



## Effect of Vacomil on Some Reproductive and Biochemical Profiles of Male Pigeons

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### ABSTRACT

The use of fungicides has increased over the years in order to improve the yield of many crops. In this aspect, the possible side effects of 'Vacomil plus 50' on reproductive and biochemical parameters of male pigeons has been investigated. The molecule was applied by gavage with a dose of 2 mg/l/j (D1) and 4 mg/l/j (D2) for 35 days. Results have showed slight increase in total body weight of pigeons treated to vacomil during the period of the experiment, but it decreased in pigeons of D2 during the last week. Liver weight was weakly reduced in both doses of vacomil, whereas those of testes were slightly decreased only in pigeons treated with the weak dose. Pigeons treated with fungicide have showed a narrow variation in thyroxine concentration throughout the experiment, but the concentration was overturned at the end of the fifth week. Compared to the control, glucose level has softly declined from the second week, except the fifth week where it was almost the same as that of the control. The fifth week has showed a non-significant increase in the level of triglycerides in the group exposed to D2, after it was lower in both doses at the beginning of the experiment. The concentration of cholesterol has been moderately affected by both doses during the experimental period when compared to the control.

**Keywords:** Fungicide, pigeons, Vacomil, reproduction, thyroxine.

### INTRODUCTION

The improvement of agricultural production has, for a long time, been associated to a rather extensive use of pesticides. Nonetheless, an uncountable number of sides effects do seem to become even uncountable in terms of both human and animals health and, thereby leading to many disruptions of physiological processes<sup>1-3</sup>.

For instance, many pesticides have been listed as confirmed or possible endocrine disrupting chemicals by U.S. Environmental Protection Agency<sup>4</sup>. However, the disorders generated by the endocrine disruptive pesticides can be temporary or permanent. It can provoke reproductive anomalies by reducing the fertility and fecundity, gonad dysfunction and also congenital malformations in offspring.

Among the males, the main effects on fertility reveal a temporary fall in sperm concentration and its quality, a stronger cryptorchism, hypospadias and a modification of the sex ratio<sup>5</sup>.

In Algeria 400 pesticides are homologated and the use of such chemicals becomes a serious problem of concerns<sup>6,7</sup>. Vacomil plus 50, is a fungicide, having a wide range of uses including many fungal diseases of vegetables, fruits and field crops, is one of those substances widely used in agricultural. Vacomil plus 50 is composed of a systemic fungicide "Metalaxy" and of "Oxychlorure". From a chemical point of view, Vacomil is: methyl N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-DL-alanine and that is soluble at 65% in methanol at 20 °C Its LD50 in rats is estimated at 669 mg/kg when given *per os*. In the present

investigation the toxic effect of Vacomil on reproductive and hormonal profiles in domestic pigeons *Columba livia* has been investigated.

### MATERIALS AND METHODS

Twenty four (24) adult male pigeons were used in this experiment. They were housed in appropriate and specific cages (60 x 54 x 52 cm) and acclimatized for 15 days before being divided into three groups of 5 each. Group 1 served as control while groups 2 and 3 were treated with the fungicide.

The fungicide was dissolved in tap water for the desired doses and then each animal received 1ml *per os* of a solution containing the fungicide at a dose of 2 and 4 mg/l/day for five weeks.

The experiment was conducted under controlled laboratory conditions of long photoperiods (20L: 4D), temperature (20-22 °C) and humidity (65%).

Blood samples were collected at weekly intervals; body weight was also recorded at 7 days interval, before the animals being decapitated at the end of the experiment.

Blood was collected in EDTA coated tubes, stored at -4°C till used for biochemical and hormonal parameters. The testes were immediately removed and weighted.

Plasma cholesterol and triglycerides concentration were measured according to the procedure of Naito<sup>8</sup> and Young<sup>9</sup>, Plasma glucose was estimated by the method of Kaplan<sup>10</sup>, while thyroxin level was evaluated by ELISA method<sup>11</sup>.



Results are represented as means  $\pm$ SD, and comparison between the treated groups and the control was made by Student t-test using Mini Tab Program.

## RESULTS AND DISCUSSION

### Biometrics Parameters

Results of pigeons exposed to two doses of Vacomil (2 mg/l and 4 mg/l) during 35 days were presented in Figures 1-6. The mean total body weight varied along the experimental period according to the treatments, where the important reduction was observed in the dose 2 (245.09 $\pm$ 122.22 g) at the end of the experiment (Fig. 1). On the other hand, in the control and dose 1 (2 mg/l of Vacomil), the mean total body weight varied slightly along the experimental period.

In this work, it appears that a long photoperiod associated with an exposure to vacomil, have disrupted the reproductive function in male pigeons.

In the first period, it seems that some variations in total body weight were registered in birds of the three groups.

Such variations are unlikely related neither to the applied photoperiod, nor to the exposure to vacomil, but it might be dependent to the laboratory rearing conditions and also to the competition between individuals. It has been observed in this context an aggressive behavior, where some injuries were noted at the levels of heads, even for birds of the control group.

This competition has also a nutritional origin, which allows observing some differences in individuals' body weights of all groups<sup>12</sup>.

Furthermore, there was a strong negative relationship between the female persistent organic pollutants (POP) concentrations and growth-rate in top predator birds<sup>13</sup>.

Certain changes in the weights of liver, spleen, testes and heart, accompanied with a slight variation in the brain weight were observed after the sacrifice of pigeons (Fig. 2).

Testicular weights decreased slightly in pigeons treated with the weak dose of 2 mg/l Vacomil. Contrary, liver weight decreased in the dose 1 and dose 2 of Vacomil to reach the values of 5.65 $\pm$ 1.28 g and 6.06 $\pm$ 0.33 g, respectively. Heart weight was higher in birds intoxicated with the fungicide than that of the control.

Organs' weights have also varied according to the dose and to the applied photoperiodic programme. In this manner, the decrease in liver weight of birds intoxicated with the fungicide has possibly two origins; one is related to the detoxifying mechanism of hepatocytes, which has exhausted the energy sources of glycogen stocked in this organ<sup>14</sup>.

The second origin might come from the direct intoxication of fungicide on hepatocytes, provoking however a destruction to hepatocytes. The increase of liver weight was accompanied with a tumor in mice<sup>15</sup>.

The second observation was a slight increase in the heart weight of dose 2 birds, meanwhile brain and spleen weights have not been affected by the fungicide.

Testicular weight was slightly reduced after being exposed to dose 1 of vacomil with a photoperiod programme of (20 L: 04 D).

In birds, gonads' body weights are affected by the starting of the seasonal reproduction, which is manifested by an increase in testicular volume.

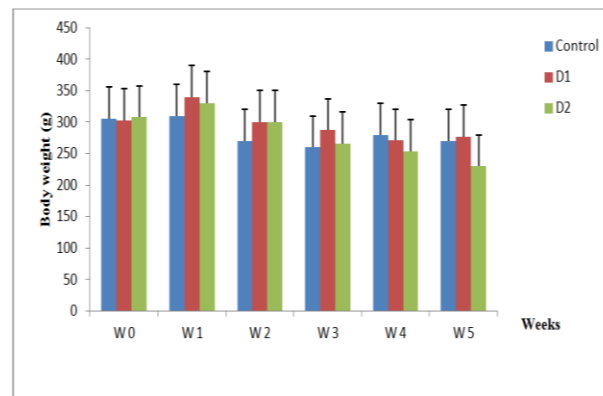
It seems that vacomil has inhibited the histological and cellular modifications of testes needed at the beginning of the sexual season.

The photoperiod programme applied on birds receiving dose 1 and 2 was expected to boost testicular weight much more than that of the control, as it was reported earlier that light could stimulate the volume of testes<sup>16</sup>.

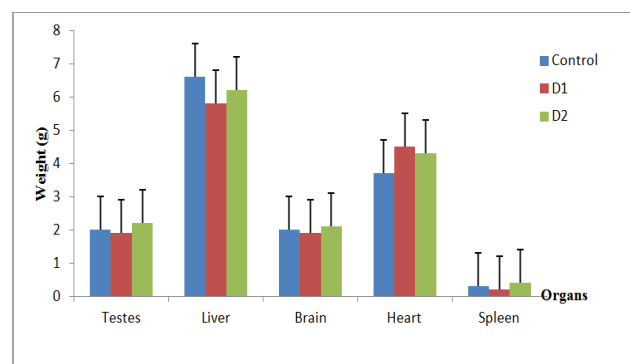
This result is in-line with that of Maalem<sup>17</sup> who used another fungicide (manebe) and observed a decrease in testicular weight, so testis is a target organ for fungicides<sup>18</sup>.

Spleen body weight has not been varied in the 3 groups along the experimental period.

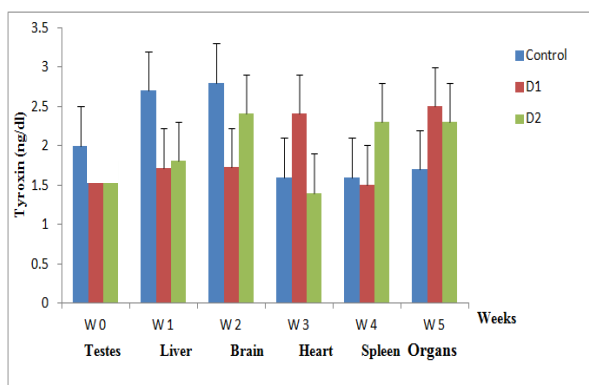
Spleen is the site of broken red blood, and hence the presence of high dose of fungicide could be the origin of elevated splenic activity, leading to a rise of the organ weight.



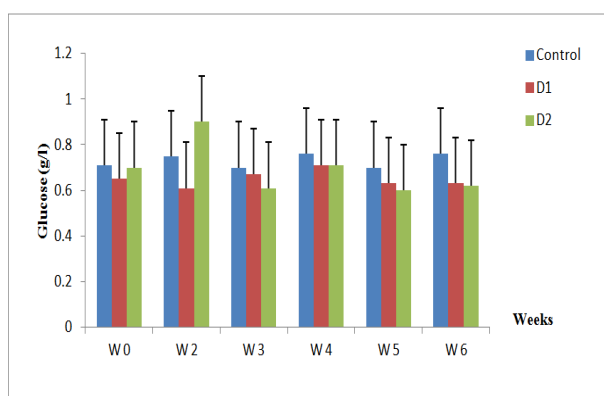
**Figure 1:** Mean Body Weight (g) of Pigeons treated with two doses of Vacomil.



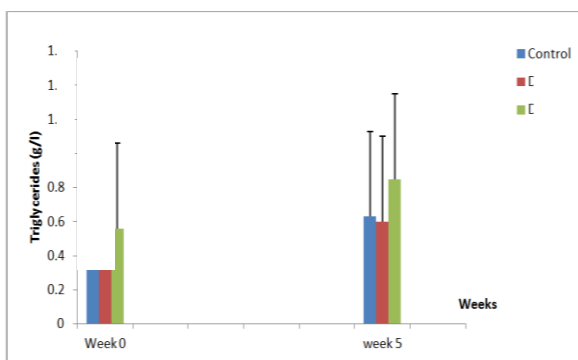
**Figure 2:** Mean Organs' Weights (g) of Pigeons treated with two doses of Vacomil.



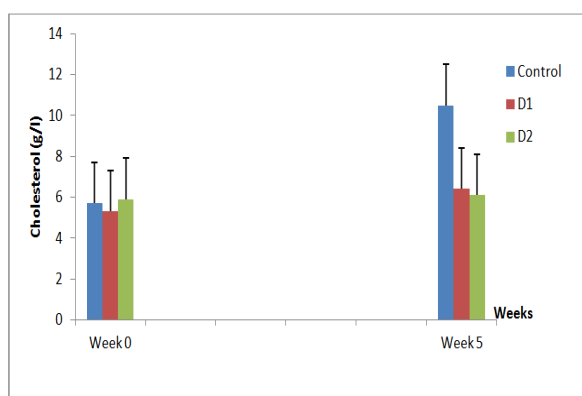
**Figure 3:** Mean Thyroxine Concentration (ng/dl) of Pigeons treated with two doses of Vacomil.



**Figure 4:** Mean Glucose Concentration (ng/dl) of Pigeons treated with two doses of Vacomil.



**Figure 5:** Mean Triglycerides Concentration (ng/dl) of Pigeons treated with two doses of Vacomil.



**Figure 6:** Mean Cholesterol Concentration (ng/dl) of Pigeons treated with two doses of Vacomil.

### Biochemical Parameters

Thyroxine profile varied along the experimental period according to the fungicide dose and according also to the photoperiodic programme (Fig. 3).

From the first week, the mean level of T4 was lower in pigeons of the treated groups compared to the control. In contrast, at the end of the fifth week, the opposite effect was observed, where the thyroxine levels were higher in pigeons exposed to fungicide.

During the first week, the concentration of plasma glucose was slightly lower in birds of dose 1 and higher in dose 2.

However, from the third week the level of glucose was in decline in birds exposed to fungicide, but such decline was within the physiological range.

At the beginning, the level of triglycerides was the same in all groups. However, it slightly declined in pigeons of dose 1 and rose in dose 2 at the end of the experiment (Fig. 5).

The concentration of cholesterol was the similar in pigeons of D 1 and D 2 during the first week (Fig. 6).

Besides, there was a remarkable reduction in cholesterol level during the fifth week in the two treated groups.

The implication of thyroid hormones in seasonal reproduction regulation has been demonstrated in many bird species<sup>19</sup>.

In this manner, thyroidectomy inhibit the refractory phase in the studied species as that of the Japanese quail and starlings<sup>20</sup>.

Results issued from the current study reveal that vacomil has provoked a decrease in thyroxine level during the second and the third week, but it increased at the end of the fifth week by both doses.

Such results are in line with that of glucose concentration, which was reduced at the end of the experiment.

Hence, thyroxine is a producing energy hormone, where it catabolises nutrients including glucose. Accordingly, in order to achieve a full cycle, thyroxine level should be elevated<sup>21</sup>. Birds breeding at the contaminated site have experienced the highest levels of stress hormones and oxidative stress, with the lowest immunoglobulin levels compared to the two the non contaminated sites<sup>22</sup>.

The concentration of triglycerides was slightly lower in birds of dose 1, which might confirm the oxidizing role of thyroxine. On the other hand the remarkable increase of triglycerides level of birds exposed to dose 2 than that of the control is possibly due to the inhibition of enzymes responsible on the triglycerides hydrolysis.

There was in the current study a weak variation in the concentration of triglycerides and cholesterol of pigeons exposed to the strong dose of vacomil at the end of the experimental period.

Recently, no correlation was established between the concentration of persistent organic pollutant in birds from geographically distinct North Atlantic areas and blood plasma clinical-chemical parameters including cholesterol<sup>23</sup>.

Moreover, plasma organohalogenated contaminants were positively correlated with plasma cholesterol level in golden eagle and white-tailed eagle nestlings during three consecutive breeding seasons<sup>24</sup>.

## CONCLUSION

Results of the present investigation reveal that the administration of vacomil with the dose of 2mg and 4mg/Kg provoked slight decrease in liver and total body weight.

The concentrations of thyroxine and triglycerides have increased non-significantly, accompanied with a slight decrease of glucose and cholesterol levels at the fifth week.

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