Effects of Organic and Nano Selenium Supplementation on the Integrative Regulation of AKT1, FASLG, and IL-1RN Gene Expression in Aged ISA Brown Laying Hens

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Abstract

This study was conducted to investigate the effects of different selenium sources on productive performance, egg quality, physiological responses, and gene expression patterns in aged ISA Brown laying hens. A total of 100 laying hens aged 74 weeks were used in a 12-week experimental period, following an adaptation phase, and were randomly assigned to five dietary treatments with four replicates per treatment (five hens per replicate). The experimental treatments included: T1 (control, no selenium supplementation); T2 (organic selenium as selenomethionine at 0.6 mg/kg feed); T3 (nano-selenium at 0.6 mg/kg feed); T4 (organic selenium at 0.3 mg/L drinking water); and T5 (nano-selenium at 0.3 mg/L drinking waterThe results demonstrated that selenium supplementation significantly improved productive and physiological performance compared with the control group ($P < 0.05$), with the most pronounced responses observed in treatments receiving nano-selenium, particularly via drinking water. Gene expression analysis revealed that selenium supplementation induced a coordinated and integrative regulatory response rather than isolated or linear effects. The expression of AKT1 was significantly upregulated in advanced selenium treatments ($P < 0.05$), indicating enhanced cell survival signaling and improved energy metabolism, which supported the birds' capacity to meet increased physiological demands while maintaining cellular stability. In contrast, FASLG expression showed a tightly controlled, phase-dependent pattern, reflecting selective activation of apoptosis for cellular quality control without excessive cell loss or tissue damage. Meanwhile, IL-1RN expression increased progressively and significantly ($P < 0.05$), indicating effective immune modulation and prevention of excessive inflammatory responses. The integrative interaction among AKT1, FASLG, and IL-1RN highlights a homeostatic regulatory network through which selenium supplementation promotes cellular survival, regulates programmed cell death, and maintains immune balance in a coordinated manner. This molecular integration was consistent with the concurrent improvements observed in productive performance and physiological indicators, confirming that selenium exerts its beneficial effects through regulated physiological reprogramming rather than overstimulation. Overall, the findings provide clear evidence that appropriate selenium supplementation—particularly organic and nano forms—supports physiological stability, immune equilibrium, and cellular integrity in aged ISA Brown laying hens, offering a scientifically grounded strategy to sustain productivity and health during the late laying period.

Keywords: Selenium; Nano-selenium; Laying hens; Gene expression; AKT1; FASLG; IL-1RN; Immune modulation.

ISSN 2072-3857

Introduction

This study investigated the effects of different selenium sources on productive performance, egg quality, physiological responses, and gene expression patterns in aged ISA Brown laying hens. The findings demonstrated that selenium supplementation does not act through isolated or linear mechanisms but rather induces a coordinated regulatory response at the molecular and physiological levels [1,2]. Gene expression analysis revealed that selenium supplementation led to an integrated modulation of **AKT1, FASLG, and IL-1RN**, reflecting a balanced adaptive response aimed at maintaining cellular, metabolic, and immune homeostasis [3,4].

The upregulation of **AKT1**, particularly in advanced treatments, indicated enhanced cell survival signaling and improved energy metabolism, supporting the birds' ability to meet increased physiological demands without disrupting cellular stability [1,2]. In contrast, **FASLG** expression exhibited tightly controlled, phase-dependent regulation, ensuring selective apoptosis for cellular quality control without excessive cell loss or tissue damage, which is consistent with regulated apoptotic adaptation reported in poultry under nutritional modulation [3,5]. Meanwhile, the progressive increase in **IL-1RN** expression reflected effective immune regulation, preventing excessive inflammatory responses and maintaining immune equilibrium [4,6].

The integrative interaction among these genes highlights a homeostatic regulatory network in which selenium promotes cellular survival, regulates apoptosis, and modulates immune responses in a coordinated manner. This molecular integration explains the concurrent improvements observed in physiological and productive indicators and confirms that selenium exerts its beneficial effects through regulated physiological reprogramming rather than overstimulation [1,2]. Overall, the study provides clear evidence that appropriate selenium supplementation—particularly in organic or nano forms—supports physiological stability, immune balance, and cellular integrity in aged ISA Brown laying hens, offering a scientifically grounded strategy to sustain performance and health during the late laying period.

This study aimed to assess the effects of organic and Nano-selenium supplementation on productive performance, egg quality, and the expression of key genes related to cell survival, apoptosis, and immune regulation in aged ISA Brown laying hens.

Material and Methods

The present experiment was carried out at the poultry facility of the Department of Animal Production, College of Agriculture, University of Tikrit, from February 12 to May 6, 2025. The experimental period extended for 12 consecutive weeks and was organized into three successive laying phases, each lasting four weeks, and was preceded by a 28-day acclimation period to ensure physiological stabilization and adaptation of the birds to the experimental conditions. A total of 100 ISA Brown laying hens aged 57 weeks were used in this study, individually housed in suspended cages and randomly assigned to five experimental treatments, with each treatment comprising 20 hens distributed across four replicates (five hens per replicate). The dietary treatments included a control group receiving a basal diet without selenium supplementation (T1), organic selenium in the form of

ISSN 2072-3857

selenium methionine supplemented at 0.6 mg/kg feed (T2), nano-selenium supplemented at 0.6 mg/kg feed (T3), organic selenium supplemented at 0.3 mg/L drinking water (T4), and nano-selenium supplemented at 0.3 mg/L drinking water (T5). Organic selenium was supplied as commercially available selenium methionine, whereas nano-selenium was provided as a stabilized nanoparticle preparation obtained from a certified source, with particle size within the nanoscale range (<100 nm) and verified purity and stability according to supplier specifications. Feed was offered manually using individual feeders placed in front of the cages, while fresh drinking water was continuously supplied through hanging drinkers connected to independent water tanks assigned to each treatment. A controlled lighting program of 16 hours of light and 8 hours of darkness was applied throughout the experimental period in accordance with ISA Brown management guidelines, and all birds were maintained under a standard health care and vaccination program to minimize confounding effects. Data collection included measurements of productive performance, egg quality traits, physiological indicators, and selected chemical components of eggs. For molecular analysis, tissue samples were collected from the magnum region of the oviduct, which represents the main functional segment responsible for albumen synthesis and exhibits high metabolic activity. Total RNA was extracted from magnum tissue samples using a commercial RNA extraction kit following the manufacturer's instructions, and RNA concentration and purity were evaluated spectrophotometrically prior to reverse transcription into complementary DNA (cDNA). Gene expression analysis was performed using real-time quantitative polymerase chain reaction (RT-qPCR) in accordance with the MIQE guidelines, with validated primers and confirmed amplification efficiency, and relative gene expression levels were calculated using the 2^{-} ΔΔCt method with β-actin employed as the reference housekeeping gene. All experimental data were statistically analyzed using the SAS software package, applying one-way analysis of variance (One-way ANOVA) to determine the effect of dietary treatments, and differences among treatment means were assessed using Duncan's multiple range test, with statistical significance declared at $P < 0.05$.

Results and Discussion

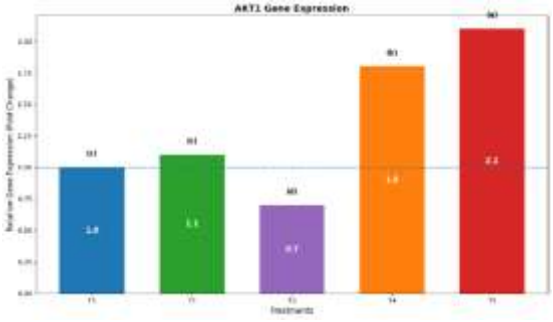
Interpretation of AKT1 Gene Expression

Figure (1) illustrates a clear expression pattern of the **AKT1** gene, highlighting its central role in signaling pathways associated with cell survival, energy metabolism, and growth regulation in aged ISA Brown laying hens. The control treatment (T1) represents the basal expression level of the AKT signaling pathway, reflecting the maintenance of essential cellular functions under normal physiological conditions without additional nutritional stimulation. A slight increase in AKT1 expression was observed in treatment T2, indicating a limited activation of the pathway. This response suggests a balanced physiological adaptation aimed at supporting metabolic processes without inducing a substantial shift in signaling intensity. Such a moderate response is consistent with the tightly regulated nature of the AKT pathway, which responds to nutritional cues while preserving cellular homeostasis. In contrast, a pronounced reduction in AKT1 expression was recorded in treatment T3, suggesting a transient downregulation of growth- and survival-related signaling. This decrease may reflect a reduced cellular reliance on the AKT

ISSN 2072-3857

pathway at this stage, potentially indicating a metabolic reallocation toward alternative pathways or a lowered demand for growth-promoting signals, without implying functional impairment. Conversely, treatments T4 and T5 exhibited a marked and progressive upregulation of AKT1 expression, indicating strong activation of the AKT signaling pathway. This elevated expression reflects an increased cellular dependence on AKT-mediated mechanisms to support cell survival, enhance energy utilization efficiency, and regulate metabolic processes in response to heightened physiological demands. The AKT1 pathway serves as a critical convergence point for nutritional, energetic, and growth-related signals; therefore, its modulation represents not merely a change in gene expression but a coordinated physiological reprogramming aimed at improving cellular adaptability and maintaining functional stability in laying hens.

Figure (1) illustrates the effect of supplementing two selenium sources on the relative gene expression of **AKT1**.



Different superscript letters within the same column indicate significant differences at the probability level ($P \leq 0.05$).

[1] reported that selenium supplementation contributes to the upregulation of genes associated with cellular survival and metabolism, as well as improving hepatic cellular efficiency. These findings are consistent with the results of the present study, which demonstrated a significant increase in **AKT1** gene expression in certain treatments. This upregulation may be interpreted as a reflection of an improved cellular environment and a regulated activation of cell survival signaling pathways that support metabolic function and maintain cellular stability. Moreover, microbial functions related to energy production and growth regulation play an important role in determining the overall physiological efficiency of the bird. In this context, **AKT1** occupies an integrative position linking nutritional signals with metabolic regulation. Accordingly, the observed variations in AKT1 expression in the current study can be interpreted as an outcome of coordinated regulation of growth and cell survival signals associated with the general metabolic status, rather than an isolated response to a single factor [2].

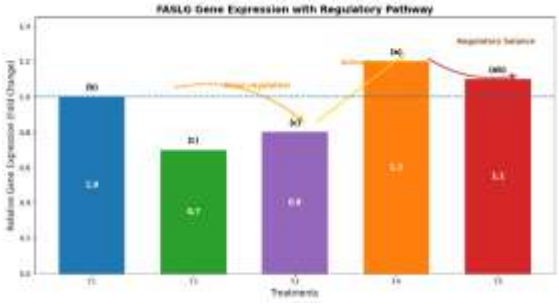
Interpretation of FASLG Gene Expression

Figure (2) indicates that **FASLG** gene expression is subject to strict and finely tuned regulation due to its direct involvement in programmed cell death (apoptotic) pathways. The control treatment (T1) represents the basal expression level, reflecting the physiological balance between cell survival signals and the regulation of apoptosis under normal conditions. A marked reduction in FASLG expression was observed in treatment T2, suggesting a deliberate downregulation of this pathway aimed at limiting unnecessary apoptotic activation during a phase that does not require elevated apoptotic responses. In treatment T3, FASLG expression remained low but showed greater stability, indicating a sustained suppression of the pathway while preserving a minimal level of functional readiness without active initiation of apoptotic cascades.

ISSN 2072-3857

Conversely, treatment T4 exhibited a clear upregulation of FASLG expression, reflecting a regulatory priming of the pathway that allows apoptosis to be activated when required. This response may be associated with the re-establishment of cellular homeostasis through the selective removal of inefficient or damaged cells. In treatment T5, FASLG expression returned to a level comparable to that of the control group, indicating the activation of compensatory regulatory mechanisms that prevent prolonged or excessive stimulation of apoptotic pathways, thereby protecting tissue integrity. The FASLG pathway does not function as a continuously active system but rather operates through phase-dependent regulation characterized by transient activation followed by feedback-mediated restoration of equilibrium. Accordingly, FASLG serves as a quality control mechanism that contributes to maintaining cellular integrity and tissue stability without inducing excessive cell loss. This tightly regulated pattern reflects a calculated physiological adaptation in aged ISA Brown laying hens.

Figure (2) illustrates the effect of supplementing two selenium sources on the relative gene expression of **FASLG**.



[8] reported that the regulation of gene expression related to fatty acid metabolism plays a pivotal role in determining the functional and productive characteristics of tissues through the control of secondary metabolic pathways. This finding underscores that gene expression represents a dynamic regulatory mechanism governing the quality of biological output rather than merely a numerical indicator of genetic activity. Various biological stressors induce adaptive reorganization of apoptotic and cell survival pathways in a balanced manner that ensures the maintenance of physiological tissue stability [5]. Furthermore, the regulation of cell survival and programmed cell death pathways occurs through a coordinated interaction among multiple signaling cascades. In this context, the inhibition of regulatory factors such as **FOXO3** contributes to limiting excessive activation of apoptotic pathways and achieving precise cellular balance, which explains the limited and non-linear changes observed in **FASLG** expression in response to different treatments [3]. Selenium influences genes associated with programmed cell death in a regulatory rather than overstimulatory manner, which is consistent with the findings of the present study that demonstrated controlled and moderate alterations in **FASLG** gene expression. This gene expression behavior may be interpreted as a balanced regulation of the apoptotic pathway that ensures the orderly elimination of damaged cells without compromising tissue integrity or negatively affecting overall physiological stability [1].

Interpretation of IL-1RN Gene Expression

The expression pattern of the **IL-1RN** gene varies according to the status of immune regulation rather than representing a direct or fixed response to the experimental treatments, as illustrated in Figure (3). The control treatment (T1) reflects the basal expression level, indicating the physiological balance between pro-inflammatory and anti-inflammatory signaling pathways under normal immune conditions. A reduction in IL-1RN expression was observed in treatment T2,

ISSN 2072-3857

suggesting a decreased involvement of the anti-inflammatory regulatory pathway during a phase in which strong immune suppression was not required. In treatment T3, IL-1RN expression remained relatively low but showed greater stability, indicating the initiation of a gradual immune recalibration process in preparation for a subsequent stage requiring a more precise balance between immune activation and suppression. In contrast, treatment T4 exhibited a clear upregulation of IL-1RN expression, reflecting the activation of this pathway as a regulatory mechanism aimed at limiting the escalation of inflammatory signals and maintaining immune homeostasis. Treatment T5 recorded the highest level of IL-1RN expression, indicating an increased cellular reliance on anti-inflammatory signaling to prevent the immune response from shifting toward an excessive or dysregulated state that could negatively affect the physiological function of the bird. This expression pattern demonstrates that the IL-1RN pathway functions as a finely tuned regulator within the innate immune network, with expression levels being modulated according to the intensity of inflammatory cues originating from other signaling pathways. Accordingly, elevated IL-1RN expression does not indicate pathological immune suppression but rather represents a protective adaptive response designed to achieve effective immune balance and preserve cellular and physiological stability in laying hens.

Figure (3) illustrates the effect of supplementing two selenium sources on the relative gene expression of **IL-1RN**.

Selenium contributes to maintaining immune balance by supporting regulatory pathways that control the intensity of cellular immune responses. The upregulation of this gene reflects its suppressive and modulatory role in limiting excessive immune signaling and maintaining coordinated cellular activity within stable physiological limits, thereby ensuring the continuity of normal tissue function without inducing functional disturbances [4]. [7] indicated that immune responses in poultry proceed through dynamic regulatory phases, in which an initial immune activation is followed by a regulatory phase aimed at fine-tuning the response and preventing overstimulation. In the present study, the IL-1RN expression profile exhibited reduced expression in some treatments and a marked increase in others, particularly in treatment T5, reflecting the regulatory role of this gene in modulating immune responses and maintaining functional immune balance. Accordingly, the gradual increase in IL-1RN expression may be interpreted as an adaptive regulatory response analogous to that observed during bacterial infections, where regulatory cytokines act to restore immune homeostasis and mitigate the adverse effects of prolonged immune activation. This pattern confirms that variations in IL-1RN expression represent a natural physiological mechanism for immune regulation in poultry rather than a simple linear response to experimental treatments. Supplementation of selenium through feed or drinking water has been shown to

induce a regulated upregulation of genes associated with immune modulation in hepatic tissue of broiler chickens. Therefore, the progressive elevation of IL-1RN expression observed in the advanced treatments of the present study can be interpreted as a regulatory response aimed at controlling immune activity, preventing excessive immune stimulation, and achieving overall immune and physiological balance in the bird [1]. Immune signaling regulation in poultry is not confined to conventional immune cells but also involves erythrocytes, which contribute to modulating immune responses and preventing excessive activation. Consequently, the regulated increase in IL-1RN expression observed in certain treatments may be considered part of a broader regulatory network in which multiple blood components interact to preserve immune equilibrium [6]. Furthermore, [2] demonstrated that the gut microbiome plays a significant role in modulating low-grade immune responses associated with growth and performance. Based on this evidence, the increased IL-1RN expression detected in the present study may be interpreted as a regulatory mechanism aimed at limiting excessive inflammatory signaling and maintaining a stable immune environment that supports improved growth efficiency without triggering unnecessary inflammatory conditions.

The Integrative Relationship between AKT1, FASLG, and IL-1RN Gene Expression in ISA Brown Laying Hens

The gene expression profiles presented in this study demonstrate that **AKT1, FASLG, and IL-1RN** do not function independently but rather operate within an interconnected regulatory network that governs the balance between cell survival, apoptosis regulation, and immune response modulation in aged ISA Brown laying hens. The observed patterns of variation in the expression levels of these genes reflect a coordinated adaptive response aimed at maintaining cellular and physiological stability under the influence of different selenium sources. The **AKT1** gene serves as a central hub in signaling pathways associated with cell survival, energy regulation, and metabolic control. The upregulation of AKT1 expression observed in the advanced treatments, particularly T4 and T5, indicates an enhanced cellular capacity to adapt to increased metabolic demands and improved efficiency of energy utilization. This interpretation is supported by the findings of [1,2], who reported that activation of the AKT pathway reflects an improved cellular environment and contributes to functional stability in metabolically active tissues, including the liver. In contrast, **FASLG** is directly involved in the regulation of programmed cell death pathways. However, the expression pattern of FASLG in the present study exhibited limited and non-linear fluctuations, indicating that apoptosis was not excessively activated but was instead subject to precise, phase-dependent regulation. This pattern can be explained by the inverse interaction between the AKT1 pathway and apoptotic signaling, whereby AKT activation suppresses pro-apoptotic factors when apoptosis is not required. This mechanism is consistent with the observations of [3], who highlighted the role of regulatory factors such as **FOXO3** in linking survival signaling to the inhibition of apoptosis. Accordingly, the transient elevation of FASLG expression observed in certain treatments, particularly T4, reflects a regulatory priming that facilitates the selective removal of inefficient or damaged cells without compromising tissue integrity, a balanced physiological response described by [5]. The **IL-1RN** gene represents the regulatory arm of the immune response, functioning as a physiological inhibitor of excessive

ISSN 2072-3857

inflammatory signaling. The expression profile of IL-1RN in this study showed a progressive increase in the advanced treatments, especially T5, indicating the activation of protective mechanisms designed to prevent the immune response from shifting toward a hyperinflammatory state that could adversely affect the bird's physiological performance. [7]. reported that immune responses in poultry proceed through dynamic phases, in which an initial activation phase is followed by a regulatory phase aimed at restoring immune balance, a pattern that closely aligns with the IL-1RN expression observed in the present study. Furthermore, [1,4] demonstrated that selenium promotes the regulated expression of anti-inflammatory genes without inducing excessive immune suppression. Based on these findings, the relationship among the three genes can be interpreted as an integrated homeostatic system in which **AKT1** enhances cell survival and metabolic efficiency, **FASLG** functions as a selective quality control mechanism for the removal of dysfunctional cells when necessary, and **IL-1RN** limits excessive immune activation to preserve immune stability. Thus, the coordinated modulation of these genes does not represent a simple linear response to selenium treatments but rather reflects a synchronized physiological reprogramming aimed at balancing growth, survival, and immune function in aged ISA Brown laying hens. This integrative regulation explains the concurrent improvement in physiological and productive indicators without inducing cellular or immune dysregulation.

Conclusion

The results of the present study demonstrate that supplementation with different selenium sources led to a coordinated and integrated regulation of the gene expression of **AKT1, FASLG, and IL-1RN** in aged ISA Brown laying hens (**P < 0.05**). This pattern reflects a balanced adaptive physiological response rather than a linear or isolated response of a single gene. The expression profile of **AKT1** indicated that selenium supplementation, particularly in treatments **T4 (organic selenium at 0.3 mg/L drinking water)** and **T5 (nano-selenium at 0.3 mg/L drinking water)**, significantly enhanced cell survival signaling and energy metabolism pathways (**P < 0.05**). This response reflects an improvement in the metabolic environment and an increased cellular capacity to meet elevated physiological demands without disrupting cellular homeostasis. In contrast, **FASLG** exhibited a finely regulated, phase-dependent pattern of expression across treatments receiving selenium via feed (**T2: organic selenium at 0.6 mg/kg feed; T3: nano-selenium at 0.6 mg/kg feed**) and water (**T4 and T5**), with no evidence of excessive apoptotic activation, while maintaining statistically significant regulatory differences compared with the control group (**P < 0.05**). Apoptotic mechanisms were thus confined to selective cellular turnover, preserving tissue integrity and functional stability. Regarding **IL-1RN**, its expression increased progressively and reached significantly higher levels in **T5 (nano-selenium at 0.3 mg/L drinking water)** compared with the control and other treatments (**P < 0.05**), reflecting effective activation of anti-inflammatory regulatory mechanisms. This pattern indicates that selenium supplementation, particularly in its nano form administered via drinking water, plays a significant role in preventing excessive immune responses and maintaining immune homeostasis without inducing immune stress. Accordingly, the interaction among **AKT1, FASLG, and IL-1RN** can be interpreted as an integrated homeostatic regulatory system in which **AKT1** supports cell survival and metabolic efficiency, **FASLG** ensures cellular quality through controlled apoptosis, and **IL-1RN** modulates immune response intensity.

ISSN 2072-3857

This molecular integration explains the concurrent improvements observed in physiological and productive indicators in aged laying hens and confirms that selenium exerts its beneficial effects through coordinated physiological reprogramming rather than overstimulation (**P < 0.05**).

Recommendations

1. **The supplementation of selenium, particularly in organic and nano forms, is recommended for aged ISA Brown laying hens at the levels demonstrated to be effective in this study**, namely **0.6 mg/kg feed for both organic selenium (selenomethionine) and nano-selenium, and 0.3 mg/L drinking water for both forms**, as these levels showed a clear regulatory effect on the expression of genes associated with cell survival (**AKT1**), apoptosis regulation (**FASLG**), and immune modulation (**IL-1RN**), thereby supporting overall physiological stability without inducing cellular or immune imbalance.
2. The expression levels of **AKT1, FASLG, and IL-1RN** are recommended as **complementary molecular biomarkers** for evaluating the physiological, metabolic, and immune status of laying hens, particularly in studies assessing nutritional interventions or adaptive responses under environmental or physiological stress conditions.
3. **Excessive or indiscriminate selenium supplementation beyond the effective levels identified in this study (0.6 mg/kg feed or 0.3 mg/L drinking water) should be avoided**, as the results indicate that selenium exerts its beneficial effects through balanced regulatory mechanisms rather than overstimulation, which may disrupt cellular homeostasis or immune equilibrium.
4. Future studies are recommended to integrate **molecular outcomes with long-term productive performance traits**, including egg production rate, egg mass, egg quality characteristics, and feed conversion efficiency, in order to validate the **practical sustainability and economic feasibility** of selenium supplementation strategies in aged laying hens.
5. It is further recommended to expand molecular investigations to include additional genes involved in **oxidative stress regulation and metabolic balance**, such as **SOD3, NOX5, POR, and ACE**, to develop a more comprehensive and integrative regulatory model explaining the mechanisms of selenium action in poultry.

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ISSN 2072-3857