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Effects of Cypermethrin on Growth, Biochemical and Reproductive Parameters in Female Japanese Quails (*Coturnix japonica*)

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Abstract

The present study was highlighted to evaluate the effects of cypermethrin on growth and reproductive parameters on female Japanese quails. Seventy-two female Japanese quails aged 5 weeks, were divided into four groups of 18 birds each and subjected to the following treatments; treatment 0 (T0) received no concentration of cypermethrin in its feed; treatment 1 (T1) received 130mg of cypermethrin/kg of feed; treatment 2 (T2) received 285 mg of cypermethrin/kg of feed; treatment 3 (T3) received 655 mg of cypermethrin/kg of feed. The results revealed that exposure to cypermethrin caused a decrease in the body weight gain in a dose-dependent manner. This insecticide caused a significant ($P<0.05$) increase in the weight of the kidney and the liver of treated groups compared to the control treatment. Cypermethrin 36% also significantly ($P<0.05$) increased the level AST, ALT, and creatinine of the treated groups compared to the control group. Reproductive parameters revealed a significant ($P<0.05$) decrease in the hatching rate of treatment 3 (T3) compared to the control. In addition, the level of serum estradiol significantly decreased in the treated groups compared to the control group, this decrease was in a dose-dependent manner. Histopathological analyses revealed hepatic necrosis, cellular infiltration, and vascular congestion in the liver of the treated groups. Meanwhile in the kidney histopathological examination revealed glomerulonephritis, leucocytic infiltration, and mesangial expansion. Indeed, cypermethrin has an adverse effect on body weight gain, caused hepatotoxicity, nephrotoxicity as well as reproductive toxicity in female quails.

Keywords: Japanese quail, Cypermethrin, Biochemical parameters, Hepatotoxicity, Reproductive toxicity

Introduction

The global population is rapidly increasing, it was estimated at 7.35 billion in 2015 and will approximately be 8.5 billion in 2030 (1). As a result of the increased population, food production capacity faces a declining ratio of arable land available to the population. Thus, the provision of adequate food supply to such a population remains a challenge for many countries and institutions (2). Sustainable and intensive agricultural production then uses pesticides to prevent, control or destroy pests in order to increase crop production and maximize yield. Besides this role, pesticides are also useful in the elimination of vector-borne diseases (3). Pesticides are classified into organochlorines, organophosphorus, carbamates, neonicotinoids, and pyrethroids. Due to the ban of long-lasting organochlorine pesticides, Pyrethroids are among the latest developed pesticide groups. However, the misuse of pesticides will result in the destruction of natural vegetation and are therefore harmful to non-targeted organisms which are of concern to public health (4). Some pesticides have adverse effects on reproduction, such as the case of cypermethrin (5).

Cypermethrin is the most common synthetic pyrethroids with high insecticidal activity and is presented in the form of an emulsifiable concentrate or wettable powder (6). Cypermethrin is used to control a wide range of pests including moth pests of cereal, fruit, and vegetable crops. Cypermethrin is also used for environmental and hygienic purposes such as control of insect pests in stores, industrial buildings, houses, laboratories, and means of transports (6). In veterinary medicine, Cypermethrin serves as a means to control flies and other insects in the animal houses and on animals thus ectoparasiticide, as well as in public health for the control of insects (mosquitoes, houseflies, cockroaches, etc.) (7). Because of so much usage, this pesticide may get into the human system or non-intended animals, through direct or indirect exposure. Interestingly, cypermethrin residues have been found in many food commodities including cereals, fresh vegetables, water sources, and aquatic animals (8).

Invertebrates and invertebrates, cypermethrin acts mainly on the nervous system. Cypermethrin is a stomach poison and a contact insecticide (6). Mechanistically it can induce damage to the

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voltage-dependent sodium channel, causing sodium channels to stay open much longer than normal and inhibit ATPase enzymes involved in the movement of ions against a concentration gradient which are regulated by active transport. These molecular disturbances may prompt certain toxicity of cypermethrin at the macroscopic level in a living organism (8).

Cypermethrin has been used worldwide since 1977, and for different purposes in Africa since 1980 (9). Having in mind the properties and extensive use in significant quantities of cypermethrin, there is a risk of the presence of residues in the food for humans and animals. Its presence is possible in environmental and occupational settings, thus cypermethrin is a potential contaminant of human and animal food. In order to determine safety limits in the light of the potential adverse biological effects, it is therefore important to test for the presence of cypermethrin in human and animal foods and to detect possible adverse effects on humans and other beneficial organisms (mammals, birds, fish).

Much has been done on the adverse effects of cypermethrin on mammals but very little has been done on birds, the reason for this present study. The aim of this study is to determine the potential adverse reproductive effects of dietary cypermethrin on female quails. Specifically, on

- Growth parameters: Feed intake, live body weight, body weight gain
- Toxic indicators: weights and measurements of vital organs (kidney, liver and heart), and toxic biomarkers (AST, ALT, Creatinine, Urea)
- Reproductive parameters: Estradiol concentration in blood, fertility rate, and hatchability rate

Material and Methods

Ethical approval

Experimental protocols used in this study were approved by the ethic comity of the school of veterinary medicine and sciences, University of Ngaoundere, Cameroon and strictly conformed with the internationally accepted standard ethical guidelines for laboratory animal use and care as described in the European Community guidelines; EEC Directive 86/609/EEC, of the 24th November 1986.

Study Area

The present study was carried out from May to November 2019 in Ngaoundere which is the capital of the Adamawa Region of Cameroon. Ngaoundere is a cosmopolitan town located on the Adamawa plateau (7°- 8° N and 13°- 14°E) in the Sudano-Guinean ecological zone of Cameroon.

Birds

Seventy-two female Japanese quails (Coturnix japonica) aged 5weeks from a local farm were used to carry out this study. The birds were randomly allotted to four groups. They were identified by cage card and leg band numbers.

Husbandry Conditions

The birds were housed under standard Laboratory conditions: 10 – 12 filtered air changes per hour, room temperature: 17 – 27 °C, relative humidity: 50 – 75%, with 12-hour light and 12-hour dark cycle.

Lodging

The birds were kept in a galvanized wire mesh cage (size: L70 x W 90 x H 22 cm) arranged in 3 tier system and with detachable litter trays, egg rolling facilities, and provisions for feeding and watering in feed and water dispensers. They were handled according to ethical guidelines of the Cameroon National Veterinary Laboratory.

Feeding

The birds were fed with feed containing 20.11% of crude protein and 2902,70 kcal of metabolizable energy.

Pesticide used

The pesticide used was Cypermethrin 36 % (360 g/L), commercially called Cigogne. It was obtained from Louis Dreyfus Commodities Cameroon.

Experimental design

Seventy-two (72) female quails were divided into 12 batches of 6 quails each. Each of the 4 experimental rations 0, 130, 285, 655 mg/kg of feed corresponding to T0, T1, T2, T3 were attributed to 3 batches in a completely randomized design (The 4 treatments were repeated 3 times each). Water and feed were given ad libitum throughout the assay meanwhile the different groups were conducted under similar environmental and managerial conditions (lodging, feeding, and prophylaxis).

Data collection and studied parameters

During the assay (before sacrifice) data was collected on feed intake, live body weight, body weight gain, and temperature. After sacrifice, blood samples were collected for biochemical dosage, weights, and organ measurement (heart, liver, ovary, and kidney), then histological analysis of the ovaries, livers, and kidneys.

Growth parameters

Feed Intake: A certain quantity of feed was weighed (using a 1g precision electronic scale and 5kg capacity) and distributed to every experimental unit of the assay. The remains were weighed, and the weekly feed intake of animals from each experiment unit was obtained by differentiating between the amount of feed distributed and the remains.

Toxic indicators

Temperature: The body temperature of animals in every experimental unit was taken every two days. It was taken at the level of the rectum with an electronic thermometer.

Weight of detoxifying organs and the heart: After sacrificing the animals, the liver, the heart, and the kidney were collected and the fatty tissues removed then weighed on a 0.01g precision electronic scale.

Biochemical markers of the hepatic and renal functions: Creatinine, urea, aspartate aminotransferase (AST), alanine aminotransferase (ALT) was quantified in the serum using an automated analyzer (Cobas C311). This was done by the kinetic method according to the technical kit form (Roche).

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Reproductive parameters

Upon removal of the chicks from the hatchers all unhatched eggs were opened and examined to determine fertility percentage, hatchability percentage of total eggs.

Fertility rate: The fertility rate was determined based on the total eggs set. Percentage fertility was expressed as:

$$\textbf{Fertility}\ (\%) = 100\ \text{x}\frac{\text{No. of hatched eggs} + \text{No. of eggs containing embryo}}{\text{total No. incubated eggs}}$$

Hatchability: This was expressed on the basis of total eggs set.

$$\textbf{Hatchibility of total eggs}\ (\%) = 100\ \text{x}\frac{\text{No. of hatched chicks}}{\text{total No. incubated eggs}}$$

Concentration of serum estradiol

The serum concentration of estradiol was determined with the help of the Biorex diagnostic ELISA kit (Park Antrim, United Kingdom). It was done according to the ELISA procedure explained by the commercial kit. The concentrations of Estradiol were determined by extrapolating from the absorbance of the standard linked to the ELISA.

Histopathological analysis

The liver and left kidney were excised from all rats and fixed in 10% neutral formalin buffer. Tissue sections (5-µm thick) were cut and stained with hematoxylin and eosin for histopathological studies.

Statistical analysis

Values are presented as Mean ± SD. ANOVA was performed for comparison with the posthoc Duncan test to compare the level of significance between the control and experimental groups. A value of $p \leq 0.05$ was considered statistically significant. Statistical analyses were performed with the aid of SPSS for Windows software program (Release 25.0).

Results

Feed intake

Figure 1 shows the weekly variation of feed intake on quails with respect to the different concentrations of cypermethrin. The weekly feed intake evolved in an irregular manner. It increased generally for all the treatments with the control treatment having the highest values. Certain weeks (2 and 4) of the assay had control treatment with a significantly higher feed intake compared to the others. At the end of the assay (week 6) the obtained results revealed that there were no significant differences between the treatments ($P \geq 0.05$), the highest value was obtained in the control group (180.03±16.4g) meanwhile the lowest was obtained in the T3 group (70.36±14.02). We observed a drastic increase in feed intake between week1 (72.21±7.40g) and week 2 (164.09±14.47g).

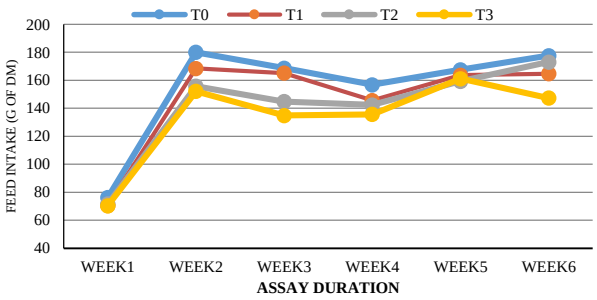

Figure 1. Variation of feed intake of female quails throughout the treatment period. T0, T1, T2 and T3 corresponding to groups having 0, 130, 285 and 655 mg of Cypermethrin /kg of feed.

Live Bodyweight

There was a general increase in life body weight throughout the assay except for the last week where we had a decrease in LBW. The control group had a higher LBW in the first, fourth and fifth week, followed by the T1 treatment (Figure 2). All the same there was no significant differences in weight between the different treatment ($P>0.05$).

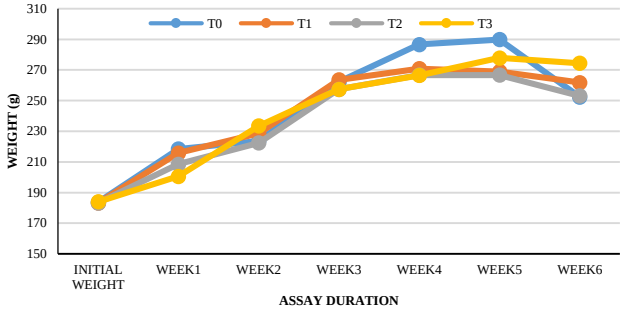

Figure 2. Variation of live body weight with respect to different doses of cypermethrin. T0, T1, T2 and T3 corresponding to groups having 0, 130, 285 and 655 mg of Cypermethrin /kg of feed.

Bodyweight gain

The bodyweight gain reduced in the treated groups in a dose related manner. We observed a 135.52% terminal weight at the control treatment, that's 82.93 g gain of bodyweight which is the highest bodyweight gain of the assay. The least bodyweight gain of bodyweight was observed in the T3 group which had a bodyweight gain of 74.82 g (Table 1).

Table 1. The effect of Cypermethrin on body weight gain for over 6 weeks of exposure

Treatment	Initial Weight	Terminal Weight	Body Weight Gain	Initial Weight	Terminal Weight
T0	189.93±3.68	100.00	272.38±8.79	135.52	82.93
T1	189.20±4.84	100.0M	269.76±6.98	142.57	80.56
T2	190.41±9.09	100.0	268.11±10.07	140.80	75.65
T3	192.61±5.89	100.0	267.43±12.56	140.40	74.82

T0, T1, T2 and T3 corresponding to groups having 0, 130, 285 and 655 mg of Cypermethrin /kg of feed. Values are expressed as means ± SD.

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Nephrotoxic biomarkers

The serum concentration of creatinine was obtained least in the control treatment and was comparable to that of the T1 treatment (10.23±0.60 mg/dl) and T2 (10.51±0.84 mg/dl). However, the serum concentration of creatinine is significantly higher in the T3 (0.87±0.18 mg/dl) group than in the control group (0.87±0.18 mg/dl) (Table 2).

The serum concentration of urea was comparable to the control treatment and the other treatments, however, there was an increase in the cypermethrin-treated groups though not significant (P>0.05) with reference to the control group.

Table 2. Effects of cypermethrin exposure on nephrotoxic biomarkers

Treatment	Urea (mg/dl)	Creatinine (mg/dl)
T0	9.86±0 .29[a]	0.41±0.18[a]
T1	10.23±0.60[a]	0.50±0.23[a]
T2	10.51±0,84[a]	0.84±0.27[b]
T3	11.43±0.46[a]	0.87±0.18[b]

Means with the same superscript (a, b) in the same column do not significantly (P>0.05) differ from each other. T0, T1, T2 and T3 corresponding to groups having 0, 130, 285 and 655 mg of Cypermethrin /kg of feed. Values are expressed as means ± SD.

Hepatotoxicity biomarkers

There was a significant difference (P>0.05) in the serum concentration of ALT between the treatment and the control group (7.84±11.65). We observed an increase in the concentration of ALT with respect to the cypermethrin-treated groups in a dose-dependent manner.

There was a significant difference between the control and the groups exposed to cypermethrin. The highest value of serum concentration of AST was obtained in the T3 group (143.06±42.57 U/L), with the control group (57.70±52.85 U/L) having the lowest serum concentration of AST. However, no significant (P>0.05) difference was obtained between the T3, T2, and T1 treatments (Table 3).

Table 3. Effects of cypermethrin exposure on hepatotoxic biomarkers

Treatment	AST (U/L)	ALT (U/L)
T0	57.70±52.85[a]	7.84±11.65[a]
T1	141.53±65.31[b]	12.38±6.51[b]
T2	115.70±62.36[ab]	14.12±6.65[b]
T3	143.06±42.57[b]	14.12±6.65[b]

Means with the same superscript (a, b) in the same column do not significantly (P>0.05) differ from each other. T0, T1, T2 and T3 corresponding to groups having 0, 130, 285 and 655 mg of Cypermethrin /kg of feed. Values are expressed as means ± SD.

Weight of detoxifying organs (kidney and liver) and heart

The heart weights were significantly (P<0.05) different between the control group and the cypermethrin-treated groups. The control group being significantly (P<0.05) higher than the treated group. The heart was heaviest in the control (2.02±0.38g) group meanwhile the least weight was obtained in the T3 group (1.62±0.22g).

The weight of the kidney was significantly (P<0.05) different between the control group (1.23±0.15g) and the treated groups. The weight of the kidney was obtained lowest in the control group and highest in the T3 (1.82±0.44g) group. Hence, the weight of the kidney increases in a dose-dependent manner.

From the results obtained, there was a significant (P<0.05) difference in the weight of the liver between the control and the groups exposed to cypermethrin. The control group (4.78±1.08g) having the least weight meanwhile the heaviest liver was obtained in the T3 (7.20±1.91g) group. Hence the weight of the liver increased in a dose-dependent manner (Table 4).

Table 4. Effects of cypermethrin on the weight of detoxifying organs

Treatment	Heart (G)	Kidney (G)	Liver (G)
T0	2.02±0.24[b]	1.23±0.15[a]	4.78±1.08[a]
T1	1.69±0.46[a]	1.59±0.35[ab]	5.78±1.45[ab]
T2	1.64±0.38[a]	1.80±0.58[b]	5.96±1.6[ab]
T3	1.62±0.22[a]	1.82±0.44[b]	7.20±1.91[b]

Means with the same superscript (a, b, c) in the same column do not significantly (P>0.05) differ from each other. T0, T1, T2 and T3 corresponding to groups having 0, 130, 285 and 655 mg of Cypermethrin /kg of feed. Values are expressed as means ± SD.

Effect of cypermethrin on reproductive traits

Fertility rate and Hatching rate

The fertility rate decreases in a dose-dependent manner. The control (91.61%) treatment had the highest fertility rate and the T3 (82.43%) group recorded the least fertility rate. However, fertility rates were comparable to the control and test groups in all doses. There was a slight decrease in the fertility ratio of T1, T2, and T3 when compared to the control groups (T0). Therefore, no significant difference (P>0.05) between the treated and the control groups (Table 5).

We observed a reduction in the hatching rate with an increase in the cypermethrin dose (Table 5). The hatching rates were lower in the treated doses compared to the control group. Evidently, the control group having a hatching rate significantly (P<0.05) higher than the T2 (58.22%) and T3 (49.04%) groups.

Table 5. Effects of Cypermethrin on the Fertility and Hatching rates of female Japanese quails

Treatment	F.R. (%)	H.R. (%)
T0	91.61±11.0[a]	63.50±22.1[b]
T1	88.93±13.6[a]	58.22±15.3[ab]
T2	88.16±11.3[a]	51.47±20.1[a]
T3	82.43±18.3[a]	49.04±18.9[a]

Means with the same superscript (a, b) in the same column do not significantly (P>0.05) differ from each other. T0, T1, T2 and T3 corresponding to groups having 0, 130, 285 and 655 mg of Cypermethrin /kg of feed. Values are expressed as means ± SD.

Estradiol Serum concentration

The estradiol concentration in female quails reduced in a dose-dependent manner with respect to the treatments, with the highest been in the control group and the lowest in the T3 group. There was a significant (P<0.05) difference between the control group (217 pg/mL) and the cypermethrin-treated female quails of the T3 group (445 pg/mL). However, the serum estradiol concentrations were comparable between the treated groups with no significant difference between them (Figure 3).

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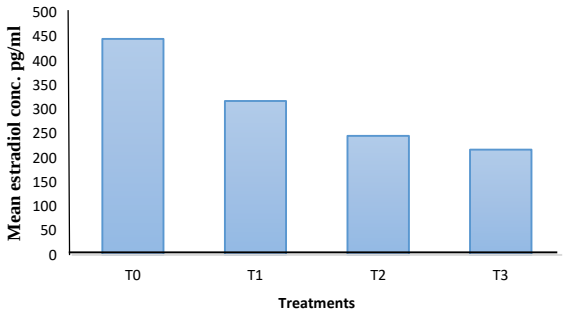

Figure 3. Variation of serum oestradiol concentration in cypermethrin-treated female quail

Cypermethrin effect on histological structure of the liver and the kidney

The histological structure of liver

The histological section of the control group (T0) showed normal architecture of the hepatic parenchyma tissue with a centro-lobular vein and normal hepatocytes. That of T1 showed touched hepatic parenchyma with hepatic steatosis and vascular congestion besides the centro-lobular vein of the liver. That of T2 showed touched hepatic parenchyma with hepatic necrosis, hepatic steatosis, and vascular congestion besides the Centro lobular vein. The T3 showed touched hepatic parenchyma with hepatic steatosis, hepatic necrosis, and vascular congestion besides the Centro-lobular vein (Figure 4).

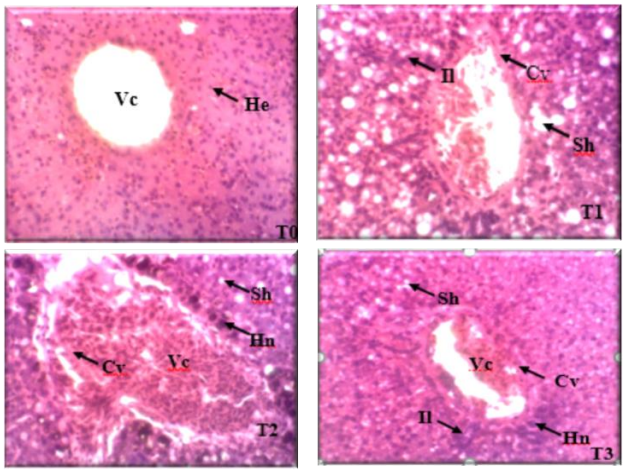

Figure 4. Microphotography of the liver of female Japanese quails (hematoxyline-eosine X 200) T0 T1, T2 and T3 corresponding to groups having 0, 130, 285 and 655 mg of Cypermethrin /kg of feed. Vc=Centro-lobular vein; He=Hepatocyte; Sh=Hepatic steatosis; Il=Leucocytic infiltration; Hn=Hepatocytic necrosis; Cv=Vascular Congestion; T0, T1, T2 and T3 corresponding to groups having 0, 130, 285 and 655 mg of Cypermethrin /kg of feed.

Histological structure of kidney

The histological analysis of the kidney of the animal in the control group showed a normal disposition of the principal elements of the kidney (Glomerulus, proximal convoluted tubule, and the distal convoluted tubule) equally the case of the T1 group. The T2 group showed a mesangial infiltration besides the glomerulus of the kidney. The T3 group showed a touched kidney with leucocyte infiltration and glomerulonephritis (Figure 5).

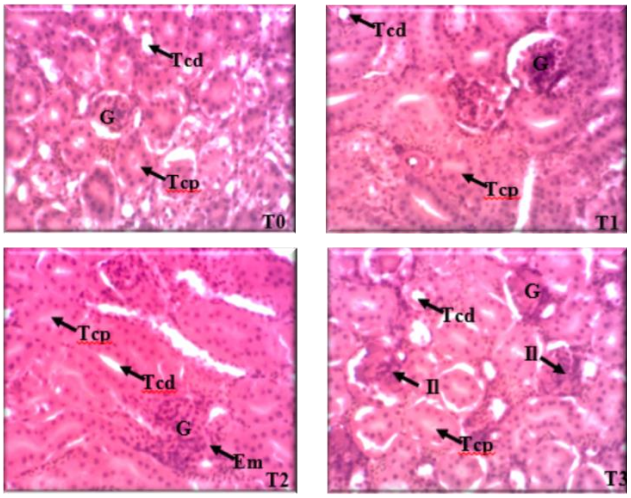

Figure 5. Microphotography of the kidney of female Japanese quails (hematoxyline-eosine X 200) T0, T1, T2 and T3 corresponding to 0, 130, 285 and 655 mg of Cypermethrin /kg of feed. G=Glomerulus; Tcd=Distal Convoluted tubule; Tcp=Proximal convoluted tubule; Em=Mesangial Expansion; Il=leucocytic Infiltration; T0, T1, T2 and T3 corresponding to groups having 0, 130, 285 and 655 mg of Cypermethrin /kg of feed.

Discussion

In this study, it is evident that cypermethrin administered mixed with feed for 42 days (all three doses) did not significantly affect the feed intake of the quails. The small differences registered, were not statistically significant when compared to the control. This statistical non-significance could be explained by the sexual maturity of the hens during the assay, hence nutritive demands increase irrespective of the physiological conditions. Female Japanese quails enter sexual maturity at the age of 6weeks. These findings are in agreement with the results obtained by other authors in similar studies on cypermethrin using chickens (7). These results are as well in line with other researchers who used deltamethrin on laying hens (10, 11). This contradicts Vemo et al., (12) who observed a significant increase in feed intake on guinea pigs treated with cypermethrin at doses of 92, 137.5, and 275 mg/ml/kg of body weight for 90 days with respect to the control group. Equally, Ngoula et al. (13) observed an increase in feed intake in rats exposed to 62,5 and 125 mg/kg of methylpirimiphos (organophosphate) meanwhile Yahia (14) observed a significant decrease in feed intake on Wistar rats exposed to mancozebe (dithiocarbamate) (500 and 1000mg/kg of feed) for 8 weeks.

Despite unlimited access to feed, there was a decrease in live body weight and body weight gain in quails exposed to cypermethrin with respect to those of the control group. This decrease in body weight gain is not directly linked to the feed intake which had no significant difference between the control group and those exposed to cypermethrin. The harmful effect of

cypermethrin could be due to poor absorption of feed at the level of the gastrointestinal tract as well, it would appear that this reduction in live body weight is a clear indication of general toxicity in Japanese quails. These results are in line with those obtained by Herman et al. (15) on quails exposed to an insecticide commercialized as Antouka super® (Pirimiphos-methyl 16% and Permethrin 3%) at doses of 37.5, 56.25, and 75 mg/kg. Equally, Elbetieha et al. (16) observed a decrease in body weight gain in rats exposed to 18.93 and 36.66 mg/animal/day of cypermethrin for 3 weeks. Rats exposed to topical permethrin for 10 days showed a decrease in live body weight with respect to those of the control (17). According to Djeffal (18), administering 8mg/kg of methomyl on rats caused a significant decrease in the body weight gain in the exposed units with respect to the control units. Equally, Ngoula et al. (13) observed a decrease in live body weight in rats exposed to 62.5 and 125 mg/kg of Methyl-pirimiphos (an organophosphate insecticide). However, some studies contradict these results, the case of Guinea pigs treated with cypermethrin at doses of 92, 137.5, and 275 mg/ml/kg of body weight for 90 days (12). Equally according to Yahia (14) on Wistar rats observed an increase in body weight in groups treated with 500 and 1000 mg/kg for 8 weeks.

Evaluating the weight of detoxifying organs is of great importance in the assessment of the potential toxicity of a substance (19). The kidney and the liver are greatly associated with the metabolism and the excretion of toxic substances like pesticides (20). The liver is the most sensible and most susceptible organ to a xenobiotic, it has an important role in the metabolism and excretion of insecticides (20). In this study, the weight of the liver increased significantly in quails exposed to cypermethrin, this concords with the results obtained by Mossa et al. (20) on rats treated with 13.8mg of cypermethrin per body weight. The increase in the weight of the liver could be attributed to the increase in its detoxifying intensity on toxic compounds (21). In addition, this damage could be due to an increase in the permeability of hepatocytes due to oxidative stress caused by cypermethrin (12). These results are equally in accordance with those of Vemo et al. (12) who obtained an increase in the weight of liver of guinea pigs exposed to cypermethrin at doses of 92, 137.5, and 275 mg/kg of body weight. Equally, Mossa et al. (20) in rats were exposed to 13.8mg of cypermethrin per body weight. Studies on other pesticides show similar results. That's the case for Djeffal (18) who observed an increase in liver weight in rats treated with 8mg of methomyl/kg of body weight. As well, Dragica et al. (22) observed an increase in the weight of the liver of rats exposed to 25, 100, and 400 ppm of carbofuran for 90 days. Yahia (14) observed on Wistar rats an increase in the weight of the liver in different groups treated for 8 weeks with mancozebe (250, 500, and 1000 mg/kg). Boulakoud-Chouabia (23) equally observed an increase in the weight of the liver of rabbits treated with Vacomil at a dose of 27.5 mg/kg for 21 days. On the other hand, female rats which were exposed to 50mg/kg of cypermethrin for a period of 2 to 4 weeks revealed a significant decrease in liver weight (24). Hussien et al. (25) obtained similar results when rats were exposed to 500mg/kg of cypermethrin for 4 weeks. Li et al. (26) also observed a decrease in liver weight in rats exposed to 60mg/kg of body weight of cypermethrin. This decrease could be due to the destruction of hepatocytes or secondly by a mechanism of detoxifying hepatocytes hence using the glycogen which is the main energy source in this organ.

The results which were obtained from this study show a significant increase in renal weight of quails exposed to cypermethrin compared to the control group. This may be explained by the hypertrophy of the tissue of this organ due to the toxic effect of cypermethrin. This result is similar to that observed by Djeffal (18) in rats submitted to methomyl and that of Li et al. (26) on rats exposed to 60mg/kg of LBW of cypermethrin. Mossa and Abbassy (27) studied the adverse effects of exposure to formulated chlorpyrifos-ethyl and chlorpyrifos-methyl on male rats for 90 consecutive days. They found a significant decrease in body weight gain and an increase in relative liver and kidney weights in treated rats. Chlorpyrifos exposure caused a decrease in cell proliferation and cell membrane damage in rabbit kidneys, rat, and murine livers (28). Changes in kidney weight may reflect renal toxicity, tubular hypertrophy, or chronic progressive nephropathy (29). On the other hand, Rezzag and Serouti (30) observed a decrease in kidney weight in rabbits exposed to metribuzine which is contradictory to our present study, this reduction in weight could be explained by the damage of this organ by cypermethrin before its excretion.

Our result showed a significant decrease in the weight of the heart in the groups treated with cypermethrin compared to the control group. This is in agreement with that obtained by Boulakoud-Chouabia (23) who observed a decrease in the weight of the heart of birds exposed to 4mg/l of vacomil. Equally, these results are similar to those obtained by Aslam et al. (31) on broilers chicken exposed to cypermethrin at the dose of 600mg/kg of LBW for 30 days. Mossa and Abbassy (27) observed an increase in heart weight due to the exposition of male mice to methylchlorpyrifos and ethyl chlorpyrifos for 90 days which contradicts the result we obtained. Equally, El-Tawil (32) observes an increase in heart weight in mice treated with chlorpyrifos (10mg/kg bw.) for 12 weeks.

The attack of the liver and kidneys can be more appreciated by evaluating biochemical parameters (33). Generally, increased enzyme concentrations are a measure of recent organ damage rather than decreased organ function. The increase of plasma AST and ALT activity is the most specific indicator of muscle and liver cell damage (34). The significant increases in plasma AST activity could therefore be the most specific indicators of liver lesions in Japanese quail intoxicated by cypermethrin. In this assay, the quails exposed to cypermethrin observed a significant increase in the concentrations of AST, ALT, and Creatinine, however, there was no increase in the urea concentration.

Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) are enzymes produced in the cytoplasm of hepatocytes and in the muscles. They are used in clinical studies to assess the damage to the liver (33). The significant increase in ALAT and ASAT levels is in line with many other studies on pesticides like that of Ibiang et al. (35) on mice exposed to 100mg/kg of body weight of deltamethrin. Equally, El-Shemi et al. (36) showed an increase in AST and ALT on goats treated with 6 and 12 mg/kg of LBW of cypermethrin. This increase might be due to the intensification of the secretory activity of the liver (37). It could also be explained by the alteration of the membrane permeability of hepatocytes, leading to the escape of tissue enzymes into the hepatic plasma (38). On the other hand,

the results obtained in this study are contradictory to Rezzag and Serouti (30) on rabbits entubed with metribuzine. The increases in plasma AST activity were the most specific indicators of liver disease in the racing pigeons intoxicated by ethylene glycol (34).

The above findings were confirmed by histopathological changes in the liver under the intoxication effect of cypermethrin. This study observed marked damage of the liver tissues in the form of hepatic steatosis, vascular congestion of the centro-lobular vein, and hepatic necrosis affecting quails exposed to cypermethrin. The changes in this histoarchitecture of the liver could be due to the toxic effect of cypermethrin which touches the structure of hepatocytes causing its dysfunction. These results are similar to those of Khaldoun-Oularbi et al. (39), who observed vascular congestion and cellular filtration in mice exposed to abamectine (insecticide) at the dose of 2,13mg/animal/day for 28 days. Equally Djeffal (18) observed congestion at the level of the centro-lobular vein of the liver, in rats treated with 8mg/kg of body weight of methomyl. Mamun et al. (40) observed leucocytic infiltration and congestion of the blood vessel in the liver of mice exposed to carbamate. Goel et al. (41) described marked alterations of hepatic pathology after 8 weeks of treatment in chlorpyrifos intoxicated rats.

The kidneys being the major detoxifying organs for many xenobiotics are frequently susceptible to the nephrotoxic effects. The elevated serum level of creatinine is considered to be the marker of renal dysfunction (42). The administration of cypermethrin significantly increased the serum creatinine level in test animals as compared to control animals. The increase in creatinine also correlated closely with the histopathological changes in the kidney, which showed marked leucocytic infiltration, congestion, and glomerulonephritis. This is similar to Common et al. (43) who observed histopathological changes in the kidney of chicken exposed to imidaclopirid (IMC) at the unique dose of 104.1 mg/kg bw. The renal injuries in these studies were attributed to a direct action of the cypermethrin, causing tubular cell necrosis and mesangial expansion. The kidneys are the major detoxifying organs for most xenobiotics hence frequently susceptible to the nephrotoxic effects. Although histopathologic examination showed lesions in kidneys exposed to cypermethrin, the level of urea remained significantly unchanged between the treated groups and the control groups. This result contradicts the result of Common et al. (43) who showed that serum uric acid of imicaprid-treated chicken decreased in a dose-dependent manner.

In the present study, quails fed with cypermethrin in the diet for 6 weeks did not show any effects on the fertility rate. The fertility rate was comparable to the control and test groups in all doses. The fertility rate can be reduced through exposure to cholinesterase-inhibiting compounds and through a decrease in feed consumption (44, 45), and potentially through altered hormone levels. The treatment period was probably not long enough for the difference to be significant between the control group and the treated groups. This result is consistent with the result of Prakash et al. (46) who showed that the fertility rate of female Japanese quails fed with 1000 ppm of dietary endosulfan 35% (insecticide) for 20 weeks did not affect the fertility rate. The fertility rate may record a significant decrease if both mates (female and male) are exposed for lengthier period.

The results obtained from this study showed a significantly higher hatching rate in the control group compared to the treated group. These results are in line with that obtained by Herman et al. (15) who showed a decrease in hatching rate on quails exposed to Antouka Super® (cypermethrin) with respect to the control. The hatching rate may have reduced through exposure to cholinesterase-inhibiting compounds and through decrease feed intake.

The result of this study indicates a significant decrease in serum estrogen levels in adult female quails when treated with cypermethrin. In agreement with our findings, Victoria and Hope (47) reported that treatment of 24 female rats with cypermethrin at doses of 0, 15, 30, and 50 mg/kg observed a decrease in the estrogen level across the treated groups. According to the present study, Cypermethrin, a type II pyrethroid, may be responsible for the decrease in the estrogen level since pyrethroids have an affinity for androgen or estrogen receptors. One of the pathways in the synthesis of estrogen in the ovaries is the conversion of androgens (testosterone and androstenedione) into estrogens by enzyme aromatase in the granulosa cells; activity stimulated by FSH (48). This is in line with the findings of Trif et al., (49) who reported that the decrease in estrogen level could be the consequence of a decrease in FSH concentration as a result of chromium exposure which led to a decrease in aromatase in the granulosa cells and androgen transformation into estrogen.

Conclusion

Cypermethrin 36% (CYGOYNE) was assessed for its impact on biochemical parameters and reproductive toxicity in female Japanese quails. The observed increase in kidney and liver weight of cypermethrin-treated female quails are clear indications of nephrotoxicity and hepatotoxicity. As well, the observed increase in transaminase enzymes (AST and ALT) indicate recent damages to the liver which are in line with the increase in weight of the liver and confirm hepatotoxicity as well as an increase in the concentration of creatinine. However, there was no significant increase in the concentration of urea. The histopathological findings of these two organs (liver and kidney) helped in the assessment of the pathological changes of this organ. This clearly indicates the reproductive toxicity of cypermethrin on female Japanese quails.

Conflict of interest

The authors declare that there is no conflict of interest.

Authors' contribution

All authors of this study have a complete contribution for data collection, data analyses and manuscript writing.

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