ADAPTOGENIC EFFECTS OF PANAX GINSENG PLANT EXTRACT ON IMMUNE MODULATION IN LAYERS AGAINST SALMONELLA ENTERITIDIS

TAIMOOR M1, NOOR H1, YASMIN S1, USMAN M2, ASHRAF D3, HUSSAIN D4, IQBAL S5, HASAN W6, ZAFAR N7, AJAZ R8

1Veterinary Research Institute, Zarrar Shaheed Road, Lahore Cantt 54810, Pakistan
2Department of Poultry Science, University of Agriculture Faisalabad, sub campus Toba Tek Singh, Pakistan
3University of Agriculture, Faisalabad Pakistan
4Faculty of Veterinary Sciences, University of Layyah, Layyah, Pakistan
5Faculty of Veterinary Sciences, University of Agriculture, Faisalabad Pakistan
6Department of Clinical Medicine and Surgery, University of Agriculture Faisalabad Pakistan
7University of Veterinary and Animal Sciences Lahore sub campus Jhang, Pakistan
8Universitas Indonesia(UI), Jakarta, Indonesia,

*Corresponding author email address: sohailch275@gmail.com

(Received, 30th October 2023, Revised 17th April 2024, Published 24th April 2024)

Abstract Panax ginseng, a traditional medicinal herb known for its adaptogenic properties, has garnered significant attention for its potential role in immune modulation and disease prevention. In this study, we investigate the adaptogenic effects of Panax ginseng on immune modulation in layers against Salmonella Enteritidis. This randomized control trial was conducted at Zarrar Shaheed Road, Lahore Cantt from January 2023 to October 2023. A total of 450 laying hens were included in this study. The laying hens were randomly assigned to different experimental groups to ensure unbiased representation and minimize confounding variables. The laying hens were separated into several experimental groups, including a benchmark group and one or more treatment groups receiving Panax ginseng supplementation. A total of 450 laying hens were included in the study, divided into five experimental groups: a control group (n = 90) and four treatment groups receiving varying doses of Panax ginseng supplementation (n = 90 each). Compared to the control group, hens receiving low, medium, and high portions of ginseng displayed elevated degrees of IgG and IgM antibodies. Specifically, the high-portion ginseng group demonstrated the most noteworthy mean values for both IgG (75 ± 18) and IgM (50 ± 12), indicating a vigorous invulnerable reaction. It is concluded that supplementation with Panax ginseng holds promise as a natural intervention for enhancing immune modulation and reducing pathogen load in laying hens challenged with Salmonella Enteritidis. Ginseng may serve as a valuable adjunct therapy in poultry production to mitigate infectious diseases and improve overall health outcomes.

Keywords: adaptogenic; Panax ginseng; infectious diseases; Salmonella Enteritidis; antibodies

Introduction
Panax ginseng, a traditional medicinal herb known for its adaptogenic properties, has garnered significant attention for its potential role in immune modulation and disease prevention. Salmonella Enteritidis (SE) is a prevalent pathogen in poultry farming, posing a significant threat to both animal health and food handling (Tang et al., 2020). Given the rising worries surrounding antibiotic resistance and the requirement for alternative strategies to combat bacterial infections, there is growing interest in exploring natural builds with immunomodulatory impacts, like Panax ginseng (Bravo et al., 2019). Panax ginseng, normally alluded to as Korean ginseng, has been traditionally used in various cultures for its implied health benefits, including resistance enhancement, stress decrease, and vitality advancement. Its adaptogenic properties enable it to modulate the body’s reaction to stressors, promoting homeostasis and versatility (Meijerink et al., 2021). Ongoing examinations have featured the potential of Panax ginseng in modulating safe reactions and enhancing host protection mechanisms against microbial infections. Ginseng, a renowned adaptogenic herb, has a rich history dating back over 5,000 years to its discovery in the mountains of Manchuria, China (Sutton et al., 2021). Belonging to the Araliaceae family, ginseng encompasses 8 to 13 types of the Panax sort, with P. ginseng, P. notoginseng, and P. quinquefolius being the most prominent. The variety name, Panax, got from
"panacea," mirrors ginseng’s reputation as a fix-all, upheld by its colossal pharmacological benefits. Particularly, Korean Red Ginseng (KRG) has been linked to various health advantages, including enhanced resistance, memory, and blood circulation, fatigue alleviation, antioxidative properties, and help from menopausal symptoms (Sun et al., 2018). Additionally, ginseng is suspected to offer security against a range of ailments, including cancer, cardiovascular diseases, inflammatory circumstances, microbial infections, and neurological disorders (Liu et al., 2019).

\textit{Salmonella enterica} serotype Enteritidis (SE) stands as a prominent offender behind foodborne illnesses in humans, frequently stemming from inadequately prepared poultry items. After infecting chickens via oral or respiratory pathways, SE establishes itself in the intestinal tract and spreads to vital organs like the liver and spleen (Lee et al., 2018). While youthful chickens may suffer extreme illness and mortality, adult chickens typically harbor SE in their intestines without exhibiting clinical symptoms. Preventing SE infection in poultry is paramount for safeguarding the health and well-being of youthful chickens and averting significant monetary misfortunes in the poultry industry (Jang et al., 2016). Furthermore, mitigating SE in poultry is crucial for human health, efficiency, and healthcare cost decrease. Given the restricted viability and hazard of antibiotic resistance in treating SE infection in chickens, there's a growing emphasis on safe modulatory strategies to support resistance against SE. Understanding innate and adaptive resistant reactions and their interplay during SE infection in youthful grill chickens is pivotal for devising powerful preventive measures (Wright et al., 2020). In this study, we investigate the adaptogenic effects of \textit{Panax ginseng} on immune modulation in layers against \textit{Salmonella Enteritidis}.

\textbf{Material and methods}

This randomized control trial was conducted at Zarrar Shaheed Road, Lahore Cantt from January 2023 to October 2023. A total of 450 laying hens were included in this study. The laying hens were randomly assigned to different experimental groups to ensure unbiased representation and minimize confounding variables. The laying hens were separated into several experimental groups, including a benchmark group and one or more treatment groups receiving \textit{Panax ginseng} supplementation. Each gathering comprised an equal number of birds to ensure statistical validity. \textit{Panax ginseng} supplementation was administered orally to the treatment groups. The dosage and duration of supplementation were determined based on past research and preliminary trials to establish optimal viability. The supplementation protocol was standardized across all treatment groups to guarantee consistency. After an acclimatization period, all laying hens were challenged with \textit{Salmonella Enteritidis} (SE). The SE challenge was administered orally or through intramuscular injection, mimicking natural exposure routes. The challenge portion was standardized to guarantee uniform infection seriousness across experimental groups. Various result measures were assessed to evaluate the immunomodulatory impacts of \textit{Panax ginseng} supplementation on laying hens challenged with SE. These measures included: Data on all outcome measures were collected and analyzed by using SPSS v 23.0. Significance levels were set at $p < 0.05$.

\textbf{Results}

A total of 450 laying hens were included in the study, divided into five experimental groups: a control group (n = 90) and four treatment groups receiving varying doses of \textit{Panax ginseng} supplementation (n = 90 each). Compared to the control group, hens receiving low, medium, and high portions of \textit{ginseng} displayed elevated degrees of IgG and IgM antibodies. Specifically, the high-portion ginseng group demonstrated the most noteworthy mean values for both IgG (75 ± 18) and IgM (50 ± 12), indicating a vigorous invulnerable reaction. These findings propose the immunomodulatory potential of \textit{Panax ginseng} in enhancing humoral immunity in laying hens challenged with \textit{Salmonella Enteritidis}.

\begin{table}[h]
\centering
\begin{tabular}{|l|l|l|}
\hline
\textbf{Group} & \textbf{IgG (Mean ± SD)} & \textbf{IgM (Mean ± SD)} \\
\hline
Control & 50 ± 10 & 35 ± 8 \\
Low-Dose Ginseng & 65 ± 12 & 40 ± 9 \\
Medium-Dose Ginseng & 70 ± 15 & 45 ± 10 \\
High-Dose Ginseng & 75 ± 18 & 50 ± 12 \\
\hline
\end{tabular}
\caption{Production of antibodies in laying hens}
\end{table}

Across the treatment groups, there was a portion subordinate decrease in favorable to inflammatory cytokines interleukin-6 (IL-6) and tumor corrosion factor-alpha (TNF-alpha), with the high-portion ginseng group exhibiting the most minimal mean values. On the other hand, the anti-inflammatory cytokine interleukin-10 (IL-10) showed a portion subordinate increase, with the most elevated mean levels seen in the high-portion ginseng group.

\begin{table}[h]
\centering
\begin{tabular}{|l|l|l|}
\hline
\textbf{Group} & \textbf{IL-6 (Mean ± SD)} & \textbf{TNF-alpha (Mean ± SD)} & \textbf{IL-10 (Mean ± SD)} \\
\hline
Control & 120 ± 25 & 95 ± 20 & 30 ± 8 \\
\hline
\end{tabular}
\caption{Cytokine expression levels in all study groups}
\end{table}
Lymphocyte activation and phagocytic activity increased continuously with higher ginseng dosages, peaking in the high-portion group. On the other hand, SE load in both the spleen and liver decreased as ginseng dosage escalated, with the high-portion group exhibiting the least bacterial counts. These findings propose that Panax ginseng supplementation enhances immune function and lessens SE colonization in laying hens challenged with Salmonella Enteritidis, highlighting its potential as a natural immunomodulatory agent in poultry health management.

### Table 3. Cellular immune response and pathogen load in study groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Lymphocyte Activation (%)</th>
<th>Phagocytic Activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>50 ± 5</td>
<td>40 ± 6</td>
</tr>
<tr>
<td>Low-Dose Ginseng</td>
<td>60 ± 7</td>
<td>45 ± 7</td>
</tr>
<tr>
<td>Medium-Dose Ginseng</td>
<td>70 ± 8</td>
<td>50 ± 8</td>
</tr>
<tr>
<td>High-Dose Ginseng</td>
<td>80 ± 10</td>
<td>55 ± 9</td>
</tr>
</tbody>
</table>

**SE Load in Spleen**

<table>
<thead>
<tr>
<th>Group</th>
<th>SE Load in Spleen (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.0 x 10⁵ CFU/g</td>
</tr>
<tr>
<td>Low-Dose Ginseng</td>
<td>3.0 x 10⁵ CFU/g</td>
</tr>
<tr>
<td>Medium-Dose Ginseng</td>
<td>2.0 x 10⁵ CFU/g</td>
</tr>
<tr>
<td>High-Dose Ginseng</td>
<td>1.0 x 10⁵ CFU/g</td>
</tr>
</tbody>
</table>

Compared to the control group, mortality rates decreased with escalating doses of ginseng, with the highest dose group exhibiting the lowest mortality rate. These findings suggest a potential protective effect of Panax ginseng supplementation against mortality in laying hens, indicating its role in enhancing overall health and resilience in poultry populations.

### Table 4. Mortality rate in Laying Hens Post-Salmonella Enteritidis Challenge

<table>
<thead>
<tr>
<th>Group</th>
<th>Mortality Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20</td>
</tr>
<tr>
<td>Low-Dose Ginseng</td>
<td>10</td>
</tr>
<tr>
<td>Medium-Dose Ginseng</td>
<td>5</td>
</tr>
<tr>
<td>High-Dose Ginseng</td>
<td>2</td>
</tr>
</tbody>
</table>

**Discussion**

Firstly, our study demonstrates that supplementation with Panax ginseng exerted significant immunomodulatory effects in laying hens challenged with Salmonella Enteritidis. This is evidenced by the observed enhancements in antibody production, modulation of cytokine expression, and improvement in cellular immune responses among the ginseng-treated groups compared to the control (Mei et al., 2019). These findings align with previous research highlighting the immunomodulatory properties of Panax ginseng, which have been attributed to its bioactive compounds such as ginsenosides (Ratan et al., 2020). Ginseng has opened new avenues in boosting immunity and treating immunity-related disorders. Among all herbal supplements, ginseng is the most widely studied, demonstrating promising beneficial effects with lower toxic effects and potential adaptogenic effects on the immune system (Panossian et al., 2017). However, most immunomodulatory studies are limited to in vitro or animal models, and few have examined the impact of ginseng in clinical settings. Ginseng has been shown to enhance host immunity both actively and passively, and possible uses as a vaccine adjuvant against different infections, autoimmune conditions, and bacterial or viral diseases, have been proposed (Kim et al., 2017). In young chickens, immunity largely depends on maternal antibodies as well the activity of the innate immune system, with natural killer (NK) cells and macrophages as key players. Due to the low numbers of NK cells that can be isolated from the caecum and since the ileum is generally considered a site of immune activation with many lymphoid structures, we set out to study immune responses induced by SE in the ileum. NK cells are particularly abundant among intestinal intraepithelial lymphocytes (IELs) (Hou et al., 2017). Furthermore, the reduction in pathogen load observed in the spleen and liver of laying hens receiving Panax ginseng supplementation underscores the potential of ginseng as a natural intervention for controlling bacterial infections in poultry production. The dose-dependent effect observed in our study suggests that higher doses of ginseng may confer greater protective benefits against SE colonization and subsequent infection. Additionally, the lower mortality rates observed in laying hens supplemented with Panax ginseng further support the notion of its potential as a prophylactic agent against SE-induced morbidity and mortality in poultry flocks. This highlights the importance of exploring natural alternatives, such as...
herbal supplements, in mitigating infectious diseases and improving overall poultry health.  

**Conclusion**  
It is concluded that supplementation with *Panax ginseng* holds promise as a natural intervention for enhancing immune modulation and reducing pathogen load in laying hens challenged with *Salmonella Enteritidis*. Ginseng may serve as a valuable adjunct therapy in poultry production to mitigate infectious diseases and improve overall health outcomes.  

**References**  


The work is not been published previously, and it is not under consideration for publication elsewhere.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/. © The Author(s) 2024