



Research Article

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The effects of two insecticides “Imidacloprid and Chlorpyrifos” on male quail Coturnix japonica

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ABSTRACT

Birds may be at risk of poisoning by insecticides sprayed in the fields. To evaluate this risk, 40 male quails (*Coturnix japonica*) were randomly divided into five equal groups, and then daily exposed to oral concentrations of Chlorpyrifos (1/10 and 1/20) and Imidacloprid (1/10 and 1/20), according to the agricultural doses used, for seven consecutive weeks. Total body weight, testis volume and plasma concentrations of triglycerides, cholesterol, glucose, total proteins, thyroxin and testosterone were determined at the first, the third and the seventh weeks. Results indicated a significant decrease in total body weight, accompanied with a significant increase of testicular volume in all treated groups. No changes were noted concerning the level of triglycerides during the experimental period with all doses. A clear augmentation of cholesterol level was seen in the first and the seventh week of birds treated with Chlorpyrifos and Imidacloprid, respectively. Glucose concentration has increased generally at the beginning of the experiment, but it declined during the last week in both insecticides applied. A significant decrease of total proteins level of all exposed groups during the first week, whereas in the third week their levels were low only with (1/20) Chlorpyrifos and (1/10) Imidacloprid. Thyroxin concentration in Imidacloprid exposed groups was noticeably higher in the higher dose and lower in the lower dose after one week, while that of testosterone level was only low in Chlorpyrifos doses at the end of the experiment, despite the increased testicular volume. To conclude, both insecticides has affected quails' reproduction with different degrees.

Keywords: *Imidacloprid, Chlorpyrifos, reproduction, pesticides, Coturnix japonica*

INTRODUCTION

Insecticides are being used extensively all over the world in the field of agriculture and public health to control insects, weeds, animals and vectors of the diseases [1]. Most of authorized pesticides are neurotoxins, which cause in non-target organisms some behavioral changes such as reduced food consumption, weaker reproductive success and lesser ability to avoid predation [2]. Chlorpyrifos (CP) is a broad-spectrum organophosphate insecticide that is classified as moderately toxic. Neurotoxicity is one of its main toxic manifestations and can occur after long-term

exposure to subclinical doses of chlorpyrifos as well as after acute intoxication [3]. In addition, increasing evidence from animal and human studies indicates that chlorpyrifos is a developmental neurotoxicant [4]. The main mechanism of acute intoxication is due to the inhibition of acetylcholinesterase, which is responsible for the degradation of the neurotransmitter acetylcholine. The resultant cholinergic over activity leads to the manifestations of neurotoxicity [5]. CP has also been reported to cause a reduction in the bacterial, fungal and actinomycete population of the soil and it is known to inhibit nitrogen mineralization in soil [6].

The mechanism for acute toxicity of Imidacloprid Organophosphates is found in their reversible inhibition of acetylcholinesterase, which is a key enzyme in the function of the nervous and neuromuscular systems [7]. Imidacloprid (IM) is a systemic insecticide belonging to the family of neonicotinoids and it is currently the first insecticide and the second agrochemical most used in the world [8]. This pesticide acts by binding to specific nicotinic acetylcholine receptors, thus interfering with the transmission of nerve impulses. The European Union declared the use of three neonicotinoid insecticides (Imidacloprid, thiamethoxam and clothianidin) for seed coating, soil treatment and foliar treatment, due to its toxicity on pollinators, but their use for seed treatment of winter cereals, as well as after crop flowering and in crops harvested before flowering, continues to be approved (Regulation 485/2013). AM oral acute LD50 for birds vary from 31 mg/kg in the Japanese quail *Coturnix japonica* to 152 mg/kg in the bobwhite quail *Colinus virginianus* [9]. Animal studies confirm the relatively low toxicity in vertebrates when compared to insects [10]. Neonicotinoids now represent the largest selling class of insecticide and seed treatments on the global market [11]. Pesticide residues and their metabolites often infiltrate through the soil surface into the ground water and cause widespread contamination of aquatic ecosystems [12].

Birds have been used as sentinel species for environmental contaminants exposure owing to their higher trophic position, widespread distribution and sensitivity to environmental changes [13]. Even birds that mainly consume grains depend on insects to feed their brood, such that the accumulation of neonicotinoids in the environment and their presence in insects may well have a knock-on effect on birds. Predatory birds have been used as bioindicators of environmental pollution because of their high trophic status in the foodweb and their vulnerability to the effects of pollutants including POPs [14]. In the field, there are some reported cases of wild bird mortalities, due to the ingestion of seeds contaminated with Imidacloprid [15].

Recent studies have pointed out that a major cause of bird population declines is the use of pesticides, either because of indirect effects on habitat and food supply, or because of direct toxic effects on the birds' health [16]. A greater probability of lethality in birds occurs when the ratio between the LD50 and the estimated field exposure dose is low. Pesticides with higher LD50 or lower risk of exposure can produce a range of sub-lethal effects such as loss of physical condition, immunosuppression, neurological impairments or endocrine disruption [17]. All these effects may ultimately affect the survival or the reproduction and therefore affect population dynamics.

Coturnix japonica is a well-suited model species for the study of insecticide risk assessment [18]. because of its fast growth rate, small size, early sexual maturity, easy to maintain in the laboratory, and relatively small food consumption [19]. It has been also reported that this species responds physiologically to chlorpyrifos exposure [20], and exhibits metabolic plasticity during the first days after hatching [21].

In this context, this study aims to investigate the effect of organophosphorus (CP) and neonico-tinoid (IM), widely used on agriculture, on the growth and development of gonads and also on certain biochemical and hormonal markers of the quail *Coturnix japonica*.

MATERIALS ET METHODS

Animal's treatment

Fourty male quails *Coturnix japonica* were captured before the breeding season early October from Oum EL-Bouaghi (East Algeria). Animals were reared in metal cages (100 x 100 x 100 cm), equipped with feeders and water. Before the establishment of experimental plots, quails were acclimated 10 days under standard laboratory conditions with food and water ad libitum (Food based on wheat and bread crumbs).

After the acclimation period, animals were weighted, kept under a photoperiod of 14 hours light and 6 hours dark (14L: 06D) and divided into five equal groups as follow: Group 1 (T) served as control received mineral water; Group 2 and 3 received C1/10 and C1/20 of LD50 of Clorofet, the agricultural form of Chlorpyrifos). Group 4 and 5 received D1/10 and D1/20 of LD50 of Commando (the agricultural form of Imidacloprid). Animals received a daily oral volume of 0.5 ml.

Sample collections

After one, three and seven weeks; males blood samples were collected from wing vein in hepa-rinized tubes, then centrifuged at 1500 rpm for 20 minutes. In parallel, the testes were observed by dissection technique that is specific for birds [22]. on the right side of bird, between the last pair of ribs and intercostales muscle, a small incision of about 2 cm is performed using sharp chisel and a pair of spacer with clamp, the membranes surrounding the gonads.

Testes volumes were examined in situ and the testicular volume was calculated using the follow-ing formula:

$$V (\text{mm}^3) = 4/3 a^2 b$$

V: testicular volume in mm³

a: half-width of gonad in mm

b: length of the half-gonad in mm

Biochemical Analysis

Plasma cholesterol, triglycerides, glucose and total proteins were measured by spectrophotometry using commercial kits (Spinreact, Spain). Testosterone and Thyroxin (T4) were measured by ELISA method (Omega Diagnostics, United Kingdom).

Statistical analysis

The results in this study were expressed as the means \pm SD. The significance of the differences in mean values among the control and treated groups was evaluated following ANOVA test with one classification criteria using Minitab software version (13).

RESULTS AND DISCUSSION

Body and testicular weights

Total body weight of quails treated with Chlorpyrifos and Imidacloprid in all groups during (1st, 3rd and 7th weeks) of the experiment are shown in figure 1. A significant decrease in total body of quails treated with 1/10 and 1/20 of Imidacloprid in the third week, and 1/10 and 1/20 of Chlorpyrifos in the seventh week were observed compared to the control.

Compared to the control, a significant increase in testicular volume of group 2 and 3 was observed after the third and the seventh week (Figure 2).

Biochemical markers

No significant difference was recorded in triglyceride's concentrations of all treated groups compared to the control group (Figure 3).

In Figure 4, a significant augmentation was observed in cholesterol level in the C1/10 and C1/20 birds during the first week, and in the D1/10 and D1/20 groups during the third and the seventh week compared to the control.

There was a significant reduction in glucose concentration in the C1/10 group and a significant elevation in C1/20 group after one-week treatment. Moreover, the D1/10 and D1/20 has increased glucose level significantly at the end of the experimental period (Figure 5).

As shown in Figure 6, a progressive decrease in the concentrations of total proteins was seen in all treated groups after one week, and in the C1/20 and D1/20 groups after three weeks. Contrary, an increase in total protein levels was recorded in the strong doses (C1/10 and D1/10) after three-week period compared to the control.

The only significant increase of thyroxin concentrations was in the groups treated with Imidacloprid at the beginning of the experiment compared to the control (Figure 7).

Concerning testosterone, its concentration was remarkably declined in the two groups of bird treated with Chlorpyrifos at the seventh week compared to the control (Figure 8).

The results of this study show that the administration of two agricultural pesticides, Chlorpyrifos and Imidacloprid affect the average total body weight of quails and certain biochemical parameters.

The gonadal volume response of quails appeared greater in all groups in full the experience. It is in fact of long photoperiod (14L: 6D), which has the effect of increasing the volume of gonads and start the reproductive cycle [23]. The primary effect of a long photoperiod in birds involves the stimulation of synthesis and secretion of hypothalamic hormone GnRH to induce the release of gonadotropins adenohipophyseal, LH and FSH, and

accordingly spermatogenesis [24]. However, sexual activity ends in vast majority of birds before the summer, when the photoperiod is longer. The phase of sexual cycle, said photorefractoriness, is characterized by gonadal involution, a decrease in plasma secretion of gonadotropins and thyroxin (T4) [24]. The decrease in body weight in all treated groups is in total agreement with other studies. [26] have reported that the decrease in body weight correlated with the decrease in food intake of rats treated with Imidacloprid this reduction is due to the toxic potential of this insecticide.

Concerning the level of plasma total proteins, a decrease in all treated groups was observed. [27] have also reported a decrease in serum proteins levels in goats treated with Cypermethrin, they postulated that this decrease might be related to the altered enzymatic activity and the hepatic impaired protein synthesis through the production of reactive oxygen species during the metabolism of Cypermethrin. Serum proteins can also be lowered because of the leakage from the altered kidneys, which have been previously reported [28].

The decrease in plasma glucose in C1/10 and C1/20 groups during the first and the seventh weeks is similar with the results reported by [29], who observed a decrease in plasma glucose after 14 and 28 days of sublethal doses of Imidacloprid to leghorn chickens. In the actual experiment, glucose level has taken the same trend as that of total body weight. Thyroid hormones are important and mediators in basal metabolism rates. [30] have detected a decrease in plasma glucose in adult partridges exposed to low doses of Imidacloprid, which might be explained by the reduction in food consumption. On the other hand, [31] have showed an increase in plasma glucose of *Cotunix japonica* treated by 1/20 LD50 of Malathion, because of the accelerated glycogenolysis. However, no significant difference in plasma glucose was observed in birds treated with Imidacloprid along the experimental period.

The clear increase of plasma cholesterol rate at the end of the experiment when quails were given both D1/10 and D1/20, indicates that the insecticide effect can be seen clearly after a long exposure time. Accordingly, [32] reported an augmentation in cholesterol and triglycerides levels after quails' treatment with malathion for 8 weeks compared to individuals treated for shorter periods. Such increase could be attributed to the blockage of liver bile duct causing reduction of its secretion (Kalender et al., 2005), and/or to the hyperadrenal activity induced by malathion treatment [34]. However, the toxic effects of C1/10 and C1/20 were observed earlier during the first week by increasing plasma cholesterol significantly.

Thyroid gland, through its hormones (thyroxin/T4 and triiodothyronine/T3), not only regulates metabolic activities, but also maintains reproductive homeostasis [35] in a variety of animals. Circulating concentration of thyroid hormones are regulated by hypothalamic-pituitary-thyroid (HPT) axis through a negative feedback response. They regulate the gonadal growth/development and reproductive axis in mammals [36]. In birds, positive regulation though has been reported for temperate zone birds, such as Japanese quail [37]; there are varied reports on thyroid regulation of reproductive axis in tropical zone birds, such as estrildid finches [38]. The involvement of thyroid hormones in regulating the function of reproduction in birds has been studied extensively during the thyroidectomy [39] and during the photo refractoriness [40] starling.

Surprisingly, plasma thyroxin level has increased with the strong dose (D1/10) and decreased with the weak dose (D1/20) earlier, followed with a slight decrease by the two doses at the end of the experiment. The increased T4 level in D1/10 quails might be originated from the alteration of HPT axis or to the decreased conversion of T4 to T3 in epithelial cells.

Recently, acute high dose of Imidacloprid was reported to cause thyroid lesions to laboratory rodents [41]. Accordingly, Mancozeb acute high concentration was able to inhibit enzyme thy-roid peroxidase, affect the thyroid gland histopathology of rats [42]. Maneb and zineb were re-ported to disrupt the activity of pituitary–thyroid axis [43].

Testosterone hormone regulates the reproductive and aggressive behaviors in male vertebrates, and in many northern latitude bird species, where circulating concentrations of this hormone are greatly elevated during the breeding season [44]. Plasma testosterone levels decreased in both C/10 and C1/20 treated groups in the seventh week, which indicate the strong effect of this pesticide on the reproductive axis. Similarly, [45] have reported a reduction in serum testosterone of *Cotunix japonica* exposed to different doses of the fungicide Atrazine for six weeks, which was related to the testicular decreased steroid-genesis at leydig cells [46].

CONCLUSION

The present data show that the administration of two doses of Chlorpyrifos and Imidacloprid to quails for seven weeks have decreased total body weight and increased testicular volume. The perturbation of biochemical markers was observed with a clear augmentation of cholesterol concentration, accompanied with a decrease in glucose and some perturbations of total proteins level along the experimental period. Thyroxin concentration was only affected in the presence of Im-idacloprid at the beginning of the experiment, while testosterone level was remarkably decreased when quails were exposed to both doses of Chlorpyrifos during the seventh week, despite the significant augmentation of testicular volume. Accordingly, care must be taken into account to avoid birds and human exposure to Chlorpyrifos and Imidacloprid, widely used in agriculture.

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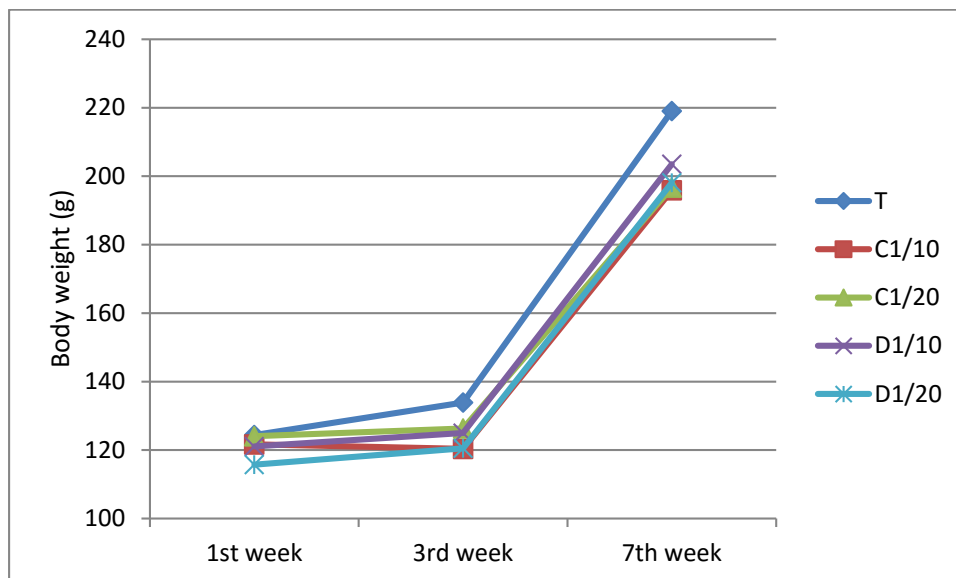


Figure 1: Variation of body weight after 1, 3 and 7 weeks of treatment in control (T) and treated groups (C1/10, C1/20, D1/10 and D1/20) (mean \pm SD, n=8).

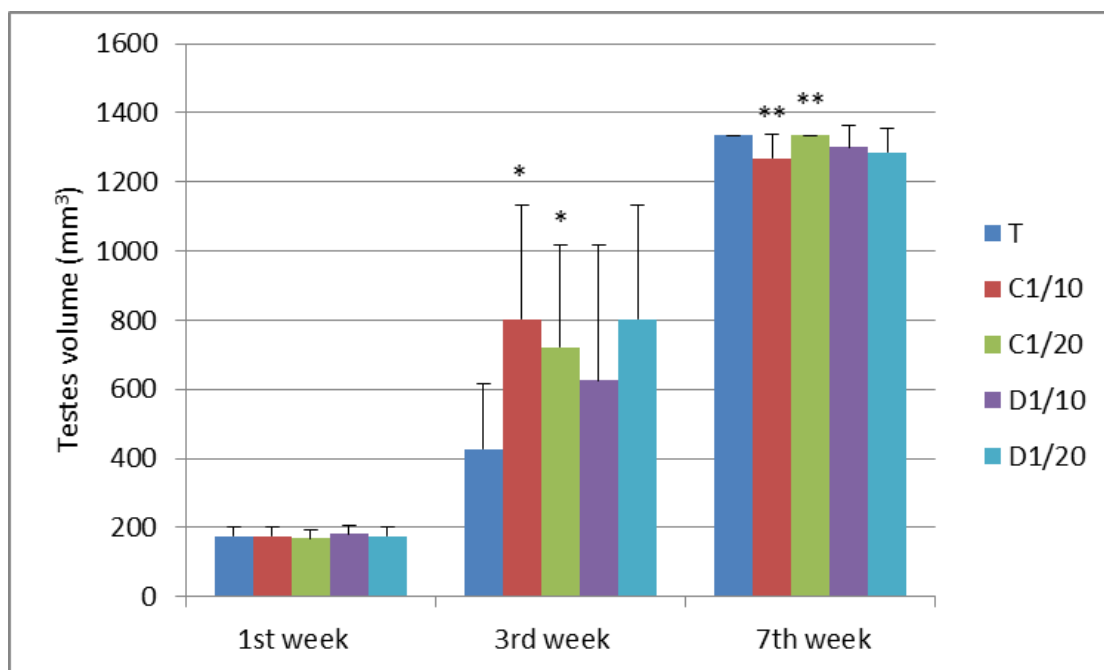


Figure 2: Testes volume variations after 1, 3 and 7 weeks of treatment in control (T) and treated groups (C1/10, C1/20, D1/10 and D1/20) (mean \pm SD, n=8)

(P<0.05) * ;(P<0,01) **

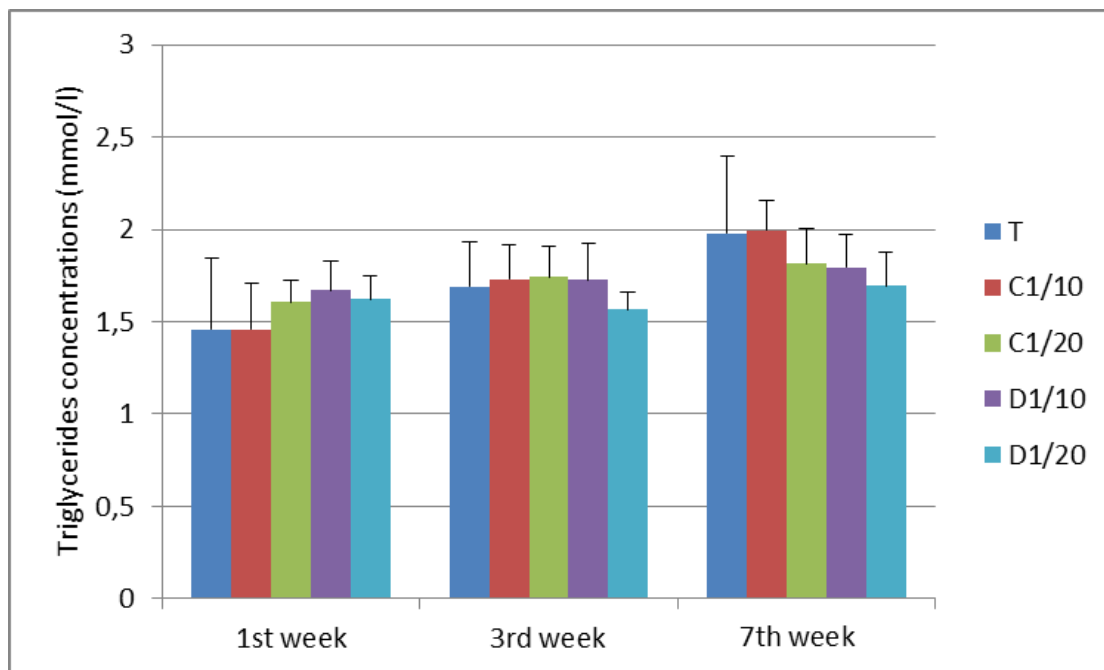


Figure 3: Variation of triglycerides concentrations after 1, 3 and 7 weeks of treatment in control (T) and treated groups (C1/10, C1/20, D1/10 and D1/20) (mean ± SD, n=8)

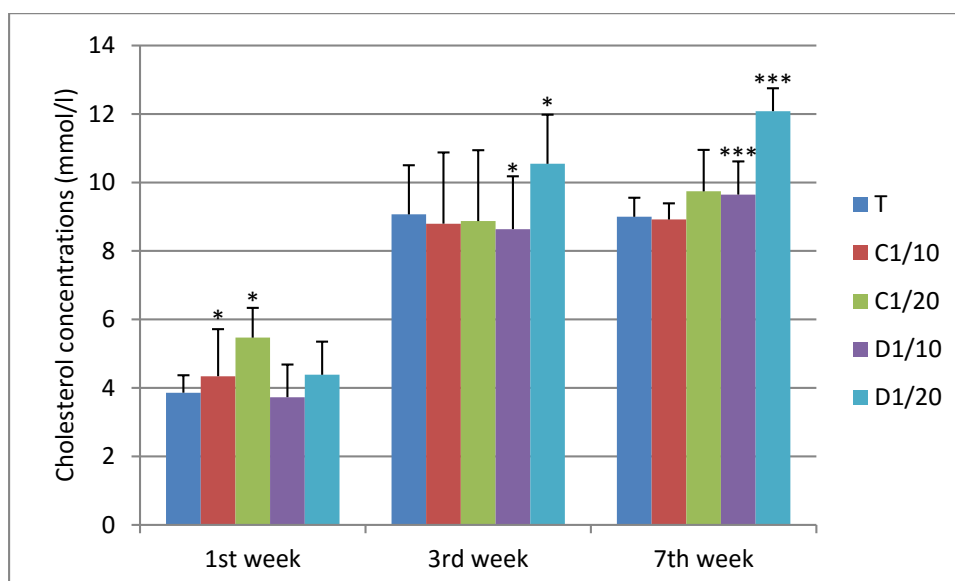


Figure 4: Variation of cholesterol concentrations after 1, 3 and 7 weeks of treatment in control (T) and treated groups (C1/10, C1/20, D1/10 and D1/20) (mean ± SD, n=8) (P<0.05) * ;(P<0,001) ***

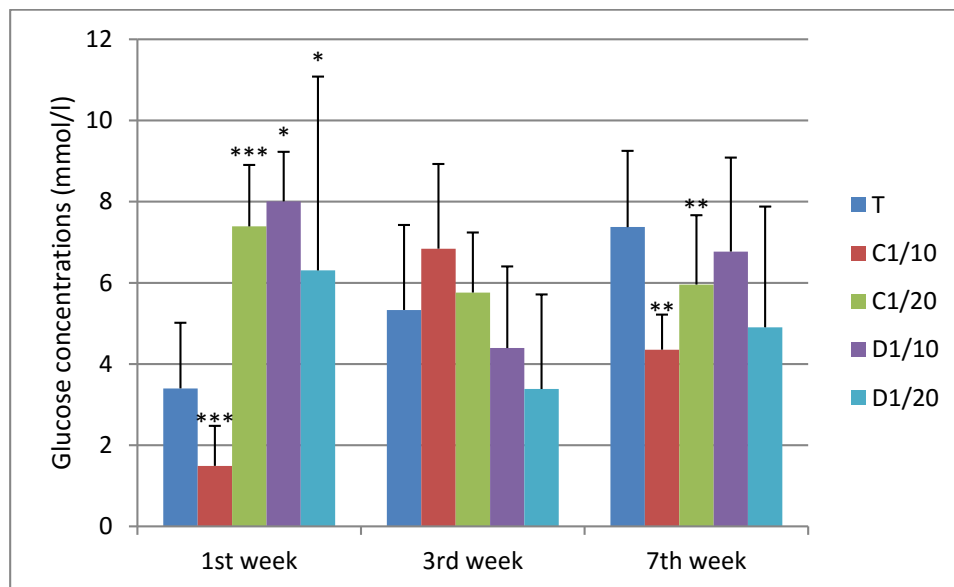


Figure 5: Variation of glucose concentrations after 1, 3 and 7 weeks of treatment in control (T) and treated groups (C1/10, C1/20, D1/10 and D1/20) (mean ± SD, n=8) (P<0.05) * ;(P<0,01) ** ;(P<0,001) ***

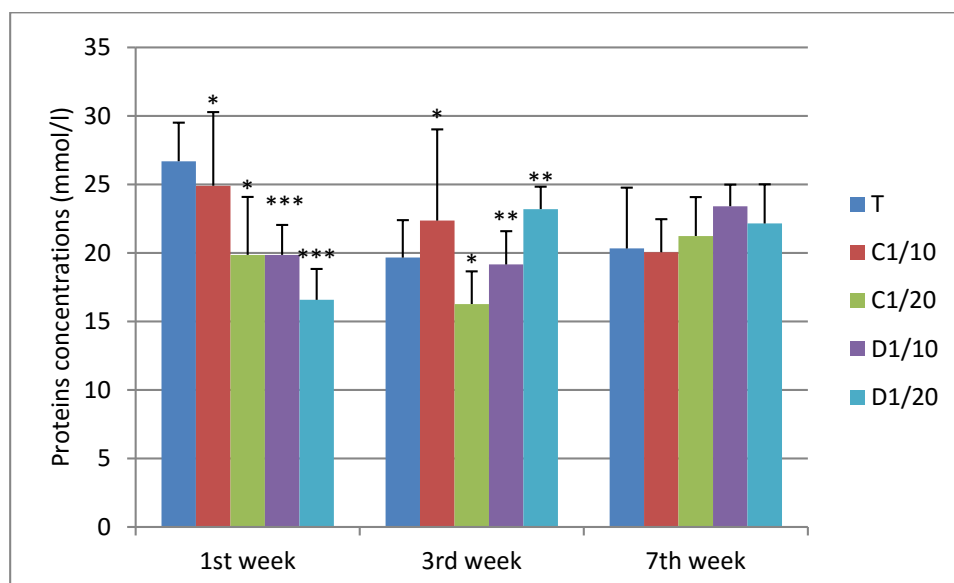


Figure 6: Variation of total proteins concentrations after 1, 3 and 7 weeks of treatment in control (T) and treated groups (C1/10, C1/20, D1/10 and D1/20) (mean ± SD, n=8) (P<0.05) * ;(P<0,01) ** ;(P<0,001) ***

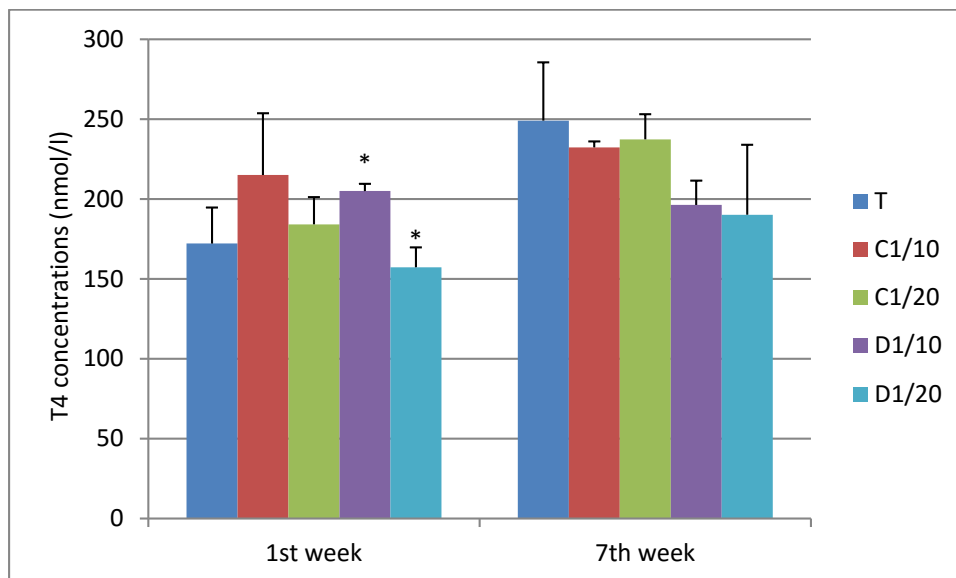


Figure 7: Variation of T4 concentrations after 1, 3 and 7 weeks of treatment in control (T) and treated groups (C1/10, C1/20, D1/10 and D1/20) (mean ± SD, n=8) (P<0.05) *

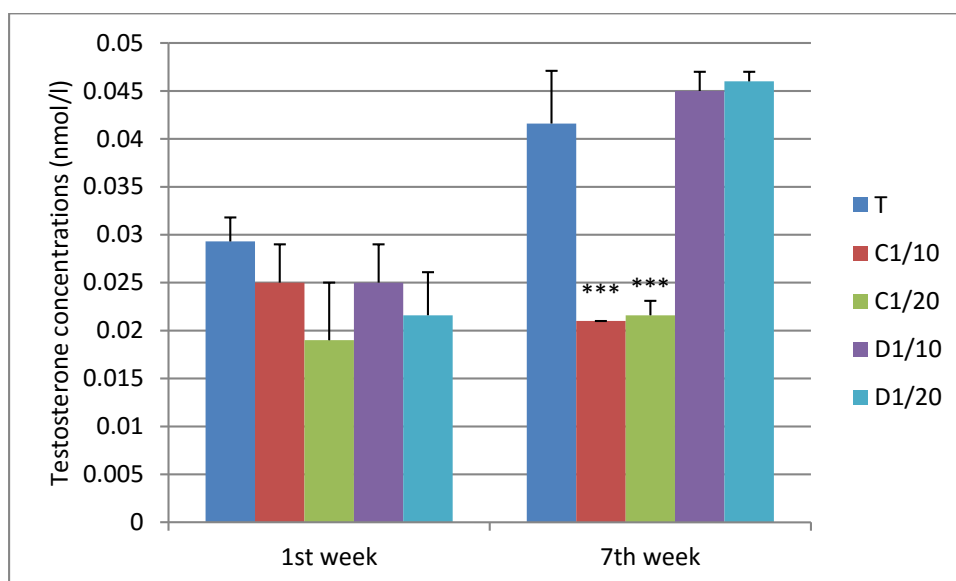


Figure 8: Variation of the testosterone concentrations after 1 and 7 weeks of treatment in control (T) and treated groups (C1/10, C1/20, D1/10 and D1/20) (mean ± SD, n=8) (P<0,001) ***