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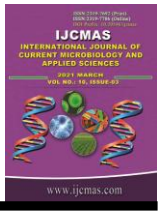

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Effect of Supplementing Neem, Ginger and Garlic Powder on Immune Response of Commercial Layers

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A B S T R A C T

An experiment was conducted to study the effect of supplementing neem, ginger and garlic powder on immune response in commercial layers. Five hundred layer birds of twenty eight weeks age were distributed into 25 replicates of 20 birds each. Basal diet T_1 and the experimental diets were prepared by incorporating garlic powder@ 0.5% (T2), neem powder @ 0.5% (T3), ginger powder @ 1% (T4) and garlic @ 0.5%, neem @ 0.5% and ginger powder @ 1% in combination (T5). The duration of the experiment was 20 weeks divided into 5 phases of 4 weeks each. The serum samples collected on each phase were assayed for antibody titre against Newcastle disease virus (ND) and Infectious bursal disease virus (IBD) using haemagglutination inhibition test and ELISA, respectively. At peak production stage (45th week) of the experiment, two birds from each replicate in different treatment groups respectively was sacrificed and the weight lymphoid organs such as spleen and thymus were taken during the slaughter of the birds. Inclusion of neem, ginger and garlic individually and also in combination improved the immune response against Newcastle disease but there was no effect on immune response against Infectious bursal disease. Feeding of neem, ginger and garlic powder revealed non-significant effect($P>0.05$) on weight of lymphoid organs. It was concluded that feeding neem, ginger and garlic powder was beneficial in improving immune response against the Newcastle disease.

Introduction

The fast growing nature of poultry birds and their shorter generation interval has been associated over the years with the use of antibiotic growth promoters at sub-therapeutic doses in poultry feeds in order to improve the production performance by reducing the subclinical infection (Nettleton 1991). Other benefits of antibiotic growth promoter compounds include control of zoonotic pathogens such as *Salmonella*, *Campylobacter*, *Escherichia coli* and *Enterococci* species in the gut (NOAH 2001).

Although birds raised with the antibiotic growth promoters achieved good performance, their potential side effects became a real public health problem worldwide (Bager. 1998) and led to the ban of these products by the European Union in January 2006.

This decision has therefore stimulated a search for alternatives. Plant derived additives have been proven to control pathogens due to their antimicrobial activity (Dorman and Deans 2000). Medicinal plants and probiotics are currently considered safe and offer a possible alternative to satisfy customer demands and current market challenges.

Bacillus subtilis, Staphylococcus aureus, Proteus vulgaris, Salmonella typhi and *Pseudomonas aeruginosa* were susceptible for the antibacterial activity of the methanol extract of *A.indica* leaves (Grover *et al.*, 2011). Ginger is desirable for greater productivity in poultry, increased palatability of feed, nutrient utilization, appetite stimulation, increased gastric juice flow.

Hence it can be used as a substitute for antibiotic growth promoters (Owen and Amakiri, 2012). Garlic (*Allium sativum*) with its anti-bacterial, anti-viral, anti-fungal, and anti-parasitic properties, recognized as one of the most important plant species used for organic poultry production..

Thus, the objective of this study was to investigate the effect of supplementing neem, ginger and garlic powder on immune response in commercial layers.

Materials and Methods

A total of five hundred layer birds of twenty eight week age were selected and the birds were reared by feeding the basal diet for 2 weeks for acclimatization. The birds were allocated into five treatment groups with 20 birds per replicate and 100 birds per treatment. The birds are fed in two phases, layer phase I and layer phase II diets as per BIS (2007). From 31st week the birds were fed with the respective experimental diet till the completion of the experiment at 50th week. The trial duration was for 20 weeks (31st week to 50th week) and this duration was divided into five phases of 4 weeks each.

The basal diet (control–T1) was formulated as per the standard recommendations of BIS (2007) nutrient requirements. The treatment group T2, T3 and T4 was supplemented with garlic powder 0.5 per cent, neem powder 0.5per cent and ginger powder 1per cent respectively.

The treatment group T5 was supplemented with garlic powder 0.5per cent + neem powder 0.5per cent + ginger powder 1per cent.

Immune response against Newcastle disease and Infectious bursal disease

The serum samples collected on each phase were assayed for antibody titres against Newcastle disease virus (ND) and Infectious bursal disease virus (IBD) using haem agglutination inhibition test and ELISA, respectively.

Immune organ weight

The weight lymphoid organs such as spleen, bursa of fabricius and thymus were taken during the slaughter of the birds on 45th week (peak stage of the experiment) of the experiment and expressed as per cent of live body weight.

$$\text{Organ weight (grams / kg live body weight)} = \frac{\text{Weight of organ (g)}}{\text{Live weight before slaughter (g)}} \times 100$$

Results and Discussion

Antibody titre against Newcastle disease

In the first phase antibody titre against Newcastle disease (log_{10} HI titre) were 1.322 (T1), 1.331 (T2), 1.364 (T3), 1.366 (T4) and 1.377 (T5), respectively. In second phase of the experiment antibody titre (log_{10} HI titre) was significantly higher ($P \leq 0.05$) in T2, T3, T4 and T5 when compared to the control group and the titre values were 1.324 (T1), 1.378 (T2), 1.421 (T3), 1.420 (T4) and 1.422 (T5), respectively. In the third phase of the experiment antibody titre values (log_{10} HI titre) were 1.311 (T1), 1.396 (T2), 1.409 (T3), 1.412 (T4) and 1.446 (T5) and the antibody titre was significantly higher ($P \leq 0.05$) in T2, T3, T4 and T5 when compared to the control group. In fourth phase of the experiment antibody titre (log_{10} HI titre) was significantly higher ($P \leq 0.05$) in T2, T3, T4 and T5 when compared to the control group and the titre values were 1.331 (T1), 1.409 (T2), 1.411 (T3), 1.398 (T4) and 1.439 (T5), respectively. In the fifth phase of the experiment antibody titre (log_{10} HI titre) values were 1.298 (T1), 1.388 (T2), 1.428 (T3), 1.401 (T4), 1.421 (T5) and the antibody titre was significantly higher ($P \leq 0.05$) in T2, T3, T4 and T5 when compared to the control group. The results of Antibody titre against Newcastle disease are represented in the table 1.

The study is in agreement with Garba *et al.,* (2013) who supplemented garlic and neem leaf aqueous extracts to broilers at the rate of 5 g each per litre of water individually and also in combination at the rate of 2.5 g each per litre of water and observed significant improvement ($P \leq 0.05$) in the antibody titre.

The significant increase recorded in this study could be attributed to the effects of garlic and neem on B- lymphocyte which differentiate into memory cells and plasma cells.

Antibody titre against Infectious bursal disease

In first phase, the average IBDV titre under different treatment groups viz., T1, T2, T3, T4 and T5 were 1659.43, 1766.65, 1841.66, 1898.88 and 1913.56, respectively. The average IBDV titre in second phase of experiment under different treatment groups were 1602.21 (T1), 2178.32 (T2), 2254.88 (T3), 2107.76 (T4) and 2456.76 (T5). In third phase of the experiment the IBDV titre under different treatment groups viz., T1, T2, T3, T4 and T5 were 1578.32, 2210.58, 2190.21, 2278.76 and 2512.87, respectively. The average IBDV titre in fourth phase of experiment under different treatment groups were 1615.12 (T1), 2098.67 (T2), 2167.87 (T3), 2145.76 (T4) and 2490.90 (T5).

In last phase of the experiment the average IBDV titre under different treatment groups viz., T1, T2, T3, T4 and T5 were 1589.32, 2130.98, 2069.76, 2170.76 and 2521.87, respectively.

The statistical analysis revealed non-significant ($P > 0.05$) difference in IBDV titre among different treatment groups during all phases of the experiment. The results of Antibody titre against Infectious bursal diseases are represented in the table 2.

The present study is in disagreement with the Jawad *et al.,* (2013) who supplemented neem leaves at the rate of 2 %, 4 % and 6 %. They observed significant improvement ($P < 0.05$) in the antibody titre against the IBD in the groups fed with neem leaves compared to the control group at 14^{th}, $21^{st,}$ and 42^{nd} day. Dissimilar results are also observed by Ghasemi *et al.,* (2013)who supplemented ginger oil to the broiler birds at the rate of 100 mg/ kg and 200 mg/ kg of feed and observed significant improvement ($P < 0.05$) in the antibody titre against IBD and IB.

Table.1 Effect of feeding neem, ginger and garlic powder on immunological response (NDV log HI titre) of commercial layers on phase wise manner

Experimental group	Description of the treatment	NDV log HI titre					
		1st phase	2nd phase	3rd phase	4th phase	5th phase	Average
T_1	Control	1.322 ± 0.30	1.324 ± 0.18[b]	1.311 ± 0.14[b]	1.331 ± 0.23[b]	1.298 ± 0.11[b]	1.313 ± 0.27[b]
T_2	Control + garlic powder 0.5%	1.331 ± 0.17	1.378 ± 0.15[a]	1.396 ± 0.19[a]	1.409 ± 0.23[a]	1.388 ± 0.54[a]	1.392 ± 0.16[a]
T_3	Control + neem powder 0.5%	1.364 ± 0.12	1.421 ± 0.34[a]	1.409 ± 0.21[a]	1.411 ± 0.15[a]	1.428 ± 0.23[a]	1.416 ± 0.11[a]
T_4	Control + ginger powder 1%	1.366 ± 0.22	1.420± 0.23[a]	1.412 ± 0.24[a]	1.398 ± 0.15[a]	1.401 ± 0.19[a]	1.405 ± 0.21[a]
T_5	Control + garlic 0.5% + neem 0.5%+ ginger powder 1%	1.377 ± 0.18	1.422 ± 0.21[a]	1.446 ± 0.25[a]	1.439 ± 0.11[a]	1.421 ±0.32[a]	1.429 ± 0.18[a]

a, b means in the same column with no common superscript differ significantly ($p \leq 0.05$)

Table.2 Effect of feeding neem, ginger and garlic powder on immunological response IBDV titre (ELISA) of commercial layers on phase wise manner

Experimental group	Description of the treatment	IBDV titre (ELISA)					
		1st phase	2nd phase	3rd phase	4th phase	5th phase	Average
T_1	Control	1659.43 ± 288.30	1602.21 ± 116.21	1578.32 ± 142.43	1615.12 ± 160.32	1589.32 ± 148.76	1588.60 ± 245.76
T_2	Control + garlic powder 0.5%	1766.65 ± 163.80	2178.32 ± 154.54	2210.58 ± 176.43	2098.67 ± 198.43	2130.98 ± 231.98	2156.59 ± 167.87
T_3	Control + neem powder 0.5%	1841.66 ± 359.74	2254.88 ± 213.76	2190.21 ± 160.98	2167.87 ± 176.33	2069.76 ± 267.87	2203.45 ± 111.89
T_4	Control + ginger powder 1%	1898.88 ± 231.06	2107.76 ± 325.89	2278.76 ± 213.78	2145.76 ± 165.98	2170.76 ± 176.89	2159.98 ± 149.09
T_5	Control + garlic 0.5% + neem 0.5%+ ginger powder 1%	1913.56 ± 291.49	2456.76 ± 154.78	2512.87 ± 176.76	2490.90± 410.98	2521.87 ± 321.90	2478.79 ± 310.88

Table.3 Effect of feeding neem, ginger and garlic powder on immune organ weight (g/100g body weight) of commercial layers

Experimental group	Description of the treatment	Immune organs weight (g/100g body weight)	
		Spleen	Thymus
T_1	Control	0.129 ± 0.150	0.340 ± 0.047[b]
T_2	Control + garlic powder 0.5%	0.121 ± 0.009	0.352 ± 0.017[b]
T_3	Control + neem powder 0.5%	0.133 ± 0.019	0.366 ± 0.027[b]
T_4	Control + ginger powder 1%	0.127 ± 0.107	0.373 ± 0.047[b]
T_5	Control + garlic 0.5% + neem 0.5%+ ginger powder 1%	0.135 ± 0.009	0.418 ± 0.040[a]

a, b means in the same column with no common superscript differ significantly ($p \leq 0.05$)

Immune organ weight

There was no significant difference ($P > 0.05$) in the weight of the spleen (g/100g body weight) among all the treatment groups compared to the control group. The weight of the spleen in T1, T2, T3, T4 are T5 are 0.129, 0.121, 0.133, 0.127 and 0.135, respectively.

The weight of thymus (g/100g body weight) was significantly highest ($P \leq 0.05$) in the birds of the treatment group which was fed with combination of neem, ginger and garlic powder compared to all other groups and thymus weight in T1, T2, T3, T4 are T5 are 0.340, 0.352, 0.366, 0.373 and 0.418, respectively and there was no significant difference among T2, T3, T4 and T5 with control. The results of immune organ weight are represented in the table 3.

The findings are in agreement with Khatum *et al.,* (2013) who supplemented tulsi and neem leaves at the rate of 1, 2 and 3 ml/ litre of drinking water and observed no significant difference ($P > 0.05$)in the weight of the spleen. The results are dissimilar with the findings of Ghasemi *et al.,* (2013) who supplemented ginger oil to the broiler birds at the rate of 100 mg/ kg and 200 mg/ kg of feed and significant improvement ($P \leq 0.05$) in the weight of bursa fabricius and thymus in the groups fed with ginger essential oil compared to the control group because of the modulation of immune system activity in broilers by phytogenic products is mainly associated with improving the gut ecosystem and reducing the production of growth depressing toxins by intestinal microflora.

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