scanning electron microscopy revealed that red selenium nanoparticles formed quasi-spherical aggregates with smooth surfaces and a relatively narrow size distribution, with a mean diameter of approximately 218 ± 24 nm, characteristic of amorphous systems produced under kinetically controlled conditions. In contrast, grey selenium nanoparticles displayed highly anisotropic, needle-like nanostructures with mean lengths of about 575 ± 202 nm and widths of 33.9 ± 11 nm, reflecting directional crystal growth typical of trigonal selenium. These pronounced morphological differences suggest that the thermal treatment induced a transition from amorphous to crystalline selenium at the nanoscale.

Energy-dispersive X-ray spectroscopy confirmed selenium as the dominant element in both nanoparticle forms, with no detectable impurities, indicating that the observed differences arose from structural rearrangement rather than changes in elemental composition. X-ray diffraction analysis supported this interpretation, demonstrating that red selenium nanoparticles were amorphous, as evidenced by broad diffuse scattering and the absence of sharp Bragg peaks, whereas grey nanoparticles exhibited multiple intense reflections corresponding to crystalline trigonal selenium, confirming high crystallinity and long-range atomic order.

Raman spectroscopy provided additional insight into allotrope-dependent atomic organization. Red selenium nanoparticles showed broad bands in the 250–255 cm^{-1} region, characteristic of disordered amorphous selenium, while grey selenium nanoparticles exhibited a sharp and intense band near 233 cm^{-1}, corresponding to the A_1 vibrational mode of trigonal selenium associated with Se–Se stretching along helical chains. These results were fully consistent with the XRD findings and clearly distinguished the two structural states.

Fluorescence spectroscopy further highlighted the optical consequences of allotropy. Red selenium nanoparticles exhibited weak fluorescence, with emission maxima around 430–450 nm upon excitation near 380 nm, indicating limited electronic transitions associated with amorphous structure. In contrast, grey selenium nanoparticles displayed much stronger, excitation-dependent fluorescence in the same spectral region, reflecting their crystalline nature and well-defined electronic states. The pronounced difference in optical behaviour not only confirmed allotrope identity but also suggested that structural ordering plays a key role in governing surface and electronic properties of selenium nanoparticles.

Together, these physicochemical results demonstrate that red and grey selenium nanoparticles represent two distinct nanoscale systems with fundamentally different structural and optical signatures. Importantly, these differences were directly mirrored in their biological performance, underscoring the close link between nanoparticle structure and function.

From a biological perspective, a central conclusion is that both selenium nanoparticle forms are metabolically active in Japanese quails. Grey crystalline selenium, often considered biologically inert, was clearly shown to accumulate in tissues, enhance hepatic glutathione peroxidase activity, and contribute to selenium retention. These findings indicate that grey selenium nanoparticles participate in selenium metabolism in vivo. Compared with red selenium nanoparticles, grey SeNPs exhibited different patterns of tissue accumulation and retention, suggesting a distinct physiological behaviour rather than biological inactivity.

Red amorphous selenium nanoparticles, however, emerged as the more efficient source for rapid systemic selenium delivery. In the second experiment, birds receiving red selenium nanoparticles at 5 mg/kg exhibited the highest selenium concentrations in

metabolically active compartments, reaching approximately 263 μg/kg in the red blood cell fraction, 129 μg/kg in the liver, and 197 μg/kg in breast muscle. These values clearly demonstrate strong dose-dependent systemic availability. The enhanced deposition was accompanied by the most pronounced increase in hepatic glutathione peroxidase activity and serum total antioxidant capacity, indicating efficient incorporation into selenoproteins and robust support of antioxidant defence.

Grey selenium nanoparticles showed a different deposition profile. At the similar high dose, they induced marked selenium accumulation, particularly in the spleen and testis, while producing liver selenium levels comparable to those of red nanoparticles. At the moderate dose (0.5 mg/kg), grey nanoparticles resulted in lower selenium concentrations in circulating and immune tissues than red nanoparticles but maintained similar levels in muscle and ocular tissues. These patterns indicate that grey selenium nanoparticles possess lower overall bioavailability but exhibit selective tissue targeting, particularly toward immune- and reproduction-related organs.

Another important conclusion is the strong dose dependence of selenium retention and clearance. After a 7-day withdrawal period, moderate supplementation levels resulted in the highest selenium retention, reaching approximately 91% for red and 88% for grey selenium nanoparticles. In contrast, supranutritional supplementation led to markedly reduced retention, decreasing to 78% for high-dose red and only 57% for high-dose grey nanoparticles. These results demonstrate that higher selenium intake does not ensure sustained selenium status and that excessive tissue saturation may activate regulatory mechanisms that accelerate selenium clearance to maintain homeostasis.

The withdrawal data also confirmed allotrope-dependent kinetic behaviour. Red selenium nanoparticles provided higher residual selenium levels in key metabolic compartments, reflecting their rapid uptake and incorporation, whereas grey selenium nanoparticles displayed slower release and lower residual retention, consistent with a sustained-release profile. Thus, the influence of nanoparticle structure extends beyond the supplementation period and shapes post-dietary selenium homeostasis.

Organ-specific selenium distribution was another consistent observation in the present study. Higher selenium concentrations were generally detected in metabolically active tissues such as the liver, red blood fraction, and breast muscle, whereas selenium levels in the kidney and testis remained comparatively stable across treatments,

suggesting the presence of physiological regulation that limits excessive accumulation in sensitive organs. Ocular tissues consistently showed relatively high selenium concentrations regardless of nanoparticle form or dose, which may reflect the strong requirement for selenium-dependent antioxidant protection in retinal tissues. Although both nanoparticle forms increased selenium levels in several tissues, some differences in the distribution pattern between red and grey selenium nanoparticles were observed, indicating that nanoparticle form may influence selenium deposition in specific organs.

Growth performance and feed intake were not adversely affected by selenium nanoparticle supplementation in either experiment, even at the highest dietary levels applied. In the first experiment, red selenium nanoparticles at 0.5 mg/kg showed the highest average body weight among treatments, whereas feed intake remained comparable across groups. These observations indicate that selenium nanoparticle supplementation did not negatively influence feeding behaviour or general physiological condition under the experimental conditions.

Taken together, the results demonstrate that selenium nanoparticle allotropy critically governs the balance between absorption, tissue targeting, antioxidant utilization, and clearance in Japanese quails. Red amorphous selenium nanoparticles are characterized by rapid bioavailability, high systemic distribution, and strong antioxidant efficacy, whereas grey crystalline selenium nanoparticles exhibit selective tissue accumulation, and different retention behaviour following the withdrawal period. Both forms are biologically active, but their distinct physiological behaviours must be considered when selecting selenium sources for nutritional applications.

In summary, this work establishes that nanoscale structural features of selenium nanoparticles determine their physicochemical identity and, in turn, their biological fate and function in vivo. By integrating detailed material characterization with physiological evaluation, it provides clear evidence that structure–function relationships are central to nano-selenium nutrition. These findings advance understanding of selenium metabolism in poultry and offer a scientifically grounded basis for optimizing selenium supplementation using nanoparticle-based sources.

6. NEW SCIENTIFIC RESULTS

1. In adult male Japanese quails, supplementation with amorphous red and crystalline grey selenium nanoparticles resulted in markedly different biological responses, including selenium bioavailability red SeNPs increased mean of tissue selenium to 162.3 µg/kg, whereas grey SeNPs reached 151.9 µg/kg, tissue distribution, antioxidant activity, and post-withdrawal kinetics, demonstrating that selenium nanoparticle allotropy governs in vivo selenium metabolism.
2. Grey crystalline selenium nanoparticles accumulated in specific organs at 5 mg/kg, particularly the spleen increased from 49.3 to 135.2 µg/kg and testis selenium from 119.0 to 151.0 µg/kg, and affect hepatic glutathione peroxidase activity depending on dosage, indicating biological activity rather than inertness.
3. Red amorphous SeNPs produced the highest selenium concentrations in metabolically active tissues. At 5 mg/kg, selenium increased to 263.2 µg/kg in the red blood fraction, 128.9 µg/kg in the liver, and 196.9 µg/kg in breast muscle and enhance antioxidant capacity suggesting their effectiveness as a selenium source.
4. After dietary withdrawal, moderate red and grey selenium nanoparticle supplementation (0.5 mg/kg) resulted in the highest selenium retention approximately 91% and 88% of selenium, respectively, whereas supranutritional doses led to accelerated selenium depletion to 22% and 43% respectively, demonstrating a clear dose-dependent regulation of selenium homeostasis and showing that higher intake does not ensure sustained selenium status.
5. Selenium deposition followed organ-specific and form-dependent patterns, with preferential accumulation of red selenium nanoparticles in metabolically active tissues (+34% in liver, +58% in blood, and +10% in muscles) and targeted deposition of grey selenium nanoparticles in spleen (+174%) and testis (+27%), revealing regulated, allotrope-dependent selenium targeting in Japanese quails.

7. PRACTICAL RESULTS

1. Red amorphous selenium nanoparticles showed high bioavailability and strong antioxidant efficacy; therefore, they can be applied as an efficient selenium source to rapidly improve selenium status and antioxidant defence in poultry, particularly under intensive production or oxidative stress conditions.
2. The demonstration that grey crystalline selenium nanoparticles are bioactive rather than inert indicates that they may serve as stable, sustained-release selenium sources. The red-to-grey transformation does not eliminate biological activity, supporting the practical feasibility and shelf stability of nano-selenium supplements.
3. The dose-dependent retention and clearance patterns indicate that moderate supplementation levels ensure the most efficient selenium utilization, whereas excessive doses lead to increased clearance and reduced long-term efficiency. This provides a practical basis for optimizing dietary selenium inclusion.
4. The organ-specific selenium targeting suggests that nanoparticle form can be selected according to physiological needs, with red SeNPs favouring systemic tissues and grey SeNPs supporting immune- and reproduction-related organs.
5. The absence of adverse effects on growth performance and feed intake confirms that selenium nanoparticles can be safely incorporated into poultry diets under controlled conditions, supporting their potential use in commercial feed formulations.
6. Finally, this study provides a scientific basis for further research on nano-selenium, including investigations of gene expression and molecular mechanisms, particularly selenoprotein- and antioxidant-related pathways, to clarify how nanoparticle allotropy regulates selenium metabolism. The results also support extending this approach to other poultry species and livestock. Moreover, exploring combined supplementation strategies, such as selenium nanoparticles with vitamin E or other bioactive compounds at varying doses, may reveal synergistic effects and improve nutritional efficiency.

8. SUMMARY

Selenium (Se) is an essential trace element involved in antioxidant defence, immune regulation, and maintenance of redox homeostasis through its incorporation into selenoproteins. In poultry nutrition, selenium supplementation is widely practized; however, the biological efficiency of selenium strongly depends on its chemical form. Selenium nanoparticles (SeNPs) have attracted increasing interest as alternative selenium sources due to their high surface reactivity, enhanced bioavailability, and potentially lower toxicity compared with conventional inorganic and organic forms. Despite growing application of SeNPs, limited information is available on how selenium allotropy at the nanoscale influences physicochemical properties and in vivo biological responses, and whether crystalline grey selenium should be considered biologically inert.

The present doctoral work was undertaken to investigate whether red (amorphous) and grey (crystalline) selenium nanoparticles differ in their structural characteristics and biological behaviour in adult male Japanese quails (Coturnix japonica). The main objective was to elucidate the relationship between nanoparticle allotropy and selenium bioavailability, tissue distribution, antioxidant activity, and post-withdrawal retention.

Selenium nanoparticles were synthesized in aqueous solution by chemical reduction of sodium selenite using ascorbic acid. Red SeNPs were obtained directly from the reduction process, while grey SeNPs were produced through controlled thermal transformation of red SeNPs suspensions at 85 °C. The resulting nanoparticles were purified and freeze-dried when required, providing both liquid suspensions and solid powders for physicochemical analyses and biological applications.

A comprehensive multitechnique characterization approach was employed to define the allotrope-dependent properties of the synthesized SeNPs. Scanning electron microscopy revealed that red SeNPs formed quasi-spherical aggregates with relatively uniform particle dimensions, whereas grey SeNPs exhibited elongated, needle-like nanostructures with high aspect ratios. Energy-dispersive X-ray spectroscopy confirmed selenium as the dominant element in both nanoparticle systems without detectable impurities. X-ray diffraction analysis demonstrated the amorphous nature of red SeNPs and the highly crystalline trigonal structure of grey SeNPs. Raman spectroscopy further supported these findings, with broad bands around 250–255 cm^{-1} for red SeNPs and a sharp band near 233 cm^{-1} for grey SeNPs. Fluorescence spectroscopy revealed weak optical activity for

red SeNPs and markedly stronger, excitation-dependent fluorescence for grey SeNPs. These results confirmed that red and grey SeNPs represent two structurally and optically distinct selenium allotropes at the nanoscale.

The biological relevance of these differences was evaluated through two controlled feeding trials in adult male Japanese quails. In the first experiment, twenty quails were fed for 28 days with diets supplemented with low doses of red or grey SeNPs (0.05 and 0.5 mg/kg), alongside an unsupplemented control group. Growth performance, organ indices, and selenium distribution in multiple tissues were assessed. Dietary supplementation with red SeNPs at 0.5 mg/kg produced the highest average body weight among treatments, whereas red SeNPs at 0.05 mg/kg resulted in the lowest body weight. Feed intake was not significantly affected by selenium supplementation, indicating that selenium treatments did not influence appetite or feed consumption. The relative liver weight was significantly increased in birds receiving red SeNPs at 0.05 mg/kg, while spleen indices remained unaffected across all treatments, suggesting selective organ sensitivity and absence of systemic toxicity.

Tissue selenium analysis in the first experiment revealed pronounced allotrope- and dose-dependent differences. The highest hepatic selenium concentration was observed in quails supplemented with grey SeNPs at 0.5 mg/kg, followed by red SeNPs at the similar dose, indicating that, despite its crystalline nature, grey selenium nanoparticles are not biologically inert and can be efficiently accumulated in metabolically active organs. Selenium levels in the Red blood cellular fraction were highest in birds receiving red SeNPs at 0.5 mg/kg and grey SeNPs at 0.05 mg/kg, demonstrating that grey SeNPs can achieve substantial cellular selenium incorporation even at lower doses. These findings directly challenge the common assumption that crystalline grey selenium is poorly bioavailable or physiologically inactive.

In contrast, selenium concentrations in the kidney and testis were not significantly affected by treatment, indicating tight physiological regulation in these sensitive organs. Notably, ocular tissues displayed the highest selenium concentrations in the control group, while SeNPs supplementation significantly reduced eye selenium levels, suggesting strong homeostatic control of selenium incorporation into ocular tissues. In the breast muscle, the highest selenium concentration was achieved with low-dose red

SeNPs, whereas high-dose red SeNPs resulted in the lowest muscle selenium levels, indicating dose-dependent redistribution and possible saturation effects.

In the second experiment, sixty quails were supplemented for 28 days with red or grey SeNPs at 0.5 and 5 mg/kg, followed by a 7-day withdrawal period. Growth performance and feed intake were not significantly affected ($p > 0.05$), even at the high dose, indicating normal feeding behaviour and absence of overt selenium toxicity.

Selenium deposition after 28 days of supplementation showed pronounced allotrope- and dose-dependent patterns. The highest selenium concentrations were observed in birds receiving high-dose red SeNPs (T2), reaching 263.18 ± 26 µg/kg in the red blood cell fraction, 128.86 ± 1.6 µg/kg in the liver, and 196.93 ± 6.2 µg/kg in breast muscle, demonstrating strong systemic distribution and dose-dependent accumulation. The lower red SeNPs dose (T1) also increased selenium levels in spleen, blood, and muscle compared with the control. Grey SeNPs exhibited a distinct deposition pattern: at 0.5 mg/kg (T3), selenium accumulation in spleen and testis remained low (spleen as low as 39.10 ± 1 µg/kg), whereas at 5 mg/kg (T4), marked increases were observed specifically in spleen and testis, with liver selenium levels comparable to those of T2. Kidney selenium showed moderate variation, with significant elevation only in T2, while selenium concentrations in the eyes remained relatively stable across all treatments. High-dose groups (T2 and T4) also exhibited the highest body selenium contents.

Direct comparison at the similar dose (0.5 mg/kg) revealed that red SeNPs produced a more uniform selenium distribution (eyes ≥ RBF ≥ breast muscle > visceral organs), whereas grey SeNPs resulted in reduced selenium incorporation into circulating and immune tissues but maintained similar levels in muscle and eyes. These findings indicate that grey SeNPs generally show lower overall bioavailability than red SeNPs, yet they are clearly bioactive and capable of effective tissue incorporation, contradicting the assumption that crystalline grey selenium is biologically inert.

Antioxidant analyses further demonstrated structure-dependent biological effects. Hepatic glutathione peroxidase (GPx) activity increased significantly in high-dose groups, with the greatest enhancement observed in T2 (red SeNP, 5 mg/kg) and similarly elevated activity in T4 (grey SeNP, 5 mg/kg), indicating a strong dose-dependent GPx response irrespective of form. In contrast, hepatic SOD activity remained unchanged across treatments. Serum SOD activity was significantly lower in all supplemented

groups compared with the control, suggesting reduced systemic oxidative demand. Liver total antioxidant capacity (TAC) was not significantly affected, whereas serum TAC differed among treatments, with the highest value observed in T2, and a significant increase also in T3 compared with the control. Overall, red SeNPs at 5 mg/kg exerted the strongest antioxidant enhancement, while grey SeNPs also improved antioxidant status but with comparatively lower efficacy at high doses.

The withdrawal phase revealed clear differences in selenium retention and depletion patterns. Control birds maintained a stable selenium balance (96% retention, 3.9% depletion). Among supplemented groups, low-dose treatments achieved the highest retention (91% in T1 and 88% in T3), indicating efficient incorporation and maintenance. In contrast, high-dose groups exhibited reduced retention (78% in T2 and only 57% in T4), reflecting saturation of tissue selenium pools and activation of regulatory clearance mechanisms. Red SeNPs showed the highest overall retention at low dose, while grey SeNPs closely followed, confirming that both forms are metabolically active, but that clearance is primarily governed by dose.

Importantly, although red SeNPs displayed superior bioavailability and antioxidant enhancement, grey SeNPs also demonstrated notable biological activity. The persistence of selenium in tissues and the maintained antioxidant responses indicate that the red-to-grey transformation represents structural stabilization rather than a loss of biological activity. Grey crystalline SeNPs therefore maintained measurable selenium accumulation and antioxidant responses in vivo, indicating that the crystalline form remains physiologically active despite its greater structural stability.

Collectively, the findings of this doctoral work demonstrate that selenium nanoparticle allotropy critically governs selenium bioavailability, tissue targeting, antioxidant utilization, and selenium retention after dietary withdrawal in Japanese quails. Red amorphous SeNPs provide rapid and high selenium availability and strong antioxidant enhancement, while grey crystalline SeNPs, although generally less bioavailable, are clearly not inert and offer distinct tissue accumulation and retention behaviour.

The current study integrates nanomaterial synthesis, advanced physicochemical characterization, and in vivo nutritional evaluation to provide novel insight into the structure–function relationships of selenium nanoparticles. The results establish a scientific basis for optimizing nano-selenium supplementation strategies in poultry

nutrition and deepen understanding of how nanoscale allotropy modulates trace element behaviour in biological systems.

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10. PUBLICATIONS IN THE FIELD OF RESEARCH

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Registry number: DEENK/214/2026.PL
Subject: PhD Publication List

Candidate: Aya Ferroudj
Doctoral School: Doctoral School of Animal Husbandry
MTMT ID: 10085943

List of publications related to the dissertation

Foreign language scientific articles in Hungarian journals (1)

1. **Ferroudj, A.**, Muthu, A., Sári, D., Seresné Törős, G., Béni, Á., El-Ramady, H., Prokisch, J.: Developing an egg model for selenium nanoparticle testing.
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2. **Ferroudj, A.**, Csik, A., Prokisch, J.: Allotrope-Dependent Physicochemical and Optical Properties of Red and Grey Selenium Nanoparticles.
RSC Adv. 22 (16), 1-8, 2026. ISSN: 2046-2069.
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3. **Ferroudj, A.**, El-Ramady, H., Prokisch, J.: Applications of Nano-Selenium in the Poultry Industry: An Overview.
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4. **Ferroudj, A.**, Muthu, A., Sári, D., Seresné Törős, G., Béni, Á., Czeglédi, L., Knop, R., El-Ramady, H., Prokisch, J.: Comparative Study of Red and Grey Selenium Nanoparticles on Organ-Specific Selenium Deposition and Growth Performance in Japanese Quails.
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10. **Ferroudj, A.**, Prokisch, J.: Organ-specific disposition of selenium nanoparticles in adult Japanese quails: ef fects of dietary supplementation.
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List of other publications

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11. Seresné Törős, G., Alibrahem, W., Helu, N. K., Jevcsák, S., **Ferroudj, A.**, Prokisch, J.: Acrylamide in Food: From Maillard Reaction to Public Health Concern.
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Total IF of journals (all publications): 82,8
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23 April, 2026

List of Figures

List of Tables

11. STATEMENTS

Statement

I wrote this thesis in the framework of the University of Debrecen Doctoral School of Animal Science for the purpose of obtaining a doctoral degree (PhD) at the University of Debrecen.

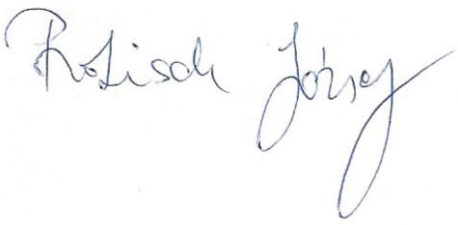
Debrecen, 2026. April 28

FERROUDJ AYA
PhD candidate

Statement

We hereby certify that the doctoral candidate **FERROUDJ AYA** has carried out his work under our supervision within the framework of the above-mentioned Doctoral School between 2022-2026. The candidate has made a decisive contribution to the results of the thesis through his independent creative work, and the thesis is the candidate's independent work. We recommend that the thesis be accepted.

Debrecen, 2026. April 28



Prof. Dr. József Prokisch
Supervisor

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