Evaluation of immunopotentiating effect of medicinal plant products in commercial layer flock vaccinated against Newcastle disease

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ABSTRACT
The present study was undertaken to evaluate the immunopotentiating effect of medicinal plant products such as Withania somnifera, Tinospora cordifolia, Allium sativum and Azadirachta indica in commercial layer flock vaccinated against Newcastle disease and production parameters. The Haemagglutination inhibition (HI) titre values in all the groups were above the protective level throughout the study period. Similar results were obtained in ELISA. The cell mediated immune response was assessed by Leukocyte Migration Inhibition Test (LMIT) and there was a significant mean per cent inhibition in treatment groups. There was no change in egg production, egg shell thickness, albumen index, Haugh unit and yolk index between treatment and control groups. In conclusion, W. somnifera (Ashwagandha, Amukkara kizhangu), (1%), T. Cordifolia (Guduchi, Seendhil kodi), (1%), Azadirachta indica (Neem, Veebmu) (0.2%) and A. sativum (Garlic, Poondu), (0.3%) can be used as an immunostimulant in poultry against Newcastle disease vaccination without affecting the egg production and egg qualities.

Key words: Allium sativum, Azadirachta indica, Immunostimulant, Newcastle disease, Poultry, Tinospora cordifolia, Withania somnifera.

INTRODUCTION
India is the fifth largest producer of eggs and ninth largest producer of poultry meat in the world, producing over 34 billion eggs and about 600,000 tonnes of poultry meat. Over the past decade the poultry industry in India has contributed approximately 100 billion rupees to the Gross National Product (GNP) (Vetrivel and Kumarmangalam, 2010). Economic losses due to diseases are scaled to 10 to 20% of the gross value of production in poultry industries of developed countries, and are likely to be higher in developing countries (FAO, 2012). Outbreak of ND results in alarming economic losses mainly due to ‘vaccine failure’ even after programmed vaccination schedules have been used. Both cellular and humoral response have been suggested to play important roles in the host defense against ND virus infection (Al-Shahery et al., 2008). To overcome this problem, modulation of micro-environment of the immune system seems to be essential. Plants such as Ocimum sanctum (Thulasi), Tinospora cordifolia (Guduchi, Seendhil kodi), Asparagus racemosus, Withania somnifera (Ashwagandha, Amukkara kizhangu), Azadirachta indica (Neem, Veembu), Curcuma longa (Turmeric), Panax ginseng (Ginseng), Picrorhiza kurroa (Katukhurohani) possess immunomodulatory activity. Rita et al. (2011) showed the determinative role of extracts of Ashwagandha (W. somnifera) as herbal feed additives in obtaining higher humoral and cell mediated immune responses providing better protection level against infections in protecting the immunodeficient chickens against infections. The alcoholic and aqueous extracts of T. cordifolia have been tested successfully for immunomodulatory activity (Ganguly and Prasad, 2011). Hanieh et al. (2010) suggested that dietary Allium sativum have a potential to enhance the immune functions against NDV given at the rate of 10 g/kg diet in White Leghorn chickens. Azadirachta indica (Neem) possess immunomodulatory property (Durrani et al., 2008). So, the present study was undertaken to evaluate the immuno- potentiating effect of Withania somnifera (Ashwagandha), Tinospora cordifolia (Gulancha), Allium sativum (Garlic) and Azadirachta indica (Neem) in commercial layer flock vaccinated against Newcastle disease and also assess the production performance in layers.

MATERIALS AND METHODS
The freeze dried Ranikhet disease virus (LaSota strain) was obtained from Ventri Biologicals, Pune was used as the source of ND virus antigen. The ND standard positive serum obtained from Avian Disease Diagnosis and Surveillance Laboratory, Namakkal was used as standard positive control serum in the HI test. Standard negative control serum was obtained from birds unvaccinated against Newcastle disease which were maintained at commercial layer farm in Thindamangalam, Namakkal. Flock screen antibody ELISA kits were procured from IDEXX, USA for detecting

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antibodies against ND virus (NDV). Crude extract of W. somnifera root powder and T. cordifolia stem powder was purchased from Hi-herbs Extracts Udyog, Bangalore. Crude extract of A. sativum was purchased from Zelang Group (American Branch) Inc, China. These were sun-dried before use. The neem leaves were collected from neem trees in and around Namakkal district. The leaves were spread evenly and dried for four days, until they become crispy and still retaining greenish colour. The dried leaves were pulsed before use.

The experimental layer birds were divided into five groups viz., A, B, C, D and E and each group comprises of 24 birds (four replicates in each group) and reared at the commercial poultry farm, Thindamangalam on caged system and standard vaccination schedule followed (Table 1).

<table>
<thead>
<tr>
<th>Table 1: Vaccination schedule.</th>
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<tbody>
<tr>
<td><strong>Age of vaccination</strong></td>
<td><strong>Strain used</strong></td>
<td><strong>Route of administration</strong></td>
</tr>
<tr>
<td>5th day</td>
<td>F1</td>
<td>Eye drops</td>
</tr>
<tr>
<td>28th day</td>
<td>La Sota</td>
<td>Eye drops</td>
</tr>
<tr>
<td>56th day</td>
<td>RDVK</td>
<td>Subcutaneous</td>
</tr>
<tr>
<td>20th week</td>
<td>R,B</td>
<td>Intramuscular</td>
</tr>
<tr>
<td>25th week</td>
<td>R,B</td>
<td>Intramuscular</td>
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</tbody>
</table>

The experimental groups are presented in Table 2. Serum samples were collected in each group at 2 weeks interval depending on the period at which immunisation was carried out. Blood samples (5 ml) were collected by vein puncture under the wing. The serum samples were separated by centrifugation and heat inactivation done at 56°C for 30 minutes and stored at -20°C for further use. Egg samples were collected at fortnight interval and processed.

The Haemagglutination Inhibition (HI) test was carried out as per the method described by OIE (2009) using Ranikhet disease virus LaSota strain as the HA antigen.

Enzyme linked immunosorbent assay was carried out as per the method described by manufacturer’s instruction.

Leukocyte migration inhibition test protocol was carried out as per the procedure described by Swain et al. (2000). Migration inhibition was calculated using the following formula.

\[
\text{Per cent migration} = \frac{\text{Average migration of cells in the presence of antigen}}{\text{Average migration of cells in the absence of antigen}} \times 100
\]

Per cent inhibition = 100 – per cent migration

Generally, greater than 20% inhibition in the presence of antigen represents significant leukocyte inhibition activity.

<table>
<thead>
<tr>
<th>Table 2: Experimental groups.</th>
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<tr>
<td><strong>Groups</strong></td>
<td><strong>Number of birds</strong></td>
</tr>
<tr>
<td>A</td>
<td>24</td>
</tr>
<tr>
<td>B</td>
<td>24</td>
</tr>
<tr>
<td>C</td>
<td>24</td>
</tr>
<tr>
<td>D</td>
<td>24</td>
</tr>
<tr>
<td>E</td>
<td>24</td>
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The eggs were collected at fortnight interval, egg number, egg weight, Haugh unit and internal qualities were assessed.

Data were statistically analysed by using the oneway ANOVA with Tukey’s test to identify significant differences between the means. Using SPSS ver 11.5.

**RESULTS AND DISCUSSION**

The humoral immune response of birds fed with medicinal plant products was assessed by HI test at fortnight intervals from 20 to 40 weeks. The result showed that all birds under the trial have responded to R,B vaccination given at 20th and 25th weeks of age and the titre values gradually increased from 20th to 40th week of age. This is in accordance with the report of Lambrecht et al. (2004) who reported that the success of vaccination in a flock is only monitored by demonstrating a rising antibody titer within a few days after vaccination.

The mean HI titre during the study period in group A, B, C, D and E were 234.66, 208.48, 212.36, 195.87 and 102.78 respectively. The mean HI titre values ranged from 85.33 to 426.66, 53.33 to 341.33, 53.33 to 341.33, 85.33 to 341.33 and 53.33 to 149.33 from 20 to 40 weeks in group A, B, C, D and E, respectively. The result showed that there was a significant difference (P<0.01) in mean HI titre values between treatment and control groups. This indicates that feeding of medicinal plant products had an immunopotentiating effect following vaccination. This is in accordance with earlier works Hanieh et al. (2010) and Landy et al. (2011).

The humoral immune response of birds fed with medicinal plant products was assessed by ELISA at fortnight intervals from 20 to 40 weeks. The mean ELISA titre during the study period in group A, B, C, D and E were 14323.75±521.72, 15144.20±485.87, 14028.76±688.53, 13231.05±535.39, 9241.11±213.21, respectively. The ELISA titre values ranged from 10554.60±241.49 to 18392.60 ±138 1.08, 12024.80±7 102.78 respectively. The mean HI titre during the study period in group A, B, C, D and E were 234.66, 208.48, 212.36, 195.87 and 102.78 respectively. The mean HI titre values ranged from 85.33 to 426.66, 53.33 to 341.33, 53.33 to 341.33, 85.33 to 341.33 and 53.33 to 149.33 from 20 to 40 weeks in group A, B, C, D and E, respectively. The result showed that there was a significant difference (P<0.01) in mean ELISA titre values between treatment and control groups. This indicates that feeding of medicinal plant products had an immunopotentiating effect following vaccination. This is in accordance with earlier works Hanieh et al. (2010) and Landy et al. (2011).
The cell mediated immune response was assessed by LMIT at fortnight intervals from 20 to 40 weeks. In LMIT, mean per cent inhibition during the study period in groups A, B, C, D and E were 23.11±0.92, 22.40±0.82, 21.13±0.28, 21.41±0.52 and 18.55±0.25 respectively. The per cent inhibition values during the study period ranged from 17.70±0.20 to 29.80±0.45, 17.25±0.25 to 28.05±0.85, 17.45±0.50 to 27.95±0.45, 17.55±0.05 to 25.15±0.35 and 17.40±0.20 to 20.15±0.50 in group A, B, C, D and E respectively. The highest per cent inhibition was observed in group A, B, C and D at the age between 38 and 40 weeks, but in group E it was observed on 32nd week of age. The result showed that there was a significant mean per cent inhibition in treatment (group A and C) and control groups (P<0.01). All the treatment groups had more than 20 per cent inhibition. This is in accordance with Agarwal and Reynolds (1991) who reported that 21 per cent or more and greater than 20% inhibition in the presence of ND antigen represent significant leukocyte inhibition factor activity.

The Immunomodulatory property of Withania somnifera may be due to presence of active glycowithanolides (Qamar Uddin et al, 2012) and Immunomodulatory property of Tinospora cordifolia may be due to the phytochemical compounds like Cordifolioside A, Cordioside and Ecdysteron (Priyanka Mishra et al, 2014).

The mean hen day egg production in group A, B, C, D and E were 20.43±0.25, 20.71±0.56, 20.79±0.36, 21.29±0.55 and 21.50±0.25 respectively. There was no significant difference in hen day egg production between the treatment and control groups. The mean egg weight (grams) in group A, B, C, D and E were 60.39±0.49, 60.51±0.47, 59.34±0.40, 59.42±0.35 and 58.98±0.34 respectively. There was no significant difference in egg weight between the treatment and control groups. The mean egg shell thickness (mm) in group A, B, C, D and E were 0.342±0.001, 0.341±0.001, 0.343±0.001, 0.344±0.001 and 0.342±0.001 respectively. This indicates that there was no significant difference in shell thickness between treatment and control groups. The mean albumen index in group A, B, C, D and E were 0.109±0.000, 0.112±0.000, 0.107±0.002, 0.108±0.001 and 0.105±0.001 respectively. This indicates there was no significant difference in mean albumen index between treatment and control groups. The mean yolk index in group A, B, C, D and E were 0.442±0.001, 0.442±0.001, 0.441±0.003, 0.441±0.001 and 0.440±0.001 respectively. This indicates there was no significant difference in mean yolk index between treatment and control groups.

In this study, there was no significant difference in hen day egg production between the treatment and control groups. This is contradictory to the findings of Qureshi et al. (2011) who reported that the supplementation of W. somnifera, A. sativum and A. indica respectively in layer diet showed significant increase in egg production.

In conclusion, addition of W. somnifera, T. cordifolia Azadirachta indica and A. sativum improves the humoral and cell mediated immune response against Newcastle disease vaccination without affecting the production parameter and egg qualities. So, W. Somnifera (1%), T. Cordifolia (1%) Azadirachta indica (0.2%) and A. Sativum (0.3%) can be used as an immunostimulant in poultry against Newcastle disease vaccination.

REFERENCES


