

ADVERSE EFFECT ON IMMUNITY AND PERFORMANCE OF BROILER CHICKENS DIPPED IN SOME PESTICIDES

(With 6 Tables & one Fig.)

By

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تأثير مبيدات الحشرات وكفاءة كفاية البدارى عند التغطيس فى بعض المبيدات الحشرية

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تم دراسة مدى الاستجابة المناعية وتغيرات الدم فى بدارى الدجاج بعد التغطيس فى بعض مبيدات الآفات (الكربوفوران والسيبرمترين)
أوضحت النتائج انخفاض مستوى الاجسام المناعية بأستخدام اختبار تلازن الدم المضاد وكذلك عدم مقاومة الدجاج لفيروس النيوكاس شديد الضراوه فى اختبار تحدى المناعه بعد التحصين ضد هذا الفيروس . وقد تبين أن مبيد الكربوفوران كان أكثر تأثيراً على الجهاز المناعى للدجاج من مبيد السيبرمترين.
كذلك حدوث اختزال فى عدد خلايا الغده الصعترية وكيس الفبريشيس عند استعمال الكربوفوران أكثر من السيبرمترين.
تم دراسة تغيرات الدم المصاحبه لاستخدام هذين المبيدين بالمقارنه بضابط التجربه . حدث تغيير فى الوزن النسبى فى الدجاج المعامل لأعضاء المناعيه (الطحال - الغده الصعترية - كيس فبريشيس) .
أوضحت النتائج أن الدجاج المعامل بكلا المبيدين الحشريين أقل قدره على بناء جهاز مناعى سليم وأكثر قابليه لتعرضها للأمراض بالمقارنه بالدجاج الغير معامل بهذين المبيدي .

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SUMMARY

Humoral and cellular immunologic responses as well as haemogram studies of the chickens were investigated. after dipping in carbamate insecticide, carbofuran and pyrethroid insecticide, cypermethrin. Carbofuran exerts humoral immunotropic effect indicated by lowering hemagglutination inhibition (HI) antibody titre against Newcastle disease (ND) vaccinated chickens and lowering resistance against virulent Newcastle disease (NDV) challenge virus. Cypermethrin treated group chickens showed a slight immunogenic effect in comparison with the control group. Cellular immunity represented by lymphocytes, thymocytes and spleenocytes numbers were dramatically reduced in the carbofuran and cypermethrin birds in relation to nontreated control ones. Haematological examination was run parallelly with immunological investigations. Haemoglobin percentage (Hb), packed cell volume (PCV), red blood cell count (RBCs), and white blood cell count (WRCs) were measured. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated. The weekly body weight gain and organ-body relative weight of bursa of Fabricius (BF), thymus and spleen were significantly reduced in carbofuran treated chickens than in cypermethrin treated ones. Our data indicates that chickens exposed to carbofuran and cypermethrin insecticides are less able to mount a normal antibody response and cellular immunity to viral vaccines as well as adverse effect on hematological parameters.

INTRODUCTION

External parasites of poultry are arthropods that live on and/or in the skin and feathers. Ectoparasites of poultry are of economic importance and are distributed over the whole world, especially in tropical and subtropical countries. In Egypt, ectoparasites represents one of the most and major economic diseases of poultry.

The immune system as a target of chemical toxicants has only recently gained concern and importance (SHARMA, 1988).

This has been brought about by an increased awareness of safety with chemical substances and also by growing knowledge in the field of immunology. Yet in a short time, a large number of chemicals have been tested for their immunotoxic potential. A variety of reviews have appeared on the effect of chemicals on the immunologic system (SHARMA, 1981 a&b; SHARMA and REDDY, 1987).

Many of the chemicals evaluated have immunotoxic effects at some conditions or levels of exposure. Of the environmental chemicals, pesticides were some of the first ones to be investigated for their immunomodulatory effects (STREET and SHARMA, 1975 and DESCOTES, 1986).

The synthetic and natural pyrethroid and carbamate insecticides were the main ectoparasitic and fly control chemicals used to direct application to poultry (ARENDS, 1991). According to LOOMIS (1984), among the pyrethroides and carbamate compounds that can be employed successfully and safely on birds are carbofuran and cypermethrin.

Synthetic pyrethroids are the new generation of pesticides that are being developed as good substitutes for unwarranted organochlorine and toxic organophosphorus insecticides (AKHTAR, 1984). Cypermethrin is one of the important members of this family, which is also known by other names, e.g., Polytrin, Stockade, Flocord, Ripcord and Cymbush.

Cypermethrin was first described by Dr. M. ELLIOTT in 1974 and was introduced commercially in 1977 as an emulsifiable concentrate to be used against a wide range of insect pests (ELLIOTT 1977).

Baron (1991) cited that the potential for specific immunotoxic effects has been investigated for relatively few carbamate insecticides. Chronic dietary studies performed to meet registration requirements, have generally effects on endpoints related to immune function. Although some laboratory animals and in vitro systems have shown treatment related changes in immunological parameters at nontoxic dosage of carbamates.

carbofuran is one of the important members of carbamates family, which is also known by other names, e.g., Brifur, Crisfuran, Cristofuran, Curaterr, Furadan, Pillarfuran and Yaltox. Carbofuran was developed in the 1960's and introduced in 1976 as a systemic and contact broad-spectrum, long-residual insecticide and nematicide for use in Agriculture and Veterinary medicine (BARON, 1991).

According To Dean et al. (1989) the immune system functions is resistance to infectious agents, homeostasis of leucocyte maturation, immunoglobulin production and immune

surveillance against arising neoplastic cells. Cells of the immune system providing these functions arise from pluripotent stem cells within the bone marrow, where they undergo highly controlled proliferation and differentiation before giving rise to functionally mature cells. In chicken, antibody-mediated immunity is dependent on the normal development of the bursa of Fabricius (GLICK, 1970).

The interaction of environmental chemical or drugs with lymphoid tissues may alter the delicate balance of the immune system and result in undesirable effects. Of the number of effects of pesticides, the ability to impair the immune system is of profound interest. There are limited data on the biological effects of carbofuran and cypermethrin pesticides particularly the involvement of immune organs. Hence, in the present investigation, the effects of these pesticides in chicken have been studied. The purpose of administering carbofuran and cypermethrin in the present study was to determine their potential for disrupting bursal development and future antibody production. Also studied their effect on body weight, organ-body weight ratio, immunological and haematological parameters in chickens.

MATERIAL and METHODS

I-Vaccines:

A commercial ND Hitchner B₁, LaSota ND live vaccines (Intervet Holland) and Oil emulsion inactivated ND vaccine (Virbac Lab. France) were used for vaccination of chicks against NDV.

Evaluation of ND vaccination was based on the HT-antibody response and protection against challenge with virulent NDV.

II-Chickens:

200 one-day-old balady chicks purchased from private farm, reared in strict isolation and weighed weekly. Chicks were vaccinated against NDV at 4 days old intraocularly by Hitchner B₁, 14 days old in drinking water using LaSota ND vaccine and with oil emulsion ND vaccine intramuscularly at 35 days old. Vaccinal dose was used as recommended according to manufacturer's directions.

III- Virulent NDV:

Virulent NDV previously isolated and characterized (9th egg passage) from the department of poultry diseases, Faculty of Vet. Med., Assiut University was used in challenge test. The mean death time of minimal lethal dose was 52 hours and the intracerebral pathogenicity index in one day chicks was 1.82.

IV- Hemagglutination (HA) antigen:

Allantoic fluid collected from embryonated chicken eggs previously infected via allantoic sac with LaSota ND vaccine was used in HI test. HA titer was 1: 2048.

V-ND-hyperimmune serum:

ND hyperimmune serum from immunized chickens (HI titre 1: 1280) was used as control serum in HI-test.

VI-HI-test:

A micro HI-test carried out after ALLAN and GOUGH (1974) using 4 HA units of NDV and 0.75% chicken erythrocytes. HI-titers were individually determined and then the geometric mean HI-titers of different groups were calculated.

VII-Pesticides:

Cypermethrin: (R,S-o-cyno-3-phenoxybenzyl 2,2-dimethyl (1R, 1S)-Cis, rans-3-(2,2-dichlorovinyl) cyclopropane carboxylate.

Carbofuran: 2, 3-dihydro-2,2-dimethyl-7-benzofuranyl N-methyl carbamate.

Both pesticides were purchased from chem service company, west chester, USA as a technical product, containing 98% of an active component.

VIII-Insecticide dipping:

At 7-week-old, 130 vaccinated chickens were classified into three equal groups each of 60 birds. The birds in the 1st and 2nd groups were dipped after other week for three times in carbofuran and cypermethrin solution respectively as recommended by manufacturer's. Birds in the 3rd group were dipped parallelly in plain water as nontreated control one.

IX-Blood samples:

Immediately before each dipping and also two weeks after the last dipping, 10 birds from each group were bled by cardiac puncture. The blood was collected into two tubes. One with heparin anticoagulant for haematological studies and the other without heparin anticoagulant for serum collection.

X-Postmortem examination and organ weight:

Along with blood sample collection, ten birds of each group were weighed individually and examined for postmortem findings. The internal organs (bursa of Fabricious, thymus, spleen, liver and kidneys) were removed and individually weighed for determining the relative weights of these organs. Since the animal size affects organ size, organ weights are expressed as weight per unit of body weight according to the organ weight index cited by BRONISZ *et al.* (1992). Splenocytes, thymocytes and bursal lymphocytes were counted after adaptation of one-

cell suspension of spleen, thymus and BF by placing in Petri dishes containing Hank's balanced salt solution (HBSS) and gently make cell suspension by teasing and mashing the tissue with barrel of 5 or 10 ml syringe (BLAYLOCK, 1990).

XI-Challenge test:

Two weeks after the last dipping, 10 birds from each group were challenged with 0.1 ml virulent NDV (containing $10^{6.2}$ ELD₅₀) per bird by instillation intraocularly. The birds were observed for 2 weeks. The response to challenge was recorded (morbidity and/or mortality). Morbidity was defined as the presence of clinical signs of ND (depression, diarrhoea and nervous disorders).

XII-Hematological examinations:

They were performed to detect the possible changes in hemogram according to the official methods adopted by CAMPBELL (1988).

RESULTS

HI-test:

Geometric mean of HI-antibody titers in insecticide treated and non treated groups were illustrated in Fig.1. Sharply decrease in HI-titers was observed in carbofuran treated group after 2nd and 3rd dipping, while was slight in the cypermethrin treated group.

Challenge tests:

Morbidity and mortality of challenged treated and non treated chickens are summarized in Table (1).

Postmortem examination:

No remarkable specific macro-postmortem changes were observed in treated and non treated groups except reduction in organ size as presented in Table (2).

Hematological examination:

The picture of blood which include RBCs, WBCs, Hb concentration and PCV are presented in Table (3). Calculated MCV, MCH and MCHC are presented in the same table.

Number of splenocytes, thymocytes and bursal lymphocytes in treated and non treated groups are presented in Table (4).

Body and organ weights:

Table (5) summarize the body weight of different chicken groups.

Table (6) summarize the body weight gain of chickens treated with cypermethrin and carbofuran in comparison to non treated control one, while the organ relative weights are presented in Table (2).

DISCUSSION

Pesticides are widely used for control of external parasites. The growing demand for pesticides has intensified the search for new preparations of higher effectiveness and better tolerance. Synthetic pyrethroids have almost fully met these requirements. Pharmacologic activity of pyrethroids is manifested by their neurotoxic and circulatory effects. There are scarce data, however, on their conceivable effect upon immunological system. The so far obtained findings are only fragmentary and concern mainly an induction of lymphatic structures atrophy, most commonly in the sphere of immunological proliferation or deficiency in their function (DEAN and THURMOND, 1986; DESCOTES, 1986 and STELZER and GORDON, 1984).

From the findings obtained cypermethrin follows the same way as the other members of pyrethroid group of pesticides. Cypermethrin exerts slight immunotropic effect to the experimental chickens. The immunosuppressive component was to prevail. Thus under the influence of immunization with Newcastle disease vaccine the titre of antibody was slightly low in the group of birds exposed to cypermethrin in comparison with the control birds. Not surprising as either the prevailing suppressive effect, since as early as in 1984 Stelzer and Cordon found in vitro experiments that differently substituted other pyrethroid derivatives (Deltamethrin, Permethrin, Allethrin and Phenprothrin) suppressed mitogenic response of murine splenocytes stimulated with Con. A and LPS.

Weight of lymphatic organs - spleen thymus and BF - was reduced under the effect of cypermethrin. GLOMET (1982) did not observe any deviations from the normal in the organs of animals with long-term exposure to deltamethrin.

GIELDANOWSKI *et al.* (1991) studied the effect of 2-isopropoxyphenyl methyl carbamate (Unden) as a member of carbamate group of insecticides. He stated that studies concerning the influence of carbamate pesticides on the immunological system are scarce and fragmentary.

Our results concerning the immunosuppressive effect of carbofuran to chicken were in agreement with the results obtained by GIELDANOWSKI *et al.* (1991) who found that unden exerted an immunotropic effect mainly suppressive on C57 Bl/6 mice and C57 Bl/6 X DBA/2/F1/ Iiw hybrids.

As illustrated in Fig.1 HI antibody titers were slightly decreased in pyrethroid treated group chickens (two weeks after the 3rd dipping) i.e geometric mean HI titers was 7.8 Log₂ in comparison with nontreated control one (GM HI-titers was 8.9

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Log₂). Sharply decrease in GM-HI-titers was observed in carbofuran treated chickens (two weeks after the 2nd dipping) i.e GM-HI-titers was 6.4 Log₂ while it was 8.9 Log₂ in control one. The more obvious effect on GM-HI titers was noticed two weeks post the 3rd dipping in carbofuran treated chickens. (GM-HI-titers was 3.7 Log₂); while GM-HI-titers was 8.9 Log₂ in non-treated control chickens. A variety of reviews have appeared on the effect of pesticides on the immune system (SHARMA, 1981a; SHARMA, 1981b and SHARMA and REDDY, 1987).

The results of challenge test are correlated with result of HI-test as chickens with GM-HI-titers was 8.9 Log₂ and 7.8 Log₂ in nontreated control and cypermethrin treated chickens were completely protected against challenge with virulent NDV, while chickens in the carbofuran treated group with GM-HI-titers 3.7 Log₂ were susceptible to virulent NDV as indicated by clinical signs and mortalities (Table 6). the relation between HI-titers levels and challenge response reported in this study were in agreement with Box, 1975 who stated that challenge by NDV will not affect the chickens whose HI antibody levels are Log₂ 5 or more and at levels below this, mortality will be seen in challenged birds.

It is known that carbamates influence the activity of cholinesterase and non specific esterase. As a result of blocking by these pesticides, of non specific esterase particularly in the cells of reticulo-endothelial system; an impairment of their function as well as disturbances in the normal functioning of immune processes may occur.

The reduction in body weight of chickens under experiment suggest over toxicity due to pesticide treatment. The hematological studies constitute certain health indices and disclose the possible reaction of blood and blood-forming organs to the tested pesticides. Reduction in hemoglobin content suggested to be due to intra-erythrocytic depletion and due to the reduction in total RBC count. Increase in the number of WBC count could be attributed to the induction of pathological effect of pesticides. Increased WBC counts were found with pesticide treatment in birds (MANDAL *et al.*, 1986).

in conclusion, exposure of chickens to certain pesticide leads to impairment of immune system and subsequently vaccination failure and increase the susceptibility of chickens to various diseases.

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Table (1): Morbidity and Mortality in pesticides treated and nontreated chickens post challenge with virulent NDV.

Group	1		2		3		4		5		6		7		8		9		10		11		12		13		14		15		Total					
	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b						
Control non treated	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Cypermethrin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Carbofuran	0	0	0	0	0	0	1	0	3	1	2	2	5	3	1	2	1	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13	8

a = Number of birds showing morbidity (depression, inappetance, diarrhoea and nervous signs).
 b = Number of dead birds.

Table (2): Effect of Cypermethrin and Carbofuran on the organ relative weight of chicken.

Treatment	Organ	Time after 1st dipping							
		Two weeks		Four weeks		Six weeks		Eight weeks	
Control	Spleen	0.231±0.012	0.230±0.015	0.166±0.018	0.289±0.021	0.166±0.018	0.233±0.011	0.185±0.015	0.201±0.012
	Thymus	0.267±0.015	0.514±0.022	0.233±0.011	0.257±0.030	0.233±0.011	0.301±0.014	0.306±0.007	0.306±0.007
	B. F	0.276±0.003	0.211±0.013	0.132±0.015	0.184±0.010	0.132±0.015	0.189±0.017	0.134±0.010	0.134±0.010
	Liver	4.641±0.253	4.159±0.351	3.832±0.201	4.471±0.155	3.832±0.201	4.093±0.173	4.244±0.095	4.244±0.095
Cypermethrin	Kidney	1.365±0.085	1.578±0.120	1.952±0.131	1.582±0.051	1.952±0.131	1.372±0.075	1.604±0.065	1.604±0.065
	Spleen	0.289±0.021	0.166±0.018	0.166±0.018	0.289±0.021	0.166±0.018	0.301±0.014	0.306±0.007	0.306±0.007
	Thymus	0.257±0.030	0.233±0.011	0.233±0.011	0.257±0.030	0.233±0.011	0.189±0.017	0.134±0.010	0.134±0.010
	B. F	0.184±0.010	0.132±0.015	0.132±0.015	0.184±0.010	0.132±0.015	4.093±0.173	4.244±0.095	4.244±0.095
Carbofuran	Liver	4.471±0.155	3.832±0.201	1.952±0.131	4.471±0.155	3.832±0.201	1.372±0.075	1.604±0.065	1.604±0.065
	Kidney	1.582±0.051	1.952±0.131	1.952±0.131	1.582±0.051	1.952±0.131	0.182±0.013	0.155±0.015	0.155±0.015
	Spleen	0.278±0.019	0.117±0.005	0.117±0.005	0.278±0.019	0.117±0.005	0.202±0.010	0.300±0.018	0.300±0.018
	Thymus	0.243±0.011	0.097±0.005	0.097±0.005	0.243±0.011	0.097±0.005	0.109±0.015	0.111±0.009	0.111±0.009
Carbofuran	B. F	0.125±0.020	0.188±0.021	0.188±0.021	0.125±0.020	0.188±0.021	4.300±0.195	4.225±0.331	4.225±0.331
	Liver	4.606±0.310	3.875±0.213	3.875±0.213	4.606±0.310	3.875±0.213	1.196±0.056	1.731±0.071	1.731±0.071
	Kidney	1.507±0.087	1.217±0.132	1.217±0.132	1.507±0.087	1.217±0.132	0.155±0.015	0.155±0.015	0.155±0.015

Table (3): Effect of cypermethrin and carbofuran on haemogram of chicken.

Time after exposure	Pird treatment	Hb (g%)	PCV (%)	RBCs (10 ⁶ /mm ³)	WBCs (10 ³ /mm ³)	MCV (fl)	NOI (pg)	Hctc (g/dl)
Two weeks	Control	9.75±0.850	29.500±2.500	3.177±0.259	15.976±0.580	92.955±6.550	30.689±1.550	23.051±2.500
	Cypermethrin	7.23±0.525	27.330±1.950	3.143±0.185	17.167±0.931	86.955±6.970	23.003±2.300	26.45±2.350
	Carbofuran	7.33±0.610	26.800±2.100	2.570±0.210	17.067±0.950	100.375±8.000	27.453±1.850	27.351±2.950
Four weeks	Control	9.210±0.650	31.700±2.650	3.650±0.130	16.531±1.150	86.849±7.150	25.233±2.150	29.054±1.800
	Cypermethrin	7.430±0.650	29.300±2.310	3.074±0.250	17.350±0.960	92.062±6.910	24.170±2.000	26.254±2.100
	Carbofuran	7.170±0.800	29.100±2.500	3.010±0.175	17.130±1.211	93.355±7.350	23.921±1.970	25.515±1.950
Six weeks	Control	8.250±0.740	29.000±2.350	2.945±0.175	16.900±1.050	98.472±7.351	23.014±2.350	29.489±2.350
	Cypermethrin	8.33±0.590	29.700±1.830	2.937±0.190	16.700±1.210	92.708±8.120	28.853±1.950	31.199±2.850
	Carbofuran	7.50±0.610	26.330±2.000	2.773±0.085	16.700±0.950	94.951±6.150	27.047±1.500	29.685±1.980
Eight weeks	Control	9.00±0.590	29.000±1.750	3.030±0.210	17.800±1.330	95.710±5.350	29.703±2.300	31.034±2.560
	Cypermethrin	7.370±0.430	25.300±1.850	2.863±0.195	17.800±1.550	91.862±6.750	27.489±1.730	29.924±2.650
	Carbofuran	7.830±0.450	25.670±1.500	2.753±0.173	16.950±1.650	93.244±6.500	28.642±2.110	30.503±2.310

Table (4): Effect of cypermethrin and carbofuran on the number of splenocytes, Bursa lymphocytes (B-lymphocytes) and thymocytes.

Time after exposure	Pird treatment	Splenocytes (10 ⁶ /mg spleen)	B-lymphocytes (10 ⁶ /mg B.f)	Thymocytes (10 ⁶ /mg thymus)
Two weeks	Control	2.985±0.130	2.034±0.125	4.24±0.170
	Cypermethrin	1.758±0.035	1.155±0.100	3.406±0.195
	Carbofuran	1.724±0.095	0.999±0.055	2.133±0.176
Four weeks	Control	2.660±0.150	2.500±0.099	3.350±0.189
	Cypermethrin	1.850±0.110	1.650±0.085	3.130±2.135
	Carbofuran	1.900±0.130	1.400±0.055	2.300±0.143
Six weeks	Control	2.575±0.095	2.421±0.075	3.255±0.098
	Cypermethrin	1.100±0.075	1.642±0.095	2.68±0.110
	Carbofuran	0.950±0.090	0.975±0.066	1.950±0.075
Eight weeks	Control	2.435±0.150	1.950±0.100	3.560±0.132
	Cypermethrin	1.547±0.075	1.040±0.090	3.878±0.150
	Carbofuran	1.136±0.090	0.915±0.095	2.528±0.065

Table (5): Effect of cypermethrin and carbofuran on body weight of chicken.

Old age	Control group		Cypermethrin group		Carbofuran group	
	Mean±S.E	Min. Max.	Mean±S.E	Min. Max.	Mean±S.E	Min. Max.
One day	35.896±0.395	29.100 45.400	---	---	---	---
1 weeks	56.30±1.221	35.200 75.500	---	---	---	---
2 weeks	73.731±1.472	51.400 100.000	---	---	---	---
3 weeks	103.223±2.354	69.000 141.000	---	---	---	---
4 weeks	129.591±3.345	82.000 193.700	---	---	---	---
5 weeks	149.077±4.607	93.200 228.000	---	---	---	---
6 weeks	194.395±5.299	113.000 292.000	---	---	---	---
2 weeks*	193.800±11.274	117.400 253.000	291.350±13.143	190.000 372.000	251.300±12.540	153.500 326.000
4 weeks*	253.000±13.934	172.000 353.000	336.038±9.325	293.300 411.000	318.05±19.536	215.500 610.000
6 weeks*	335.513±15.319	249.000 405.000	450.413±11.232	324.000 620.000	401.056±24.000	312.000 575.000
8 weeks*	516.667±21.200	465.000 605.000	576.364±29.083	425.000 690.000	593.033±17.904	440.000 610.000

* Time in weeks after 1st dipping.

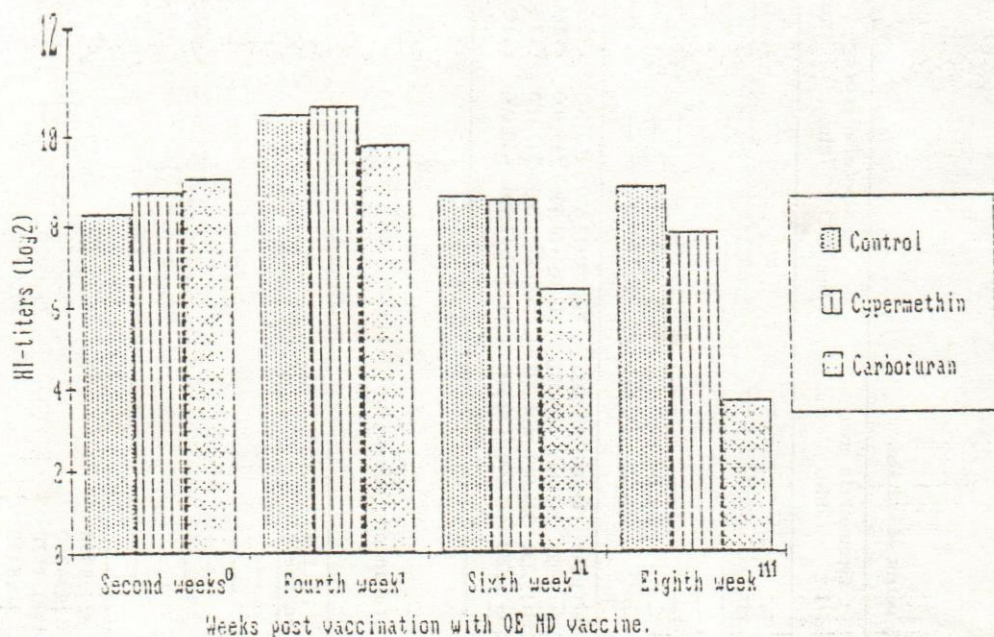
Table (6): Effect of Cypermethrin and Carbofuran on body weight gain of chicken.

Day old	Control		Cypermethrin (%)		Carbofuran	
	Control	Cypermethrin	Cypermethrin	Carbofuran	Control	Carbofuran
One week	53.2430	---	---	---	---	---
2 weeks	29.8010	---	---	---	---	---
3 weeks	39.9990	---	---	---	---	---
4 weeks	25.5450	---	---	---	---	---
5 weeks	14.2650	---	---	---	---	---
6 weeks	31.2900	---	---	---	---	---
2 weeks*	39.1140	23.8990	23.8990	23.8990	23.5210	23.5210
4 weeks*	35.7070	19.4380	19.4380	19.4380	26.5630	26.5630
6 weeks*	37.5710	30.1570	30.1570	30.1570	26.0970	26.0970
8 weeks*	53.9930	27.8480	27.8480	27.8480	30.9430	30.9430

* Time in weeks after 1st dipping.

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Fig. (1): HI-titers (Log₂) response of ND vaccinated chickens treated with Pesticides.



0 = Sera were collected two weeks after vaccination with OE vaccine and immediately before the 1st dipping in pesticides.

1 = Sera were collected four weeks after vaccination with OE vaccine and immediately before the 2nd dipping in pesticides.

11 = Sera were collected six weeks after vaccination with OE vaccine and immediately before the 3rd dipping in pesticides.

III = Sera were collected eight weeks after vaccination with OE vaccine and immediately before the last dipping in pesticides.