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EFFECT OF COUMARIN SUPPLEMENTATION TO GROWING RABBIT DIETS ON ALLEVIATION THE TOXICITY OF AFLATOXIN B_1

Amerá A.A. Helal*, S.A. Shehata, A.E. Naser and M.S. Ayyat

Animal Prod. Dept., Fac. Agric., Zagazig Univ., Egypt

ABSTRACT

Forty two New Zealand White (NZW) male rabbits with average body weight 685.4±11.7 g were randomly assigned to 6 groups (7 animals in each) in a 3 x 2 factorial design to evaluate the ability of coumarin on alleviation the toxicity of aflatoxin B_1. Rabbits of the first 3 groups were given basal diet (contained 17% yellow corn, 35% clover hay, 10% barley, 13% soybean meal, 20% wheat bran, 3.0% molasses, 0.20% sodium chloride, 0.40% vitamins and minerals, 1.0% premix and 0.40% limestone) without aflatoxin B_1, while the other 3 groups were given the basal diet containing 0.25 ppm aflatoxin B_1. Within each the previous two groups they were divided to other 3 subgroups: the first group fed diet without any treatment (control), the second and third groups were supplemented with 2.5 and 5 g coumarin/kg diet, respectively. Feeding rabbits contaminated diet with aflatoxin B_1 were significantly reduced daily feed intake, feed conversion, and digestibility of crude protein. Also, red blood cells count (RBCs) as well as hemoglobin, total protein and albumin concentrations were decreased in blood rabbits feed with contaminated diet. This in turn was reflected as a reduction in total and daily body weight gain and an increase in mortality rate. Adding coumarin at rate of 2.5 or 5g / kg diet contaminated with aflatoxin B_1 were significantly ($P<0.05$) reduced the negative effects of aflatoxin B_1 on total body weight and daily weight gain, feed intake, feed conversion, digestibility of nutrients (organic matter, crude protein, crude fiber, ether extract and nitrogen free extract), blood parameters (hematokrite %, red blood cells count, total protein and albumin concentration, aspertate aminotransferase and alanine aminotransferase activities), mortality rate, residue of aflatoxin B_1 in rabbit thigh muscles and economical efficiency. There was no significant difference between the two tested coumarin levels. In conclusion, adding coumarin (2.5 g coumarin/ Kg diet) to aflatoxin B_1 contaminated rabbit diet was safe and practical method to minimize aflatoxin B_1 toxicity.

Key words: Rabbits, aflatoxin, coumarin, growth performance, digestibility, blood, aflatoxin residue.

INTRODUCTION

Aflatoxins are naturally occurring toxins produced in grains and other feedstuffs both before and after harvest by toxigenic strains of the fungi *Aspergillus flavus* and *Aspergillus parasiticus* (Handan and Güleray, 2005). In the family of aflatoxins, aflatoxin B_1 is the most prevalent and toxic for human and land animals, mostly by its strong carcinogenic, mutagenic and teratogenic effects (IARC, 1993 and Han *et al.*, 2008). Aflatoxin constitutes a real threat to the health of livestock as well as humans by their continuing intermittent occurrence in both feeds and foods (Robens and Richard, 1992). Several factors may enhance the occurrence of mycotoxin in the human diet in developing countries. These include eating habits, existing marketing problems which encourage long storage periods, the pre and post harvest practices that encourage build up of moisture and thus encourage mould growth, ignorance and poverty. This is aggravated by the fact that there are no strict regulations that impose limits on the concentration of mycotoxins in crops that is marketed in these countries as well as lack of relevant technology required in monitoring fungi and mycotoxins in the grains (Wilkister and

* **Corresponding author: Tel. :** +201095950678
E-mail address: helalamera@yahoo.com

Nyaora, 2008). Aflatoxin B_1 is of primary concern because it is the most abundant and the most toxic. Acute or chronic aflatoxicosis may occur depending on the dietary concentration of toxins. Rabbits are extremely sensitive to aflatoxin. The acute oral single dose median lethal dose is about 0.3 mg kg−1 body weight (Nèwberne and Butler, 1969), among the lowest of any animal species. Moderate to severe death losses can be encountered with diets containing even low concentrations of toxin; <100 ppb; (Krihsna *et al.*, 1991). Acute aflatoxin poisoning (aflatoxin B_1 daily doses >0.04 mg kg−1 body weight) causes a prolonged blood-clotting time, extensive liver damage and death from liver failure (Clark *et al.*, 1980, 1982, 1986).

The mechanism of action of aflatoxin B_1 on the cell is mediated through the production of free radicals and reactive oxygen species (Baynes, 1991 and Van Dam *et al.*, 1995). Amstad *et al.* (1984) showed that aflatoxin B_1 could stimulate the release of free radicals resulting in chromosomal damages. So, reactive oxygen species may in part be responsible for the carcinogenic activity of aflatoxin B_1 (Shen *et al.*, 1996).

The beneficial effect of coumarin may be due to: 1- Reduction aflatoxin B_1-DNA adducts formation by both liver and intestinal microsomes (coumarin enhanced aflatoxicol formation therefore decrease aflatoxin B_1-DNA adducts, because direct interaction of aflatoxicol-epoxide with DNA is minor compared with aflatoxin B_1-epoxide; Loveland *et al.*, 1987, 2- Enhancement of glutathione S-transferase (GST) activity in the intestine to conjugate aflatoxin B_1, 3- Suppression of p450 enzyme activity in the liver and enhancement of GST in the intestine (Tulayakul *et al.*, 2007), 4- Improving liver function (Gilani and Janbaz, 1993) and body health (Maucher *et al.*, 1994; Hoult and Paya, 1996; Pillai *et al.*, 1999; Devienne *et al.*, 2005) and 5- Increasing the digestibility of crude protein and ether extract (Ko *et al.*, 2006).

More than 1300 coumarins have been identified from natural sources especially green plants (Hoult and Paya, 1996). Coumarins are antioxidants, contain the parent nucleus of benzo-ά-pyrone and occur in plants like tonaka beans, sweet clover, wood ruff, cassia leaf (Lake *et al.*, 1989). Also, it was in the variety of plant families like Loganiaceae (Bhattacharyya *et al.*, 2008), Orchidaceae, Leguminaceae, Rutaceae, Umbeliferae and Labiatae (Vyas *et al.*, 2009). Synthetic coumarin (4-methyl-7 hydroxy coumarin) derived from resorcinol and ethyl aceto-acetate in the presence of concentrated sulphuric acid is structurally close to scopoletin, being a coumarin derivative. Coumarin has anti cancer, antibacterial, anticoagulants, anti-thrombotic and anti-mutagenic effect (Hoult and Paya, 1996; Pillai *et al.*, 1999; Khan *et al.*, 2005; Bhattacharyya *et al.*, 2009 and Prince *et al.*, 2009). Coumarin was used for reduction aflatoxin B_1 toxicity in pigs (Tulayakul *et al.*, 2007), rat (Kelly *et al.*, 2000) and Nile tilapia fish (Shehata and Mohamed, 2012).

The present investigation was under taken to study alleviation of afltoxin B_1 toxicity in growing rabbit diets by different levels of coumarin.

MATERIALS AND METHODS

The experimental work was carried out in Animal Production Department, Faculty of Agriculture, Zagazig University, Egypt. Forty two New Zealand White (NZW) male rabbits with average body weight 685.4±11.7 g were randomly assigned to 6 groups (7 animals in each) in a 2 X 3 factorial design. Rabbits of the first 3 groups were given basal diet without aflatoxin B_1, while the other 3 groups were given basal diets containing 0.25 ppm aflatoxin B_1. Within each the previous two groups they were divided to other 3 subgroups: the first group fed diet without any treatment (control), the second and third groups were supplemented with 2.5 and 5 g coumarin/kg diet, respectively.

Coumarin was prepared according to method of (Furniss *et al.*, 1978) which summarized as follow: one liter of concentrated sulphuric acid in a 3 liter nicked flask, immerse the flask in ice bath, add a solution of 100 g (0.91 mol) of resorcenol in 134 g (130.5 ml, 1.03mol) of ethyl aceto-acetate drop wise and stirring 2 hrs., keep the reaction mixture at room temperature for about 18 hrs., then pour it with vigorous stirring into a mixture of crushed ice and water, collect the crude yield (yield = 155 g, 97%

concentration), recrystallization in ethanol 95% and air dried.

Aspergillus flavus MD 341 was obtained from the Central Lab. of Residues in Agriculture Products, Agriculture Pesticides Residues Centre, Dokki, Egypt, for production of aflatoxin B_1 on liquid media (2% yeast extract and 20% sucrose). The aflatoxin concentration was determined using the method of AOAC (1990). The media was found to contain aflatoxin B_1 alone. The media sprayed on diet to obtain 0.25 ppm aflatoxin B_1. Rabbits were housed in individual cages under the same managerial, hygienic and environmental conditions all over the experimental period (7 weeks). The used basal diet was contained 17% yellow corn, 35% clover hay, 10% barley, 13% soybean meal, 20% wheat bran, 3.0% molasses, 0.20% sodium chloride, 0.40% vitamins and minerals, 1.0% premix and 0.40% limestone. These components were reflected as: 88% organic matter, 17.3% crude protein, 13.42% crude fiber, 2.89 ether extract, 54.39 nitrogen free extract and 12% ash.

Daily fresh water was available all time. At the last week of the trial, feed intake and feces excreted of 4 rabbits from each treatment were daily recorded for digestibility trials. At the end of the experimental feeding period, blood samples of 4 rabbits were collected at slaughter time to estimate blood parameters. Hemoglobin and hematokrite concentrations as well as red blood cells count were determined. Also, serum total protein, albumin, aspertate amino transferase (AST) and alanine amino transferase (ALT) were analyzed using commercial kits purchased from Diamond Diagnostics Company, Egypt. Also, the internal organs (liver, kidneys, heart and lungs) were removed from the body, and then weighted. Proximate analysis of feed, feces and residue of aflatoxin in thigh muscles were determined according to AOAC (1990).

Economic evaluation was calculated according to Ayyat (1991) as the following equation: Final margin (Profit) = Income from body gain weight - feed cost. Relative margin = Final margin X Survival rate (Ayyat *et al.*, unpublished equation). Other overhead costs were assumed constant. Price of one kg of diet was 2.012 LE (Egyptian pound = 0.185 US$) and price of selling of one kg live body weight of rabbits was 22.0 LE and the price of one kg coumarin was 520 LE.

Data of the trial was statistically analyzed using General Linear Model Program of SAS (2002). Resulted data of growth performance, digestibility, blood components and pre-slaughter live body weight were analyzed using factorial analysis of variance, according to the following model:

Model 1: $Y_{ijk} = \mu + A_i + C_j + AC_{ij} + e_{ijk}$

Where:

Y_{ijk} = an observation, μ is the overall mean, A is the fixed effect of aflatoxin (i = 1 ...2), C is the fixed effect of treatments (j = 1 ...3), AC is the fixed effect of the interaction between aflatoxin and coumarin and e_{ijk} is random error. Significant differences between treatments were tested with Duncan's multiple range test (Duncan, 1955).

Data of carcass and internal organs were statistically analyzed by analysis of covariance (factorial experiment) according to the following model:

Model 2: $Y_{ijk} = \mu + S_i + A_j + AC_{ij} + b(X-x) + e_{ijk}$

Where:

Y_{ijk}, μ, A_i, C_j, AC_{ij} and e_{ijk} were as defined in the Model 1, b = partial linear regression coefficients of Y_{ij} on slaughter weight, X = value of slaughter weight and x = overall average of slaughter weight.

RESULTS AND DISCUSSION

Growth Performance and Feed Conversion

Final body weight, daily gain, feed intake and feed conversion affected significantly ($P<0.001$) with aflatoxin contaminated diets (Table 1). Final body weight, daily gain and feed intake were decreased by 16.86, 23.74 and 15.60%, respectively among rabbit groups fed on diets contaminated with 0.25 ppm afltoxin when compared with the control group (fed diets

Table 1. Growth performance and feed conversion of growing rabbits as affected by aflatoxin B_1 toxicity and coumarin supplementation to reduce its effect and their interactions

Items	Initial weight (g)	Final weight (g)	Daily body weight gain (g)	Daily feed intake (g)	Feed conversion (feed/gain)
Effect of aflatoxin					
0.00 ppm	688.76±12.16	2251.90±20.18	31.89±0.43	111.83±3.40	3.643±0.082
0.25 ppm	681.95±20.21	1872.33±85.81	24.32±1.69	94.38±4.66	4.273±0.224
Significance	NS	***	***	***	***
Effect of coumarin					
0.0 g/Kg diet	686.14±12.91	1759.57^{c}±107.84	21.91^{c}±2.16	85.77^{c}±4.45	4.454^{a}±0.306
2.5 g /Kg diet	679.36±26.86	2124.29^{b}±45.77	29.48^{b}±0.80	104.82^{b}±3.98	3.694^{b}±0.136
5.0 g/Kg diet	690.57±19.85	2302.50^{a}±27.16	32.91^{a}±0.43	118.73^{a}±4.07	3.727^{b}±0.118
Significance	NS	***	***	***	***
Interaction effect between aflatoxin and coumarin					
0.00 ppm aflatoxin					
0.0 g/Kg diet	691.00±18.91	2145.29^{b}±11.02	29.67^{d}±0.40	98.26±4.23	3.503^{b}±0.146
2.5 g /Kg diet	687.00±19.29	2265.71^{a}±22.73	32.20^{b}±0.27	113.47±3.66	3.641^{b}±0.125
5.0 g/Kg diet	688.29±27.36	2344.71^{a}±8.59	33.80^{a}±0.41	123.77±5.40	3.783^{b}±0.157
0.25 ppm aflatoxin					
0.0 g/Kg diet	681.29±18.89	1373.86^{d}±26.14	14.17^{f}±0.32	73.29±3.99	5.401^{a}±0.290
2.5 g /Kg diet	671.71±52.30	1982.86^{c}±43.50	26.76^{e}±0.50	96.16±5.51	3.746^{b}±0.254
5.0 g/Kg diet	692.86±30.94	2260.29^{a}±50.29	32.03^{c}±0.61	113.70±5.84	3.671^{b}±0.187
Significance	NS	***	***	NS	***

Means in the same column bearing different letters differ significantly ($P < 0.05$).

NS = Not significant, * $P<0.05$, ** $P<0.01$ and *** $P<0.001$.

without aflatoxin contamination), while feed conversion was impaired by 17.29%. The reduction in body weight gain by added aflatoxin is due to depression of feed intake, reduction in metabolism of feed nutrients (Cheeke and Shull, 1985 and Marai and Askar 2008), also, it might be due to detoxification process in the body utilizing glutathione enzymes (glutathione partly composed of methionine and cystein), hence this detoxification process depletes the metabolic availability of methionine leading to poor growth and feed efficiency (Devegowda *et al.*, 1998). The obtained results were agreed with the findings of Meshreky *et al.* (2007) and Shehata (2010) on rabbits.

Final body weight, daily gain, feed intake and feed conversion affected significantly ($P<0.001$) with coumarin supplementation in rabbit diets (Table 1). Final body weight, daily gain and feed intake increased by 20.73, 34.55 and 22.21%, respectively in rabbit groups fed on diets supplemented with 2.5 g coumarin/kg diet when compared with the control group (fed diets without coumarin supplementation), while the same trend for rabbits fed diets supplemented with 5 g coumarin/kg were 30.86, 50.21 and 38.43%, respectively. On the other hand fed conversion improved with 17.06 and 16.32%, respectively on rabbits fed diets supplemented with 2.5 and 5 g coumarin/ kg diet.

The interaction showed that coumarin had significant (P<0.001) effects on final body weight, daily body gain and feed conversion of rabbits (Table 1). Rabbit fed diets without or with aflatoxin B_1 contamination and supplemented with coumarin (2.5 or 5 g/kg diet) increased the final body weight and daily gain and improved feed conversion. Within aflatoxin group, the high level coumarin (5 g) supplementation recorded the best growth rate (P<0.05). These data suggest that consumption of a coumarin-containing diet provides substantial protection against the aflatoxin B_1 in rabbit. Kelly *et al.* (2000) found that the treatment with coumarin has been found to increase hepatic aldo-keto reductase activity toward aflatoxin B_1 -dialdehyde and glutathione *S*-transferase (GST) activity toward aflatoxin B_1 -8, 9-epoxide in both male and female rats.

Digestibility and Nutritive Values

Digestibility of crude protein and ether extract and nutritive value as digestible crude protein (DCP %) were significantly (P<0.01) decreased by aflatoxin B_1 treatment, while other traits insignificantly affected (Table 2). These results were in harmony with those reported by Abd El-Baki *et al.*, (2002) and Shehata, (2002 and 2010). The harmful effect of aflatoxin on digestibility and nutritive values may be due to interfering with utilization of dietary nutrients (Cheeke and Shull, 1985 and Diekman and Green, 1992).

Coumarin supplementation in rabbit diets significantly (P<0.05) increased the digestibility of crude protein, crud fiber, ether extract and nutritive value as digestible crude protein, while free nitrogen extract (NFE) was decreased (P<0.05).

The interaction between aflatoxin toxicity and coumarin supplementation insignificantly affected the digestibility traits, except the ether extract digestibility was increased significantly (P<0.05). Adding 2.5 or 5 g coumarin/kg diet improved the digestibility traits and nutritive values as DCP% (Table 2).The beneficial effect of coumarin may be due to reduction of aflatoxin effect on body function (Loveland *et al.*, 1987), improve the absorption of protein and fat (Ko *et al.*, 2006).

Also, coumarin stimulates the secretion of bile salts and lipolytic enzymes in the small intestine (Hahn, 1966). The interaction effect showed an improvement in feed utilization with contaminated diets supplemented with coumarin.

Blood Parameters

As shown in Table 3, the concentrations of hemoglobin, red blood cells count (RBCs) and total protein (P<0.001), albumin (P<0.01) and ALT (P<0.05) were decreased, while AST concentration was increased (P<0.001) with aflatoxin toxicity. These findings agreed with those reported by Nowar *et al.* (1996), Abd El-Baki *et al.* (2002) and Shehata (2002 and 2010). Decreasing of serum protein may be attributed to degeneration of endoplasmic reticulum and inhibition of protein synthesis (Srivastava, 1984). The activity of aspertate amino transferase (AST) enzyme was increased by aflatoxin B_1. These results agreed with those reported by Zaky *et al.* (2000) and Shehata (2010). Increasing AST activities may be due to hepatocellular necrosis or increasing the permeability of cell membrane (Zaky *et al.*, 2000).

Generally, addition of coumarin (2.5 or 5 g/kg diet) significantly (P<0.05) increased the RBCs count, hematokrite, total protein and albumin concentrations, but decreased AST and ALT concentration (Table 3).

The interaction between aflatoxin toxicity and coumarin significantly affected the RBCs count and hematokrite (P<0.05), total protein, albumin and AST (P<0.01) concentrations (Table 3). Within aflatoxin groups, coumarin supplementation in rabbit diets increased the RBCs count and the concentrations of each hematokrite, total protein, and albumin and decreased the concentration AST. These results may be due to the beneficial effect of coumarin on reduction aflatoxin effect and improve body organs function as mentioned above.

Carcass and Internal Organs

Pre-slaughter weight affected significantly (P<0.001) with aflatoxin contamination, coumarin supplementation and the interaction between them (Table 4). However, analysis of covariance indicated that adjusted carcass and

Table 2. Digestibility and nutritive value of the experimental diets as affected by aflatoxin B_1 toxicity and coumarin supplementation to reduce its effect and their interactions

Items	Digestibility Coefficient (%)						Nutritive values (%)	
	DM	OM	CP	CF	EE	NFE	TDN	DCP
Effect of aflatoxin								
0.00 ppm	72.26±1.51	76.24±1.24	83.39^{a}±1.23	46.61±1.67	82.79^{b}±2.98	78.18±1.58	68.59±0.96	14.43^{a}±0.21
0.25 ppm	71.50±1.31	76.06±1.13	78.77^{b}±0.97	43.09±2.47	89.58^{a}±1.37	78.05±1.51	67.69±0.95	13.63^{b}±0.17
Significance	NS	NS	**	NS	**	NS	NS	**
Coumarin effect								
0.0 g/Kg diet	71.49±.1.28	75.14±1.02	78.41^{b}±1.33	39.02^{b}±2.14	80.68^{b}±3.56	81.87^{a}±0.70	68.57±0.68	13.57^{b}±0.23
2.5 g /Kg diet	72.38±1.71	77.20±1.35	83.68^{a}±1.06	48.75^{a}±2.29	89.78^{a}±2.68	76.33^{b}±1.77	68.37±1.14	14.48^{a}±0.18
5.0 g/Kg diet	71.78±2.24	76.11±1.88	81.15^{ab}±1.86	46.78^{a}±1.67	88.10^{a}±1.84	76.16^{b}±1.90	67.47±1.60	14.04^{ab}±0.32
Significance	NS	NS	*	*	*	*	NS	*
Interaction effect between aflatoxin and coumarin								
0.00 ppm aflatoxin								
0.0 g/Kg diet	71.00±2.09	73.90±0.75	80.00±1.92	42.00±1.79	73.00^{b}±1.95	81.98±1.00	68.81±0.72	13.84±0.33
2.5 g /Kg diet	70.97±1.80	76.20±1.33	85.68±0.79	49.31±1.87	86.56^{a}±4.93	74.06±1.48	67.35±1.24	14.82±0.14
5.0 g/Kg diet	74.83±3.83	78.63±3.27	84.50±2.22	48.52±3.29	88.83^{a}±2.26	78.51±3.29	69.60±2.76	14.62±0.38
0.25 ppm aflatoxin								
0.0 g/Kg diet	71.97±1.90	76.39±1.75	76.83±1.63	36.05±3.31	88.36^{a}±0.88	81.75±1.19	68.33±1.32	13.29±0.28
2.5 g /Kg diet	73.80±3.05	78.19±2.52	81.68±1.00	48.18±4.75	93.00^{a}±1.15	78.60±2.89	69.39±1.98	14.13±0.17
5.0 g/Kg diet	68.73±1.01	73.59±0.83	77.80±1.06	45.03±0.17	87.38^{a}±3.36	73.81±1.29	65.33±0.82	13.46±0.18
Significance	NS	NS	NS	NS	*	NS	NS	NS

Means in the same column bearing different letters differ significantly ($P < 0.05$).

NS = Not significant, * $P<0.05$ and ** $P<0.01$.

Table 3. Blood parameters of growing rabbits as affected by aflatoxin B_1 toxicity and coumarin supplementation to reduce its effect and their interactions

Items	RBCs count (10^6/ml)	Hemoglobin (g/dl)	Hematokrite (%)	Total Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	AST (u/l)	ALT (u/l)
Effect of aflatoxin								
0.00 ppm	5.39±0.16	10.60±0.21	43.82±0.83	5.39 ±0.17	3.07±0.10	2.32±0.20	15.58±0.53	12.33±0.93
0.25 ppm	4.73±0.22	9.41±0.13	42.00±1.36	4.73±0.20	2.70±0.18	2.03±0.12	20.83±1.13	10.33±1.01
Significance	***	***	NS	***	**	NS	***	*
Coumarin effect								
0.0 g/Kg diet	4.45^{b}±0.24	9.79±0.35	39.86^{c}±1.57	4.45^{b}±0.26	2.40^{b}±0.21	2.05±0.22	21.38^{a}±1.80	10.67^{b}±1.45
2.5 g /Kg diet	5.19^{a}±0.08	9.88±0.18	42.52^{b}±0.62	5.19^{a}±0.12	3.16^{a}±0.10	2.03±0.14	16.25^{b}±0.59	13.33^{a}±1.25
5.0 g/Kg diet	5.54^{a}±0.25	10.34±0.35	46.35^{a}±0.65	5.53^{a}±0.19	3.08^{a}±0.12	2.45±0.24	17.00^{b}±0.90	10.00^{b}±1.00
Significance	***	NS	***	***	***	NS	***	*
Interaction effect between aflatoxin and coumarin								
0.00 ppm aflatoxin								
0.0 g/Kg diet	5.01^{b}±0.18	10.48±0.47	42.98^{ab}±1.47	5.01^{b}±0.24	2.90^{a}±0.19	2.12±0.39	17.00^{bc}±1.29	12.33±1.20
2.5 g /Kg diet	5.15^{b}±0.15	10.28±0.14	41.89^{b}±0.14	5.15^{b}±0.21	3.16^{a}±0.15	1.99±0.13	15.00^{c}±0.41	13.00±2.65
5.0 g/Kg diet	6.00^{a}±0.18	11.05±0.40	46.60^{a}±1.18	6.00^{a}±0.17	3.15^{a}±0.22	2.85±0.38	14.75^{c}±0.48	11.67±1.20
0.25 ppm aflatoxin								
0.0 g/Kg diet	3.89^{c}±0.18	9.10±0.19	36.75^{c}±1.68	3.89^{c}±0.22	1.91^{b}±0.05	1.98±0.26	25.75^{a}±0.86	9.00±1.53
2.5 g /Kg diet	5.23^{b}±0.10	9.48±0.16	43.15^{ab}±1.24	5.23^{b}±0.18	3.16^{a}±0.14	2.07±0.27	17.50^{b}±0.65	13.67±0.88
5.0 g/Kg diet	5.07^{b}±0.36	9.64±0.28	46.10^{a}±0.73	5.06^{b}±0.02	3.02^{a}±0.12	2.05±0.14	19.25^{b}±0.48	8.33±0.88
Significance	*	NS	*	**	**	NS	**	NS

Means in the same column bearing different letters differ significantly ($P < 0.05$).

NS = Not significant, * $P<0.05$, ** $P<0.01$ and *** $P<0.001$.

Table 4. Pre-slaughter, carcass and some internal organs weights of growing rabbits as affected by aflatoxin B_1 toxicity and coumarin supplementation to reduce its effect and their interactions

Items	Pre-slaughter weight (g)	Carcass weight (g)	Liver weight (g)	Kidney weigh (g)	Lung weight (g)	Heart weight (g)	Testes weight (g)
Effect of aflatoxin							
0.00 ppm	2254.33±28.089	1291.572±21.739	66.579±3.830	17.319±1.050	13.491±0.816	6.511±0.500	7.177±0.600
0.25 ppm	1879.58±119.55	1175.845±21.739	92.330±3.830	18.122±1.050	15.226±0.816	6.648±0.500	8.348±0.600
Significance	***	NS	**	NS	NS	NS	NS
Coumarin effect							
0.0 g/Kg diet	1764.88±148.20^{c}	1168.403±32.248	86.992±5.682	18.021±1.558	14.452±1.210	5.732±0.7423	7.985±0.891
2.5 g /Kg diet	2126.50±67.80^{b}	1255.392±16.954	77.612±2.987	17.264±0.819	14.367±0.636	6.629±0.390	7.626±0.462
5.0 g/Kg diet	2309.50±45.69^{a}	1277.330±27.600	73.759±4.863	17.878±1.334	14.256±1.036	7.378±0.635	7.677±0.762
Significance	***	NS	NS	NS	NS	NS	NS
Interaction effect between aflatoxin and coumarin							
0.00 ppm aflatoxin							
0.0 g/Kg diet	2151.75±18.70^{bc}	1253.445±23.995	72.981±4.228	17.874±1.159	14.567±0.900	6.537±0.552	6.925±0.663
2.5 g /Kg diet	2266.75±42.49^{ab}	1286.738±29.266	66.620±5.157	16.717±1.414	12.161±1.098	6.357±0.674	6.729±0.808
5.0 g/Kg diet	2344.50±15.95^{a}	1334.533±34.277	60.135±6.039	17.366±1.656	13.745±1.286	6.639±0.789	7.877±0.947
0.25 ppm aflatoxin							
0.0 g/Kg diet	1378.00±48.58^{d}	1083.361±67.722	101.003±11.932	18.167±3.272	14.337±2.542	4.926±1.559	9.045±1.871
2.5 g /Kg diet	1986.25±80.81^{c}	1224.047±23.873	88.604±4.206	17.811±1.154	16.573±0.896	6.901±0.550	8.523±0.659
5.0 g/Kg diet	2274.50±93.13^{ab}	1220.126±29.725	87.382±5.237	18.389±1.436	14.767±1.116	8.116±0.684	7.476±0.821
Significance	***	NS	NS	NS	NS	NS	NS
Regulation on slaughter weight	----------	**	*	NS	NS	NS	NS

non-carcass components were insignificantly affected with aflatoxin toxicity, except the adjusted liver weight increased significantly ($P<0.01$). Increase liver weight may be due to accumulation of fat in liver (fatty liver). This accumulation is due to failure of transfer synthesized lipids from liver.

Analysis of covariance of carcass and non-carcass components relatively to live body weight at slaughter did not show any significantt effects for the coumarin supplementation in rabbit diets (Table 4). Also, the analysis of covariance of carcass and non-carcass components did not show any significantt effects for the interaction between dietary aflatoxin toxicity and coumarin supplementation in rabbit diets.

Residue of Aflatoxin in Thigh Muscles

The residue of aflatoxin as aflatoxin B_1 (Table 5) was decreased by coumarin addition, this may be due to increasing aflatoxin excretion from the body and reducing organs aflatoxin B_1-DNA adducts (Loveland *et al.*, 1987). Based on the results obtained in this study it can be concluded that the coumarin dietary supplementations reduced and ameliorate the hazards of aflatoxin pollution in rabbit farms.

The mortality rate was increased up to 71.42% in rabbits fed aflatoxin B_1 contaminated diet without coumarin supplementation in comparison with 14.29% in control and other rabbit groups fed diets supplemented with coumarin. The incidence of death may be due to the disturbance of organs function and decreased the immune responsiveness (Lovell, 1991).

Economical Efficiency

Feed cost and income from gain per rabbit were increased with coumarin supplementation within each aflatoxin groups (Table 5). Final margin was decreased with coumarin supplementation in rabbits fed diets without aflatixin contamination, while it was increased by 64.47 and 9.69% in rabbits fed diets contaminated with aflatixin and supplemented with 2.5 and 5 g coumarin/kg diet, respectively (Table 5). The relative margin (relatively to the mortality rate) was increased by 393.48 and 229.13%, respectively in rabbit groups fed diet contaminated with 0.25 ppm afaltoxin and supplemented with 2.5 and 5 g coumarin/kg diet.

Table 5. Economical visibility of growing rabbits as affected by aflatoxin B_1 toxicity and coumarin supplementation to reduce its effect and their interactions

Treatments	Mortality rate (%)	Aflatoxin B_1 residue (ppb)	Total feed intake (g)	Feed cost (LE)/Rabbit	Total gain (g)	Income from gain (LE)/ Rabbit	Final margin (LE)/ Rabbit	Relative margin LE/ Rabbit
0.00 ppm aflatoxin								
0.0 g/Kg diet	14.29	---	4814.74	9.69	1453.83	31.98	22.30	19.11
2.5 g /Kg diet	14.29	---	5560.03	18.41	1577.8	34.71	16.30	13.97
5.0 g/Kg diet	14.29	---	6064.73	27.97	1656.2	36.44	8.47	7.26
0.25 ppm aflatoxin								
0.0 g/Kg diet	71.42	732.75	3591.21	7.23	694.33	15.28	8.05	2.30
2.5 g /Kg diet	14.29	122.19	4711.84	15.61	1311.24	28.85	13.24	11.35
5.0 g/Kg diet	14.29	86.40	5571.3	25.69	1569.47	34.53	8.83	7.57

Conclusion

Results indicated that adding coumarin especially at 2.5g/ kg contaminated diet with aflatoxin B_1 to growing rabbits was safe and practical method to minimize aflatoxin B_1 toxicity.

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تأثير إضافة الكيومارين لعلائق الأرانب النامية لتخفيف سمية الأفلاتوكسين ب١

أميرة عبد المحسن عبد الشافي هلال - صبري عبد الحافظ محمد شحاتة
عبـــد المجيـــد الســـــيد نصـــر – محمـــد صلاح الدين محمد عياط

قسم الإنتاج الحيواني - كلية الزراعة - جامعة الزقازيق - مصر

قسم عشوائيا عدد ٤٢ من ذكور الأرانب النيوزلاندى الأبيض (متوسط وزن الأرنب ٦٨٥,٤±١١,٧ جم) إلى ٦ مجاميع (٧ حيوانات بكل مجموعة) لتصميم تجربة عامليه ٣×٢ لتقييم قدرة الكيومارين على تخفيف أثر سمية الأفلاتوكسين ب١ الموجود فى عليقه الأرانب النامية . أعطيت أرانب الثلاث مجاميع الأولى عليقة أساسية خالية من الأفلاتوكسين بينما أعطيت أرانب الثلاث مجاميع الأخرى عليقة تحتوى على ٠,٢٥ جزء فى المليون أفلاتوكسين ب١ ، قسمت الحيوانات داخل كلا المجموعتين السابقين إلى ثلاث تحت مجاميع ، غُذيت المجموعة الأولى منهم على عليقة خالية من أى معاملة (للمقارنة) بينما عوملت المجموعة الثانية والثالثة بإضافة الكيومارين فى العليقة بمعدل ٢,٥ ، ٥ جم/ كجم عليقة، على التوالي. أدت تغذية الأرانب بعليقة ملوثة بالأفلاتوكسين ب١ إلى خفض معنوي فى كل من كمية المأكول اليومي ، التحول الغذائي ، هضم البروتين الخام ، وكذلك فى عدد كرات الدم الحمراء وتركيز الهيموجلوبين والبروتين الكلى والألبيومين فى الدم ، وهذا انعكس كنقص فى معدل الزيادة اليومية والكلية فى الوزن وكزيادة فى نسبة نفوق الحيوانات، أظهرت النتائج أن إضافة الكيومارين بمعدل ٢,٥ أو ٥ جم لكل كيلو جرام عليقة ملوثة بالأفلاتوكسين ب١ أدت إلى تقليل معنوي (على مستوى ٥ %) للتأثيرات السلبية للأفلاتوكسين ب١ على كل من: الزيادة اليومية والإجمالية فى جسم الحيوان ، معدل التغذية، التحول الغذائى ، هضم العناصر (مادة عضوية ، بروتين خام ، ألياف خام ، نيتروجين حر ومستخلص الإثير) ، قياسات الدم (هيماتوكريت ، عدد كرات الدم الحمراء ، تركيز البروتين الكلى والألبيومين ، ونشاط إنزيمات AST و ALT)، النسبة المئوية للحيوانات النافقة ، المتبقي فى أنسجة الحيوان من الأفلاتوكسين ب١ ، العائد الاقتصادي ، ولم تلاحظ فروق معنوية بين أثر كلا التركيزين المختبرين من الكيومارين، فأن إضافة الكيومارين لعليقه الأرانب الملوثة بالأفلاتوكسين ب١ (خصوصاً بتركيز ٢,٥ جم / كجم عليقه) وسيلة آمنة وطريقة عملية لتقليل سمية الأفلاتوكسين ب١ للحد الأدنى.

المحكمـــــون:
١- أ.د. **إيمان حنفـــي محمـــود مقلد** أستاذ الإنتاج الحيواني – كلية الزراعة – جامعة المنصورة.
٢- أ.د. **جمال الدين على عبدالرحمن** أستاذ تغذية الحيوان - كلية الزراعة جامعة الزقازيق.