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Medicago sativa Seeds as a Natural Source of Isoflavones to Counteract the Toxicity of Contaminated Broiler Rations

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Abstract: To reduce the eco-physiological effects of pesticide-residual in poultry feedstuffs one-day broiler chicks were fed rations contaminated with 5, 10 and 20 mg/kg carbofuran (CF) or 10 and 20 mg/kg CF + 0.5 or 1% *Medicago sativa* seeds as a source of isoflavones (IF). Results revealed that CF was toxic to growing broilers at any given dose. Carbofuran caused a non-significant reduction in chicks' body weight and feed intake compared with control group while, the addition of IF to the diets reduced CF hazardous effect and improved birds' performance and increased body weight. Feed intake decreasing was a correlated with deterioration in feed conversion ratio, whereas, IF resulted in avoid CF hazardous effect and improvement these parameters. Blood hemoglobin, serum total protein, acetyl-cholinesterase enzyme activity, growth hormone and triiodothyronine hormone (T_3) were significantly decreased after CF treatment. IF-antioxidant effect led to influence the improvement rates of physiological performance of chicks, whereas, growth hormone level showed a significant increase when adding IF in diets of chicks. It could be concluded that use of *Medicago sativa* seeds as a source of Isoflavones IF minimized CF hazardous effects on most of the tested parameters and this may be attributed to the vital antioxidant role of IF as an antioxidant in preventing pesticide toxicity during exposure to environmental pollution.

Key words: Isoflavones · Carbofuran · Pesticide · Triiodothyronine hormone · Broiler

INTRODUCTION

The application of pesticides to manage pests in land and water has posed potential health hazard to livestock and wildlife including fishes, mammals, birds and humans. N-substituted esters of carbamic acid commonly known as carbamates comprise second major group of synthetic insecticides being utilized worldwide for agriculture [1]. The mechanism of action of these compounds is the inhibition of cholinesterase activity [2]. Carbofuran (2,3-dihydro-2,2-dimethylbenzofuran-7-methylcarbamate) (CF) was first used commercially in 1967 and it is widely used in agriculture mainly in crops and for keeping crops during storage in silo and it has received intensive concern not only due to its heavy use but also due to its highly oral toxicity [3, 4]. The toxic potential of insecticides to birds in the field was affected by the residual or the degradation of residues over time [5] and poultry can be affected by them during eating or drinking contaminated feedstuffs or water, these hazards accumulate in poultry bodies and may interfere with its biochemical functions [6]. Yousef *et al*., [7] reported that treated rabbits with sub-lethal doses of CF conducted to decrease plasma total protein, Albumin and globulin, while plasma glucose was increased compared to control. When Mallard ducklings were fed CF at 0.25 mg/kg BW for 10 days, growth retardation and lower blood acetylcholinesterase (ChE) were observed [8]. Also, the

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deleterious effects of CMs were alterations in the enzymes activities such as alkaline phosphatase, acetylcholinesterase, hepatic marker enzymes and lactate dehydrogenase (LDH) [1]. *Medicago sativa* commonly known as Lucerne or alfalfa, the pharmacological active substances present in *Medicago sativa* include acids, alkaloids, amino acids, isoflavonoids, vitamins, pectin and minerals [9]. Isoflavonoids are a diverse group of natural products that have been ascribed estrogenic, antioxidant and anticancer, which impact animal and human health [10]. Plasma growth hormone was increased significantly in ewes and sheep when treated with isoflavones [11].

The aim of the present study was to reduce the eco-physiological effects of dietary pesticide residues on productive performance of broiler chicks by using (IF) as an antioxidant to counteract the hazardous pesticide-residues' effects.

MATERIALS AND METHODS

One-day old 240 Hubbard commercial broiler chicks were randomly divided into 8 groups of 30 chicks with 3 replicates of 10 chicks each. Chicks were kept in caged wire floor batteries in a controlled environmental house during six weeks of experimental period. Groups 2, 3 and 6 were fed diet contaminated with 5, 10 and 20 mg/kg Carbofuran (CF). Groups 4 and 5 were given a diet contaminated with 10 mg/kg CF plus 0.5 or 1% *Medicago sativa* seeds as a source of isoflavones (IF), while groups 7 and 8 were treated in a similar manner except that CF level was 20 mg/kg. Whereas, group one served as a control. Experimental diets were formulated to provide chicks with 22.4% protein and 3160 kcal/kg. Feed and water were provided *ad libitum*. Body weight (BW) (of each bird) and feed intake (FI) (of each replicate under each treated group) were recorded weekly. Feed conversion was calculated as g feed intake /g body weight gain. At sixth week of age, blood samples were collected from five birds (randomly chosen) from each treatment group to obtain plasma or serum, which stored at –20 ºC for determination the level of total protein (STP) concentration as (g/dl), Albumin (Alb) concentration as (g/dl), Globulin (Glob) concentration as (g/dl), plasma glucose (PG) concentration as (mg/dl), total cholesterol concentration as (mg/dl), serum total triglycerides (TrGl) concentration as (mg/dl), plasma uric acid concentration as (mg/dl), the activity of Aspartate aminotransferase (AST) and the Alanine aminotransferase (ALT) as (U/L), serum lactate dehydrogenase activity (LDH) as (U/L), serum alkalinphosphatase (ALP) activity (U/L), acetylcholinesterase (ACHE) activity (U/L), growth hormone (GH) (U/L)and triiodothyronine (T3) (ng/ml).

Statistical Analysis: Data were analyzed by analysis of variance using the general linear model procedure (Proc GLM; SAS institute) [12]. Differences among means were determined using Duncan test [13].

RESULTS AND DISCUSSION

The hazardous effects of Carbofuran (CF) and alleviating effects of Isoflavones (IF) on growth performance of broilers are summarized in Table 1. Data indicated that presence of CF in diet exerted deleterious effect on chick's growth performance, whereas there was a gradual but not significant decrease in body weight (BW), body weight gain (BWG) and feed intake (FI) that was dose-dependent. The hazard effect of CF on BWG and feed intake was reflected on with deterioration in feed conversion ratio (FC) for chicks' groups fed rations contaminated with CF. On the other hand, the addition of *Medicago sativa* seeds as a natural IF source conducted to reduce the hazardous effects of CF and enhance chick's growth with improvement in the appetite for feed consumption but the BW did not reach the values of the control group. The reduction in chicks' body weight and body weight gain in CF treated groups was correlated with decreasing growth hormone (GH) secretion from pituitary gland (as shown in Table 4), whereas GH level as (ng/ml) was decreased significantly ($P<0.01$) with increasing CF level compared to the control group and this effect was CF-dose dependent. Thus the overall means of GH as a percentage of the control were -12.55, -32.38 and -61.13% for 5, 10 and 20 ppm CF, respectively. While, the presence of IF source at 0.5% and 1.0% in CF's rations (10 or 20 ppm CF) alleviate the toxic effect of CF and stimulated GH secretion from pituitary gland, which enhanced the growth and this effect was significantly ($P<0.01$) with 1.0% IF source only but it was still lower than the control mean. Similar results were reported by Rahman *et al*., [14], who found that treated White leghorn chicks with CF for 21 days caused a reduction in BW and feed intake. This reduction in live BW was probably due to impaired food conversion in treated animals. Increase live BW and feed intake in groups treated with CF plus IF source may be attributed to the vital role of IF as antioxidant factor [15]. As well as, plasma growth hormone was increased significantly in ewes and sheep that treated with isoflavones [11].

Table 1: Body weight (BW), Body weight gain (BWG), Feed intake and Feed conversion (FC) of broiler chickens fed rations contaminated with CF at different doses with or without IF source.

	Measurements			
Treatments	BW (g)	BWG (g)	Feed intake (g)	FC (g feed/g gain)
Control	1761.67±63.7	1710.73±68.2	3115.14±20.7	1.82±0.14
5 mg CF	1729.72±38.7	1678.55±54.7	3164.28±28.5	1.88±0.15
10 mg CF	1726.11±47.8	1675.28±47.2	3097.92±18.6	1.84±0.14
10 mg CF +0.05% MS	1750.00±36.0	1699.56±36.2	3042.06±24.7	1.79±0.13
10 mg CF +1.0% MS	1759.17±56.2	1708.23±64.1	3133.20±32.9	1.83±0.12
20 mg CF	1647.08±39.9	1595.75±41.8	2913.54±21.8	1.82±0.11
20 mg CF +0.05% MS	1673.33±46.4	1622.55±52.8	3005.52±21.8	1.85±0.15
20 mg CF +1.0% MS	1679.72±50.9	1629.16±56.3	3006.78±438	1.84±0.11

abc means in the same row having different superscripts are significantly different ($p< 0.05$).

Table 2: Hemoglobin content (Hb), Red blood cell count (RBC), Plasma glucose concentration (Glu) and Serum total protein, Albumin (Alb) and Globulin (Glob) concentration of broiler chickens fed rations contaminated with CF at different doses with or without IF source

	Measurements					
Treatments	Hb (g/dl)	RBC*10^6	TP (g/dl)	Albu (g/dl)	Glob (g/dl)	Glu (mg/dl)
Control	17.83^{a}±0.5	2.73^{a}±0.03	3.99^{a}±0.08	1.54^{a}±0.03	2.45^{a}±0.08	285.6^{e}±10.0
5 mg CF	13.03^{b}±0.8	2.48^{a}±0.12	2.77^{c}±0.04	1.23^{c}±0.01	1.54^{bc}±0.04	445.7^{d}±10.3
10 mg CF	9.20^{de}±0. 2	1.83^{b}±0.25	2.43^{d}±0.09	1.06^{d}±0.02	1.37^{cd}±0.09	530.8^{b}±5.5
10 mg CF +0.05% MS	9.47^{d}±0.5	1.94^{ab}±0.08	2.66^{cd}±0.04	1.18^{cd}±0.02	1.48^{c}±0.04	450.1^{d}±9.0
10 mg CF +1.0% MS	10.40^{c}±0.4	2.29^{a}±0.21	3.06^{b}±0.04	1.35^{b}±0.03	1.71^{b}±0.06	350.4^{e}±3.4
20 mg CF	8.53^{e}±0.1	1.76^{c}±0.20	2.01^{e}±0.07	0.77^{f}±0.04	1.24^{d}±0.07	650.3^{a}±12.4
20 mg CF +0.05% MS	9.70^{cd}±0.2	1.80^{b}±0.02	2.55^{d}±0.02	0.93^{e}±0.02	1.62^{bc}±0.03	483.3^{c}±3.2
20 mg CF +1.0% MS	9.97^{c}±0.4	1.92^{ab}±0.18	2.75^{c}±0.05	1.11^{d}±0.02	1.64^{bc}±0.06	454.6^{d}±3.0

^{abc} means in the same row having different superscripts are significantly different ($p< 0.05$).

Data presented in Table 2 illustrated that hemoglobin (Hb) concentration (g/dl) and red blood cell count (RBC) were significantly ($P<0.01$) decreased in CF treated groups compared to control group and this decrease was CF-dose dependent. The decreasing effect as a percentage from control mean was 15.32, 37.96 and 41.46% in HB content and 2.04, 25.30 and 27.75% in RBC count for 5, 10 and 20 ppm CF, respectively. These data refer to that CF resulted in anemia for birds through its effect on bone marrow caused alteration in wall characteristics of RBC and/or synthesis of these cells. Addition of IF source to the CF's rations resulted in a significant increase in Hb concentration and RBC count and the level of 1% of IF source had higher effect than 0.5% level but this effect was still lower than the control mean. According to Gupta *et al*. [16] the administration of CF to the male albino mice caused a significant reduction in HB and RBCs and the reduction in HB content was due to the reduction in the total number of RBCs. Also, bone marrow depression was observed in mice after administration of pesticide. Moreover, treated rabbits with IF in combination with cypermythrin pesticides minimized the toxic of pesticide [17].

Data presented in Table 2 illustrated that serum total protein (TP) albumin (Alb) and globulin (Glob) as (g/dl) were significantly decreased ($P<0.01$) in a dose-dependent manner with CF treatment compared with control. Addition of IF to the CF-contaminated rations resulted in a significant increase ($P<0.01$) in TP compared to the CF-treated groups and the level of 1% of IF source had higher effect than 0.5% level but remained lower than the control. The reduction of TP, Alb and Glob in birds treated with CF could be attributed to the changes in protein metabolism and in part to the damaging effect of CF on liver cells that confirmed by increasing the activities of serum transaminases activities. This was in accordance with the finding of Faisal *et al*. [18], who observed that liver enzymes activities (AST and ALT) tend to rise suggesting some liver damage in mammals and birds. The increase in serum transaminases activities was also related to amino acid imbalance that initiates protein catabolism [19]. Shakoori *et al*. [20] maintained that the decrease serum TP may be due to reduce protein synthesis, increase proteolytic activity or protein degradation. From the previous studies, administration of CF caused a significant reduction in TP of male rabbits [7].

Table 3: Total cholesterol (Chol), total triglycerides (Tr Gly)), Plasma uric acid concentration and transaminase activities (AST and ALT) of broiler chickens fed rations contaminated with CF at different doses with or without IF source.

	Measurements				
Treatments	Chol (mg/dl)	Tr Gly (mg/dl)	Uric acid (mg/dl)	AST (U/L)	ALT (U/L)
Control	168.67^{d}±7.2	54.84^{d}±1.3	8.28^{e}±0.14	3.06^{e}±0.05	258.24^{f}±9.2
5 mg CF	260.89^{c}±3.5	60.26^{c}±1.0	9.72^{c}±0.15	3.50^{d}±0.07	288.18^{d}±12.9
10 mg CF	295.00^{b}±2.6	74.61^{b}±1.7	11.19^{b}±0.09	4.23^{b}±0.14	303.36^{c}±12.4
10 mg CF +0.05% MS	188.89^{d}±6.8	63.97^{c}±1.7	9.94^{c}±0.10	3.96^{c}±0.04	292.01^{d}±14.2
10 mg CF +1.0% MS	140.11^{e}±4.1	54.45^{d}±0.9	8.98^{d}±0.08	3.43^{e}±0.10	276.86^{e}±11.7
20 mg CF	306.22^{a}±10.8	89.61^{a}±1.3	15.04^{a}±0.19	5.29^{a}±0.07	327.93^{a}±12.8
20 mg CF +0.05% MS	294.11^{b}±2.2	74.13^{b}±1.4	11.00^{b}±0.35	4.64^{b}±0.11	313.3^{b}±12.9
20 mg CF +1.0% MS	176.78^{d}±5.5	64.45^{c}±2.1	8.77^{de}±0.19	3.57^{d}±0.09	293.82^{d}±13.3

^{abc} means in the same row having different superscripts are significantly different ($p< 0.05$)

Moreover, IF in combination with cypermythrin (Carbamate pesticide) reduced the toxicity of pesticide in male rabbits [17]. The improvement effects in TP, Albu and Glob contents due to addition of IF source to the CF-rations may be attributed to the vital role of IF as an antioxidant factor that protects the animal against CF-induced damage. This finding was in agreement with those obtained by Sierens *et al* [21], who found that IF may be able to function as an antioxidant, scavenging potentially harmful free radical.

Plasma glucose level as (g/dl) was significantly increased in a dose-dependent manner (P<0.01) in CF-treated groups compared with control resulting in hyperglycemia. On the other hand, the use of IF source (0.5 or 1.0%) with CF alleviated the negatively effect of CF on Plasma glucose and decreased its concentration in blood, this decrease was IF-level dependent. The negative effect of CF on blood glucose metabolism may be reflected on blood lipid metabolism and activate the conversion of glucose skeleton carbon to lipids, whereas, the plasma triglycerides (TGl) concentration as (mg/dl) was significantly increased (P<0.01) taking the same trend of blood glucose, whether with CF or CF plus IF (Table 3). These results are in accordance with the finding of Rahman *et al*. [14], who found that plasma glucose was significantly increased when white leghorn chicks treated with CF. The change in carbohydrate metabolism induced by CF treatment can be correlated with the effect of CF toxicity on hepatic enzyme systems, which are intimately involved in glucose production, storage and metabolism and/or correlated with the effect on endocrine activity of the pancreas (insulin activity). In addition, hyperglycemic status may be due to glycogenolysis in liver or due to gluconeogenesis through breaking down protein [22]. Moreover, the supplementation of IF in diet decreased the side effect of CF on the carbohydrate metabolism. On the other hand, TGl was significantly increased in rats that treated with carbaryl [23]. While, treated rabbits with IF caused a significantly decrease in plasma Tgl [17]. From data in Table 3 it could be concluded that CF had a highly significant effect (P<0.01) on plasma cholesterol (Chol) concentration (mg/dl), whereas, plasma cholesterol content was adversely increased compared to the control group and this effect was CF-dose dependent. While, based on CF dosage, adding IF source at any level resulted in significantly decrease (P<0.01) in plasma cholesterol level. Moreover, the level of 1% IF plus 10 ppm CF had the lowest cholesterol mean than the other treated groups or the control. Guclu *et al*. [24] found that plasma cholesterol content and egg yolk cholesterol content were decreased in laying quails that fed diet containing alfalfa meal as IF source.

The hazardous effect of CF on serum uric acid concentration (mg/dl) (Table 3) showed a significant increase (P<0.01) in serum uric acid that was increased with increase CF-dosage in diet. This effect was 117.39, 135.14 and 181.64% from the control mean. While, the addition IF source to the CF ration resulted in a positive effect on CF hazard effect and significantly reduced (P<0.01) serum uric acid concentration compared to the CF groups. Increase serum uric acid concentration in the CF groups may be due to the increase of protein catabolism, whereas, the data showed a decrease in serum protein concentration with increase transaminases enzymes activities levels (AST and ALT) that initiates protein catabolism and/or the toxicity effect of CF on the kidney caused histopathological changes in renal, which caused a disturbance in the transportation of biochemical constituent in the kidney. Our finding is in agreement with Almdal and Vilstrup [25], who declared that the exposure to pesticides induced elevation of serum uric acid content, which is considered as significant marker of renal dysfunction. Also, blood uric acid is known to be correlated with an increase protein catabolism and more to

Table 4: Serum lactate dehydrogenase (LDH), alkalinphosphatase (ALP), acetylcholinesterase (ACHE) activities, growth hormone (GH) concentration and total triiodothyronine (T3) concentration of broiler chickens fed diets containing DDGS at different levels with or without commercial enzymes supplementation

	Measurements				
Treatments	LDH	ALP (U/L)	ACHE (U/L)	T3 (ng/ml)	GH (ng/ml)
Control	219.02^{d}±3.2	365.7±10.9	1476.4^{a}±4.0	1.35^{a}±0.05	2.47^{a}±0.06
5 mg CF	244.84c±1.1	377.9±10.4	1230.4^{c}±7.3	1.29^{a}±0.02	2.16^{bc}±0.04
10 mg CF	261.71^{b}±1.4	380.5±9.0	1121.0^{d}±6.4	1.18^{b}±0.02	1.67^{c}±0.04
10 mg CF +0.05% MS	245.22^{c}±1.5	378.8±10.4	1189.1^{c}±3.8	1.19^{b}±0.01	1.85^{c}±0.07
10 mg CF +1.0% MS	231.81^{d}±2.0	377.9±8.4	1300.3^{b}±7.5	1.30^{a}±0.02	2.36^{b}±0.01
20 mg CF	297.70^{a}±3.2	397.0±11.2	1000.3^{e}±5.2	0.92^{c}±0.04	0.96^{d}±0.07
20 mg CF +0.05% MS	259.93^{b}±3.5	396.8±10.9	1093.8^{d}±5.1	0.98^{c}±0.05	1.00^{d}±0.03
20 mg CF +1.0% MS	247.29^{c}±2.1	389.3±10.2	1170.5^{cd}±7.0	1.17^{bc}±0.02	1.94^{c}±0.02

^{abc} means in the same row having different superscripts are significantly different ($p< 0.05$)

efficient conversion of ammonia to urea as a result of increase synthesis of enzymes involved in urea production and then to uric acid [26]. On the other hand, supplementation of IF protected the kidney function and prevent the damage in the nephrotubules, thus, the presence of IF with CF alleviate its harmful effect.

Data illustrated in Table 3 showed that CF at any doses increased significantly ($P<0.01$) serum transaminases enzymes (AST and ALT) activities (U/L) compared to the control group and this effect was CF-dose dependent. Meanwhile, addition of IF source to CF ration (10 and 20 ppm) revealed a significant reduction ($P<0.01$) in serum AST and ALT activities compared to the CF treated groups. Increase AST and ALT levels indicate that there is an active transamination of amino acids and operation of keto acids, which are probably fed into tricarboxylic acid cycle for oxidation [27]. The increments of AST and ALT activities in plasma are mainly due to the leakage of these enzymes from the hepatic cytosol into the blood [28] and the alteration in the activities of these enzymes indicated liver damage and disruption of normal liver function [20]. Results, also, showed that IF minimize the hazardous effect of CF on AST and ALT activities. These results are in a good agreement with Cho *et al*. [29], who concluded that there was a dose-dependent decrease in AST in rats that administrated with IF.

From data in Table 4 it can be concluded that contaminated feed with CF at any dose resulted in a highly significant increase in serum lactate dehydrogenase (LDH) activity (U/L) and this effect was CF-dose. On the other side, the combination between CF and IF caused a significant decrease in serum LDH activity compared to the CF treated group or control one, except the group treated with 10 ppm CF plus 1.0% IF, which did not differ significantly from the control group. These findings are in a good agreement with the results of Gupta *et al*. [30], who found that an acute dose of CF (1.5 mg/kg BW) in rats evoked severe toxic manifestation characteristics. A 24-hours time course following CF administration indicated a two-fold increase in the activity of total LDH in serum. In the present study results showed that IF supplementation minimized the hazardous effect of CF on serum LDH activity and IF could favorably modify the metabolism of CF and cause free radical scavenging activity [15]. The enzymatic activity of ALP (U/L) was gradually and non-significantly increased with increase CF dosage compared to the control group (Table 4). Otherwise, the presence of IF in the contaminated rations counteracted the toxicity effect of CF and decreased ALP activity but the level was still higher than detected in control group. Increasing ALP activity may be due to the breakdown of the transport system membranes, an inhibitory effect on cell growth and proliferation [31]. Also, increasing ALP was attributed to its release from damaged hepatic cell [32]. Also, ALP was significantly increased compared to the control when rats treated with sub-chronic exposure of CF in drinking water [33]. Rations treated with CF at any doses caused a dose-dependent and significant reduction ($P<0.01$) in serum acetylcholine esterase (ACHE) activity compared to control. While, the combination of CF with IF source at any levels caused highly significant increase ($P<0.01$) in ACHE activity compared with CF-treated group but remained significantly lower than control. Our findings are in agreement with the results of Yousef *et al*. [7], who found that ACHE activity was significantly decreased after treated New Zealand white rabbits with CF. As well as, when mallard ducklings were fed Diet containing CF for 10 days, lower ACHE was observed [8]. Serum ACHE activity was significantly decreased compared to the control after treatment rats with sub-chronic exposure of CF in drinking water [33]. Our data revealed that IF may be

act as an antioxidant that protects against CF-induced damage. IF could favorably modify the metabolism of CF and confirming the beneficial effects of IF to the animals CF-contaminated diets and cause free radicals scavenging activity [15]. Data presented in Table 4 indicated that there was a significant decrease ($P<0.01$) in serum triiodothyronine (T_3) due to CF treatments compared with control, except the 5 mg/kg group that did not differ from control. However, adding IF source to the CF chicks' rations caused a significant increase ($P<0.01$) in serum T_3 compared to the CF-treated group. CF works as a thyroid inhibitor through a direct action on thyroid gland and/or indirectly through the hypothalamic-pituitary-thyroid axis [34]. A decline in plasma protein-pound iodine (PBI) in fishes exposure to CF and this effect conducted to the limit synthesis and or release of T3 by the thyroid follicles leading to its atrophy [35]. On the other hand, adding IF plus CF decreased the toxic effect of CF on the thyroid hormone activity. Isoflavones could favorably modify the metabolism of CF in CF-contaminated diets and invoke free radicals scavenging activity [15].

CONCLUSION

It could be concluded that using Isoflavones (IF) in combination with Carbofuran (CF) minimized CF hazardous effects on most of the tested parameters and this may be attributed to the vital role of IF as an antioxidant in preventing pesticide toxicity during exposure to environmental pollution.

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