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# Effect of individual and combined toxicity of chlorpyrifos and acetamiprid on growth parameters in broilers

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**Abstract**
The present study was conducted to investigate the effect of individual and combined toxicity of chlorpyrifos and acetamiprid on growth parameters in broilers in different doses at various intervals. The study was conducted on 252, day old broiler birds in month of March 2016. All the acetamiprid treated birds resulted in a significant ($P \leq 0.05$) decrease in feed consumption, body weight, body weight gain and increased FCR. Chlorpyrifos treated birds showed increased weight gain and lowered FCR in individual doses however combined toxicity groups showed decreased weight gain and increased FCR. Highest mortality was recorded during 1st week in group G (3 birds). Adverse effects of chlorpyrifos and acetamiprid toxicity were supported by the varying degree of toxic signs in birds of treatment groups. The study indicated the adverse effects of these insecticides, either alone or in combination on growth parameters in broilers.

**Keywords:** broilers, chlorpyrifos, acetamiprid, growth parameter

## Introduction
Organophosphorus and neonicotnoids are extensively used in agriculture to control crop pests and in livestock to control ectoparasites [1, 2]. The increased use of pesticides in crop protection increases the possibility of feed contamination and the consequent exposure of poultry to these products. Animals intended for human food may absorb insecticides from residues in their feed, water or during direct/indirect exposure in the course of pest control [3]. Exposure of poultry to pesticides often results in numerous health hazards and economic losses, in addition to posing a threat to public health, due to the presence of pesticide residues in poultry meat [4].
Among the several organophosphorus insecticides, chlorpyrifos [o, o-diethyl-o-(3,5,6-trichloro-2-pyridinol) phosphoro-thionate] is a broad spectrum insecticide which particularly affects the cholinesterase enzyme system. The continuous and indiscriminate use of chlorpyrifos insecticide leaves residues in poultry feed ingredients [5]. Poultry birds with infestation of lice and mites are also treated with insecticides. Chlorpyrifos is also applied to the soil surrounding or beneath buildings as protection against termites including chicken houses [1].
Acetamiprid is a member of neonicotinoid synthetic chlorinated insecticide family that has been recently introduced in the market [2]. It acts as agonists on the nicotinic acetyl choline receptors. Acetamiprid is used against insects that have gained resistance to organophosphate, carbamate and synthetic pyrethroid [6].
Co-exposure to chemical mixtures often results in alterations in biological responses as compared to those caused by exposure to individual chemicals [7]. Therefore, the challenge of toxicological consequence due to interaction between chlorpyrifos and acetamiprid prompted us to undertake the present study in broiler birds. Also, the data on growth parameters due to combined effect of chlorpyrifos and acetamiprid in broilers is lacking, the present study was undertaken to investigate the individual and combined effect of these insecticides on growth parameters in broilers.

## Materials and Methods
The present study was conducted on day old broiler birds at Poultry research centre, Post graduate institute of Veterinary and Animal Sciences, Akola, India. The birds were provided

with standard feed and clean water *ad libitum* and were acclimatized for seven days prior to the commencement of the study. The chlorpyrifos (purity 98.06 %) and acetamiprid (purity 97.00 %), used in this study were obtained from the Krishi Rasayan Export Pvt. Ltd. Samba, Jammu.

The experimental trial was approved by the Institutional Animal Ethics Committee, India and conducted under its guidelines. Two hundred and fifty two birds (n=36/group) were divided into seven groups viz, group I (Control) while groups II and III birds were given chlorpyrifos @ 50 and 100 mg kg-1, respectively, groups IV and V birds were given acetamiprid @ 100 and 200 mg kg-1, respectively, group VI birds were given chlorpyrifos @ 50 and acetamiprid @ 200 mg kg-1 and group VII birds were given chlorpyrifos @ 100 and acetamiprid @ 100 mg kg-1, daily in feed for a period of 4 weeks starting from 8$^{th}$ day and 7 days withdrawal period (5$^{th}$ week) was given during which no dietary treatment was provided. Birds of each group were observed daily for clinical symptoms and mortality if any, during experimental period of 5 weeks. Weight of individual bird was recorded on weekly basis up to 5$^{th}$ week and weekly weight gain of individual bird was calculated. Also feed consumed by birds of each group was recorded to calculate feed conversion ratio (FCR).

**Statistical analysis**

Statistical analysis of the experimental data generated was done as per Snedecor and Cochran using one way ANOVA test [8].

**Results and Discussion**

**Clinical observations:** The experimental birds from control group were apparently healthy throughout the experimental period. However, clinical signs were noticed in birds of all the treatment groups after 24 hrs of feeding except birds from group II. They exhibited clinical signs like less feed and water intake, not willing to move. Subsequently, they exhibited hock sitting posture, unable to get up, resting on sternum with backward stretching of legs and imbalance in standing. Birds from groups V, VI and VII showed clinical symptoms of closed eyes, dropping of wings and open mouth breathing from second week post intoxication. Birds from all the treatment groups showed diarrhoea from first week onward. The above symptoms were observed in all the treatment groups, more pronounced in groups III, V, VI and VII but to the lesser extent in groups II and IV birds.

The cholinergic signs observed in chlorpyrifos intoxicated chickens were similar with those reported in chickens intoxicated with organophosphate insecticides.

Several workers reported various clinical signs *viz*. slugishness, listlessness, depression, dysponea, reduced activity, hair loss, closed eyes/dropped eyes, chickens sitting on hocks, open mouth breathing, tremors, diarrohea, salivation, chickens lying on one side, paralysis during chlorpyrifos toxicity [10, 11, 12].

Respiratory distress, diarrhoea, depression and dullness in mice given acetamiprid were reported earlier however, clinical signs of toxicity were not observed after subchronic oral toxicity of acetamiprid in Wistar rats [13, 14].

**Mortality pattern:** No mortality was recorded in control group and group IV whereas, other treatment groups showed mortality (Table 1) from 1$^{st}$ week of experiment. However during the 1$^{st}$ week, mortality of 2, 1, 2, 1 and 3 in groups II, III, V, VI and VII respectively was observed. No mortality was recorded during the 2$^{nd}$ week of experiment in any of the treatment groups whereas, during 3$^{nd}$ week of treatment, mortality of 2, 1 and 1 in groups III, VI and VII respectively was recorded. During 4$^{th}$ week of treatment, 1 bird from group II, 1 bird from group VI and 1 birds from group VII succumbed. Highest mortality was recorded during the first week of treatment. These deaths were due to starvation as the birds were unable to get up and take feed and water. No Mortality was observed in group IV birds whereas; highest mortality was recorded in group VII birds as compared to groups II, III, V and VI throughout the experimental period of four weeks (1$^{st}$ to 4$^{th}$). During 5$^{th}$ week of experiment, 1 bird from group III and 2 birds from group VII succumbed whereas; no mortality was recorded in rest of the treatment groups during which no dietary treatment was given.

Present finding of mortality in chlorpyrifos treated birds is in accordance with earlier worker who recorded 3, 7 and 10% mortality in female chickens which were fed chlorpyrifos in the diet at 25, 50 and 200 ppm for 52 weeks [15]. Literature scanned did not reveal any information related to mortality of bird due to acetamiprid toxicity.

Avian species lack detoxifying acetylcholinesterase (A-esterase) activity and have low microsomal monooxygenase detoxifying activity in the liver, which renders them more susceptible to organophosphate poisoning than mammals [16].

**Table 1:** Mortality pattern in different groups

| Weeks | I | II | III | IV | V | VI | VII | Total |
|---|---|---|---|---|---|---|---|---|
| 1$^{st}$ week | 0 | 2 | 1 | 0 | 2 | 1 | 3 | 9 |
| 2$^{nd}$ week | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3$^{rd}$ week | 0 | 0 | 2 | 0 | 0 | 1 | 1 | 4 |
| 4$^{th}$ week | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 3 |
| Total | 0 | 3 | 3 | 0 | 2 | 3 | 5 | 16 |
| % mortality | 0 | 8.33 | 8.33 | 0 | 5.55 | 8.33 | 13.88 | |
| 5$^{th}$ week | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 3 |
| % mortality | 0 | 0 | 3.33 | 0 | 0 | 0 | 6.66 | |

**Body weights (g) and body weight gain (g)**

The weekly mean body weight and weekly body weight gain of treated and control chickens are summarized in tables 2 and 3 respectively. Reduction in the body weight was observed in the broiler birds fed with acetamiprid at all doses as well as in the birds fed with the combination of chlorpyrifos and acetamiprid (groups VI and VII) as compared to control group from first week of treatment. However, it was observed that there was significant increase ($P \leq 0.05$) in mean body weight of birds fed with 50 ppm of chlorpyrifos alone (group II)) as compared to control group from first week onwards to the completion of the experiment whereas the birds fed with 100 ppm of chlorpyrifos alone (group III) showed comparable values of mean body weight with that of control group. Rate of weight gain was less in treated birds in comparison to the control except groups II and III which were fed with chlorpyrifos alone during the entire period of experiment. Statistical analysis revealed significant increase ($P \leq 0.05$) in

mean body weight gain in birds of groups IV and V at the end of 2nd week of experiment in comparision to control. However, at the end of 5th week all the treatment groups showed increased mean body weight gain as compared to control indicating withdrawal effect. The non-significant difference in body weight in chlorpyrifos fed birds was reported earlier [17]. Decreased body weight or body weight gain in chlorpyrifos treated birds was reported by several workers [11, 18, 19]. Contrary to these observations, present study revealed increased body weights in chlorpyrifos treated birds. Increase in rat body weight after chronic exposure to chlorpyrifos was reported earlier [20]. They opined that chlorpyrifos have favourable effect on body weight gain. Organophosphate pesticides may induce accelerated differentiation of immature adipocytes into mature fat cells which might be one of the potential mechanisms of weight gain. Organophosphate pesticides have been shown to have endocrine effects that can result in weight gain also.

The finding of decreased body weight in acetamiprid treated birds is in conformity with many research reports in rats [13, 14, 21].

**Feed consumption (g) and FCR**

The values for weekly feed consumption and FCR are depicted in tables 4 and 5 respectively. Feed consumption was lowered in all the treated groups from 1st week onwards up to the end of 3rd week whereas from 4th week feed consumption was increased in treated groups in comparison to control. Feed conversion ratio (FCR) of chlorpyrifos and acetamiprid fed birds (II, III, IV and V) was lower up to the end of 3rd week of experiment in comparison with untreated control. However, during 4th and 5th week of experiment FCR was higher in groups II,III, IV and V. FCR of combined chlorpyrifos and acetamiprid fed groups (VI and VII) was higher at all the periods of experiment in comparision to untreated control and groups fed with either chlorpyrifos or acetamiprid alone at all doses.

The Feed conversion ratio was higher in combined toxicity groups indicating that higher FCR in these groups reflected in lower body weights. Decrease in feed consumption during chlorpyrifos toxicity is in accordance with the findings of earlier research reports in broilers [11, 18, 22]. However any appreciable change in the feed consumption of birds treated with chlorpyrifos was not observed by earlier worker [23]. Increased FCR in chlorpyrifos treated broilers was reported earlier [12]. Decrease in feed consumption due to acetamiprid toxicity was observed earlier in rats [14, 21]. Literature scanned did not reveal any information related to correlation of combined toxicity of chlorpyrifos and acetamiprid with growth parameters.

**Conclusion**

This study indicated that subacute exposure of chlorpyrifos @ 100 ppm acetamiprid @ 100 ppm in feed has adverse effect on growth parameters in broilers. The present study also indicated the adverse effects of these insecticides, either alone or in combination on mortality in broilers with improvement after providing withdrawal period of seven days.

**Table 2:** Mean weekly body weights per bird in different groups (g) during experimental period from 1st to 5th week (Mean±S.E.)

| Groups | Baseline data | 1st week | 2nd week | 3rd week | 4th week | 5th week |
|---|---|---|---|---|---|---|
| I | 122.528±2.01 | 320.500 $^{b}$ ± 6.75 | 490.417 $^{c}$ ±11.59 | 864.667 $^{bc}$ ±16.07 | 1343.167 $^{b}$ ± 1.88 | 1689.233 $^{ab}$ ± 29.35 |
| II | 120.556±2.38 | 356.486 $^{a}$ ± 6.77 | 596.600 $^{a}$ ±12.73 | 978.771 $^{a}$ ± 22.82 | 1479.235 $^{a}$ ± 1.71 | 1840.929 $^{a}$ ±45.96 |
| III | 125.306±1.51 | 325.314 $^{b}$ ± 5.50 | 559.114 $^{b}$ ±10.34 | 909.515 $^{b}$ ± 18.18 | 1365.030 $^{b}$ ± 7.54 | 1745.440 $^{ab}$ ± 38.35 |
| IV | 122.278±2.36 | 293.875 $^{c}$ ± 5.49 | 489.250 $^{c}$ ±5.20 | 862.000 $^{bc}$ ± 16.57 | 1301.750 $^{b}$ ± 8.16 | 1640.318 $^{b}$ ±33.36 |
| V | 125.972±1.77 | 279.657 $^{c}$ ± 4.03 | 485.114 $^{c}$ ±7.85 | 849.657 $^{c}$ ± 14.59 | 1200.686 $^{c}$ ± 1.92 | 1638.071 $^{b}$ ±35.62 |
| VI | 124.972±1.60 | 240.543 $^{d}$ ± 4.68 | 407.657 $^{d}$ ±9.99 | 755.471 $^{d}$ ± 23.32 | 1141.967 $^{cd}$ ± 2.69 | 1604.154 $^{b}$ ±48.88 |
| VII | 125.139±1.97 | 251.364 $^{d}$ ± 4.82 | 399.273 $^{d}$ ±10.23 | 729.938 $^{d}$ ± 15.78 | 1071.259 $^{d}$ ± 3.82 | 903.630 $^{c}$ ±36.93 |
| Significance ** | | ** | ** | ** | ** ** | |

**$P \leq 0.01$, Mean values with common alphabet as superscript do not differ significantly, $P \leq 0.05$

**Table 3:** Mean weekly body weight gain per bird in different groups (g) during experimental period from 1st to 5th week (Mean±S.E.)

| Groups | 1st week | 2nd week | 3rd week | 4th week | 5th week |
|---|---|---|---|---|---|
| I | 197.972 $^{b}$ ±4.80 | 169.917 $^{c}$ ±5.04 | 374.250 $^{a}$ ± 5.10 | 478.500 $^{ab}$ ± 7.92 | 385.700 $^{c}$ ± 14.61 |
| II | 213.657 $^{a}$ ±8.49 | 240.114 $^{a}$ ±6.10 | 379.235 $^{a}$ ± 10.30 | 507.412 $^{a}$ ± 10.81 | 414.214 $^{c}$ ± 18.06 |
| III | 199.514 $^{b}$ ±3.92 | 231.909 $^{a}$ ±5.21 | 355.424 $^{ab}$ ± 8.02 | 455.515 $^{bc}$ ± 10.61 | 441.920 $^{bc}$ ± 13.49 |
| IV | 174.625 $^{c}$ ±3.54 | 195.375 $^{b}$ ±1.15 | 372.750 $^{a}$ ± 11.47 | 439.750 $^{cd}$ ± 12.16 | 423.409 $^{bc}$ ± 14.91 |
| V | 151.914 $^{d}$ ±2.21 | 205.457 $^{b}$ ±3.97 | 364.543 $^{a}$ ± 6.90 | 351.029 $^{e}$ ± 8.08 | 481.500 $^{ab}$ ± 19.13 |
| VI | 112.286 $^{e}$ ±2.83 | 165.235 $^{c}$ ±5.31 | 331.433 $^{bc}$ ± 11.56 | 417.333 $^{d}$ ± 13.88 | 507.808 $^{a}$ ± 24.97 |
| VII | 128.121 $^{f}$ ±3.05 | 146.000 $^{d}$ ±5.38 | 323.000 $^{c}$ ± 5.28 | 366.407 $^{e}$ ± 9.49 | 433.294 $^{bc}$ ± 19.71 |
| Significance ** | ** | ** | ** | ** | |

**$P \leq 0.01$, Mean values with common alphabet as superscript do not differ significantly, $p<0.05$

**Table 4**: Weekly feed consumption (g) per bird in different groups during experimental period from 1st to 5th week (Mean±S.E.)

| Groups | 1st week | 2nd week | 3rd week | 4th week | 5th week | Cumulative mean |
|---|---|---|---|---|---|---|
| I | 477.14 | 470.47 | 704.67 | 661.61 | 789.47 | 3103.36±63.57 |
| II | 392.11 | 482.17 | 686.74 | 806.82 | 994.43 | 3362.28±109.09 |
| III | 353.54 | 417.54 | 733.27 | 758.85 | 1097.76 | 3360.97±134.29 |
| IV | 355.56 | 425.38 | 662.81 | 789.44 | 1141.82 | 3375.01±141.01 |
| V | 322.17 | 469.60 | 685.66 | 717.94 | 997.50 | 3192.87±115.64 |
| VI | 322.54 | 465.51 | 727.88 | 752.24 | 1008.62 | 3276.80±119.96 |
| VII | 361.36 | 447.97 | 676.84 | 898.37 | 1545.29 | 3929.84±212.19 |

**Table 5:** Feed Conversion Ratio (FCR) in different groups during experimental period from 1st to 5th week

| Groups | 1st week | 2nd week | 3rd week | 4th week | 5th week | Pooled mean |
|---|---|---|---|---|---|---|
| I | 2.41 | 2.77 | 1.88 | 1.24 | 2.05 | 2.07±0.259 |
| II | 1.84 | 2.01 | 1.86 | 1.40 | 2.40 | 1.86±0.170 |
| III | 1.77 | 1.91 | 2.06 | 1.42 | 2.48 | 1.93±0.175 |
| IV | 2.04 | 2.18 | 1.78 | 1.38 | 2.70 | 2.01±0.218 |
| V | 2.12 | 2.29 | 1.88 | 1.70 | 2.07 | 2.00±0.101 |
| VI | 2.87 | 2.90 | 2.49 | 1.69 | 1.99 | 2.37±0.232 |
| VII | 2.82 | 3.16 | 2.48 | 1.99 | 3.57 | 2.81±0.272 |

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