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**Research Article**

# Immunomodulatory activity of *Withania somnifera* against Newcastle disease in Aseel chicken

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**Abstract**

**The regular outbreak of Newcastle disease (ND), irrespective of vaccination of Aseel chickens, results in huge economic loss to the farming community. Hence, the present study was conducted to evaluate the immunomodulatory activity of *W. somnifera* (Ashwagandha) root powder against ND and its effects on growth rate and oxidative status in Aseel chickens. One hundred chicks were randomly divided into 5 treatment groups, Control ($T_1$), ND Vaccinated ($T_2$), and ND Vaccinated + *W. somnifera* @ 0.25 % ($T_3$), + *W. somnifera* - 0.5 % ($T_4$) and + *W. somnifera* - 1 % ($T_5$) of ten birds each with one replicate respectively for four months. ND pellet vaccine was administered orally on 15th and 90th day. The hemagglutination inhibition (HI) test on different intervals, cell mediated immunity assessment through delayed cutaneous hypersensitivity test, leucogram analysis and, lymphoid organ weights were measured. The antioxidant activity was calculated using superoxide dismutase and catalase enzymes estimation and production performance was also measured. The HI titers of $T_4$ were 4.50±0.30, 3.50±0.30 and 5.17±0.42 on 45th, 90th and 112th day, respectively, which was significantly (P>0.05) higher on day 112 and higher mean skin thickness (P>0.05) and lymphoid organ weight also recorded. The additive effects like superior antioxidant activity and better production performance were also found in $T_4$ group. Hence, it was concluded that *W. somnifera* @ 0.50% could improve the disease resistance power of Aseel chicken against ND and the supplementation yields profit through better feed conversion ratio.**

**Keywords:** Aseel Chicken, Immunity, Newcastle disease, Production performance, *W. somnifera*

**Highlights**

- The supplementation of *W. somnifera* root powder @ 0.50% could be recommended to improve the humoral and cell mediated immunity response of Aseel chicken against Newcastle disease.
- *W. somnifera* could be used as a growth promoter since better feed conversion ratio was noticed in herb supplemented group.
- Also, the antioxidant activity of *W. somnifera* was observed in the current study.

## INTRODUCTION

Newcastle disease virus (NDV) causes a highly contagious and economically important disease known as the Newcastle disease (ND) in domestic poultry. Consequently, the World Organization for Animal Health has included ND among the list of diseases that require immediate notification upon recognition (OIE, 2012). Many species of migratory birds carry the low-virulence Newcastle disease virus subclinically, and wild birds also carry the organism and spread the virus to domestic poultry, which makes containment and control a more difficult task (Bansal *et al*., 2022).

Generally, the chicken breeds reared in India are adapted to local conditions, including the climate, disease prevalence, and feed intake. These factors lead to the development of genetic traits related to immunocompetence and disease resistance, and adaptability to the tropical environment (Jaiswal *et al*., 2014). However, the variability in the magnitude of the expression pattern of chicken interferon-inducible transmembrane protein (chIFITMs) mRNA between Aseel and Kadaknath chickens was reported (Malarmathi *et al*., 2023) for Newcastle disease infections. Since, the chicken interferon-inducible transmembrane protein (chIFITM) genes usually function by preventing the entry of viruses into host cells, inhibiting viral replication, and controlling the viral load (Smith *et al*., 2013), its reduced expression in the Aseel chicken than Kadaknath breed might results in lower disease resistance (Malarmathi *et al*.,

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2023). Also, virulent NDV is still endemic in many regions of the world, especially in low- and middle-income countries, impacting the livelihood of millions of people dependent on poultry for food (Hu *et al*., 2022). Further, the immunosuppression was also recorded when the birds were susceptible to protozoan and other bacterial infections (Abdu *et al*., 2001).

Hence, to overcome all these hurdles, the Aseel chicken must be supplemented regularly with an immunomodulator or immunostimulating herbal compound since herbs are expected to show fewer side effects, which could maintain a healthy ND titer level and prevent infections. In this context, the present study was designed to find out the immunomodulatory activity of *Withania somnifera* (Ashwagandha) along with ND vaccination using local strain-specific Oral Pellets in Aseel chicken along with antioxidant and production performance activities.

## MATERIALS AND METHODS

**Collection, extraction and phytochemical analysis of *W. somnifera*:** *W. somnifera* herb was collected and authenticated with the help of a botanist and the dried root powder was processed for extract preparation (Vikramachakravarthi and Selvaraju, 2017). The qualitative phytochemical analysis of aqueous and ethanolic extracts of *W. somnifera* was carried out at the laboratory of Ethno Veterinary Herbal Research Centre for Poultry, Namakkal, Tamil Nadu (Vikramachakravarthi *et al*., 2022). The quantitative estimation of total alkaloids content (Harborne, 1973), total phenols (Singleton *et al*., 1999) and total flavonoids (Chang *et al*., 2002) content in the ethanolic extract of *W. somnifera* was estimated using double beam UV-Visible spectrophotometer (Model: Systronics 2202).

***In vivo* study:** The biological experiment was conducted on a hundred-day-old female Aseel cross chicks that were procured and randomly divided into five treatment groups of ten birds, each with one replicate, respectively, as per the approval of the government-nominated ethical committee. The uniformity in the average body weight of chicks (56±0.76 g) was followed before the commencement of the experiment, and the birds were housed according to their groups in separate cages. The treatment groups were non-vaccinated control ($T_1$), ND vaccinated control ($T_2$), ND vaccinated birds supplemented with *W. somnifera* root powder @ 0.25% ($T_3$), ND vaccinated birds supplemented with *W. somnifera* root powder @ 0.5% ($T_4$), ND Vaccinated birds supplemented with *W. somnifera* root powder @ 1% in feed ($T_5$). The herbs were supplemented in the feed using separate feeders for each group from the 15$^{th}$ day to the 112$^{th}$ day of the experiment. The TANUVAS Oral Pellet vaccine (Reetha *et al*., 2016) granules (live D 58 isolate of Newcastle disease virus) were used to immunize the Aseel chicken as per the prescribed schedule on 15$^{th}$ day (First dose) and 90$^{th}$ day (Second dose) (Vikramachakravarthi and Arivuchelvan, 2023).

**Humoral mediated immunity assessment:** The blood samples were collected on 15$^{th}$ day before vaccination, 45$^{th}$ day, 90$^{th}$ day and 112$^{th}$ day for HI titer estimation to know the humoral mediated immunity activity. HI test was performed as per the guideline (Alexander, 1988) and results were expressed as log 2.

**Cell mediated immunity assessment:** Cutaneous hypersensitivity test was performed at the end of experiment on 112$^{th}$ day (Haribabu *et al*., 1993) to study the cell mediated immunity activity of *W. somnifera*. After the measurement of skin thickness of interdigital space in between third and fourth digit of all groups (Zero hour) using an electronic digital caliper, the birds were sensitized with intradermal injection of 0.05 mL of 2% Dinitrochloro benzene (Sigma). The skin thickness (mm) was measured at 24$^{th}$ and 48$^{th}$ hour after injection to find out the efficacy of cell mediated immunity. No mortality was observed in the herbal treatment groups, and on the last day of the experiment, one bird per replicate was chosen at random, weighed, and sacrificed by cervical dislocation.

**Lymphoid organs weight, antioxidant activity and production performance activity assessment:** The lymphoid organs (thymus, spleen, and bursa of fabricius) were carefully dissected and weighed. Their relative weights were determined as percentages of the live weight. The antioxidant activity was determined by estimating the superoxide dismutase (SOD), catalase (CAT) and total reduced glutathione (GSH) content (Surai *et al*., 2019) using commercially available kits (Sigma - Aldrich) in double beam UV/ Visible spectrophotometer (Model: Systronics 2202) in serum. The production performance parameters, *viz*., feed intake and body weight gain, were measured at regular intervals (4$^{th}$ week, 8$^{th}$ week, 12$^{th}$ week and 16$^{th}$ week) for the estimation of feed conversion ratio (FCR).

**Statistical analysis:** The results were analyzed statistically in one way ANOVA using SPSS software

version 20. Experimental data was statically analysed in a complete randomised design (Snedecor and Cochran, 2004) using a one-way ANOVA and significant differences among means from triplicate analysis at (P<0.05) were determined by Duncan's multiple range tests.

## RESULTS

**Phytochemical analysis of *W. somnifera*:** The results of qualitative phytochemical analysis of both extracts of *W. somnifera* showed the presence of alkaloids, phenols, flavonoids, terpenoids, steroids and saponin. The quantitative phytochemical analysis of *W. somnifera* showed the concentration of alkaloids (0.97±0.23 mg/g), flavonoids (16.20±0.16 mg of rutin/g) and phenols (52.00±0.42 mg of gallic acid equivalent/g) in the extract.

**Humoral mediated immunity assessment:** HI titer values were estimated against ND virus in all the groups on 15th, 45th, 90th and 112th day and presented in Table 1.

The significant difference was not observed between the control and treatment groups on 15th day. On the 45th, 90th and 112th day, all the herbal treatment groups showed higher HI titer values than the control and vaccinated groups.

**Cell mediated immunity assessment:** The results of cutaneous hypersensitivity test are shown in Table 2. *W. somnifera* treatment groups showed significantly higher mean skin thickness than control at 24 hours and 48 hours after 2,4, Dinitrochlorobenzene (DNCB) injection.

The results of the leucogram estimated on the 112th day are shown in Table 3. All treatment groups were significantly (P<0.05) revealed higher total leucocyte count, neutrophil, and lymphocyte count than the control and vaccinated group.

**Table 1. Effect of supplementation of *W. somnifera* on haemagglutination inhibition titer (log 2) in Aseel chicken (Mean ± SE)**

| Group/Days | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ |
|---|---|---|---|---|---|
| 15th day | $1.50^{a}$±0.41 | $1.50^{a}$±0.41 | $1.50^{a}$±0.41 | $1.50^{a}$±0.41 | $1.50^{a}$±0.41 |
| 45th day | $1.17^{a}$±0.30 | $4.00^{bc}$±0.20 | $4.00^{bc}$±0.20 | $4.50^{c}$±0.22 | $4.50^{c}$±0.22 |
| 90th day | $1.17^{a}$±0.30 | $3.00^{b}$±0.25 | $3.50^{c}$±0.34 | $3.50^{c}$±0.30 | $3.67^{cd}$±0.21 |
| 112th day | $1.17^{a}$±0.30 | $4.10^{bc}$±0.22 | $5.00^{cd}$±0.30 | $5.17^{cd}$±0.42 | $5.33^{d}$±0.42 |

Values are Mean ± Standard Error of one replicate; Means with different superscripts vary significantly (p<0.05) within a column.

**Table 2. Effect of supplementation of *W. somnifera* on skin thickness (mm) with DNCB test in Aseel chicken (n=10) (Mean ± SE)**

| Group/hour | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ |
|---|---|---|---|---|---|
| O hour | $1.118^{a}$±0.08 | $1.115^{a}$±0.34 | $1.118^{a}$±0.08 | $1.115^{a}$±0.34 | $1.118^{a}$±0.46 |
| 24 hours | $2.15^{a}$±0.23 | $2.47^{a}$±0.24 | $3.24^{b}$±0.13 | $3.69^{c}$±0.28 | $3.71^{c}$±0.07 |
| 48 hours | $2.03^{a}$±0.22 | $2.45^{ab}$±0.17 | $2.71^{bc}$±0.86 | $3.38^{d}$±0.21 | $3.45^{d}$±0.19 |

Values are Mean ± Standard Error of one replicate; Means with different superscripts vary significantly (p<0.05) within a column.

**Table 3. Effect of supplementation of *W. somnifera* on leucogram on 114th day in Aseel Chicken (n=10) (Mean ± SE)**

| Group/ Cell (/ µL) | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ |
|---|---|---|---|---|---|
| Heterophil | $8914.80^{a}$±1.4 | $8919.20^{a}$±1.6 | $9424.21^{c}$±1.8 | $9602.56^{d}$±2.4 | $9877.28^{e}$±1.8 |
| Lymphocyte | $14158.80^{a}$±1.34 | $14164.40^{a}$±1.42 | $14939.52^{b}$±1.58 | $15222.24^{c}$±1.8 | $15441.95^{d}$±1.32 |
| H/L ratio | $0.63^{a}$±0.02 | $0.63^{a}$±0.02 | 0.63±0.04 | 0.63±0.02 | 0.64±0.04 |
| Monocyte | $1835.40^{bc}$±0.37 | $1836.73^{bc}$±0.17 | $1579.63^{a}$±0.62 | $1609.52^{bc}$±0.62 | $1613.75^{bcd}$±0.87 |
| Eosinophil | $734.160^{de}$±0.64 | $734.69^{de}$±0.42 | $508.69^{a}$±0.20 | $518.32^{b}$±029 | $584.29^{c}$±0.40 |
| Basophil | $576.840^{cd}$±0.54 | $575.840^{cd}$±0.24 | $321.28^{b}$±0.12 | $327.36^{b}$±0.31 | $316.05^{a}$±0.24 |
| Total cells | $26220.12^{a}$±2.86 | $26230.86^{a}$±2.12 | $26773^{b}$±3.04 | $27280^{c}$±2.92 | $27823.33^{d}$±2.81 |

Values are Mean ± Standard Error of one replicate; Means with different superscripts vary significantly (p<0.05) within a column.

**Table 4. Effect of supplementation of *W. somnifera* on weight of lymphoid organs (gram) in Aseel chicken (n=10) (Mean ± SE)**

| Group/Organ | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ |
|---|---|---|---|---|---|
| Thymus (g) | $0.658\pm0.32^{a}$ | $0.668\pm0.20^{ab}$ | $0.668\pm0.12^{ab}$ | $0.665\pm0.20^{ab}$ | $0.671\pm0.22^{ab}$ |
| Spleen (g) | $0.152\pm0.44^{a}$ | $0.169\pm0.28^{b}$ | $0.179\pm0.30^{bc}$ | $0.180\pm0.10^{bc}$ | $0.182\pm0.14^{bc}$ |
| Bursa (g) | $0.113\pm0.26^{a}$ | $0.120\pm0.12^{b}$ | $0.124\pm0.32^{bc}$ | $0.122\pm0.28^{bc}$ | $0.128\pm0.42^{bc}$ |

Values are Mean ± Standard Error of one replicate; Means with different superscripts vary significantly ($p<0.05$) within a column.

**Table 5. Effect of supplementation of *W. somnifera* on feed intake (g) in Aseel chicken (n=10) (Mean ± SE)**

| Group/hour | | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ |
|---|---|---|---|---|---|---|
| 4th week | Feed intake (g) | $416.00^{a}\pm0.86$ | $418.67^{a}\pm1.28$ | $425.67^{b}\pm1.45$ | $426.67^{b}\pm0.56$ | $429.50^{bc}\pm1.12$ |
| | Body Weight (g) | $142.17^{a}\pm0.48$ | $151.83^{b}\pm0.31$ | $179.17^{c}\pm0.48$ | $180.67^{c}\pm0.99$ | $178.17^{c}\pm0.31$ |
| | FCR | $2.93^{c}\pm0.01$ | $2.76^{b}\pm0.01$ | $2.38^{ab}\pm0.01$ | $2.36^{a}\pm0.01$ | $2.41^{ab}\pm0.01$ |
| 8th week | Feed intake (g) | $1389.67^{a}\pm0.71$ | $1398.17^{a}\pm1.30$ | $1398.67^{ab}\pm1.23$ | $1408.00^{b}\pm1.41$ | $1414.83^{bc}\pm2.46$ |
| | Body Weight (g) | $522.33^{a}\pm1.93$ | $558.67^{b}\pm2.42$ | $591.00^{c}\pm0.36$ | $602.83^{d}\pm1.72$ | $572.33^{c}\pm1.48$ |
| | FCR | $2.66^{c}\pm0.01$ | $2.50^{b}\pm0.01$ | $2.37^{b}\pm0.00$ | $2.34^{a}\pm0.01$ | $2.47^{bc}\pm0.01$ |
| 12th week | Feed intake (g) | $2637.67^{ab}\pm10.86$ | $2616.17^{a}\pm1.60$ | $2635.17^{bc}\pm2.90$ | $2620.50^{ab}\pm3.05$ | $2633.67^{bc}\pm0.76$ |
| | Body Weight (g) | $887.67^{a}\pm1.50$ | $934.67^{b}\pm0.95$ | $1009.83^{c}\pm2.23$ | $1050.83^{d}\pm3.81$ | $1026.17^{c}\pm3.31$ |
| | FCR | $2.97^{cd}\pm0.01$ | $2.80^{bc}\pm0.00$ | $2.61^{bc}\pm0.00$ | $2.49^{a}\pm0.01$ | $2.57^{bc}\pm0.01$ |
| 16th week | Feed intake (g) | $3969.0^{c}\pm8.54$ | $3951.83^{b}\pm2.34$ | $3891.33^{ab}\pm1.33$ | $3886.83^{ab}\pm1.38$ | $3886.67^{ab}\pm1.33$ |
| | Body Weight (g) | $1306.67^{a}\pm1.56$ | $1336.83^{b}\pm0.60$ | $1425.17^{c}\pm1.08$ | $1448.50^{cd}\pm9.10$ | $1443.50^{cd}\pm2.28$ |
| | FCR | $3.04^{cd}\pm0.01$ | $2.96^{bc}\pm0.00$ | $2.73^{bc}\pm0.00$ | $2.69^{a}\pm0.00$ | $2.69^{a}\pm0.02$ |

Values are Mean ± Standard Error of one replicate; Means with different superscripts vary significantly ($p<0.05$) within a column.

**Table 6. Effect of supplementation of *W. somnifera* on antioxidant activity in Aseel chicken (n=10) (Mean ± SE)**

| Parameter | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ |
|---|---|---|---|---|---|
| SOD (U/mg protein) | $1.21^{c}\pm0.012$ | $0.80^{a}\pm0.014$ | $1.11^{b}\pm0.002$ | $1.18^{c}\pm0.024$ | $1.21^{c}\pm0.042$ |
| CAT (U/mg protein) | $10.4^{c}\pm0.172$ | $7.74^{a}\pm0.183$ | $9.94^{b}\pm0.092$ | $10.5^{c}\pm0.144$ | $10.4^{c}\pm0.152$ |
| GSH (U/mg protein) | $10.3^{cd}\pm0.072$ | $8.98^{a}\pm0.072$ | $9.73^{b}\pm0.082$ | $10.2^{cd}\pm0.190$ | $10.3^{cd}\pm0.032$ |

Values are Mean ± Standard Error of one replicate; Means with different superscripts vary significantly ($p<0.05$) within a column.

**Lymphoid organs weight, antioxidant activity and production performance activity assessment:** The higher spleen relative weight was noted in *W. somnifera* treatment groups as described in Table 4. No significant effects of *W. somnifera* treatment groups were observed for the thymus and bursa of fabricius organs.

The results of production performance parameters, *viz.*, feed intake, body weight gain and feed conversion ratio, were presented in Table 5. Significant differences in body weight and feed conversion ratio were noticed between the control/vaccinated control and $T_4$ and $T_5$ groups in all weeks.

The significant antioxidant activity was observed in all the *W. somnifera* treatment groups as shown in table 6.

## DISCUSSION

The study was conducted to evaluate the immunomodulatory activity of *W. somnifera* root powder against Newcastle disease and to find out the effects of antioxidant and production performance effects in Aseel chicken.

The phytochemical analysis of *W. somnifera* detects the presence of alkaloids, phenols and flavonoids which are generally known for immunomodulatory activity in birds (Behl *et al.*, 2021). The alkaloids *viz.*, withanine, withanonine, and withanolides, which showed immunomodulatory activity (Ghosal *et al.*, 1989), were present in *W. somnifera*.

It was observed that the HI titers were increased on day 45 after primary immunization and decreased on

day 90, i.e. on the day of secondary immunization with Oral Pellet vaccine. Again, the HI titer was increased on 112th day after secondary immunization. Hence, all the doses of *W. somnifera* were effective in increasing the HI titer level, and the results are in agreement with Akotkar *et al*. (2007) in broiler chicken and Bhardwaj *et al*. (2012) in Japanese quails. The immunomodulatory activity of *W. somnifera* might be due to the presence of glycowithanolides such as sitoindoside IX (1) and sitoindoside X (Ghosal *et al*., 1989).

Also, Senthilnathan *et al*. (2006) observed the immunomodulatory effect of *W. somnifera* root powder in benzo(a) pyrene-induced lung cancer in male Swiss albino mice through improvement in the levels of immune competent cells, immune complexes and immunoglobulins. Thus, the immune potentiating activity of *W. somnifera* might be due to the activation of macrophages, increased lysosomal enzymes, and modulation of interleukin 1 and TNF-α production (Duque and Descoteaux, 2014). Hence, the increase in the humoral immunity by *W. somnifera* might be due to one or a combination of their active principles as detected in the present study.

In the cutaneous hypersensitivity test, among the *W. somnifera* groups, $T_4$ was highly significant at 24 hours after injection, whereas at 48 hours after DNCB injection, results of $T_3$, $T_4$ and $T_5$ did not differ significantly. Davis and Kuttan (2000) reported that *W. somnifera* showed cell mediated immunity stimulation activity through the generation of cytotoxic T lymphocytes in mice. The increase in the cell mediated immunity by *W. somnifera* might be due to one or a combination of their active principles as detected in the present study.

The higher total leucocyte counts, neutrophil, and lymphocyte counts were observed in all treatment groups than in control and vaccinated groups. Akotkar *et al*. (2007) reported significant ($P<0.01$) improvement in the haematological parameters like total leukocyte count, heterophils, and lymphocyte count when *W. somnifera* used at the dose rate of 0.5%, 0.75%, 1% and 1.25% than control in broilers. Also, *W. somnifera* showed a stimulating effect on the bone marrow cells (Davis and Kuttan, 2000), which might have resulted in an increased leukogram that could have contributed to the immunomodulatory activity of *W. somnifera* herb.

The results of lymphoid organ weights were in agreement with those of Azimi *et al*. (2020) in broiler chickens. The good production performance and no mortality showed the well-functioning of the immune system as indicated by larger spleen weight (Senthilnathan *et al*., 2006). The *W. somnifera* root powder (0.50%) showed higher body weight and better feed conversion ratio than other treatment groups. The higher dose supplementation did not significantly alter the body weight and feed conversion ratio in comparison to 0.50% group.

The improved feed conversion ratio might be due to the adaptogenic and antistress activity of Withanolides present in the roots of *W. somnifera* (Singh *et al*., 2003). The equivalent antioxidant activity with respect to SOD, catalase and reduced glutathione estimation was noted in the groups supplemented 0.5% and 1% *W. somnifera*, and this result was in agreement with Barnes *et al*. (2016) and Azimi *et al*. (2020) and the presence of phenolic and flavonoid compounds might have contributed to antioxidant activity.

In conclusion, the supplementation of *W. somnifera* root powder could be recommended to improve the humoral and cell mediated immunity response of Aseel chicken against Newcastle disease. Further, it also helps in improving antioxidant activity and production performances due to the presence of bioactive phytochemicals like alkaloids, phenolic and flavonoids. Hence, *W. somnifera* root powder at the dose rate of 0.50% could be an economically viable option for better immunomodulatory activity.

**Conflict of interest:** The authors declare no financial or conflict of interest regarding this study that could inappropriately influence the work.

**Author's contribution:** PV: Involved in the design of study, investigation, data generation, and preparing original draft; AA: Involved in statistical analyses, methodology and draft editing; AJ: Engaged in the idea of study, conceptualization and supervision.

**Data availability statement:** Raw data of this study are available from the corresponding author.

**Ethical approval:** The authors declare that the study was conducted based on the approval of the Institutional Animal Ethics Committee (Certificate No. IAEC/11/2014, dated 12.03.2014 of VCRI, Namakkal).

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