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Down regulation of humoral immunity in chickens due to carbendazim

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Abstract

This study was planned to investigate the effect of very low dose of carbendazim on the humoral immune response in the chicken. Sixteen adult chickens, earlier vaccinated against New Castle Disease were divided in two experimental groups. Chickens of group I served as control, while group II birds were given a feed containing 200 ppm of carbendazim, which is considered no observable effect level (NOEL) dose, for a period of 6 months. The Humoral immune response was measured by the B-lymphocyte blastogenesis assay using lipopolysaccharide as mitogen and the quantitation of IgG, IgA, IgM levels by using respective antichicken conjugates, through an ELISA method. Total serum proteins, serum gamma-globulins and globulins were measured using commercially available kits. Carbendazim significantly ($P \leqslant 0.05$) reduced both the B-lymphocyte proliferation and serum IgG, IgM and IgA levels, leading to decreased immunocompetence. At the end of experiment percent decrease in B-lymphocyte proliferation was 20.5% and that in serum IgG, IgM and IgA were 11.2, 22.9 and 28.8%, respectively. The percent decrease in total serum protein, serum gamma-globulins and serum globulins were 14.6, 18.5 and 9.7%, respectively. Results clearly indicated down regulation of humoral immunity by carbendazim at NOEL dose.

Keywords: Humoral immunity; Carbendazim; Chicken; B-Cell blastogenesis; Immunoglobulins

1. Introduction

Carbendazim, a white crystalline powder, is a systemic broad spectrum fungicide of the benzimidazol family, used to control fungi causing diseases in cereals, fruit crops, ornamental plants, chillies, cotton, cluster beans, cowpeas, sugarcane, groundnut, tobacco, vegetables and many field crops. It is absorbed through roots and leaves and acts by inhibiting both the germination and the growth of fungal mycelia. The chemical name and the molecular formula of carbendazim are methyl benzimidazol-2-yl-carbamate and $C_9H_9N_3O_2$, respectively. In 1988, the estimated global sale of carbendazim was approximately 3600 tonnes (WHO, 1993). Acute toxic effects of carbendazim are well documented. The primary source of carbendazim exposure in humans is the dietary intake (FAO/WHO, 1988), although, dermal contact and inhalation may adversely affect the human's health too. Symptoms of poisoning are vomition and eye, nose and throat irritation. Other symptoms such as hypotension, rapid pulse rate, headache and blurred vision may be seen when the subject victim has been exposed to a large amount of the fungicide. Experimentally, carbendazim is known to cause carcinogenic and teratogenic effects (Gahukar, 1999).

Actually the possible immunomodulation given by agrochemicals is gaining significance in their toxicity evaluation. The consumption of small amounts of pesticide residues through the diet might result in a decreased resistance to infectious agents, recurrence of diseases and break down of immunity developed through vaccination (Rodgers, 1996). Although the impact of carbendazim on the immune function wasn't yet a matter of study, the immunosuppressive effect of some carbamate pesticides upon the humoral and cell mediated immunity (CMI), was demonstrated even at low doses (Chauhan, 1998a; Fournier et al., 1988; Khurana et al., 1998; Luster et al., 1982). No observable

Abbreviations: ELISA, Enzyme linked immunosorbent assay; LPS, Lipopolysaccharide; MTT, 3-[4,5-Dimethylthiazol-z-yl]-2,5-diphenyl tetrazolium bromide; NCD, New castle disease; NOEL, No observable effect level; OD, Optical density; SE, Standard error; TSP, Total serum protein.

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effect level (NOEL) of carbendazim was reported to be 200 ppm (Mantovani et al., 1998). NOEL dose means a dose which if given to birds, does not show any observable adverse effects on the health of birds. The present investigation was conducted to explore the impact of carbendazim NOEL dose on the humoral immunity in chickens.

2. Materials and methods

2.1. Animals

Sixteen adult White Leghorn broiler chickens, procured from the Poultry Research Centre at the G.B. Pant University of Agriculture and Technology in Pantnagar (India), were randomly divided in two equal groups. The first one received no chemical treatment and was used as control, while the second one was offered for 6 months a diet containing 200 ppm of carbendazim (Mantovani et al., 1998). They were kept under good ventilation and hygienic conditions and allowed to free access to feed and water during the study. Birds were daily observed for any sign of toxicity. Their blood samples were taken at 10 day intervals, for a period of 180 days, for following observations.

2.2. Lymphocyte blastogenesis assay

B-Lymphocyte blastogenesis assay was carried out as described by Rai-el-Balhaa et al. (1987) with some minor modifications according to Chauhan (1998b) using RPMI-1640 as test media and lipopolysaccharide (LPS) as a mitogen. The reduction of the MTT dye (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) to formazon was used as an indicator of cell proliferation (Mosmann, 1983) and results were reported as optical density (OD) measured at wavelength of 570 nm.

2.3. Immunoglobulin assays

The serum levels of immunoglobulins (IgG, IgM and IgA) against New Castle Disease (NCD) vaccine in the serum were measured, using their respective antichicken conjugates by ELISA method (Kurstak, 1985). The absorbance was measured at a wavelength of 492 nm. Mean ELISA value was calculated by dividing the absorbance of test samples by the absorbance of negative control wells.

2.4. Total serum proteins (TSP) serum gamma-globulins and serum globulins

TSP, serum gamma-globulins and serum globulin were measured using the ready to use diagnostic test kits of Span Diagnostics Ltd., Surat, India. Results were expressed as gram of proteins per 100 ml of blood (g/dl).

2.5. Statistical analysis

Student's *t*-test was used to estimate statistically significant differences between the mean values of treated and control birds. The values were expressed as mean ± standard error (mean ± S.E.).

3. Results

The daily observations made during the entire experiment did not reveal appreciable clinical signs of toxicity in birds of each experimental group.

Mean delta OD of LPS stimulated lymphocyte cultures in control and carbendazim fed group has been shown in Table 1. A significant ($P \leqslant 0.05$) reduction in delta OD of treated birds vs control was observed after 70 days of exposure. This significant reduction was present until the ending of the experiment, where the mean delta OD in control birds was 0.442 ± 0.035, while it was 0.350 ± 0.042 in carbendazim fed birds. This shows a 21% reduction in B-lymphocyte proliferation. The mean ELISA values reflecting the effect of carbendazim on serum IgG are reported in Fig. 1. The NCD vaccine induced antibody titre decreased significantly from day 90 in pesticide treated birds and remained decreased upto the end of experiment. After 180 days, mean ELISA value was 2.14 ± 0.039 in control birds and 1.90 ± 0.031 in treated ones, a value, which is about 11.0% lower than that of control.

The mean ELISA value for IgM is presented in Fig. 2. The values showed significant reduction in carbendazim treated groups in comparison to control, starting from

Table 1
Effect of carbendazim on B-lymphocyte blastogenesis using LPS (Mean delta OD ± S.E.)

Days	Control	Carbendazim
0	0.162 ± 0.009	0.162 ± 0.009
10	0.172 ± 0.017	0.168 ± 0.016
20	0.197 ± 0.076	0.181 ± 0.046
30	0.209 ± 0.022	0.194 ± 0.023
40	0.252 ± 0.016	0.199 ± 0.035
50	0.277 ± 0.014	0.212 ± 0.013
60	0.287 ± 0.008	0.223 ± 0.006
70	0.308 ± 0.019	0.225 ± 0.028*
80	0.301 ± 0.031	0.237 ± 0.036*
90	0.317 ± 0.057	0.246 ± 0.075*
100	0.330 ± 0.046	0.257 ± 0.052*
110	0.337 ± 0.089	0.252 ± 0.044*
120	0.345 ± 0.023	0.262 ± 0.024*
130	0.339 ± 0.062	0.271 ± 0.048*
140	0.346 ± 0.012	0.292 ± 0.052*
150	0.379 ± 0.039	0.305 ± 0.043*
160	0.409 ± 0.031	0.333 ± 0.035*
170	0.425 ± 0.031	0.339 ± 0.034*
180	0.442 ± 0.035	0.351 ± 0.042*

* Significant at $P \leqslant 0.05$.

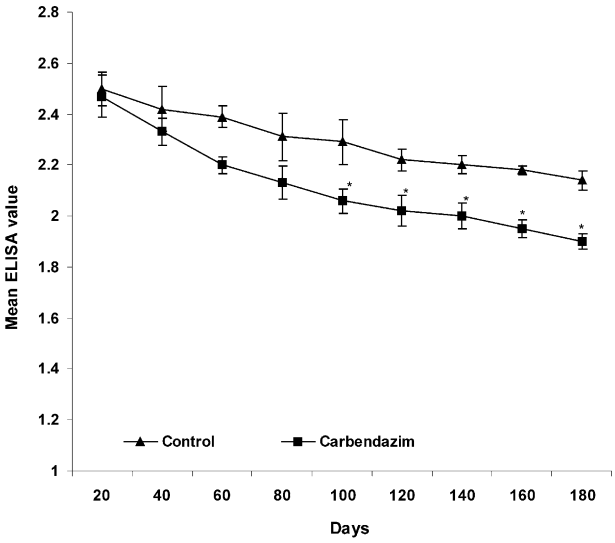

Fig. 1. Effect of carbendazim on serum IgG.

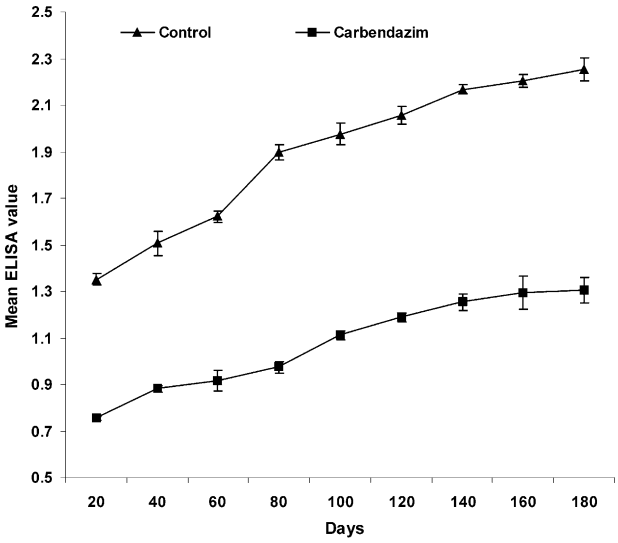

Fig. 3. Effect of carbendazim on serum IgA.

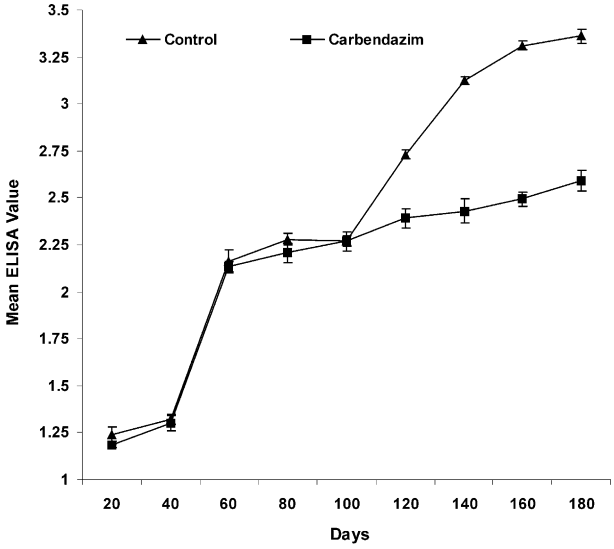

Fig. 2. Effect of carbendazim on serum IgM.

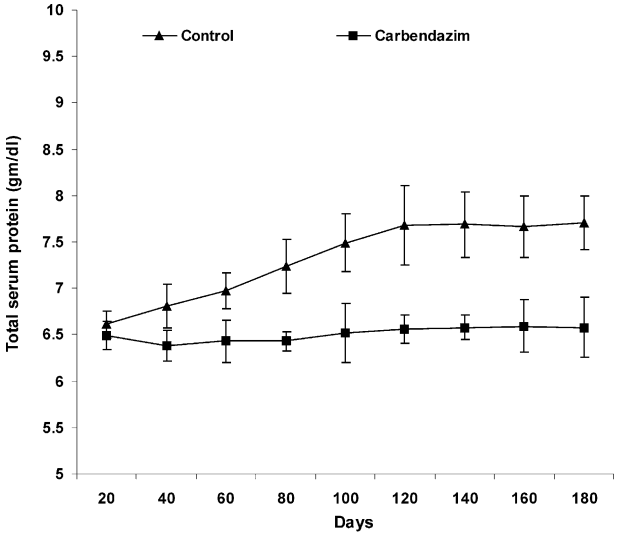

Fig. 4. Effect of carbendazim on total serum protein.

day 100 of the experiment. In chicks fed with carbendazim, the mean ELISA value at 180 day post-treatment was 2.59 ± 0.02, and significantly decreased compared to controls (3.36 ± 0.07). There was a reduction of 22.9% in mean ELISA values for IgM in carbendazim fed group in comparison to control group at the end of experiment.

The serum IgA values were decreased marginally in carbendazim treated chicks in comparison to controls since day 100 post-treatment (Fig. 3). At the end of experiment, the mean ELISA values of IgA were 2.91 ± 0.021 and 2.07 ± 0.03 for the control and the carbendazim fed group, respectively. At the end of experiment a reduction of 28.9% in serum IgA level was observed in carbendazim fed group in comparison to control group.

The values of total serum protein (TSP) and serum globulins (g/100 ml) serum are given in Figs. 4 and 5, respectively. TSP values in carbendazim fed chicks were significantly lowered ($P \leqslant 0.05$) since the day 90 post-treatment. After 6 months, TSP values were 7.71 ± 0.15 in control birds whereas in treated ones these values were 6.58 ± 0.02. The effect of carbendazim on the mean serum gamma-globulins (g/100 ml serum) is presented in Table 2. In carbendazim treated birds a significant decrease of serum γ-globulin levels vs the control value was recorded since the 80th day post-treatment and lasting for all the experiment. After 180 days serum gamma-globulins levels were 2.05 ± 0.018 and 1.67 ± 0.045 in control and carbendazim treated birds, which accounted for 18% decrease. On the other hand, the carbendazim fed birds showed serum globulin amounts of 2.22 ± 0.08 gm/100 ml serum, while in

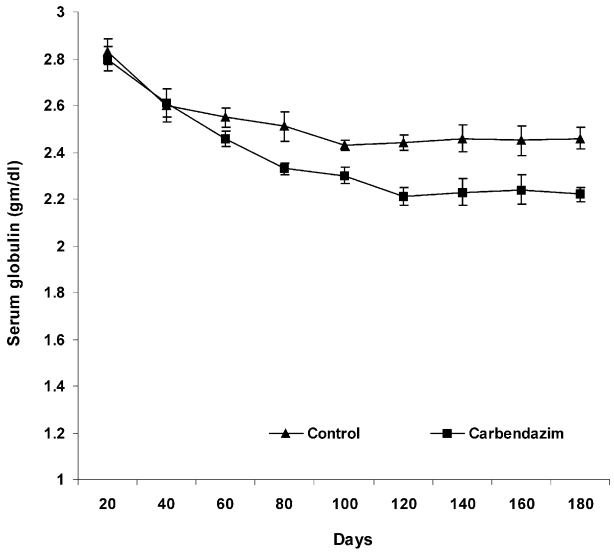

Fig. 5. Effect of carbendazim on serum globulin.

Table 2
Effect of carbendazim on serum γ-globulin (g/100 ml) in chickens (mean±S.E.)

Days	Control	Carbendazim
0	1.16±0.009	1.16±0.009
10	1.21±0.022	1.18±0.021
20	1.29±0.043	1.20±0.042
30	1.33±0.062	1.21±0.034
40	1.47±0.035	1.24±0.052
50	1.51±0.024	1.29±0.048
60	1.54±0.042	1.30±0.044
70	1.51±0.036	1.33±0.039*
80	1.58±0.029	1.37±0.025*
90	1.63±0.036	1.41±0.045*
100	1.70±0.052	1.43±0.024*
110	1.77±0.038	1.47±0.041*
120	1.82±0.022	1.51±0.024*
130	1.83±0.048	1.54±0.051*
140	1.87±0.054	1.55±0.043*
150	1.90±0.035	1.57±0.019*
160	1.94±0.052	1.61±0.032*
170	1.98±0.023	1.60±0.022*
180	2.05±0.018	1.67±0.045*

* Significant at $P \leqslant 0.05$.

controls these ones were 2.46±0.02. As shown in Fig. 5, the serum globulin contents were significantly decreased in treated birds vs. the control since day 110 post-treatment.

4. Discussion

In this study we investigated the immune competence of chickens over 6 months' dietary exposure to 200 ppm of carbendazim, which is considered NOEL dose of carbendazim (Mantovani et al., 1998). Results demonstrated that carbendazim in diet leads to suppression of the humoral immune response. Birds previously immunized with NCD vaccine and then exposed to the fungicide showed a significant decrease in mean delta OD after the stimulation with the mitogen LPS. There was a 21% reduction in B-lymphocyte proliferation which is an indicative of decreased humoral immunity. The MTT-dye method has been widely used as a marker for proliferation studies (Rai-el-Bahlaa, 1985; Denizot and Lang, 1986; Charmichael et al., 1987; Vohr, 1995). Earlier, reduced lymphocytes blastogenic response has been reported in minks and ferrets exposed to another fungicide, hexachlorobenzene (Bleavins et al., 1983). Similar effects on the lymphocyte blastogenesis were observed by Thomas and Ratajczak (1988) after the exposure to aldicarb, a pesticide which is classified as a carbamate group. Another pesticide of the carbamate group, carbaryl, has been shown to inhibit humoral immunity in mice (Wiltrout et al., 1978; Ladies et al., 1994) and lower the resistance of quails (Fournier et al., 1988). In vitro, carbaryl impaired the immune function of splenocytes (Rodgers, 1996) and inhibited proliferation of interleukin 2-dependent T-cells (Bavari et al., 1989). Carbofuran suppressed both the humoral (Fournier et al., 1988; Khurana et al., 1997) and the cellular immunity (Street and Sharma, 1975) and inhibited the expression of complement activity when added to the human serum (Casale et al., 1989). Aminocarb reduced humoral immunity and increased the virus-dependant cytolysis of macrophages (Fournier et al., 1988; Bernier et al., 1995). Previcur, another carbamate pesticide, was studied for its immunotoxicological properties and found to suppress the humoral and cell mediated immunity in mice (Elsabbagh and El-Tawil, 2001).

In the present investigation a significant reduction in NCD vaccine induced humoral immune response measured by ELISA has been observed in birds exposed to the fungicide compared to controls. A decrease in serum immunoglobulin level has been reported in animals exposed to pesticides (Street and Sharma, 1975), lindane (Saha and Banerjee, 1994), carbaryl and malathion (Bhushan, 1993). Barnett et al. (1980) recorded alterations in immunoglobulin levels in off-springs of mice as a result of prenatal exposure of carbofuran or diazinon. Since the immunoglobulins are synthesized by lymphocytes and pesticides being lymphocytotoxic, the quantitative reduction was evident because of the detrimental effect of pesticides on peripheral blood lymphocytes (Goyal et al., 1986). Reduction in serum immunoglobulins level might also be due to functional impairment of B-lymphocytes by the fungicide as evident in B-lymphocyte blastogenesis assay.

The TSP values were significantly reduced in fungicide fed birds when compared to control ones. Similarly,

decreased serum proteins were also observed in birds fed malathion (Goyal et al., 1986; Bhushan et al., 1993) and goats fed carbaryl (Wahbi et al., 1987). The γ-globulins are directly related to the antibody titre measured by ELISA, which was found to be significantly decreased in fungicide treated group. The 18.0% decrease in serum γ-globulins is an indication of lowered immunity. A decrease in serum globulin level has been reported in animals exposed to pesticides (Street and Sharma, 1975), lindane (Saha and Banerjee, 1994), to carbaryl, and malathion (Bhushan, 1993). A decrease in serum proteins due to decreases in albumin and γ- and β-globulin values was noticed in rabbits administrated DDT, too (Chung et al., 1989).

The immunosuppressive action of pesticides is due to their detrimental action on lymphoid organs. The decreased number of lymphocytes along with their decreased functional capacity has been reported in patients following pesticide intoxication (Katsenovich et al., 1981). Vigreux et al. (1998) demonstrated the DNA damaging effects of two fungicides, carbendazim and chlorothalonil by the single cell gel electrophoresis assay and the chromosomal aberration in the absence of noticeable cytotoxicity. Rodgers (1996) reported that carbamates act through an inhibition of serine esterases which modulate the generation and expression of immune response. The immunosuppression may result from direct action of acetylcholine upon the immune system or it may be secondary to the toxic chemical stress associated with cholinergic poisoning (Van Damme et al., 1987). Aldicarb has no direct effect on T cells and the mechanism of modulation is indirectly mediated through an impairment of macrophage ability to secrete interleukin-1 (IL-1), which is critical for T-cell activation. The decreased activity of T-lymphocytes might affect the T-cell dependent humoral immune response, too. In addition, the decrease in IL-1 production may also affect the production of other cytokines, including IL-2, IL-4, IL-6 and IL-8 (Luster et al., 1996; Van Damme et al., 1987; Dean et al., 1990). This will in turn affect the regulation of the interaction and function of antigen-presenting cells, T helper cells and B cells (Xie et al., 1997).

It is clearly evident from the present study that the immune system of birds is quite susceptible to the action of NOEL dose of carbendazim. Such pesticides may act directly or indirectly on lymphoid cells (Street and Sharma, 1975), altering their normal function, distribution, immunoglobulin metabolism, T-cell/B-cell/macrophage cooperation and macromolecular biosynthesis (Vos, 1977), thereby leading to immunosuppression. Thus, low doses (NOEL) of carbendazim apparently have no adverse effect, but causes severe immunosuppression, which in turn may be responsible for vaccination failures and disease outbreaks in animals (Singhal et al., 2001).

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