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Effect of Herbal Immunomodulator on Immune Organ and Immunological Parameters in Giriraja Birds

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A B S T R A C T

An experiment was conducted to evaluate the effects of Herbal immunomodulator powder and liquid form (with and without vaccine) comparing with Levamisole powder (with and without vaccine) on immune organ weights and immunological parameters in Giriraja birds. In a completely randomised design, 525 day old Giriraja chicks were assigned to seven treatments with each treatment group consisting of five replicates with 15 chicks each. The results of the present study found that the dietary supplementation group had non-significant difference in relative weights of Spleen, Bursa of Fabricicus among the various treatment groups and thymus exhibited significant difference in herbal immunomodulator powder supplemented group (T_4 and T_5) among themselves. Significant effect in antibody titer against NDV showed during 21st day of age, levamisole supplemented group (T_2 and T_3) and herbal immunomodulator powder supplemented group (T_4 and T_5) exhibited significant difference among themselves and herbal immunomodulator liquid supplemented group (T_6 and T_7) exhibited non- significant difference among themselves. The non- significant difference during 56th day of age, the titer value against IBD showed significant difference during 56th day of age in treatment groups (T_2, T_6 and T_7) and no significant difference on H: L ratio was observed.

Introduction

Poultry rearing is currently the fast growing industry in National livestock sector with its benefits in the form of increased and economic production and provision of proteinaceous food. In India, poultry industry is recognized as an important cottage as well as fast growing large commercial agriculture industry. Sustained economically viable poultry production demands the stringent control of various infectious diseases affecting the birds which result in huge economic losses to the poultry farm owners (Ganguly and Prasad, 2010). Nutrient requirements varies proportionately with the rate of growth, age of the bird, sex and genotype (breed or strain). Poultry nutritionists in India are adopting BIS or NRC recommendations in formulating rations for different age group of chicken. The carcass quality is an important characteristic from the view point of consumer's preference and acceptance of meat. Characterization and evaluation of immune parameters in Giriraja, Swarnadhara, Vanaraja and genotypes can offer knowledge that can be incorporated into breeding programmes for enhancing the natural resistance to disease in tropical and subtropical environment.

The use of natural feed additives as substitutes for antibiotic in poultry production has become an area of great interest. Medicinal plants or herbs consists of many pharmacologically active chemical compounds which have antimicrobial activity, antioxidant activity, antifungal activity, antiviral activity, anti-inflammatory effects as well as immunomodulatory properties (Toghyani *et al.,* 2015).

Materials and Methods

Five hundred and twenty five day old straight run Giriraja chicks from a single hatch were wing banded for identification, weighed and randomly distributed to seven treatment groups in Completely Randomised Design. Each treatment had five replicates with 15 birds in each replicate.

All the experimental chicks were healthy and received normal routine health care during eight weeks of the trial. The birds were provided feed and water *ad libitum*. A practical diet comprising of Yellow Maize, Soya bean meal, feed supplements and feed additives without antibiotics and coccidiostat constituted the control diet for all the seven treatment groups. The feed for the treatment group was formulated as per BIS (1994) requirements for all the nutrients except antibiotics and coccidiostat. The experimental birds in T_1 were fed with control diet without any antibiotics and coccidiostat.

The birds were administered with ND and IBD vaccines. The experimental birds in T_2 were fed as in T_1 and supplemented with Levamisole powder. The experimental birds in T_3 were fed as in T_2 and administered vaccines. The experimental birds from T_4 and T_5 were fed with Herbal Immunomodulator preparation in powder form containing Mandukaparni, Yasthi madhu, Guduchi, vriddadaru, Amalaki, Nimba and etc without and with vaccines, respectively and birds from T_6 and T_7 were fed with Herbal Immunomodulator preparation in liquid form containing Himsara, Kasani, Vasaka, Guduchi, Daraksha, Jhavuka, Shatavari etc., without and with administration of vaccines, respectively against ND and IBD.

Results and Discussion

Immunological parameters

The results of the effect of immune response of supplementing herbal immunomodulator in both powder and liquid form (with and without vaccine) and levamisole powder (with and without vaccine) on immunological parameters in Giriraja birds are presented in this article.

Immune organs (spleen, thymus and bursa)

Spleen

The results mean and standard errors of the relative weight of spleen for immunomodulators in Giriraja birds at 56th day of experimental period are presented in Table 2. Among the various treatment groups T_3 (levamisole with vaccine) showed maximum relative weight of 0.22 as against the lowest weight of 0.19 in T_6 (herbal immunomodulator liquid without vaccine) and control (T_1). Statistical analysis revealed non-significant difference in relative weights among the various treatment groups

Bursa of fabricicus

The mean and standard errors of the relative weight of bursa of Fabricicus for immunomodulators in Giriraja birds at 56th day of experimental period is presented in table 1. The relative mean weight of bursa of Fabricicus ranged from 0.056 per cent in herbal immunomodulator (T_6 and T_7) to 0.099

per cent in herbal immunomodulator (T_4). Statistical analysis revealed non-significant difference in relative weights among the various treatment groups.

Thymus

The mean and standard errors of the relative weight of thymus for immunomodulators in Giriraja birds at 56th day of experimental period is presented in table 1.

Among the various treatment groups T_3 (levamisole with vaccine) showed maximum relative weight of thymus (0.56) as against the lowest weight of 0.36 in T_5 (herbal immunomodulator powder with vaccine). The highest relative weight of 0.56 per cent recorded in levamisole with vaccine (T_3) and significantly higher compared to 0.36 observed in herbal immunomodulator powder with vaccine supplemented group (T_5) and non- significantly comparable with other treatment groups (T_1 T_2 T_4 T_6 and T_7).

However, levamisole supplemented group (T_2 and T_3) and herbal immunomodulator liquid supplemented group (T_6 and T_7) exhibited non- significant difference among themselves and herbal immunomodulator powder supplemented group (T_4 and T_5) exhibited significant difference among themselves.

Haemagglutination inhibition titer against Newcastle disease

The results of the effect of supplementing herbal immunomodulators powder and liquid form (with and without vaccine) and levamisole powder (with and without vaccine)on Haemagglutination inhibition titer against Newcastle disease vaccine after vaccinating the birds at 7th and 21st day of age are presented in tables 2. The analysis of variance remained significant (P>0.05) among various treatment groups at 7th and remained non- significant at 21st of day of vaccination.

The mean HI titer value analysed on 21st day of the experiment which were vaccinated on 7th day of age was significantly highest (23.60units) in group supplemented with levamisole with vaccine ($T_{3)}$ compared to 3.60 units recorded in group supplemented with levamisole without vaccine (T_2) and 3 units recorded in group supplemented with herbal immunomodulator powder without vaccine (T_4) and 2.80 units recorded in group supplemented with herbal immunomodulator liquid without vaccine (T_6). However, the highest titer value recorded in group supplemented with levamisole powder with vaccine (T_3) was non- significantly comparable with other treatments (T_1, T_5 and T_7). The lowest HI titer value (2.80 units) was observed in group supplemented with HIM liquid without vaccine (T_6) and significantly different from other treatment groups (T_1, T_3, T_5 and T_7). However, levamisole supplemented group (T_2 and T_3) and herbal immunomodulator powder supplemented group (T_4 and T_5) exhibited significant difference among themselves and herbal immunomodulator liquid supplemented group (T_6 and T_7) exhibited non- significant difference among themselves. However, different herbal immunomodulator administered with vaccine showed highest HI titer and comparable among themselves.

The means of HI titer value analyzed on 56th day of experiment which were vaccinated on 21st day of age for T_1, T_2, T_3, T_4, T_5, T_6 and T_7 were 13.80, 6.60, 9.40, 11.40, 9.20, 4.80 and 9.20 units, respectively.

The non significantly highest HI titer value was recorded in control (T_1) and the lowest HI titre value was observed in group supplemented with herbal immunomodulator liquid without vaccine (T_6). Apparently, the above results indicated that herbal immunomodulator and levamisole did not have any significant effect (P>0.05) on HI titer value when compared to control and

among the other treatment groups at the end of 56th day of experiment.

Antibody titer against IBD

The results of the effect of herbal immunomodulator powder and liquid form (with and without vaccine) and levamisole powder (with and without vaccine) on antibody titer against Infectious Bursal Disease vaccine after vaccinating the birds on 14th and 28th day of experiment and which were analyzed on 21st and 56th day of experiment are presented in tables 3.

The analysis of variance remained non significant (P>0.05) during 21st day of serum samples and significant (P≤0.05) during 56th day of serum samples of various treatment groups at 14th and 28th day of post immunisation.

The non-significantly lowest mean antibody titer value (1921.00) was revealed on 14th day of post immunization in group supplemented with herbal immunomodulator liquid with vaccine (T_7) and the highest mean antibody titer value (2374.60) was recorded in group supplemented with herbal immunomodulator liquid without vaccine (T_6). Statistical analysis revealed no significant difference in mean antibody titer values among different treatment groups.

The mean antibody titer value on 56th day of age was highest (2236.10) in group supplemented with herbal immunomodulator liquid with vaccine (T_7) followed by 2201.20 in group supplemented with levamisole without vaccine (T_2), 2167.30 in group supplemented with herbal immunomodulator liquid without vaccine (T_6), 2016.80 in group supplemented with herbal immunomodulator powder with vaccine (T_5), and 1829.60 in group supplemented with levamisole with vaccine (T_3). The highest antibody titer recorded in T_7 was significantly higher compared to T_4 and T_1 and non- significant with other treatment groups (T_2, T_3, T_5 and T_6).

However, levamisole supplemented group (T_2 and T_3) showed non- significantly comparable antibody titer among themselves.

The lowest antibody titre value (1721.40) was observed in group supplemented with herbal immunomodulator powder without vaccine (T_4) and non significantly comparable with 1684.90 antibody titer value observed in control (T_1), 1829.60 antibody titer value observed in group supplemented with levamisole with vaccine (T_3) and 2016.80 antibody titer value observed in group supplemented with herbal immunomodulator powder with vaccine (T_5). However, significantly different from treatment groups (T_2, T_6 and T_7). Apparently, the above results indicated that herbal immunomodulator and levamisole have significant effect (P<0.05) on antibody titer against IBD compared to control and also among various treatment groups.

Heterophill and lymphocyte ratio

The data on supplementing herbal immunomodulator powder and liquid (with and without vaccine) and levamisole powder (with and without vaccine) in Girirraja birds is presented in table 4. Statistical analysis revealed non- significant difference compared to control on heterophill and lymphocyte ratio.

Among the various treatment groups, highest H:L value (0.14) was recorded in control fed group (T_1) as against the lowest value (0.005) recorded in group supplemented with herbal immunomodulator liquid without and with vaccine (T_6 and T_7).

Immunological parameters

Immune organs

Spleen and bursa

There was no significant difference in relative weights of lymphoid organs viz., spleen and bursa in Giriraja birds due to supplementation of herbal immunomodulator powder and liquid form (with and without vaccine) and levamisole powder (with and without vaccine). The results are in contrary to the findings results of Durrani *et al.,* (2006), who found that inclusion of turmeric powder increased the spleen weight and whole giblets weight of broilers.

Thymus

There was significant difference in relative weight of lymphoid organ viz., thymus in Giriraja birds by supplementation of herbal immunomodulator powder without vaccine (T_4) and levamisole powder with vaccine (T_3). The results of present study are in agreement with the findings of Al-Sultan (2003) who found that the higher thymus weight indices were detected in birds received diet containing 0.5% turmeric.

HI titre against New Castle disease

Immunological studies using ND vaccine showed significantly higher HI titer values at 21st day of age in groups fed with the levamisole powder with vaccine (T_3) and herbal immunomodulator powder with vaccine groups (T_5) when compared to other group supplemented with herbal immunomodulator. The results are similar to Ziaran *et al.,* (2005), who reported that lower doses of plant extract in broilers had a positive antibody titer against ND, while the negative effects at higher levels.

Antibody titer against IBD

Immunological studies using IBD vaccine showed significantly higher antibody titer values at 8th weeks of age in groups fed with herbal immunomodulator liquid (T_6 and T_7) and levamisole powder without vaccine group (T_2). The analysis of variance remained non significant ($P>0.05$) during 21st day of serum samples and significant ($P\leq0.05$) during 56th day of serum samples of various treatment groups at both 14th and 28th day of age of post immunisation. Non-significantly lowest mean antibody titer value (1921.00) on 14th day of post immunization was recorded in group supplemented with herbal immunomodulator liquid with vaccine (T_7) and the highest mean antibody titer value (2374.60) was recorded in group supplemented with herbal immunomodulator liquid without vaccine (T_6). Statistical analysis revealed non-significant difference in mean antibody titer values among different treatment groups. The results of the present study are in agreement with the findings of Balwinder Singh Dhote *et al.,* (2005), investigation indicated that Immuplus (25 mg/kg body weight) has potentiating effect on the paraspecific responses against ND and IBD antigens as detected by TLC, ALC, NBT and for IL-1 and IL-2 ELISA test in chicks.

Heterophil and lymphocyte ratios

The results of the present study revealed that there was no significant difference in H:L in Giriraja fowl fed with supplementing herbal immunomodulator both in form of powder and liquid (with and without vaccine) and levamisole powder (with and without vaccine). Among the various treatment groups, highest H:L value (0.14) was recorded in control fed group (T_1) as against the lowest value (0.005) recorded in groups supplemented with herbal immunomodulator liquid without and with vaccine (T_6 and T_7).

Table.1 Effect of herbal immunomodulator and Levamisole on immune organs (gm/100gm body weight) in Giriraja birds

Treatment		Organ weight (gm/100gm body weight)		
		Spleen	**Bursa of Fabricicus**	**Thymus**
T_1	Control	0.19±0.02	0.097±0.02	0.44±0.03^{ab}
T_2	Control + levamisole without vaccine	0.21±0.01	0.094±0.04	0.47±0.04^{ab}
T_3	Control + levamisole with vaccine	0.22±0.02	0.079±0.02	0.56±0.06^{a}
T_4	Control + HIM powder without vaccine	0.21±0.02	0.099±0.01	0.49±0.04^{a}
T_5	Control + HIM powder with vaccine	0.21±0.01	0.081±0.01	0.36±0.04^{b}
T_6	Control + HIM liquid without vaccine	0.19±0.01	0.056±0.01	0.44±0.03^{ab}
T_7	Control + HIM liquid with vaccine	0.21±0.01	0.056±0.01	0.43±0.03^{ab}

Table.2 Effect of herbal immunomodulator and Levamisole on HI titre against NDV in Giriraja birds

Treatment		HI titer	
		III week	**VIII week**
T_1	Control	22.40±7.03^{a}	13.80±5.77
T_2	Control + levamisole without vaccine	3.60±0.78^{b}	6.60±1.27
T_3	Control + levamisole with vaccine	23.60±7.53^{a}	9.40±2.78
T_4	Control + HIM powder without vaccine	3.00±0.61^{b}	11.40±6.00
T_5	Control + HIM powder with vaccine	19.60±5.48^{a}	9.20±2.85
T_6	Control + HIM liquid without vaccine	2.80±0.33^{b}	4.80±0.90
T_7	Control + HIM liquid with vaccine	17.60±5.31^{ab}	9.20±1.20

Table.3 Effect of herbal immunomodulator and Levamisole on antibody titer against IBDV in Giriraja birds

Treatment		Antibody titer	
		III week	VIII week
T_1	Control	2197.90±147.98	1684.90±197.09^{b}
T_2	Control + levamisole without vaccine	2272.10±124.93	2201.20±172.07^{a}
T_3	Control + levamisole with vaccine	1977.20±169.53	1829.60±191.31^{ab}
T_4	Control + HIM powder without vaccine	2322.00±147.32	1721.40±96.73^{b}
T_5	Control + HIM powder with vaccine	2239.20±163.83	2016.80±52.50^{ab}
T_6	Control + HIM liquid without vaccine	2374.60±160.98	2167.30±127.97^{a}
T_7	Control + HIM liquid with vaccine	1921.00±199.37	2236.10±114.87^{a}

Table.4 Effect of herbal immunomodulator and Levamisole on blood parameters (H: L) in Giriraja birds

Treatment		**H:L**
T_1	Control	0.14 ± 0.04^a
T_2	Control + levamisole without vaccine	0.04 ± 0.01^b
T_3	Control + levamisole with vaccine	0.01 ± 0.004^b
T_4	Control + HIM powder without vaccine	0.01 ± 0.001^b
T_5	Control + HIM powder with vaccine	0.01 ± 0.001^b
T_6	Control + HIM liquid without vaccine	0.005 ± 0.001^b
T_7	Control + HIM liquid with vaccine	0.005 ± 0.001^b

The present findings are in accordance with the findings of Nidhi Singh *et al.*, (2008), who reported that heterophill count, as evinced by the analysis of variance found that it decreased with the supplementation of herbal formulations (amla plus turmeric powder each @ 5 gm/Kg of feed) in the feed irrespective of the seasons. This decrease in heterophil count was highly significant in seasons like summer, winter and rainy. This observation proves the immuno modulating effect of herbal preparations.

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