



RESEARCH ARTICLE

Enhancing the Effectiveness of Newcastle Disease Vaccination in the Laying Hens by Herbal Diet Supplementation

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ABSTRACT

A study was undertaken to improve the general health and immunity in laying hens. Vaccination plays a vital role in preventing diseases and in maintaining the immunity levels in hens. In this study, we tested if a diet containing herbal feed supplements namely garlic, fenugreek and bay leaves can enhance the efficacy of vaccination against Newcastle disease (ND) in laying hens. The immunity levels against ND vaccination were assessed by screening the serum samples using haemagglutination inhibition and enzyme-linked immunosorbent assay. We also estimated the blood cell count and immunoglobulin levels in the egg yolk. Our results revealed that, the combination of three herbal supplements has conferred higher levels of immunity to hens and also resulted in higher levels of immunoglobulin content in the eggs.

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INTRODUCTION

Poultry industry is growing at a phenomenal rate by increasing commercialization of poultry production systems. This increased commercialization is aided by high quality poultry breeds, balanced feed, most advanced vaccines, medicines and diagnostics. With continuous expansion of poultry production systems, the economic implications of poultry diseases have also become increasingly important at both farm and national levels. Among the diseases that affect the profitability of layer farms, avian influenza (AI), infectious bursal disease (IBD) and Newcastle disease are the important viral diseases in terms of both incidence and economic losses. In this study, an attempt was made to improve the immune status of the laying hens that are given a dose of Newcastle disease vaccination using feeds containing herbal supplements. The herbal supplements namely garlic, fenugreek seeds and bay leaves were added to the regular layer mash. Studies of Abdullah *et al.* (1989), Kyo *et al.* (2001) and Gulati *et al.* (2002) have shown that garlic (*Allium sativum*) stimulates humoral immune

response, enhances the count of macrophage and T-lymphocytes. The fenugreek seeds (*Trigonella foenum*) have been shown to contain hyperinsulinemic, hypolipidemic properties along with a property to reduce platelet aggregation (Hannan *et al.*, 2003). In southern parts of India, bay or curry leaves (*Murrakoeingii*) are traditionally included in the diet because of their anti diabetic properties. Apart from possessing anti diabetic properties, bay leaves also contain hypoglycemic principles (Yadav *et al.*, 2002). Furthermore, alkaloids from bay leaves have anti oxidative properties (Tachibana *et al.*, 2003). Kirubakaran (2003) and Narahari *et al.* (2004) reported that the use of designer feeds led to increased RBC, WBC and Hb levels in the hens, compared to the control group. The Eosinophil levels were decreased; with a proportionate increase in the Heterophil levels in hens maintained on the designer feed. Collectively, results from previous studies indicated that garlic, fenugreek and bay leaves possess health-enhancing properties. Thus, this research work was undertaken to test if the use of such herbal supplements can further enhance the immunity in laying hens, in particular toward

Newcastle disease virus. Indeed, our results demonstrate that the use of herbal supplements in the feed potentiates the immunity of layer hens against Newcastle disease virus.

MATERIALS AND METHODS

One hundred and forty four Single Comb 'Forsgate' strain White Leghorn layers aged 70 weeks, belonging to the same hatch and of uniform body size were randomly grouped into six treatments with four replicates of 6 hens each. The treatments consist of control-regular layer mash (T₁), functional feed (FF) - layer mash having oil rich sardine fish, linseed oil, mustard oil and antioxidants (T₂), FF + garlic at 6g/kg (T₃), FF+ fenugreek seeds at 6g/kg (T₄), FF+ bay leaves (Curry leaves) at 6g/kg (T₅) and FF+ 2g each of garlic, fenugreek seeds and bay leaves/kg (T₆). In order to induce active immunity, the birds were vaccinated with Newcastle Disease Vaccine, a week prior to start of the experiment. Standard managerial practices were followed throughout the experiment. The hens were fed with this experimental feed for 8 weeks of time.

Biochemical assay

In order to study the effect of herbal supplemented feeds on the immune status of the hens, the serum haemagglutination inhibition and enzyme-linked immunosorbent assay (ELISA) titres for Newcastle Diseases and the Immunoglobulin-Y (IgY) levels in the yolk were measured. At the end of the third week, blood samples were collected from one layer per replicate. The serum samples were separated from the blood, and were used to find out the HI titre level for Newcastle Disease. The serum samples were also utilized for estimation of ELISA titre for Newcastle Disease, as per the procedure of Office International des Epizooties (2000). At the end of the fourth week, one egg was collected from each replicate. Thus, a total of 24 eggs were utilized for the estimation of Immunoglobulin-Y (IgY) level in the yolk, according to

the procedure described by Polson (1980). During the fifth week of this study, blood samples taken from one bird per replicate with an anticoagulant were used for estimation of Haemoglobin, total RBC and WBC counts as well as differential count of WBC.

Data analysis

All the data collected were subjected to analysis of variance for significance according to the methods of Snedecor and Cochran (1989) for a Completely Randomized Design. The percentage values were converted to root Arcsine values and the numerical values were converted into their log values.

RESULTS AND DISCUSSION

The influence of the six dietary treatments on the parameters tested is shown in Table 1 and 2. Effect of functional feeds on the immune status of hens, measured as HI titre, ELISA and immunoglobulin-IgY, were significantly in favour of functional feeds (Table 1). Presence of immune modulators in the feed like vitamin-E, selenium and other herbal active principles might be responsible for these superior immune statuses. Significant levels of IgY production in egg yolk by birds with these herbal supplements are also indicators of immune modulating activity. Similar conclusions were drawn earlier by studies of Kirubakaran (2003) and Narahari *et al.* (2004) which showed that basil leaf meal supplementation to the designer feed produced eggs with increased IgY levels.

The results showed that the RBC and WBC counts, as well as the Hb levels were significantly ($P < 0.05$) increased in the functional feed fed hens, compared to control hens (Table 2). The lymphocyte counts were also increased significantly ($P < 0.01$), with a proportionate reduction in heterophils and monocytes in the functional feed fed hens when compared to the control group. The eosinophil and basophil counts did not show any significant variations between dietary treatments. Among

Table 1: Effect of dietary treatments on HI titre, ELISA and IgY values (n=24)

Trait	Control	FF	FF+garlic	FF+fenugreek	FF+bay leaves	FF+garlic+ fenugreek+bay	F 'value
HI titre(log ₂ values)	4.33±0.22	5.83±0.31	6.17±0.14	5.96±0.28	5.67±0.21	6.51±0.34	4.72**
ELISA(log ₂ values)	2.77±0.05	2.98±0.16	3.10±0.15	2.98±0.18	2.91±0.12	3.24±.15	2.95*
IgY (mg/g yolk)	15.33±0.09	18.47±0.08	19.38±0.07	19.67±0.05	18.42±0.09	19.51±0.07	6.23**

F-value; one way ANOVA, F-distribution test statistics values; **highly significant ($P < 0.01$); *Significant ($P < 0.05$)

Table 2: Effect of dietary treatments on RBC, WBC, Hb and differential counts of WBC (n=24)

Trait	Control	FF	FF+garlic	FF+fenugreek	FF+bay leaves	FF+garlic+ fenugreek+bay	F 'value
RBC ($\times 10^6/\text{mm}^3$)	2.45±0.11	2.57±0.01	2.95±0.04	2.82±0.12	2.64±0.01	2.81±0.04	3.11*
WBC($\times 10^3/\text{mm}^3$)	25.5±0.56	31.3±0.26	35.9±1.45	29.7±2.43	32.6±0.67	33.7±1.00	7.78**
Haemoglobin (g %)	9.13±0.21	9.41±0.23	9.66±0.33	9.53±0.31	9.43±0.31	9.45±0.22	4.19*
% Lymphocytes	66.6±1.48	73.5±1.43	73.3±1.42	72.7±1.48	72.8±1.60	73.5±1.43	15.10**
% Heterophils	19.8±0.40	16.5±0.43	16.8±0.48	17.5±0.43	17.6±0.42	17.0±0.26	3.45*
% Eosinophils	2.0±0.37	1.8±0.31	1.5±0.22	1.5±0.26	1.8±0.31	1.6±0.37	1.52NS
% Monocytes	9.9±0.21	6.3±0.33	6.6±0.42	6.8±0.31	6.2±0.31	6.2±0.31	4.69**
% Basophils	1.7±0.02	1.9±0.03	1.8±0.02	1.5±0.02	1.6±0.02	1.7±0.03	1.08NS

F-value; one way ANOVA, F-distribution test statistics values; ** highly significant ($P < 0.01$); *Significant ($P < 0.05$); NS: Not significant ($P > 0.05$)

the functional feeds, supplementation of garlic and a combination of garlic + fenugreek + bay leaves conferred the maximum immune status in hens and eggs. This result concurs with the studies of Abdullah *et al.* (1989), Kyo *et al.* (2001) and Gulati *et al.* (2002) which demonstrated that garlic stimulates humoral immune response, enhances the levels of macrophages and T-lymphocytes.

Conclusions

It could be concluded from our study that herbal-enriched functional feeds, not only improved the general health and immune status of the hens, but also led to incorporation of these health-promoting principles into the egg. It was proven from our study that garlic was the better immunomodulator than fenugreek and bay leaves. However the combination of these three herbs conferred a better immunity development. We recommend the use of herbal supplements in the feeds as a strategy to improve the overall health and immunity of laying hens.

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