

EFFECTS OF DIETARY CYPERMETHRIN ON CHICKENS

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In the following study effects of dietary cypermethrin in chickens were observed. Chickens were administered cypermethrin in the feed in nominal concentrations of 150, 300 and 600 ppm (mean measured concentrations: 130, 285 and 655 mg/kg feed) for a period of 28 days. The following parameters were monitored: clinical symptoms and mortality, feed consumption and body weight gain, liver weight, plasma ChE activity, hematological and biochemical effects. Any accumulation of cypermethrin in the liver, peritoneal fat, breast and leg muscle was monitored, as well.

The results obtained showed that cypermethrin added to the chickens feed did not significantly affect feed intake, body weight gain and liver weight. Also, cypermethrin did not adversely affect the activity of plasma ChE and ALT in the serum. Serum AST activity was significantly increased in the treated chickens, in males at 300 and 600 ppm and in females at all three tested doses. ALP activity was significantly decreased in comparison with the control, in males at doses 300 and 600 ppm, but in females only at the dose of 600 ppm. Hematological data showed that cypermethrin induced a statistically significant increase only in PLT number (both sexes, all three doses tested). The obtained results also showed that cypermethrin did not accumulate in organs and tissues of chickens.

Key words: biochemistry, chickens, cypermethrin, hematology, toxic effects

INTRODUCTION

Cypermethrin is a highly active synthetic pyrethroid with contact and stomach action. It is used as an insecticide in the control of a wide range of insects in agriculture (especially Lepidoptera, but also Coleoptera, Diptera, Hemiptera, etc), in veterinary medicine (for the control of flies and other insects in animal houses and as an animal ectoparasiticide) and in public health (control of mosquitoes, houseflies, cockroaches, etc.) (He, 1994; Miyamoto *et al.*, 1995; Tomlin, 2009). Cypermethrin is a neuropoisoning compound acting on the axons

in the peripheral and central nervous system in mammals and insects by interaction with sodium channels (WHO, 1989; Miyamoto *et al.*, 1995).

Cypermethrin has been used worldwide since 1977, and for different purposes in Serbia since 1980 (Mitić, 1980). It is still used in significant quantities. Having in mind the properties and extensive use of cypermethrin, its presence is possible in environmental and occupational settings, and there is a risk of presence of residues in the food for humans and animals. Namely, cypermethrin is a potential contaminant of human and animal food. All this points to the importance of testing for the presence of cypermethrin in human and animal foods and detecting possible adverse effects on humans and other beneficial organisms (mammals, birds, fish), in order to determine safety limits in the light of the potential adverse biological effects.

The aim of this study was to determine the potentially adverse biological effects of dietary cypermethrin on chickens, as well as the content of residues in organs and tissues.

MATERIALS AND METHODS

Experimental animals

Chickens (Arbor Acres Hybrid) of both sexes, and initial age 30 days were used in the experiment. In each group there were 12 birds (6 males and 6 females) kept in wire cages (two birds in each) at a constant temperature of $19 \pm 2^\circ\text{C}$ with a photoperiod of 12:12 hours. Feed and water were available *ad libitum*.

The birds were handled in accordance with the guidelines described by the Canadian Council on Animal Care (Olfert *et al.*, 1993).

Chemicals

Cypermethrin [(RS)- α -cyano-3-phenoxybenzyl (1RS, 3RS; 1RS, 3SR)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-carboxylate], technical, purity 93.0% (*cis-trans* isomers ratio 55:45) from FMC Corporation, USA, was used in the study. All other chemicals were standard commercial products of highest purity, purchased from different manufacturers.

Treatment

Cypermethrin in nominal concentrations of 150, 300 and 600 ppm (mean measured concentrations: 130, 285 and 655 mg/kg feed) was administered to the appropriate test groups of chickens in the feed, for a period of 28 days. Control animals were given the standard diet for chickens, cypermethrin-free. Diets containing cypermethrin were prepared every 2 days by mixing weighed amounts of the substance with standard chicken feed in a rotary mixer for 30 minutes. Samples of the prepared test diets were taken immediately after preparation (zero time) and after 2 days to determine the content, stability and homogeneity of cypermethrin in each test diet. Cypermethrin was determined by a gas-chromatographic method. Mean measured concentrations of cypermethrin ranged from 86 to 109% of the nominal concentrations and the measured concentrations were consistent throughout the study. In the assessment of adverse

effects, the following parameters were taken into consideration: feed consumption and body weight gain, liver weight, plasma ChE activity, and appropriate selected hematological and biochemical parameters.

Feed intake, body weight gain, organ weight

The chickens were weighed weekly, and the feed consumption measured daily. After 28-days, the birds were killed by cervical dislocation, blood and tissues of birds from each group taken, and prepared for further investigation. Residues of cypermethrin were determined in the liver, peritoneal fat, breast and leg muscle. Blood was subjected to hematological and biochemical analyses.

Enzyme activity

Cholinesterase (ChE) activity in plasma was determined according to the method of Ellman *et al.* (1961) and Bošković *et al.* (1984). Serum alkaline phosphatase (ALP) activity was determined according to the procedure described by McComb and Bowers (1972). Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined as described by Reitman and Frankel (1957) and Wootton (1964), respectively. In all cases, test kits from Randox Laboratories Ltd., UK, were used.

Hematology

Haemoglobin concentration (Hb), haemocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW), platelets (PLT), total white (WBC) and total red blood cell counts (RBC) were determined using a Cell Din 1700 (model CD-1700) automatic analyzer (Abbott Diagnostics, USA). Commercial test kits from Abbott were used.

Residue analysis

Cypermethrin content in the test diets and in selected organs and tissues (liver, peritoneal fat, breast and leg muscle) was determined by gas chromatography using the method described by Braun and Stanek (1982).

Statistical analysis

The results were processed using analysis of variance and Student's t-test (Vuković, 2000). The 0.05 and 0.01 levels of probability were employed as the criteria of statistical significance.

RESULTS

Feed intake, body weight gain and liver weight

The body weight gain and feed consumption of each group during the experiment are shown in Tables 1 and 2. The mean absolute and relative liver weights are given in Table 3. Cypermethrin did not significantly affect feed consumption, body weight gain or liver weight of the experimental chickens. The minor differences from the control group were not statistically significant.

Table 1. Feed consumption of chickens fed diets without and with cypermethrin for 28 days

Sex	Treatment	Dose (ppm)	Feed consumption (kg/chicken) ^a		
			Total (28 days)	Average daily	Percentage of control
Males	Control	0	4.732 ± 0.320	0.169 ± 0.020	100.00
	Cypermethrin	150	4.854 ± 0.415	0.173 ± 0.035	102.37
		300	4.956 ± 0.430	0.177 ± 0.041	104.73
		600	4.900 ± 0.390	0.175 ± 0.040	103.55
Females	Control	0	4.704 ± 0.290	0.168 ± 0.015	100.00
	Cypermethrin	150	4.564 ± 0.310	0.163 ± 0.020	97.02
		300	4.928 ± 0.420	0.176 ± 0.023	104.76
		600	4.984 ± 0.435	0.178 ± 0.028	105.95

^aMean ± SD (n=6)

Table 2. Body weight gain of chickens fed diets without and with added cypermethrin for 28 days

Sex	Treatment	Dose (ppm)	Initial weight ^a		Terminal weight ^a	
			(kg)	(%)	(kg)	(%)
Males	Control	0	1.37 ± 0.08	100.0	2.80 ± 0.08	204.4
	Cypermethrin	150	1.39 ± 0.08	100.0	2.80 ± 0.09	201.4
		300	1.34 ± 0.06	100.0	2.78 ± 0.09	204.5
		600	1.33 ± 0.07	100.0	2.69 ± 0.12	202.2
Females	Control	0	1.19 ± 0.06	100.0	2.53 ± 0.07	212.6
	Cypermethrin	150	1.20 ± 0.06	100.0	2.53 ± 0.08	210.8
		300	1.21 ± 0.07	100.0	2.59 ± 0.07	214.0
		600	1.16 ± 0.05	100.0	2.53 ± 0.06	218.1

^aMean ± SD (n=6)

Table 3. Body weight and liver weight of chickens fed diets without and with added cypermethrin for 28 days

Sex	Treatment	Dose (ppm)	Body weight ^a (kg)	Liver weight ^a (kg)	Index (%)
Males	Control	0	2.80 ± 0.15	0.124 ± 0.010	4.43
	Cypermethrin	150	2.80 ± 0.17	0.130 ± 0.015	4.64
		300	2.78 ± 0.17	0.122 ± 0.012	4.39
		600	2.69 ± 0.20	0.123 ± 0.012	4.57
Females	Control	0	2.53 ± 0.10	0.115 ± 0.009	4.54
	Cypermethrin	150	2.53 ± 0.17	0.120 ± 0.014	4.74
		300	2.59 ± 0.16	0.120 ± 0.008	4.63
		600	2.53 ± 0.17	0.118 ± 0.012	4.66

^aMean ± SD (n=6)

Table 4. Hematological data for chickens fed diets without and with added cypermethrin for 28 days^a

Sex	Treatment	Dose (ppm)	RBC (10 ¹² /L)	WBC (10 ⁹ /L)	Hb (g/L)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/L)	RDW (%)	PLT (10 ⁹ /L)	
Males	Control	0	2.62 ± 0.10	161.8 ± 53.1	135.67 ± 2.50	29.88 ± 1.20	113.98 ± 2.70	51.75 ± 1.50	454.5 ± 62.5	10.57 ± 1.10	11.5 ± 0.9	
		150	2.86 ± 0.15	158.3 ± 51.0	147.33 ± 2.75	32.70 ± 1.35	114.42 ± 2.90	51.55 ± 1.75	450.7 ± 59.1	10.45 ± 0.95	21.0 ± 2.1 ^b	
	Cypermethrin	300	2.68 ± 0.25	142.6 ± 43.9	143.33 ± 3.10	31.40 ± 1.09	117.25 ± 3.50	53.50 ± 2.15	456.7 ± 67.3	11.17 ± 1.23	44.3 ± 5.3 ^c	
		600	2.83 ± 0.22	160.4 ± 49.5	147.67 ± 2.65	32.22 ± 2.10	114.07 ± 3.25	52.28 ± 2.05	458.2 ± 71.0	10.97 ± 1.05	20.8 ± 2.0 ^b	
	Females	Control	0	2.56 ± 0.15	139.8 ± 41.1	133.00 ± 3.20	29.13 ± 1.36	114.02 ± 3.05	52.00 ± 2.45	456.2 ± 55.9	10.75 ± 2.05	13.5 ± 1.2
			150	2.70 ± 0.20	133.6 ± 37.5	138.33 ± 3.05	30.22 ± 1.90	112.28 ± 3.10	51.40 ± 2.20	457.8 ± 61.2	10.40 ± 1.90	30.0 ± 2.8 ^b
Cypermethrin		300	2.51 ± 0.15	129.2 ± 40.2	132.50 ± 2.90	28.60 ± 1.85	113.98 ± 2.70	52.85 ± 2.15	464.5 ± 69.0	10.43 ± 2.10	58.0 ± 6.3 ^c	
		600	2.68 ± 0.32	149.3 ± 47.3	139.17 ± 3.70	30.47 ± 2.05	113.73 ± 2.75	51.95 ± 3.05	456.8 ± 70.2	10.30 ± 2.07	47.2 ± 6.1 ^c	

a) Mean ± SD (n=6); b) Significantly different at p 0.05; c) Significantly different at p 0.01. Abbreviations: RBC = red blood cells; WBC = white blood cells; Hb = haemoglobin; HCT = haematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; RDW = red cell distribution width; PLT = platelets.

Table 5. Residues of cipermethrin in organs and tissues of chickens fed diets without and with added cypermethrin for 28 days

Treatment	Dose (ppm)	Residues (mg/kg) ^a			
		Liver	Peritoneal fat	Breast muscle	Leg muscle
Control	0	ND	ND	ND	ND
Cypermethrin	150	0.032 ± 0.002	0.026 ± 0.002	0.024 ± 0.005	0.018 ± 0.001
	300	0.045 ± 0.008	0.029 ± 0.004	0.019 ± 0.001	0.036 ± 0.012
	600	0.049 ± 0.008	0.024 ± 0.001	0.018 ± 0.001	0.032 ± 0.009

^aMean ± SD (n=6); ND = not detected

Enzyme activity

The effects of cypermethrin on plasma ChE activity of control and test chickens are shown in Figure 1, while the activities of ALP, AST and ALT found in serum from control and test chickens at the end of the 28-day period are given in Figure 2.

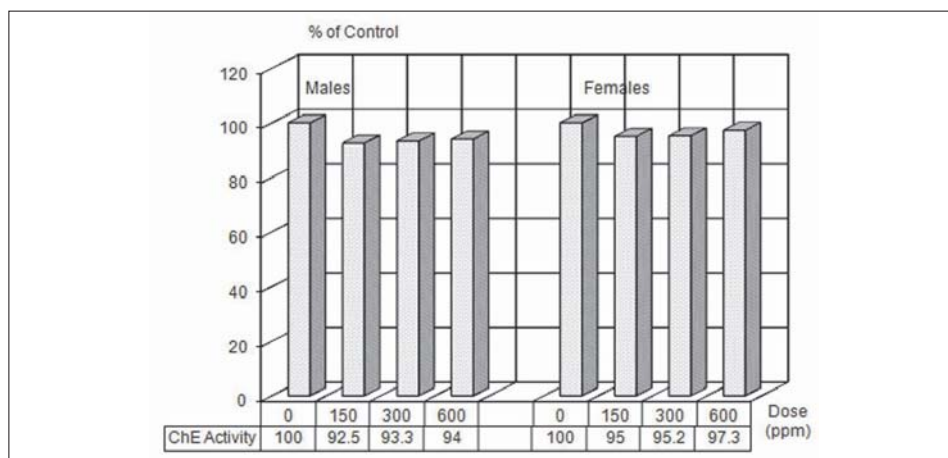


Figure 1. Plasma ChE activity of chickens fed diets without and with cypermethrin for 28 days. The data are expressed as a percentage of the control

As can be seen, all three doses led to a small decrease in plasma ChE activity in both sexes, but the differences from the value for the control group were not statistically significant. No dose of cypermethrin adversely affected serum ALT activity of the experimental chickens, but AST activity was significantly increased in female chickens at all dose levels and with 300 and 600 ppm cypermethrin in males. At the same time, ALP activity was significantly lower in male chickens which received 300 and 600 ppm, and females offered 600 ppm cypermethrin in the diet.

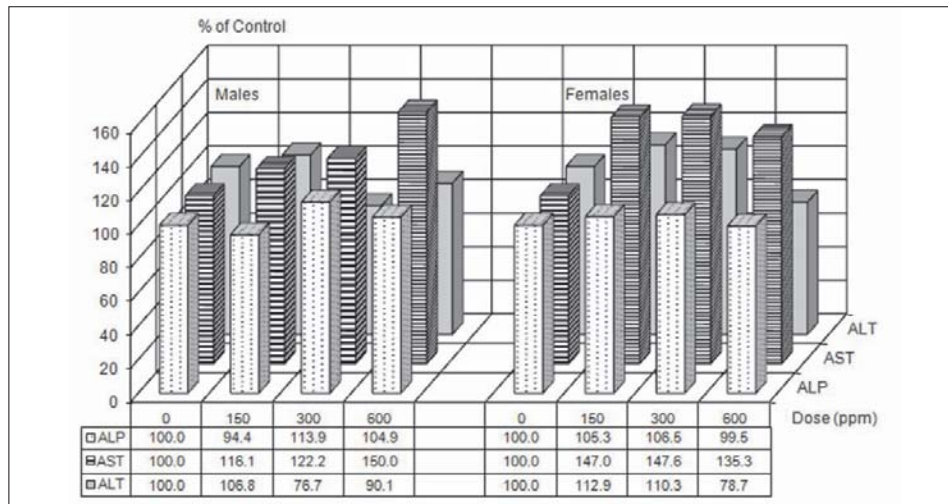


Figure 2. Serum enzyme activity in chickens fed diets without and with cypermethrin for 28 days. The data are expressed as a percentage of the control. ^aSignificantly different at $p < 0.05$ ($n=6$); ^bSignificantly different at $p < 0.01$ ($n=6$).

Abbreviations: ALP = alkaline phosphatase; AST = aspartate aminotransferase; ALT = alanine aminotransferase

Hematology

The hematological effects of cypermethrin are shown in Table 4, where it can be seen that cypermethrin at all three tested doses did not adversely affect the investigated hematological parameters in either sex, except in the case of PLT. When compared to the control group, statistically significant increase in PLT was registered in males and females at all three doses tested.

Residues

The residues of cypermethrin found in the liver, peritoneal fat, breast and leg muscle are shown in Table 5. Accumulation of cypermethrin residues in the liver and tissues during the 28-day period was low. Values for the liver (0.032 - 0.049 mg/kg) were somewhat higher than in the other investigated tissues, where quantities were in the range from 0.024 to 0.029 (peritoneal fat), 0.018 to 0.024 (breast muscle) and 0.018 to 0.036 mg/kg (leg muscle), depending of the amount of cypermethrin administered.

DISCUSSION

Cypermethrin is highly toxic to insects and also to aquatic organisms (fish and aquatic invertebrates), while it is much less toxic to birds and mammals (WHO, 1992; Gupta *et al.*, 1999; Bradberry *et al.*, 2005). It is a neurotoxic compound and in the case of poisoning in mammals, as for other pyrethroids, it causes different symptoms, such as excessive salivation, ataxia, tremor, clonic

convulsions (WHO, 1989; He, 1994). Miyamoto *et al.* (1995) found that pyrethroids in general did not affect any known enzymes in mammals, but their action was aimed at the nervous system. However, other authors (Husain *et al.*, 1994; Kaul *et al.*, 1996; Ayub Shan and Gupta, 1997) showed that some pyrethroids (permethrin, deltamethrin, fenvalerat) expressed adverse effects at higher doses of over 60 mg/kg/day in different ways, such as increased activity of aminotransferases (ALT and AST) and raised blood glucose level (permethrin), thyroid dysfunction (fenvalerat), neurochemical and neuromorphological changes indicating alterations in synaptic function (deltamethrin) (He, 1994; Seth *et al.*, 2000; Garg *et al.*, 2004). Studies on poultry are few and were more focused on investigations of metabolism and residue accumulation than on the effects on biochemical and/or hematological parameters (Akhtar *et al.*, 1987; Hutson and Stoydin, 1987; Khurana *et al.*, 1998; Garg *et al.*, 2004). However, Khurana *et al.* (1998) found that cypermethrin had an immunotoxic effect with suppression of serum globulins, gamma globulins and inhibition of specific hemagglutination-inhibition (HI), and ELISA antibodies.

In our studies on chickens it was evidenced that cypermethrin administered mixed to feed for 28 days (all three doses) did not significantly affect feed intake and body weight gain of the chickens. The small differences registered, were not statistically significant when compared to the control. These findings are in agreement with the results obtained by other authors in similar studies on cypermethrin and other pyrethroids using different experimental animals, including chickens and laying hens (WHO, 1989; Khurana *et al.*, 1998; Gupta *et al.*, 1999; Qadri *et al.*, 2006). No changes were recorded in plasma ChE activity in chickens offered diets containing cypermethrin compared to the control. However, there were some alterations in the activity of some serum enzymes exhibited as increased AST activity and decreased ALP activity in both male and female birds (Figure 2). These findings point to possible adverse effects of cypermethrin on liver functions, but there is little probability that those changes would have significant biological effects, even at the relatively high doses employed in our study. Regarding hematological parameters (Table 4) only an increase in PLT number was registered. All other tested parameters were within the normal range and did not differ significantly from control values. Thus, our results confirm those of other authors who investigated the effects of cypermethrin and other pyrethroids in poultry and mammals, and other beneficial organisms. They used different doses (in most cases lower than in our study) with different methods of application and duration of exposure (Khurana *et al.*, 1998; Garg *et al.*, 2004; Sayim *et al.*, 2005).

Cypermethrin accumulation in the liver, peritoneal fat, breast muscle and leg muscle of our chickens was low in all cases, often hardly above the detection level. In all cases the level of residues was below the MRL established in the EU, and in many other countries, including Serbia. Our findings are in agreement with those of other authors who found that residue levels were below detection limits in the organs and tissues of chickens and laying hens, and also in mammals given cypermethrin (Akhtar *et al.*, 1987; Hutson *et al.*, 1987; WHO, 1992; EMEA, 2001). These are mainly composed of the basic compound (cypermethrin) with

substantially smaller quantities of metabolites often present only at trace levels. The absence of residues in organs and tissues, as well as in final poultry products (eggs), is explained by the fact that birds, as well as mammals, have very effective mechanisms for the detoxification of cypermethrin so it is rapidly metabolized and eliminated from the organism.

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EFEKTI CIPERMETRINA U HRANI NA PILIĆE

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SADRŽAJ

U radu su ispitivani efekti cipermetrina dodatog u hranu za piliće. Pilići su dobijali cipermetrin sa hranom u nominalnim koncentracijama od 150, 300 i 600 ppm (prosečne izmerene koncentracije iznosile su 130, 285 i 655 mg/kg hrane) tokom perioda od 28 dana. Praćeni su sledeći parametri: klinički simptomi i smrtnost, uzimanje hrane i prirast telesne mase, masa jetre, aktivnost ChE u

plazmi, hematološki i biohemijski efekti. Takođe, praćena je kumulacija cipermetrina u jetri, peritonealnoj masti, mišićima grudi i nogu.

Rezultati ukazuju da cipermetrin dodat u hranu pilića nije statistički značajno uticao na uzimanje hrane, prirast telesne mase i masu jetre, kao ni na aktivnost ChE u plazmi i ALT u serumu. Statistički značajno povećanje aktivnosti AST registrovano je kod pilića iz grupa od 300 i 600 ppm (mužjaci) odnosno kod sve tri testirane doze (ženke). Statistički značajno smanjenje aktivnosti ALP registrovano je kod mužjaka iz grupa od 300 i 600 ppm, a kod ženki samo u grupi od 600 ppm. Rezultati hematoloških ispitivanja ukazuju da je cipermetrin izazvao samo statistički značajno povećanje broja trombocita (oba pola, sve tri ispitivane doze). Rezultati ispitivanja su, takođe, potvrdili da se cipermetrin ne akumulira u organima i tkivima pilića.

