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Full Length Research Paper

Immunological and productive responses in male growing white New Zealand rabbits exposed to Diazinon®

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Rabbit production plays a considerable role in solving problems of animal protein shortage. This study was conducted to investigate the effect of the dipping of male rabbits in Diazinon on immunological and growth performance. Rabbits were randomly assigned into three equal groups (*n*=20). Different treatments were administered; control group G1 (dipping in tap water); low dose of Diazinon group G2 (0.60 mg Diazinon in 1 liter water); and high dose of Diazinon group G3 (3.0 mg Diazinon in 1 liter water). Results indicated that plasma insulin like growth factor-1 (IGF-1) was lower (P<0.05) in the groups exposed to Diazinon than the control group. The plasma total protein and albumin values were significantly declined (P<0.05) gradually in a dose dependent manner. Values of IgG and IgM in blood plasma were gradually declined in rabbits in a dose dependent manner. Unlike the levels of IgM and IgG, Diazinon was found to significantly (P<0.05) elevate the bioactivity of the TNFα in blood plasma as estimated by ELISA. Dipping with Diazinon significantly decreased (P<0.05) growth rate, daily feed intake and feed efficiency. The present research unequivocally demonstrates the adverse effects of the exposure of the growing male rabbits to Diazinon on the relevant immunological parameters and the productive performance.

Key words: Diazinon, male rabbits, IgG, IgM, TNFα, growth performance.

INTRODUCTION

Rabbit production plays a considerable role in solving problems of animal protein shortage, especially in the developing countries and is an excellent animal model in the different physiological and immunological studies. Infection by external parasites diminish and limit the utilization and partitioning of nutrients to growth, also increases both the mortality and morbidity rates in growing rabbits.

Diazinon® is an Organophosphorus compounds which is being utilized and widely applied as insecticides, helminthicides, ascaricides, nematocides, fungicides and herbicides for 5 decades (Reece and Handson, 1982). Diazinon {O, O-diethyl–O-[6-methyl–2- (1-methylethyl)-4pyrimidinyl] phosphorothioate} has applications in both agriculture and animals husbandry (Grafft et al., 2002).

Diazinon® is known to cause inhibition of acetylcholinesterase (AChE) activity in the target tissues (Kappers et al., 2001). Diazinon® exerts its toxicity by binding its oxygen analog to the neuronal enzyme AChE, resulting in the accumulation of endogenous acetylcholine in nerve tissues and effectors organ (Mayer et al., 1991). Furthermore, it affects mitochondrial membrane transport in rat liver and it disturbs cytochrome P450 system in human liver (Sams et al., 2003). Little is known about the immunological toxicology of Diazinon® on rabbits, also the reflection on their growth performance.

Various toxicological conditions due to Diazinon exposure had been reported as consequences to the impairment in plasma protein levels, and a reduction in immunoglobulins, also the elevation in tumor necrosis factor (TNFα) by Iyer and Makris (2010), Yehia et al. (2007) and Iyer (2001).

The objectives of this study were to find out whether Diazinon® has a significant effect on some immunological parameters and tumor necrosis factor-α

Table 1. Chemical structure of the diets fed to the experimental rabbits.

Chemical analysis	%
Moisture	14.21
Dry matter	85.10
Ash	0.65
Crude protein	16.23
Ether extract	3.54
Crude fiber	17.10
NFE	41.11

Vitamins and minerals per 1 ml diluted in 1 liter drinking water contains: Vit. A 50000000 I.U., Vit. D3 5000000 I.U., Vit. E 40000 mg., Ascorbic acid 100000 mg., Mn 6000 mg., Zn 7200 mg., Fe 1500 mg., Cu 500 mg., I 120 mg., Se 100 mg., Co 100 mg., Mg 1000 mg., Na 14000 mg., K 7500 mg. and P 10000 mg.

responses, in addition to some productive responses in male growing white New Zealand rabbits.

MATERIALS AND METHODS

Experimental animals

This study was conducted in the Rabbit Production Unit, Faculty of Agriculture, Cairo University, Egypt throughout the period from 10 January 2010 to 15 July 2010. Sixty (60) male White New Zealand (NZW) rabbits aged seven months old were used in this study. The rabbits were reared in metal batteries with automatic drinkers. The average ambient temperature ranged between 20.5 ± 0.28 to 27.25 ± 0.13°C and relative humidity ranged between 60.00 ± 3.19 to 68.50 ± 2.9 at the rabbitry.

Experimental ration

Rabbits were fed commercial pellets diet for fattening. The chemical composition of this ration estimated according to AOAC (2000) is presented in Table 1. Feeds and water were offered *ad libitum*. Furthermore, vitamins and minerals (1 ml/ liter drinking water) were added.

Experimental design

After two weeks of adaptation to the feeds, housing and surrounded environment, the experimental rabbits were randomly divided into 3 symmetric groups, each of twenty rabbits, according to the treatment (Table 2). The initial body weights of the different experimental groups are presented in Table 2.

The stock of Diazinon® (obtained from Ciba Geigy) were diluted in tap water to prepare a low dose of Diazinon solution (0.60 mg Diazinon in 1 liter of water, utilized in G2 group) and a high dose of Diazinon solution (3.0 mg Diazinon in 1 liter of water, utilized in G3 group), each stock was prepared immediately before use. The LD50 of Diazinon in males is 250 mg/kg live body weight (According to manufacture, Merck index). The experimental groups were: 1-Control group (G1, The whole body of the animals was dipped in 10 L of tap water sparing the head for 10 s) 2-Low dose Diazinon group (G2, The whole body of the animals was dipped in 10 L of low dose Diazinon solution sparing the head for 10 s) 3-High dose Diazinon group (G3, The whole body of animals was dipped in 10 L of high dose Diazinon solution sparing the head for 10 s). The dipping of the experimental rabbits was done twice at the first and the second month of the experiment. Use of Diazinon was approved by the Animal Care Committee and meets all guidelines for its use.

Blood samples

Blood samples were taken from the marginal vein of the ears. The samples were taken by using a 21-gauge butterfly catheter and collected fortnightly into 5 ml heparenized tubes. Blood samples were centrifuged at room temperature using 3500 rpm for 15 m. The plasma was carefully withdrawn and kept in a deep freezer at -20°C pending carrying out different assays.

Blood parameters

Plasma total protein and albumin

Quantitative spectrophotometrical determination of total protein in plasma was recorded based on the principle of the Biuret reaction, copper salts in an alkaline medium, according to the method described by Gornall, et al. (1949). Albumin in plasma was quantified spectrophotometrically according to Doumas et al. (1971). Plasma globulin was calculated by deducting the value of albumin from total protein.

Plasma Insulin Like Growth factor 1 (IGF-1)

Following the procedure mentioned by Breier et al. (1991) the concentration of IGF-1 was estimated by using BioSource® IGF-1-RIA-CT kit (Catalog No. KIP1588) produced by BioSource Europe, Nivelles, Belgium. In this kit, a pre-treatment step is needed to dissociate the IGF-1 from its binding proteins (8 types) in order to improve the clinical performance of the assay because the binding proteins interfere with the radioimmunoassay for IGF-1. Cross-reactivity data provided by the manufacturer included values for IGF-1 (100%), IGF-II (0.7%), Insulin (ND) and GH (ND). The coefficient of variability of intra-assay precision was less than 9.1%, and less than 9.0% for inter-assay precision.

Humoral immune response

Immunoglobulin fraction G (IgG) and M (IgM)

A single radial immunodiffusion technique was developed to quantify plasma IgG using the immunoradiometeric plates supplied by Biocientifica S.A., Argentina according to the method described by Fahey and McKelvey (1965). An immunoprecipitation in agarose gel between the plasma IgG and anti rabbit IgG antibody is formed, whereas the antigen diffuses radially out of the well into the surrounding gel-antibody mixture and proportionally related to the amount of IgG in plasma. The IgM in plasma is precipitated radially in an agarose gel containing an anti rabbit IgM. The diameter of the expanded ring is related proportionally to the concentration of the antigen in blood.

Tumor necrosis factor (TNF-α)

An enzyme linked immunosorbent assay was developed to quantify

Table 2. Experimental conditions.

Item	G1	G2	G3
Numbers of rabbits	20	20	20
Initial body weight (LSM±SE)	1. 465 ± 13.8	1.480 ± 21.7	1.490 ± 12.0
Concentration of Diazinon per liter	0	0.6 mg	3.0 mg
Schedule of dipping	The first and the second month of the experiment		
Duration of dipping	10 s		

Table 3. Concentrations (LSM ± SE) of plasma IGF-1, total proteins, albumin, globulin, and albumin globulin ratio as affected by Diazinon exposure in white NZW male rabbits

Blood parameter	G1	G2	G3
IGF-I ng/ml	295.4^{a}±25.6	229.0^{b}±51.2	189.7^{c}±93.3
Total protein g/dL	9.51^{a} ± 0.24	8.24^{b} ± 0.30	7.13^{c} ± 0.35
Albumin g/dL	4.65^{a} ± 0.11	3.80^{a} ± 0.40	3.21^{b} ± 0.22
Globulin g/dL	4.86^{a} ± 0.11	4.44^{a} ± 0.40	3.92^{b} ± 0.22
Albumin/ globulin ratio	0.96	0.86	0.82

Within the same row, LSM with different superscripts significantly differ at P < 0.05.

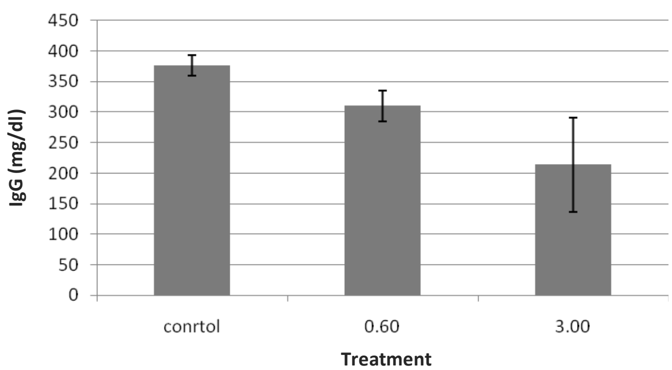

Figure 1. Concentrations (LSM ± SE) of plasma IgG as affected by rbST treatment in NZW male rabbits. Within the same row, LSM with different superscripts significantly differ at P < 0.05.

the tumor necrosis factor (TNF-α) using the kits from Orgenium Laboratories, Finland as described by Seriolo et al. (2006).

Productive performance

The initial body weights of each rabbit in the different groups were recorded after the adaptation week. Live body weights were recorded weekly after that. The feed consumption throughout the experimental period was recorded to calculate the average daily feed intake (g/day) in the test day equal to (Offering feed – Residual feed).

Statistical analysis

Data set was subjected to the analysis of variance using the general linear model of SAS (SAS, 2002). Repeated measurements (split plot in time) were adjusted according to Netter et al. (1985). The significant differences were tested according to Duncan's new multiple 't' test (Duncan, 1955).

RESULTS

Plasma Insulin-like growth factor-I (IGF-I) and Plasma Total proteins

Plasma IGF-1 in NZW male rabbits exposed to Diazinon was significantly decreased than the controls (Table 3). There were gradual significant reductions (Table 3) in the overall mean of plasma total proteins due to Diazinon exposure either the high dose or the low dose.

Plasma IgG (mg/dl), IgM (mg/dl) and TNF α (pg/ml)

Plasma concentrations of the immunological fraction IgG and IgM and the Lymphoid factor (TNF-α) are presented in Figure 1. There were significant effects due to Diazinon exposure of rabbits relevant to plasma IgG, IgM and TNF-α. Rabbits exposed to Diazinon had lower (P<0.05) plasma IgG and IgM than the control.

The lowest IgG value (214 mg/dl) was recorded for rabbits dipped for 10 s in high dose of Diazinon. Also, there was a gradual decline in the values of IgM in the blood plasma of the experimental rabbits exposed to Diazinon (Figure 2). The concentration of TNF-α was the highest (P<0.05) in the rabbits exposed to the high dose of Diazinon (Figure 3). Also, there was a slight elevation in this parameter in the blood of the rabbits exposed to the low dose of Diazinon.

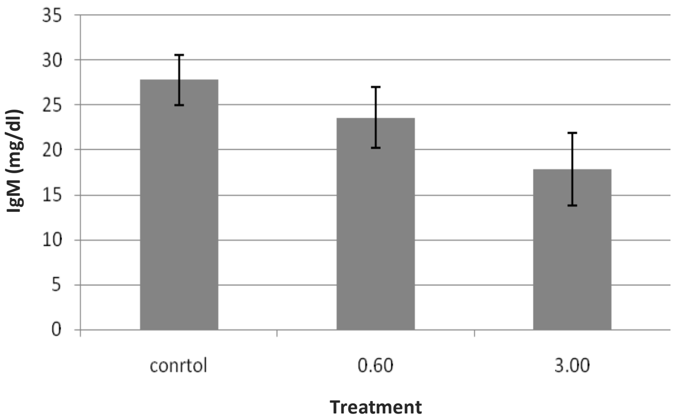

Figure 2. Concentrations (LSM ± SE) of plasma IgM as affected by rbST treatment in NZW male rabbits. Within the same row, LSM with different superscripts significantly differ at P < 0.05.

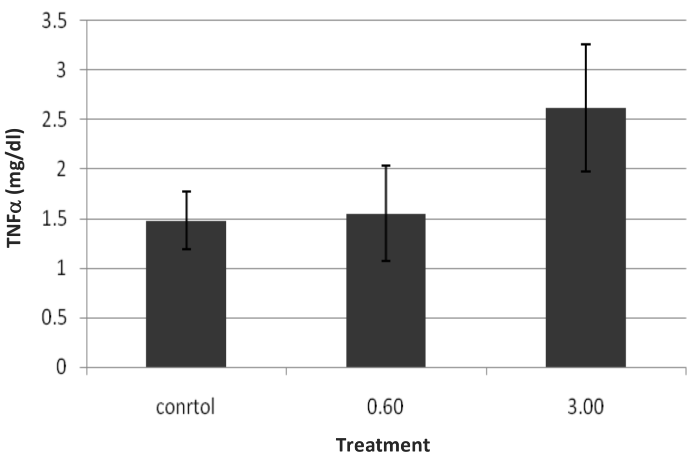

Figure 3. Concentrations (LSM ± SE) of plasma TNF-a as affected by rbST treatment in NZW male rabbits. Within the same row, LSM with different superscripts significantly differ at P < 0.05.

Live body weight and feed consumption

Overall mean effect of Diazinon exposure to NZW male rabbits was shown to be significant (P<0.05) whether on the live body weight or in the growth rate (Table 4).

Feed consumption in the test day at the beginning of the experiment (at 7^{th} months old) was almost similar in the experimental rabbits groups. Rabbits exposed to Diazinon had consumed lower feed (P<0.05) throughout the entire experiment period in comparison to that in control ones (100.40±1.09 *vs.* 129.45 ± 1.16 g/day, for groups exposed to Diazinon and control groups, respectively). Total feed consumed by rabbits exposed to Diazinon during the experimental period (7-12 months of age) was lower by 3.2 % in comparison to countercurrent ones (6940.21 vs. 7112.63 g, respectively) (Table 4).

DISCUSSION

Blood biochemistry profiles are empolyed for the diagnosis of body physiology and tissue integrity against exposure to any toxic materials (Robert et al., 2003). Plasma insulin like growth factor-1 is the mediator of growth hormone action and its concentrations is affected by any physiological disorders that could happen by the exposure to toxic material and pesticides.

The response due to Diazinon exposure was noticed to be proportionally related to the dose of Diazinon, which could be explained by the homeostatic reactions toward the toxic effect of Diazinon that could deplete body energy and then reduce serum proteins as reported by Salvatori (2004) and Yehia et al. (2007). Growth Hormone (GH) acts to control IGF-1 plasma levels and IGF-1 acts directly at the cellular level. In addition, serum IGF-1 levels were positively correlated with heavier weight and positive energy balance (Jones and Clemmons, 1995). The values of blood plasma IGF-1 in the exposed rabbits to Diazinon could explain the reduction in growth rate and other productive traits.

The gradual significant reductions in the overall mean of plasma total proteins were within the normal physiological range reported by Harkness and Wagner (1995) and Kaneko et al. (1997). They mentioned that the normal range of serum total proteins in rabbits ranges between 2.8 and 10.0 g/dL. In agreement with these results, Yehia et al. (2007) found that rabbits exposed to Diazinon had a significant lower concentrations of plasma total proteins *versus* control group.

There were significant effects due to Diazinon exposure of rabbits relevant to plasma IgG, IgM and TNF-α. Rabbits exposed to Diazinon had lower (P<0.05) plasma IgG and IgM than the control. This reduction in immunoglobulins could be used as an indicator of some physiological and immunological disorders of Diazinon exposure in the experimental rabbits. High levels of TNF-α had been reported with various pathological conditions include septic shock, autoimmuno diseases, hepatitits, leukemia, multiple scalerosis, rheumatoid arthritis and septicemia (Seriolo et al., 2007; Yehia et al., 2007).

Growth involves a complex set of metabolic events, which are genetically hormonally and environmentally controlled. Environmental stressors like the use of Diazinon as a pesticide could have an adverse effects on the growth performance of the rabbits. In agreement with the present results, Yehia et al. (2007) reported that Diazinon exposure of male rabbits had a significant reduction (P<0.05) in some relevant physiological and hematological parameters which in turn will reduce their live body weight and in their growth rate than the control. These results indicated that Diazinon exposure reduced the metabolic rate and consequently the performance of male rabbits, which could be explained from the results of IGF-1. Total feed consumed by rabbits exposed to

Diazinon during the experimental period were lower by

Table 4. Productive performance of growing NZW male rabbits as affected by Diazinon exposure.

Item	G1	G2	G3
LBW at 7th months	910.6 ± 90.4	897.0 ± 81.7	933.5 ± 44.9
LBW at 12th months	1810.0 ^{b} ± 19.9	1785.7 ^{b}±71.5	1670.0 ^{a}±71.6
DWG (7-12 months)	25.6	21.1	20.5
Mortality rate (%)	11	14	15

LBW, live body weight (g) and DWG, daily weight gain (g/day). Within the same row, LSM with different superscripts significantly differ at $P < 0.05$.

3.2% than countercurrent ones.

In conclusion the present study, declared that; (1) the exposure of white NZW growing male rabbits to Diazinon resulted an adverse effects on the relevant immunological and physiological responses, (2) The productive performance of male rabbits was altered by Diazinon exposure, which is explained by the impairment in plasma IGF-1 concentration.

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