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# Effect of Fipronil toxicity in haematological parameters in white leghorn cockerels

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**Pesticide use in the modern agriculture, animal husbandry and public health practices has increased enormously. Pesticides affect man, environment and wild life including birds. Pesticides including fungicides, herbicides and insecticides are the major class of chemicals deliberately released into the environment because of their indiscriminate use in various agricultural practices. Pesticides are present in air, soil and water which leave their residue in food chain causing deleterious effect on the health status of man and animals including poultry. After ingestion, they get into blood stream lodging themselves in various tissues causing adverse effects on health and production of livestock and poultry resulting in substantial economic losses. Fipronil was discovered and developed by Rhone-Poulenc between 1985- 1987 and placed in the market in 1993. It is the member of new and relatively small class of pesticides, the phenyl pyrazoles, which are principally chemicals with an herbicidal effect. According to data obtained from metabolic studies showed that there was potential for bioaccumulation of the Fipronil in fatty tissues, fish, fibre crops and food crops. Limited investigations have been carried on effect of fipronil in health of animals and man. Therefore present study was designed to study the effect of fipronil toxicity in white leghorn cockerels. Twenty white leg horn male chickens of one month old weighing about 150 to 200 g were procured and kept at Instructional Poultry Farm Nagla, Pantnagar. The birds in poultry shed in deep litter system under standard managemental conditions. They were randomly divided into four groups of 5 birds each. After one week of adaptation period, different doses of Fipronil were administered orally through feed at 1, 5 and 10 ppm levels to groups G1, G2 and G3 respectively for 100 days daily. The birds of group "C" served as control. After every 20 days blood was taken from each bird for study of different parameters. The experiment was conducted for a period of 100 days. All the hematological parameters viz. hemoglobin, packed cell volume, total erythrocyte count and differential leucocyte count were performed on the day of blood collection as per the standard method. Hematological parameters showed significant decrease leading to anemia and significant decrease in total leucocyte count leading to suppression in cell mediated immune response in different doses of fipronil treated birds in comparison to birds of control group.**

**Key words:** Fipronil toxicity, white leghorn cockerels, haematological parameters.

## INTRODUCTION

Pesticide use in the modern agriculture, animal husbandry and public health practices has increased enormously. Pesticides affect man, environment and wild life including birds. According to Chauhan and Singhal (2006) 90,000 MT of technical grade pesticides are used annually to control pests and plant diseases in India. The pesticides are classified as insecticides, fungicides, weedicides, herbicides, nematodicides and rodenticides; of

which insecticides constitutes 77% of the total pesticides used in different agricultural and animal husbandry practices and in public health operations. There is a gradual increase in production and consumption of pesticides during last few decades. The pesticides consumption increased from 2353 MT during 1955 to 75033 MT (technical grade) in the year 1991-1992 and which is again in the decline phase with the adoption of integrated pest management practices and the pesticides consumption level declined to the level of 43020 MT (technical grade) in the year 2003-2004. Pesticides are present in air, soil and water everywhere due to which they leave behind their residue in food chain (Ram et al., 1987; Kaushik et al., 1991) causing deleterious effect on the health status of man and animals including poultry. After ingestion, they get into blood stream lodging themselves in various tissues causing adverse effects on health and production of livestock and poultry resulting in substantial economic losses (Kaphalia and Seth, 1984). Chicken serve as good animal model for immunotoxicological risk assessment, that may be used for the determination of immune responses affected by environmental contaminants including pesticides (Linda et al., 1989). Moreover, poultry industry has made tremendous growth all over the world during the last decades. The poultry industry which was earlier classified as an unorganized sector has now turned itself as an organized sector through people, process and technology. According to Watt Executive Guide (2010) poultry industry has emerged as the most dynamic and fastest expanding segment in animal husbandry sector with 47.4 billion eggs produced by 2.4 billion layers and 3 billion broilers giving 2.25 million metric tons of poultry meat and likely to grow up to 75.6 billion eggs and 5.21 million metric tons of poultry meat by year 2012. The population of broiler and layer are 3 and 2.4 billion, respectively while per capita consumption of meat and eggs are 2.1 kg/annum and 42 eggs/annum respectively in India (Ravi, 2010) which is much less than ICMR recommendation against the requirement of 180 eggs and 10.8 kg of poultry meat (FAO, 2004). Reasons for more pesticide residue in India are indiscriminate and improportionate use of insecticide, lack of education and extension activity, inadequate literature supplied by manufacturers, want of more production and profit, lack of safer pesticides and use of banned pesticides. Keeping the above mentioned facts in mind studies on the toxicological impact of Fipronil on White Leghorn Cockerels (WLH) was planned.

## MATERIALS AND METHODS

The present study was conducted to investigate haematological effect of induced Fipronil toxicity in white leghorn cockerels. Birds were continuously observed for different clinical manifestations throughout the trial from the beginning. Measurement of body weight of birds was taken after every 20 days interval in all groups till the end of trial.

### Collection of blood samples

Blood samples for haematological examinations were collected from wing vein in sterilized disposable syringes (22 gauge needles) after proper restraining of birds. Blood samples were transferred to heparin coated tubes for haematological examinations. Collection of blood samples was done at the 20 days interval starting from day 0 during 100 days study. Approximately, 5 ml of blood per bird was collected. 125 IU/ml of blood heparin was used as anticoagulant for haematology.

One milliliter of blood was collected from each bird in clean heparin coated tubes and haematological parameters such as Total Erythrocyte Count (x $10^6$/µl blood) and Total Leucocyte Count (x $10^3$/µl blood) were determined according to the method of Natt and Herrick (1952) using poultry blood diluting fluid. Packed Cell Volume (PCV %) and haemoglobin (gm/dl) were estimated using the method of Jain (1986). Differential Leucocyte Count (DLC %) was done by preparing thin blood smear from a drop of blood without anticoagulant. The smear was air dried and stained with Leishman stained for 20 min. The leucocytes were counted by zig-zag method as described by Lucas and Jamroz (1961) and recorded on percent basis.

## RESULTS

The results of induced toxicity of Fipronil in cockerels in the following blood parameters were as follows:

### Packed cell volume (PCV)

The results of mean PCV values from different groups are presented in Table 1. There was significant ($P<0.05$) reduction in the PCV value in group G2 and G3 on as compared to the control on day 40,60, 80 and 100, whereas group G1 showed significant decrease as compared to the control after 60 days of feeding of the Fipronil.

### Hemoglobin (Hb)

The results of mean Hb values from different groups are presented in Table 2. A significant ($P<0.05$) decrease was observed in group G1, G2 and G3 as compared to the control after 40,60 and 80 days of feeding of the Fipronil.

### Total erythrocyte count (TEC)

The results of mean TEC values from different groups are

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**Table 1.** Mean packed cell volume (PCV %) in different groups of experimental birds (mean ± S.E).

| Group / Day | C (0 ppm) | G1 (1ppm) | G2 (5 ppm) | G3 (10 ppm) |
|---|---|---|---|---|
| 0 Day | 32.20±.382 | 32.21±.38 | 32.18±.383 | 32.22±.382 |
| 20 Day | 32.21±.382 | 31.8±.382 | 31.68±.4 | 31.2±.38 |
| 40 Day | 32.21±.382 | 31.5±.382 | $30.2\pm.382^{wx,ab}$ | $29.1\pm.382^{wx,ab}$ |
| 60 Day | 32.20±.382 | $29.76\pm.384^{w,abc}$ | $29.4\pm.382^{w,ab}$ | $28.1\pm.382^{wxy,ab}$ |
| 80 Day | 32.15±.385 | $28.4\pm.39^{w,abcd}$ | $28.1\pm.382^{w,abcd}$ | $27.33\pm.389^{w,abc}$ |
| 100 Day | 32.21±.382 | $27.96\pm.533^{w,abc}$ | $27.5\pm.533^{w,abcd}$ | $26.3\pm.382^{wx,abcd}$ |
| Mean | 32.20 | 30.27 | 29.84 | 29.04 |

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**Table 2.** Mean hemoglobin concentration (gm/dl) in different groups of experimental birds (mean ± S.E).

| Group / Day | C (0 ppm) | G1 (1ppm) | G2 (5 ppm) | G3 (10 ppm) |
|---|---|---|---|---|
| 0 Day | 9.16±.212 | 9.16±.212 | 9.16±.212 | 9.16±.212 |
| 20 Day | 9.17±.213 | 9.12±.212 | 8.98±.212 | 8.61±.212 |
| 40 Day | 9.15±.212 | 8.96±.212 | 8.61±.212 | $8.3\pm.212^{wx,a}$ |
| 60 Day | 9.17±.213 | 8.6±.212 | $8.24\pm.212^{w,ab}$ | $8.03\pm.212^{w,a}$ |
| 80 Day | 9.15±.212 | $8.3\pm.21^{w,abc}$ | $8\pm.212^{w,ab}$ | $7.81\pm.212^{w,ab}$ |
| 100 Day | 9.15±.212 | $7.78\pm.123^{w,abcd}$ | $7.9\pm.212^{w,abc}$ | $7.6\pm.212^{w,abc}$ |
| Mean | 9.16 | 8.65 | 8.48 | 8.25 |

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**Table 3.** Mean Total Erythrocyte Counts ($x10^6$ cells/ µl) in different groups of experimental birds (mean ± S.E).

| Group / Day | C (0 ppm) | G1 (1ppm) | G2 (5 ppm) | G3 (10 ppm) |
|---|---|---|---|---|
| 0 Day | 2.59±.035 | 2.59±.035 | 2.61±.035 | 2.58±.035 |
| 20 Day | 2.57±.035 | 2.56±.035 | 2.56±.035 | 2.48±.035 |
| 40 Day | 2.58±.035 | 2.54±.035 | $2.43\pm.035^{wx,ab}$ | $2.39\pm.035^{wx,a}$ |
| 60 Day | 2.56±.035 | $2.44\pm.035^{w,ab}$ | $2.32\pm.035^{wx,abc}$ | $2.31\pm.035^{wx,ab}$ |
| 80 Day | 2.57±.035 | $2.37\pm.035^{w,abc}$ | $2.23\pm.035^{wx,abc}$ | $2.16\pm.035^{wx,abcd}$ |
| 100 Day | 2.58±.035 | $2.25\pm.035^{w,abcd}$ | $2.14\pm.035^{wx,abcd}$ | $2\pm.035^{wxy,abcde}$ |
| Mean | 2.58 | 2.46 | 2.38 | 2.32 |

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presented in Table 3. There was no significant (P<0.05) change in among different treated groups on day 20 of the feeding trial, whereas after 40 days of feeding of Fipronil there was significant decrease in TEC values in group G2 and G3 as compared to the control. Significant decrease in G1 was observed after 60 days of feeding trial.

**Total leucocyte count (TLC)**

The results of mean TLC values from different groups are presented in Table 4. There was significant (P<0.05) decrease in the TLC in group G1, G2 and G3 as compared to control after 20 day of trial till the end of study.

**Table 4.** Mean Total Leucocyte Counts (x$10^3$ cells/ µl) in different groups of experimental birds (mean ± S.E).

| Group / Day | C (0 ppm) | G1 (1ppm) | G2 (5 ppm) | G3 (10 ppm) |
|---|---|---|---|---|
| 0 Day | 24.56±.065 | 24.56±.087 | 24.56±.060$^{wx}$ | 24.55±.112$^{wxy}$ |
| 20 Day | 24.56±.058 | 24.53±.027$^{w,b}$ | 24.3±.035$^{wx,ab}$ | 23.83±.035$^{wxy,ab}$ |
| 40 Day | 24.55±.067 | 24.46±.035$^{w,b}$ | 24±.242$^{wx,ab}$ | 23.43±.035$^{wxy,ab}$ |
| 60 Day | 24.57±.098 | 24.11±.035$^{w,abc}$ | 23.57±.098$^{wx,abc}$ | 22.77±.035$^{wxy,abc}$ |
| 80 Day | 24.57±.070 | 23.61±.104$^{w,abcd}$ | 23.17±.053$^{wx,abcd}$ | 21.91±.035$^{wxy,abcd}$ |
| 100 Day | 24.58±.137 | 23.13±.035$^{w,abcde}$ | 22.49±.035$^{wx,abcde}$ | 21.13±.035$^{wxy,abcde}$ |
| Mean | 24.56 | 24.07 | 23.68 | 22.94 |

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**Table 5.** Average values of Monocytes (%) different groups of experimental birds (mean ± S.E).

| Group / Day | C (0 ppm) | G1 (1ppm) | G2 (5 ppm) | G3 (10 ppm) |
|---|---|---|---|---|
| 0 Day | 3.2±.2 | 3.2±.2 | 3.2±.2 | 3.20±.2$^{a}$ |
| 20 Day | 3±.316 | 3.2±.2 | 3.4±.245 | 3.4±.245$^{b}$ |
| 40 Day | 3.4±.245 | 3.2±.2 | 3.2±.374 | 3.2±.2 |
| 60 Day | 3.2±.2 | 3.2±.316 | 3.2±.2 | 3.2±.2 |
| 80 Day | 3±.316 | 3±.2 | 3±.316 | 3.4±.245$^{e}$ |
| 100 Day | 3±.316 | 3.2±.2 | 3±.316 | 2.6±.245$^{abe}$ |
| Mean | 3.13 | 3.17 | 3.17 | 3.17 |

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**Table 6.** Average values of Lymphocytes (%) different groups of experimental birds (mean ± S.E).

| Group / Day | C (0 ppm) | G1 (1ppm) | G2 (5 ppm) | G3 (10 ppm) |
|---|---|---|---|---|
| 0 Day | 65.6±.245 | 65.6±.245 | 64.8±.2$^{wx}$ | 65.40±.245 |
| 20 Day | 65.6±.245 | 64.8±.372 | 63.8±.374$^{w}$ | 64±.316$^{w}$ |
| 40 Day | 65.2±.2 | 64.6±.4 | 63±.316$^{wx,a}$ | 63.2±.8$^{wx,a}$ |
| 60 Day | 65.2±.374 | 63.6±.51$^{w,a}$ | 63±.548$^{w,a}$ | 62.2±.49$^{w,ab}$ |
| 80 Day | 65.8±.374 | 63±.55$^{w,abc}$ | 62±.447$^{w,ab}$ | 61.2±.583$^{wx,abc}$ |
| 100 Day | 65.6±.245 | 62±.548$^{w,abcd}$ | 61±.316$^{w,abcd}$ | 60.2±.2$^{wx,abcd}$ |
| Mean | 65.5 | 63.93 | 62.93 | 62.7 |

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## Differential leucocyte count (DLC)

### *Monocytes*

The results of average monocyte values from different groups are presented in Table 5. There was a non significant change in all treated groups as compared to the control in the entire course of the study.

### *Lymphocytes*

The results of average lymphocyte values from different groups are presented in Table 6. A significant ($P<0.05$) decrease was noticed in group G2 and G3 on day 20 and

**Table 7.** Average values of Eosinophils (%) different groups of experimental birds (mean ± S.E).

| Group / Day | C (0 ppm) | G1 (1ppm) | G2 (5 ppm) | G3 (10 ppm) |
|---|---|---|---|---|
| 0 Day | 1.6±.4 | 1.6±.4 | 1.8±.374 | 1.6±.4 |
| 20 Day | 1.6±.4 | 1.8±.374 | 2.2±.2 | 2.2±.2 |
| 40 Day | 1.6±.4 | 2.2±.2 | 2.2±.2 | 2±.374 |
| 60 Day | 1.8±.374 | 1.8±.2 | 2.2±.2 | 1.8±.2 |
| 80 Day | 1.6±.4 | 1.8±.2 | 1.8±.374 | 2±.316 |
| 100 Day | 1.6±.4 | 1.6±.374 | 1.8±.2 | 2±.316 |
| Mean | 1.63 | 1.80 | 2 | 1.93 |

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**Table 8.** Average values of Heterophils (%) different groups of experimental birds (mean ± S.E).

| Group / Day | C (0 ppm) | G1 (1ppm) | G2 (5 ppm) | G3 (10 ppm) |
|---|---|---|---|---|
| 0 Day | 29.6±.510 | 29.2±.583 | 29.8±.583 | 29.40±.510 |
| 20 Day | 29.4±.510 | 29.8±.49$^{w}$ | 30±.894$^{wx}$ | 29.8±.583$^{wx}$ |
| 40 Day | 29.4±.510 | 29.4±.51 | 31±.70 $^{wx,}$ | 31±.632w$^{x,ab}$ |
| 60 Day | 29.4±.510 | 30.8±.735$^{a}$ | 31.2±1.114 | 32.2±.374$^{w,ab}$ |
| 80 Day | 29.2±.510 | 31.6±.4$^{w,abc}$ | 32.6±.245$^{w,ab}$ | 33±.316$^{wx,abc}$ |
| 100 Day | 29.4±.510 | 32.6±.245$^{w,abcd}$ | 33.6±.4$^{w,abcd}$ | 34.4±.4$^{wx,abcde}$ |
| Mean | 29.4 | 30.57 | 31.37 | 31.63 |

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**Table 9.** Average values of Basophils (%) different groups of experimental birds (mean ± S.E).

| Group / Day | C (0 ppm) | G1 (1ppm) | G2 (5 ppm) | G3 (10 ppm) |
|---|---|---|---|---|
| 0 Day | 0.6±.245 | 0.6±.245 | 0.6±.245 | 0.6±.245 |
| 20 Day | 0.6±.245 | 0.4±.245 | 0.6±.245 | 0.6±.245 |
| 40 Day | 0.6±.245 | 0.6±.245 | 0.6±.245 | 0.6±.245 |
| 60 Day | 0.6±.245 | 0.6±.245 | 0.4±.245 | 0.6±.245 |
| 80 Day | 0.6±.245 | 0.6±.245 | 0.6±.245 | 0.4±.245 |
| 100 Day | 0.6±.245 | 0.6±.245 | 0.4±.245 | 0.8±.245 |
| Mean | 0.6 | 0.57 | 0.53 | 0.6 |

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in all treated groups after 60 days onward as compared to the control group.

### *Eosinophils, Heterophils and Basophils*

The results of average eosinophils, hetrophils, basophils values from different groups are presented in Tables 7, 8 and 9, respectively. There was a non significant change in eosinophils and basophils in all treated groups as compared to the control in the entire course of the study. A significant ($P<0.05$) increase in the heterophils was noticed in all the treated groups on day 80 and 100 as compared to the control.

A significant (P<0.05) decrease in Hb was observed in group G1, G2 and G3 as compared to the control after 40, 60 and 80 days of feeding of Fipronil respectively. There was no significant (P<0.05) change in TEC among different treated groups on day 20 of the feeding trial, whereas after 40 days of feeding of Fipronil there was significant decrease in TEC values in group G2 and G3 as compared to the control. Significant decrease in G1 was observed after 60 days of feeding trial. There was significant (P<0.05) decrease in the TLC in group G1, G2 and G3 as compared to control after 20 day of trial till the end of study. The decrease in Hb and PCV may be due to shifting of the fluid from extravascular compartment to the intravascular compartment in order to maintain the normal cardiac output and pooling of the blood cells in the blood reservoirs such as spleen etc. may contribute to fall in Hb, PCV, TEC and TLC. Also, these effects might be due to cytotoxic effect of insecticide. A significant (P<0.05) decrease in average lymphocyte % was noticed in group G2 and G3 on day 20 and in all treated groups after 60 days onward as compared to the control group. This might be due to cytotoxic effect of insecticide given in feed.

Aughton (1993) observed decrease in the erythrocyte parameters (Hb, PCV) in rats receiving 1.5, 30 and 300 ppm in diet in a combined oncogenic and toxicity study of 104 weeks.Holmes (1991b) reported decrease in Hb concentration in male rats and slight lower PCV, mean cell volume, mean cell Hb, prothrombin time in female rats given Fipronil at 300 ppm in the diet after 12 weeks in a 13 weeks study. According to report of FAO (1997) rats were given fipronil-desulfinyl by gavage for two weeks at doses of 0, 0.3, 1, 3, or 10 mg/kg bw per day. At 1 mg/kg bw/day, pale livers and reduced leukocyte counts were observed in females.

## Conflict of Interest

The authors have not declared any conflict of interest.

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