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Effects of Exposure of Higher Doses of Cypermethrin in Layers Hens

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Abstract: Cypermethrin (CY), a type II pyrethroid has become a major class of insecticides used to control of ectoparasites that infecting caged layer hens. Although many studies have been done on toxicity of CY on mammals and fishes, little has been evaluated on chicks, especially hens. Thus, this study was carried out on forty-five, 70 weeks old laying hens to evaluate the safety of cypermethrin emulsifiable concentrate 15% (CY-EC) and cypermethrin dust (CY-D). Birds received CY-EC or CY-D applied as a spray and dust, respectively, at the rate of 3-fold higher doses than recommended by the manufacturer and hematological and serum biochemical parameters were evaluated. Results obtained to hematological and biochemical findings showed that CY-EC significantly ($p<0.05$) increased the levels of hemoglobin, MCV and MCHC while WBC reduced in the treated birds. Similarly, significantly increase in MCHC values was marked in CY-D treated birds; additionally, WBC and basophils values became significantly reduced ($p<0.05$). There were no statistical differences in the serum chemistry values between layer hens groups before or after CY-EC treatment. However, layer hens CY-D-treated presented decrease of total protein and GGT when compared with the control while glucose showed significantly high values. It can be concluded that CY-EC and CY-D affecting some hematological and biochemical parameters when applied on 3-fold higher doses than recommended by the manufacturer.

Key words: Layer hens, cypermethrin, hematology, biochemistry, toxicity

INTRODUCTION

Pyrethroids are pesticides considered of low toxicity in mammals due your rapid metabolism in the blood and liver (more than 90% is excreted as metabolites in urine within 24 h after exposure) (Eadsforth and Baldwin, 1983; Eadsforth *et al*., 1998). In birds, these compounds are eliminated two to three times faster via ester hydrolysis and oxidation (Bradbury and Coats, 1989) and this occur due to higher metabolic rates of birds (Schleier and Peterson, 2011).

Cypermethrin (CY) is considered among the most effective pyrethroid (Bradbury and Coats, 1989). However, despite to reduced toxicity, studies have shown that CY is hazardous for rodents (Yousef *et al*., 2003; Sayim *et al*., 2005), rabbits (Shah *et al*., 2007), fishes (Firat *et al*., 2011) and chickens (Anwar, 2003; Aslam *et al*., 2010; Sharaf *et al*., 2010).

Although CY is largely used in the form of dip or spray in poultry industry to control external parasites, no information is available on the toxic potency of this insecticide in exposed layer hens.

Thus, this work was conducted to assess the hematological and biochemical profile of CY-treated layer hens, in two different formulations, spray and dust to verify if CY may cause hematological and/or biochemical disturbances in hens under natural condition.

MATERIALS AND METHODS

Hens: Experiment was perform in experimental breeder farm of the Faculty of Zootechny and Food Engineering, Animal Feed Department, Pirassununga, São Paulo, Brazil (21°59'S, 47°25'W). A total of thirty mature (about 70 week of age), female Hy-Line Brown were housed in battery cages (45 x 45 x 25 cm) located in the total area of 15 m^{-2} and equipped with a nipple-drinker and front-feed bin. Food was supplied daily and water was provided *ad libitum*. The daily photoperiod was 17-h light and 7-h dark. The study was conducted under a research protocol approved by the Animal Research Ethics Committee on of the University of São Paulo (Protocol 1873/2010).

Insecticides: Cypermethrin emulsifiable concentrate 15% (CY-EC) was obtained from Sespo Industry and Commerce Ltda, Brazil; Cypermethrin dust (CY-D) was obtained from Vetanco, AR.

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Treatments: Thirty birds were divided into two equal groups and each bird served as its own control through pre and post-treatment. Birds from the CY-EC group were exposed to cypermethrin 15% EC at the rate of 3-fold higher doses than recommended by the manufacturer (9 mL of a 15% solution per 15 m^{-2}) and were sprayed to the whole body using a hand pump sprayer. Birds from the CY-D group were exposed to cypermethrin dust at the rate of 3-fold higher doses than recommended by the manufacturer (3 kg per 300 m^{-2}) and were dusting to the whole body using a backpack duster S-4 (Guarany Industry and Commerce Ltda, Brazil). The total area was 6.1 m^{-2}.

Collection and preparation of sample: Blood samples were collected on d 0 (prior to treatment) and d 7 (6 days after treatment) from a wing (brachial) vein for the estimation of various hematological and biochemical parameters. Blood samples were prevented from clotting with EDTA for hematological studies. Serum was separated by centrifugation after the blood had been coagulated and stored at -20°C before analysis.

Hematological studies: Hemoglobin was determined by the test-combination kit (Bioclin Quibasa®). The hematocrit was measured by the microhematocrit capillary method and red blood cells and white blood cells were counted with a hemocytometer with blood diluted on 0.01% of toluidine blue stain. The Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin Concentration (MCHC) were calculated from these hematological measurements. Leukocyte differential counts were made on blood films stained with Wright's stain, using average of 100 leukocytes.

Serum biochemical parameters: The serum chemistry parameters aspartate aminotransferase (AST), gama glutamiltransferase (GGT), alkaline phosphatase (ALP), creatine kinase (CK), protein, albumin, glucose, uric acid and urea were determined with an automated (spectrophotometer) chemistry analyzer SBA-200 Celm® SBA-200).

Statistical analysis: Data of hematological and biochemical parameters were submitted to test for normality of error by the Kolmogorov-Smirnov test and the homogeneity of variance through the Levene test and subsequently submitted to the univariate analysis of variance through general linear models procedure (PROC-GLM General Linear Models Procedures). The means were adjusted through the ordinary least squares method (LSMEANS - Least Square Means) and compared through the Tukey test at 5% of significance (SAS System for Windows version 9.2; SAS Institute Inc., Cary, N.C.).

RESULTS

Any clinical signs were observed (bird appearance, behavior and apparent appetite) in hens from the groups treated with CY-EC and CY-D.

Hematological changes in group treated with Cypermethrin EC: Hemoglobin, MCV and MCHC values became significantly increased in the CY-EC-treated birds while White Blood Cell Count (WBC) was reduced ($p<0.05$). Red Blood Cell count (RBC), Packed Cell Volume (PCV), monocytes, lymphocytes and heterophils values showed any significant differences in this group of birds (Table 1).

Hematological changes in group treated with Cypermethrin Dust: Similarly to CY-EC-treatment, the increase in MCHC values was marked in this group of birds. Additionally, WBC and basophils values became significantly reduced in the treated birds with cypermethrin dust (Table 2).

Biochemical changes in group treated with Cypermethrin EC: There were no statistical differences ($p<0.05$) in the serum chemistry values between layer hens groups (Table 3).

Table 1: Hemogram of layer hens treated with cypermethrin 15% EC at the rate of 3-fold higher doses than recommended by the manufacturer (9 mL of a 15% solution per 15 m^{-2})

Parameters	Control group (day 0)	Treated group (day 7)
RBC (x10^6/iL)	2.5±0.1^a	2.6±0.1^a
Hemoglobin (g/dL)	18.5±0.9^a	23.6±0.9^b
PCV (%)	26.8±0.5^a	28.3±0.7^a
MCV (fl ou x10^{-15})	85.4±10.5^a	107.8±2.4^b
MCHC (%)	69.2±3.5^a	83.3±1.4^b
WBC (x10^3/iL)	59.0±4.0^a	34.5±2.6^b
Monocytes	4.2±0.6^a	2.3±0.6^a
Lymphocytes	59.7±1.9^a	54.4±2.9^a
Basophils	1.1±0.4^a	2.6±0.6^a
Eosinophils	0.6±0.2^a	1.9±0.5^a
Heterophils	34.1±2.5^a	38.6±2.8^a

Means followed by different letters in the same line are significantly different ($p<0.05$)

Table 2: Hemogram of layer hens treated with cypermethrin dust at the rate of 3-fold higher doses than recommended by the manufacturer (3 kg per 300 m^{-2})

Parameters	Control group (day 0)	Treated group (day 7)
RBC (x10^6/iL)	2.6±0.05^a	2.6±0.1^a
Hemoglobin (g/dL)	21.8±1.2^a	25.2±1.0^a
PCV (%)	29.8±0.7^a	27.7±1.2^a
MCV (fl ou x10^{-15})	79.0±12.8^a	109.5±4.9^a
MCHC (%)	53.5±8.4^a	90.2±4.6^b
WBC (x10^3/iL)	53.8±1.9^a	35.0±1.2^b
Monocytes	4.5±0.6^a	3.5±0.6^a
Lymphocytes	60.1±1.0^a	62.1±1.6^a
Basophils	3.2±0.5^a	0.7±0.3^b
Eosinophils	0.7±0.3^a	0.7±0.4^a
Heterophils	30.7±0.1^a	33.5±1.6^a

Means followed by different letters in the same line are significantly different ($p<0.05$)

Table 3: Serum chemistry values for layer hens treated with cypermethrin 15% EC at the rate of 3-fold higher doses than recommended by the manufacturer (9 mL of a 15% solution per 15 m^{-2})

Parameters	Control group (day 0)	Treated group (day 7)
AST (UI/L)	253.8±51.1[a]	201.4±23.3[a]
GGT (UI/L)	15.8±51.2[a]	10.6±0.9[a]
ALP (UI/L)	879.4±212.9[a]	1510.3±506.5[a]
CK	2417.6±270.1[a]	2782.3±334.4[a]
Total protein (g/dL)	7.2±0.2[a]	5.7±0.5[a]
Albumin (g/dL)	2.9±0.0[a]	2.6±0.0[a]
Glucose (mg/dL)	7.2±0.2[a]	5.7±0.5[a]
Uric acid (mg/dL)	79.0±12.8[a]	109.5±4.9[a]
Urea (mg/dL)	2.3±0.4[a]	3.9±0.6[a]

Means followed by different letters in the same line are significantly different ($p<0.05$)

Table 4: Serum chemistry values for layer hens treated with cypermethrin dust at the rate of 3-fold higher doses than recommended by the manufacturer (3 kg per 300 m^{-2})

Parameters	Control group (day 0)	Treated group (day 7)
AST (UI/L)	234.4±10.9[a]	175.8±7.4[a]
GGT (UI/L)	18.1±1.8[a]	11.2±0.8[b]
FA (UI/L)	485.7±95.8[a]	1254.4±249.6[a]
CK	3004.6±392.1[a]	2780.7±407.5[a]
Total protein (g/dL)	8.8±0.4[a]	7.0±0.2[b]
Albumin (g/dL)	3.0±0.1[a]	2.8±0.0[a]
Glucose (mg/dL)	168.1±4.4[a]	197.9±3.2[b]
Uric acid (mg/dL)	6.1±0.3[a]	6.3±0.4[a]
Urea (mg/dL)	3.8±0.8[a]	1.6±0.6[a]

Means followed by different letters in the same line are significantly different ($p<0.05$)

Biochemical changes in group treated with Cypermethrin Dust: Protein and GGT in layer hens CY-D-treated showed significantly low values ($p<0.05$); however, glucose showed significantly high values when compared with the pre-treatment (control). The changes in other biochemical parameters were generally insignificant (Table 4).

DISCUSSION

Many studies have been conducted on mammalian species and fishes to investigate toxicological effects of CY and others pyrethroids (Khan *et al*., 2012). However, although this pesticide has been largely used in poultry industry, few studies have been carried out on avian species to evaluate the possible CY toxicity.

Our results indicate that treatment with CY caused significant changes in both, hematological and biochemical, parameters in layer hens.

Considering the hematological analysis, we verified that layer hens exposed to CY-EC showed an increase in hemoglobin concentrations which also has been recorded in cockerels exposed to chlorpyrifos, an organophosphate insecticide (Ojezele and Abatan, 2009). In the same manner, it was detected an increase in MCV values. Although no reports were found in the literature in avian species, increase in MCV was also observed in rats (Matsushima *et al*., 2003; Sayim *et al*., 2005), rabbits (Basir *et al*., 2011) and fishes (Adhikari *et al*., 2004) exposed to CY. Additionally, corresponding increase in Mean Corpuscular Hemoglobin Concentration (MCHC) was also noticed by Dörücü and Girgin (2001) in fishes treated with CY.

CY-EC and CY-D-treated hens in the present study show a significant decrease in total leucocyte counts. The same alteration was previously reported in chicks treated with the pyrethroid compound fenvalerate (Garg *et al*., 2004), the organophosphates chorpyrifos and methidathion (Ojezele and Abatan, 2009) and also cockerels exposed to the insecticide neonicotinoid imidacloprid (Balani *et al*., 2011). Contrarily, leukocytosis has been documented after CY treatment in broiler chicks (Sharaf *et al*., 2010).

Despite to WBC differential, effects of CY treatment in layer hens in this work did include only significant reduction on basophils values in the CY-D-treated birds. The changes in others leukocytic parameters were insignificant and these present findings are in accordance with Sharaf *et al*. (2010) in broiler chicks exposed to CY.

The main biochemical response to CY administration verified in the present study was a significant decrease in protein and glucose increase. Reduction in plasma protein was related in previous studies in young chickens exposed to methidathion and chlorpyrifos (Ojezele and Abatan, 2009); fishes exposed to deltamethrin (Ravider *et al*., 1988); rabbits exposed to cypermethrin (Lakkawar *et al*., 2006) and rats exposed to fenvalerato (El-Demerdash *et al*., 2004). Accordingly Rivarola and Balegno (1991), the plasma protein decrease in animals treated with pesticides could be attributed to changes in protein and free amino acid metabolism and their synthesis in the liver. Also, the decrease in plasma proteins could be attributed in part to the damaging effect of insecticides on liver cells (Yousef *et al*., 2006). On the other hand, reduction in plasma protein might occur because of the physiological adaptation of animal to combat stress produced by the pesticide. In fact, Bradbury *et al*. (1987) pointed out that the protein depletion in tissues may constitute a physiological mechanism and may play a role of compensatory mechanism under CY stress, to provide intermediates to the Kreb's cycle. Furthermore, to overcome the stress, the animals require high energy and this energy demand might lead to the stimulation of protein catabolism (Sancho *et al*., 1997).

The increase in glucose levels of CY-D-treated hens here verified was supported by the findings in chickens *White Leghorns* after oral administration of the carbamate pesticide isoprocarb (Rahman *et al*., 1990). The rise in blood glucose produced by pesticides may indicate disrupted carbohydrate metabolism due to enhanced breakdown of liver glycogen, possibly mediated by increase in adrenocorticotrophic and glucagon hormones and/or reduced insulin activity (Raja *et al*., 1992).

It is concluded that CY-EC and CY-D may cause hematological and biochemical disturbances in layer hens when applied on 3-fold higher doses than recommended by the manufacturer. The results of this study emphasize the importance of toxicological studies assay because layer hens are regularly exposed to insecticides for pest control.

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