THE COMPARATIVE EFFECT OF HERBAL AND ALLOPATHIC IMMUNE STIMULATORS ON THE EFFICACY OF NEWCASTLE DISEASE VACCINE IN BROILER

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ABSTRACT: The modulation of the broiler immune system by using immune-stimulating compounds provides a promising solution and it enhances the immune response to a broad range of viral and bacterial infections without causing any risk. The current in-vitro study included 18-day-old broiler chicks (Ross-308) divided into three equal groups i.e. A, B, and C comprising 6 birds each. The study was conducted to investigate the comparative efficacy of ND vaccine at days 5 and 18 orally in combination with two commercially available immune stimulators i.e. Powder Lisovit® (A blend of growth stimulators, carbohydrates, and vitamins manufactured by Biomin, Austria) administered @ 1gm/ 4 lit of drinking water twice a week for 5 weeks in group A and Liquid Virnet® (A blend of immune boosting and growth-enhancing natural herbs manufactured by Seza Pharma, Pakistan) administered @ 1ml/ lit of drinking water twice a week for 5 weeks in group B, while group C was attributed as control group and only ND vaccine was given with the same regime as in A and B. The blood sampling of all groups was done at days 0, 10, 20, and 35 for antibody titers estimation through a haem-agglutination inhibition test. The feed conversion ratio (FCR) was also calculated weekly. The mean values of antibody titers of group A and B individually were found highly significant (P=0.001) as compared to group C while the mean values of antibody titers of group A and B were non-significant (P=0.35) with each other. Similarly, the mean values of the weekly FCR of groups A and B were not significantly different (P=0.27) from each other, while the mean values of the weekly FCR of groups A and B individually were highly significant (P=0.001) as compared to the group C. The immune stimulators under study showed a significant effect on growth performance and an additive effect on ND vaccine titers. In the light of study results, it may be strongly recommended to use the immune stimulators as a routine practice to mitigate the deadly poultry diseases like ND.

Keywords: ND, Immune stimulator, Antibody titers, FCR, Virnet, Lisovit.

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INTRODUCTION

The poultry industry has become the leading supplier of efficient, high-quality animal proteins to the world (Abbas, 2020). Poultry meat and eggs provide several advantages relative to other sources of animal-based foods. Poultry meat compares favorably with other animal products in terms of protein content, amino acid balance, energy, and micronutrients (Bohler, 2017; Abbas et al., 2020). The estimated number of commercial chickens in the country in 2017 was 1,022 million birds, producing 17,083 million eggs and 1,270,000 tons of meat while directly and indirectly employing over 1.7 million people (Directorate of Poultry Research Institute, Performance Report 2014-2017). Demand for poultry meat has increased substantially over the past several decades because of the growing population and greater per-capita consumption (Abbas et al., 2020; Abbas et al., 2022; Organization for Economic Co-operation and Development, 2023). The poultry industry is the backbone of the economy contributing 50% of the total meat consumption in the country (Abbas, 2020; Chaudhary et al., 2015). Over Rs. 1981.63 billion is now spent in Pakistan's poultry industry, which currently produces 17,500 million table eggs and 1245 million kg of chicken meat annually (Abbas et al., 2023).

Newcastle disease is an avian viral disease that belongs to the Paramyxoviridae family and is highly contagious. It affects both domestic and wild bird species and is brought on by infections with virulent avian avulavirus I and results in high morbidity and mortality in birds that have not received adequate vaccinations (Absalon et al., 2019). The Newcastle disease virus (NDV) is a particularly virulent type that can present...
Experimental chances and evaluate used commonly Allium arvensis effects supplement al reliable al combination has losses, morbidity brought also practices, unfavorable immunosuppressing African America. itself. 

Eighteen-day-old chicks (Ross 308) were reared in a cage housing system for 35 days in the current study and were divided into three equal groups i.e. A, B, and C comprising six chicks in each group. According to the Ross standards, feed and water were provided in each group. Immune potentiating agents powder Lisovit®, (Biomin-Austria) @ 1gm/4liter of drinking water and liquid Virnet®, (Seza Pharma, Pakistan) @ 1ml/liter of drinking water were given twice a week along with ND vaccine (Lasota, Pfizer, USA) at the age of Day 5 and Day 18 orally to the chicks of group A and B respectively. Group C was attributed as the control group and only ND vaccine was given with the same regime as in A and B. The haem-agglutination inhibition (HI) (Two-fold serial dilution) was performed for antibody titers estimation on days 0, 10, 20, and 35 at the University Diagnostic Lab, UVAS Lahore. The average body weight and feed conversion ratio (FCR) of each group were also calculated every week.

Immune potentiating agents: Virnet liquid (Seza Pharma, Pakistan) which consists of Garlic extract 5,000,000mg, Echinacea purpurea 5,000mg, Nigella sativa 5,000mg, Mintha piperita 5,000mg, Allium cepa 5,000mg, Azadirachta indica 10,000mg, Piper nigrum 5,000mg, Ziziphus jujuba 20,000mg and powder Lysovit (Biomin-Austria) which consists of Lysosome 20.0%, Vitamin E50 SD 0.5% were used.

Sample processing: A total of 72 blood samples from all three groups were collected on days 0, 10, 20, and 35 by using 3ml disposable syringes. The blood was drawn through a wing vein/ jugular vein and collected in 2ml micro-centrifuge tubes. The samples were centrifuged for 3 minutes at 12,000 rpm. The serum was separated from the blood clot and underwent further serological procedures.

Haem-agglutination inhibition (HI): In a 96-well plate, 25 μL of PBS was added to each of the 12 wells in one row. A two-fold serial dilution of serum (25 μL) was made up to the 10th well. Next 4 HA units of ND virus were added to 11th well. The plate was then incubated at room temperature for 30 minutes. Subsequently, 1% chicken RBCs (25 μL) suspension was added to all the wells. The plate was left undisturbed for 40 minutes and agglutination was assessed by tilting the plates. A central button-shaped settling down of RBCs was indicative of antibody protection against the antigen, and titers of seven or above were regarded as the disease-protective threshold in any sample exhibiting this feature. The last well that exhibited complete inhibition of agglutination was regarded as the HI antibody titer (Rehman et al., 2017). Antibody titer of treatment and control groups was recorded on days 0,10,20 and 35 of this experiment.

**MATERIALS AND METHODS**

**Experimental Design:** The study was carried out under standard experimental conditions at the animal house, Department of Veterinary Medicine, UVAS, Lahore.
The mean body weight gain (BWG) and feed intake (FI) of each group were recorded weekly to calculate the feed conversion ratio (FCR) by using the formula as follows:

**FCR=Feed intake/weight gain:** The mortality in each group was also recorded daily.

**RESULTS**

**Feed conversion ratio (FCR):** The results of FCR in the study showed a significant difference between supplemented and control groups. The final cumulative FCR of both A and B groups were 1.560 and 1.588 respectively which were not significantly different from each other and the final cumulative FCR of group C was recorded as 1.62 which was lowest from the other two groups and was significantly lower than A and B. The week-wise and group-wise details of FCR and significances are also given in **Table. 1.**

**Antibody titers estimation:** The results of antibody titers in the study exhibited a significant difference between supplemented and non-supplemented groups. The highest antibody titer protection against NDV in the 5th week was shown by group A (7.33) followed by group B (7.16). Group C exhibited the lowest antibody titer protection (5.00) which was significantly lower than A and B. The day-wise and group-wise details of antibody titer values and significances are also given in **Table. 2.**

**Mortality:** Mortality was not found in Groups A and B while two birds were found dead in Group C in the second week of the experiment probably due to seasonal high environmental temperature-induced stress. In the post-mortem, there was no evidence of infection or illness was found.

<table>
<thead>
<tr>
<th>Groups</th>
<th>1st week</th>
<th>2nd week</th>
<th>3rd week</th>
<th>4th week</th>
<th>5th week</th>
<th>SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.947&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.062&lt;sup&gt;bd&lt;/sup&gt;</td>
<td>1.223&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.453&lt;sup&gt;bb&lt;/sup&gt;</td>
<td>1.560&lt;sup&gt;ba&lt;/sup&gt;</td>
<td>.01</td>
<td>0.001</td>
</tr>
<tr>
<td>B</td>
<td>.932&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.085&lt;sup&gt;bd&lt;/sup&gt;</td>
<td>1.243&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.475&lt;sup&gt;bb&lt;/sup&gt;</td>
<td>1.588&lt;sup&gt;ba&lt;/sup&gt;</td>
<td>.01</td>
<td>0.001</td>
</tr>
<tr>
<td>C</td>
<td>0.973&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>1.148&lt;sup&gt;ad&lt;/sup&gt;</td>
<td>1.315&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>1.573&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.625&lt;sup&gt;aa&lt;/sup&gt;</td>
<td>.01</td>
<td>0.001</td>
</tr>
<tr>
<td>SE</td>
<td>0.013</td>
<td>0.013</td>
<td>0.013</td>
<td>0.013</td>
<td>0.013</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-value</td>
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<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>A, B, C, a, b, c, d, e</sup> Mean values with different superscripts are significant at P<0.05: mean values with similar superscripts are non-significant with each other while mean values with different superscripts are significant with each other.

Abbreviations: SE Standard error
Table (2) Average HI titers of immune response against Newcastle Diseases.

<table>
<thead>
<tr>
<th>Groups</th>
<th>0 day</th>
<th>10 day</th>
<th>20 day</th>
<th>35 day</th>
<th>SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>6.333&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>6.333&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>6.667&lt;sup&gt;Ab&lt;/sup&gt;</td>
<td>7.333&lt;sup&gt;Ab&lt;/sup&gt;</td>
<td>0.144</td>
<td>0.001</td>
</tr>
<tr>
<td>B</td>
<td>6.167&lt;sup&gt;Ab&lt;/sup&gt;</td>
<td>6.167&lt;sup&gt;Ab&lt;/sup&gt;</td>
<td>6.500&lt;sup&gt;Ab&lt;/sup&gt;</td>
<td>7.167&lt;sup&gt;Ab&lt;/sup&gt;</td>
<td>0.144</td>
<td>0.001</td>
</tr>
<tr>
<td>C</td>
<td>6.167&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>5.500&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>5.167&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>5.000&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>0.144</td>
<td>0.001</td>
</tr>
<tr>
<td>SE</td>
<td>0.125</td>
<td>0.125</td>
<td>0.125</td>
<td>0.125</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>P-value</td>
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<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
</tr>
</tbody>
</table>

<sup>A, B, a,b</sup> Mean values with different superscripts are significant at P<0.05; mean values with similar superscripts are non-significant with each other while mean values with different superscripts are significant with each other. A titer value >7 is considered protective against NDV.

Abbreviations: SE Standard error.

**DISCUSSION**

Newcastle disease has occupied a drastic position in poultry production leading to massive economic losses for the poultry farmers. Therefore, the question arises whether the currently used vaccines under different vaccination schemes in the field induce effective immunity in chickens against the virulent NDV, which is reflected in mortality rates, clinical signs, postmortem lesions, level of antibody titer, and viral shedding. Additionally, one of the main causes of diseases in poultry is stress-stimulated immune system suppression (Carvalho et al., 2018). Elucidation of the immune response to NDV remains a top priority for the development of better control strategies in the face of reoccurring outbreaks. Keeping in view that immune booster remedies are in dire need to be used with vaccines to boost the immunity and performance level of poultry birds. For this purpose, the current study was designed to evaluate herbal remedies easily available in local markets. Interest in herbal medicines and their multiple beneficial utilization in poultry health and performance has been recently reviewed (Nazir et al., 2018, Yimer et al., 2019).

Finding the best beneficial and safe phytogetic feed additives as the most suitable substitute for antibiotic promoters is much more today's research topic (Muthusamy et al., 2015). Previous studies have shown that the herb in the formula has significant antiviral activity (Chiow et al., 2016). A recent systematic review of medicinal plants as a treatment option for gastrointestinal and respiratory livestock diseases showed that a high number of in vivo studies were performed on poultry (Ayrle et al., 2016). For the treatment of digestion problems and inflammation of the digestive tract, the use of a variety of medical plants has been described in recent German textbooks about veterinary herbal medicine (Brendieck-Worm. et al., 2018). Peppermint leaves have beneficial effects on antioxidant activity, abdominal fat deposition, and ammonia production in broilers (Khempaka et al., 2013). The main objective of this study was to evaluate some immunomodulating remedies that may work synergistically with the ND vaccine to enhance vaccine efficacy.

The current study conducted on antibody titer-based demonstration by the HI test method which is an excellent indicator of the immune status and disease resistance of a flock, especially to assess protective responses following vaccination because, unlike ELISA, the HI test correlates well with the more laborious virus neutralization (VN) assays (Miller et al., 2013).

Dominant antibody titer protocol produced by immune modulates against Newcastle disease better weight gain and FCR as compared to the control group exhibited a lack of adequate protection against the virus. These findings are in line with the broad idea of Iren, 2000, who discovered that the administration of immune-stimulating substances leads to an increase in immune response, which may result in increased growth rate and performance due to a decrease in the load of infectious causes and allow for maximum performance. Additionally, Williams et al., 2001 and Hashem pour et al., 2013 discovered that the increased body weight of broiler chickens treated with immune stimulants may be due to an increase in digestive enzymes such as trypsin and amylase.

Our findings about the efficacy of local immune-modulates on the performance of broiler chickens are in agreement that these have great potential to promote immunity, body weight, and FCR. The study supports our hypothesis that local plant-made remedies stimulate the immune system and serve as a significant stimulus for further exploring the potential plants’ remedies and traditional treatment for such hazardous diseases that are responsible for a massive loss in the poultry industry.

**Conclusions:** Supplementation of Immune-potentiating agents in broiler diets can be used as multipurpose agents i.e. natural growth promoters, performance enhancers, and humeral immune response promoters in broiler chickens. Based on the research findings, being a safer and more economical alternative to drugs, herbal constituents in drinking water showed a positive response in broiler chickens to enhance the immune response against N.D and growth performance which ultimately
contributed to better body weight gain, FCR, gross return, lower mortality and higher antibody titer against Newcastle disease in broiler chicks.

**Recommendations**

- Herbal immune-potentiating agents are strongly recommended in poultry diets because of their antioxidant, antiviral, and performance-enhancing properties.
- Further investigations are required concerning different potential herbs to evaluate efficacy in response to mitigating hazardous diseases in the poultry industry.

**Ethical approval:** Ethical approval to work with birds was taken from the Ethical Review Committee, University of Veterinary and Animal Sciences Lahore, Pakistan No. DR/232 dated 30-5-2023 and no bird was harmed during the study.

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