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Zearalenone-induced Deterioration in Reproductive Performance and Seminal Plasma Biochemistry of Male Rabbits

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Abstract

Zearalenone (ZEA) is a nonsteroidal mycotoxin, produced by various fungi of the Fusarium genus; Zearalenone exhibits estrogenic and anabolic properties in several animal species, including human beings. Zearalenone contamination of food is caused either by direct contamination of grains, fruits, and their product or by "carry-over" of mycotoxins and their metabolites in animal tissues, milk, and eggs after intake of contaminated feedstuff.Despite Zearalenone has toxicity and carcinogenicity.Detoxification strategies for contaminated foods and feeds to reduce or eliminate the toxic effects of ZEA by chemical, physical, and biological methods are crucial to improve food safety, prevent economic losses, and reclaim contaminated productsHowever; there is no enough data on the reproductive toxicity of Zearalenone in adult male.Therefore, the present investigation aimed to elucidate the toxicity of different doses of Zearalenone on reproductive performance, enzyme activities in seminal plasma and growth performance of males New Zealand white rabbits. Twenty rabbits were divided into 4 equal groups, first group was used as control, second group was treated with 0.5 µg/kg BW, third group was treated with 5 µg/kg BW and the fourth group was treated with 10 µg/kg BW of Zearalenone. Animals were treated orally every day for 12 weeks. Results obtained showed that Zearalenone significantly ($P < 0.05$) decreased libido (by increasing the reaction time), ejaculate volume, sperm concentration, total sperm output, sperm motility (%), total motile sperms per ejaculate (TMS), packed sperm volume (PSV), total functional sperm fraction (TFSF), normal and live sperms and semen initial fructose. While, initial hydrogen ion concentration (pH) and dead and abnormal sperms were increased ($P < 0.05$). Live body weight (LBW), feed intake (FI) and, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and acid phosphatase (ACP) were significantly ($P < 0.05$) decreased. It was suggested that Zearalenone exerted a significant adverse effect on reproductive performance of male rabbits and the effects were in a dose- dependent manner.

Keywords: Zearalenone; Reproductive toxicity; Rabbits; Fertility; Free radicals; Enzymes

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1. Introduction

In today's changing world, safety and security have generally remained basic human needs. Ensuring the safety of food has been a major focus of international and national action over the last years. Both microbiological and chemical hazards are of concern. Among chemical hazards, the contamination of food and feed by mycotoxins (toxic metabolites of fungi), fishery products by phototoxins (toxins produced by algae) and edible plant species by their plant toxins have been recently characterized by the World Health Organization (WHO) as significant sources of food borne illnesses [(1)] of these three categories of natural toxins, most attention has been directed to mycotoxins until now. In several parts of the world, mycotoxins currently represent a major food safety issue.

Mycotoxins can have a cumulative effect in humans causing cancer, acute symptoms, and immune deficiency diseases. In livestock, they can be linked to reduction in body weight gain and lower feed intake and efficiency) [(2)]. Accumulation of mycotoxins in animal tissues can be a source of exposure to humans who consume products originating from animals.

Among toxins produced by Fusarium genus species, the most frequent and the most important ones, in a great number of agricultural products, are deoxynivalenol (DON) and zearalenone (ZEA) toxins, which are known to cause serious health problems to people and animals [(3)].

Zearalenone (ZEA) is a non-steroidal estrogenic mycotoxin, produced by various fungi of the Fusarium genus, which are frequently found in cereal crops and other plant products all over the world, resulting in contamination of food and animal feed material. [(4, 5)] It exists widely in many cereal crops such as maize, barley, wheat, oats, sorghum and sesame seeds, as well as in hay and corn silage. These are all ingredients in many food products for human or animal nutrition.[(6,7)] Despite its low acute toxicity and carcinogenicity, zearalenone exhibits estrogenic and anabolic properties in several animal species, including human beings.[(8,9)]

Zearalenone contamination of food is caused either by direct contamination of grains, fruits, and their product [(8)] or by ''carry over'' of mycotoxins and their metabolites in animal tissues, milk, and eggs after intake of contaminated feedstuff [(9,10).]

Zearalenone (F-2 toxin) is a toxin produced by fungi belonging to the genus Fusarium. These fungi contaminate corn, as well as food mixtures for farm animals [(11,12)]. ZEA competes with the naturally produced hormone estradiol-17β for binding sites (estradiol receptors) in various organs in the body of both genders. ZEA can obstruct normal steroid hormone (estradiol, testosterone, progesterone) synthesis in the ovaries and testicles of livestock.
There are also data on its capability to induce adverse liver lesions with subsequent development of hepatocarcinoma.[(13)] In young male swines the toxin causes prepuce oedema, testicular atrophy and increasing of mammary gland[(14)].

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Detoxification strategies for contaminated foods and feeds to reduce or eliminate the toxic effects of ZEA by chemical, physical, and biological methods are crucial to improve food safety, prevent economic losses, and reclaim contaminated products.
Degradation and detoxification of common mycotoxins in the presence of high concentrations of ozone (o_3) have been investigated. [15]
Extrusion cooking of cereal products is being used increasingly in the food industry to convert cereals into breakfast foods, snack foods, and pet foods. Extrusion cooking is one of the fastest growing food processing operations in recent years due to several advantages over traditional methods.

In addition to improving the quality of intermediate and final processed products it may also improve food safety because of having the potential to reduce mycotoxin levels in cereals. [16] The levels of ZEA in cereal based foods were reduced significantly by extrusion processing, and reduction of 83% of ZEA in corn based foods was obtained with this process.[17].

However, there remains a need to demonstrate that the toxicity or biological activity of ZEA has been reduced or completely eliminated in cereal-based foods using extrusion processing. [16] Addition of nutritionally inert sorbents is one of the most recent approaches that have been proposed to reduce ZEA toxicity.

Most studies related to the alleviation of mycotoxicosis by the use of adsorbents are focused on aluminum silicates, and aluminum silicates containing clays.

2-Materials and Methods

2.1 Ethics statement

This study was performed in strict accordance with the recommendations of the National Research Council Guide, and all of the animal experimental procedures were approved by the Ethical and Animal Welfare Committee. Rabbits were housed in a temperature-controlled room with proper darkness-light cycles, fed with a regular diet, and maintained under the care of the Animals and feed treatments.

The experimental work of this study was carried out at Institute of Graduate Studies and Research, Alexandria University during the end of 2012 to the beginning of 2013. Twenty mature male Newzeland White rabbits aging 6 months and weighing 3.00 kg at the beginning of the experiments were used. Animals were divided randomly into four equal groups of 5 rabbits each. Rabbits were individually housed in galvanized wire cages provided with feeders and automatic stainless steel nipple drinkers where basal diet and water were offered ad libitum. The experimental groups were as follows: Group 1: was used as control. Rabbits were fed on standard diet.

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Group 2: Rabbits were fed on standard diet and given orally 5 µg/kg body weight zearalenone in corn oil. Group3: Rabbits were fed on standard diet and given orally 10 µg/kg body weight zearalenone in corn oil. Group 4: Rabbits were fed on standard diet and given orally a dose of zearalenone equivalent to the estimated daily intake (0.5 µg/kg body weight zearalenone) in corn oil.

The tested doses of zearalenone were given orally with the help of syringe directly into the eosopharyngeal regions by gavage according to the animal's body weight every other day for 12 weeks.

2.2 Semen quality

Daily feed intake and body weight were recorded weekly. Semen collection occurred weekly over the 12 weeks of the study, so 84 ejaculates obtained per treatment. Ejaculates collected using an artificial vagina and a teaser doe. The volume of each ejaculate was recorded after removal of the gel mass. A weak eosin solution was used for evaluation of sperm concentration by the improved Neubauer haemocytometer slide[18]. Total sperm output calculated by multiplying semen ejaculate volume and semen concentration. Determination of initial fructose concentration in seminal plasma carried out immediately after collection according to Mann[19]. Assessment of live and normal spermatozoa were performed using an eosin–nigrosine blue staining mixture[20]. The percentages of motile sperms were estimated by visual examination under low-power magnification (10×) using a phase-contrast microscope with heated stage. Total number of motile sperms calculated by multiplying percentage of motile sperm and total sperms outputs.

Reaction time for the buck is calculated as the time needed for mounting a doe until complete ejaculation; it measured in seconds using a stopwatch. Initial hydrogen ion concentration (pH) of semen samples was determined immediately after collection using a pH cooperative paper (Universal indicator pH 0–14 Merck, Merck KgaA, 64271 Darmstadt, Germany). Packed sperm volume (PSV) recorded. Total functional sperm fraction (TFSF) parameter was also calculated as the product of total sperm output by motility (%) by normal morphology (%). [21] Seminal plasma was obtained by centrifugation of semen samples at 860×g for 20 min at 4 ^{o}C, and was stored at−60 ^{o}Cuntil analysis.Normal sperm have an oval head with a long tail. Abnormal sperm have head, midpiece or tail defects, such as a large or a misshapen head or a crooked or a double tail.

Determination of initial fructose (IF) concentration in semen was carried out immediately after collection according to Mann[19]. Seminal plasma samples were analyzed weekly for alanine aminotransferase (ALT)[22], aspartate aminotransferase (AST)[22], alkaline phosphatase (ALP)[23], acid phosphatase (ACP)[24] lactate dehydrogenase (LDH)[25], free radicals[26] and lipids[27] of seminal fluid which were evaluated spectrophotometrically, using commercially

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available diagnostic kits supplied by BioMerieux (France) according to the manufacturer's instructions.

3. Results

3.1 Semen characteristics

Data on ejaculate volume (EV), sperm concentration and total sperm output (TSO) of rabbits treated with zearalenone. Figure 2 and 4 represent the weekly mean values of these parameters expressed as absolute values. Rabbits treated with zearalenone resulted in significant ($P<0.01$) decrease in sperm concentration compared to control group. While, treatment only significant ($P<0.01$) for total sperm output (TSO).

In semen characteristics, Over all means indicated that treatment with zearalenone caused significant ($P<0.05$) decrease on reaction time (libido), semen pH, sperm motility, percentages of dead sperms, and sperm abnormalities, live sperms, normal sperms, and sperms concentration were significantly decreased ($P<0.01$). Analysis of variance tables indicated that the last semen characteristics were significantly affected by treatment and the interactions between treatment and time ($P<0.01$), tables (3).

Data on the percentage of normal, dead sperms and semen initial fructose (IF) of rabbits as affected by treatment with zearalenone. Figures (8, 9, and 10) represent the weekly mean values of these parameters expressed as absolute values. The overall means of the activity of sperm motility (%), total motile sperms (TMS) and total functional sperm fraction (TFSF) as affected by treatment with different doses of zearalenone throughout the 12-week experimental period. Figures (8) to (10) represent the weekly mean values of these parameters expressed as absolute values.

Treatment with zearalenone caused significant ($P<0.05$) decrease in sperm motility (%), TMS and TFSF. Analysis of variance (Table 5) showed that treatment, time and the interactions between treatments and time had significant ($P<0.01$) effects on sperm motility (%), TMS and TFSF.

The overall means values of semen packed sperm volume (PSV), semen initial hydrogen ion concentration (pH) and reaction time (RT). Figure (2), (5) represent the weekly mean values of these parameters expressed as absolute values. Zearalenone treatment resulted in a significant ($P<0.05$) increase in pH and RT, while PSV was significantly ($P<0.05$) decreased compared to control group.

Analysis of variance indicated that treatment and the interactions between treatment and time had significant ($P<0.01$) effects on PSV and pH. While, time had SIGNIFICANT ($P<0.01$) effect on PSV and pH but had not significant effect on RT.

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3.2 Effect of zearalenone on seminal plasma biochemistry

Figure (5) shows the overall means of the activities changed in seminal plasma of aspartate (amino transaminase) (AST), alanine (amino transaminase) (ALT), alkaline phosphatase (ALP) and acid phosphatase (ACP) and LDH due to zearalenone treatment, throughout the 12-week experimental period. Zearalenone caused significant ($P<0.05$) increase in the activities of AST, ALT, and LDH, and caused significant ($P<0.05$) decrease in the activity of ACP, and ALP in seminal plasma as compared to control group. Analysis of variance indicated that treatment and the interactions between treatment and time had significant ($P<0.01$) effects on seminal plasma AST, ALT, ACP, ALP and LDH. Also, time had significant ($P<0.01$) effect on seminal plasma AST, ALP, ACP and LDH, and ($P<0.05$) on ALT.

3.3 Effect of zearalenone on seminal plasma total lipids

Table 8 illustrates the effect of Zearalenone on the levels of total lipids (TL) in plasma of male rabbits. The level of total lipids (TL) significantly ($P<0.05$) decreased in plasma of rabbits treated with zearalenone as compared with control group.

Analysis of variance showed that levels of TL, were significantly affected by treatment and the interactions between treatment and time ($P<0.01$). Also, level of TL, was significantly affected by time ($P<0.01$).

3.4 Effect of zearalenone on free-radicals

The effect of zearalenone on free radicals were significantly affected by treatment and the interactions between treatment and time ($P<0.01$).

Table (1) Analysis of variance for the effect of different doses of zearalenone on body weight, feed intake, water intake

S.O.V.	D.F.	M.S.		
		BW	FI	WI
Treatment (T)	3	0.20300167	985.404873**	1841.607128**
Weeks (W)	11	0.11941348	84.105197*	223.290267
T x W	33	0.01026561	22.184028	34.843614
Error	192	0.10926812	43.42700	336.15480

*Significant at P <0.05
**Significant at P <0.01

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Table (2) Analysis of variance for the effect of different doses of zearalenone on ejaculation volume, sperm motility, and dead sperms

Parameter	DF	M.S.		
		E v	S m	DEAD
Treatment (T)	3	0.20300167	2488.016667**	2124.815278 **
Weeks (W)	11	0.11941348	556.734848**	169.885985**
T x W	33	0.01026561	254.359091**	67.018308**
Error	192	0.10926812	83.51042	31.18750

*Significant at P <0.05
**Significant at P <0.01

Table (3) Analysis of variance for the effect of different doses of zearalenone on PH, live sperms, reaction time, and live sperms

Parameter	DF	M.S		
		pH	RT	LIV
Treatment (T)	3	1.19655931**	4.78333333**	1541.116667**
Weeks (W)	11	0.03933042**	1.12272727	119.122727 **
T x W	33	0.02231415**	0.42272727	80.719697**
Error	192	0.01240375	1.0937500	39.28333

*Significant at P <0.05
**Significant at P <0.01

Table (4) Analysis of variance for the effect of different doses of zearalenone on sperm concentration, total sperm output and total motile sperms.

Parameter	DF	M.S		
		conc.	TSO	TMS
Treatment (T)	3	94953.0500**	280824.2042**	216906.5944**
Weeks (W)	11	3707.3773**	1041.4466	1343.3045
T x W	33	3731.3439**	9003.4769	9367.2157**
Error	192	723.6354	5605.200	3076.071

*Significant at P <0.05
**Significant at P <0.01

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Table (5) Analysis of variance for the effect of different doses of zearalenone on total function sperm fraction, packed sperm volume and Initial fructose

Parameter	DF	M.S		
		TFSF	PSV	FRU
Treatment (T)	3	176713.1597**	247.2303333**	17509.23750**
Weeks (W)	11	1045.9102	7.1775455**	2144.78295**
T x W	33	7654.8355**	16.7631515**	584.59205
Error	192	2026.279	1.051813	541.0396

*Significant at P <0.05
**Significant at P <0.01

Table (6) Analysis of variance for the effect of different doses of zearalenone on Abnormal and normal sperms

Parameter	DF	M.S	
		ABN	NOR
Treatment (T)	3	2039.137500**	2041.793056**
Weeks (W)	11	148.001136**	146.967803**
T x W	33	68.810227**	69.650631 **
Error	192	32.38958	32.07500

*Significant at P <0.05
**Significant at P <0.01

Table (7) Analysis of variance for the effect of different doses of zearalenone on Aspartate aminotrnsferase, Alanine aminotransferase, and Acid phosphatase

Parameter	DF	M.S		
		AST	ALT	ACP
Treatment (T)	3	32.23348611**	1158.114931**	193.3413128**
Weeks (W)	11	5.30473864**	229.695678**	20.1146623**
T x W	33	0.80163763	13.641143**	2.1596582
Error	192	0.6533333	1.849188	2.767298

*Significant at P <0.05
**Significant at P <0.01

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Table (8) Analysis of variance for the effect of different doses of zearalenone on alkaline phosphatase, total lipid and lactate dehydrogenase

Parameter		ALP	TL	LDH
Treatment (T)	3	2361.614024**	71032.9042**	438553.767**
Weeks (W)	11	246.028179**	12622.9042**	11837.648**
T x W	33	62.051516**	2716.6648**	11783.542**
Error	192	19.78336	1106.2021	4330.063

*Significant at P <0.05
**Significant at P <0.01

Table (9) Analysis of variance for the effect of different doses of zearalenone on free radicals

Parameter	DF	M.S		
		FR		
Treatment (T)	3	2.12140677**		
Weeks (W)	11	0.46049911**		
T x W	33	0.06075190**		
Error	192	0.00513698		

*Significant at P <0.05
**Significant at P <0.01

4. Discussion

Body weight, feed intake and drinking water

The changes in body weight (BW), feed intake (FI) and drinking water throughout the 12-week experimental period of rabbits treated with different doses of zearalenone were expressed as absolute values and the effect was time dependent.

No significant difference in body weight attributable to toxic effect was detected in any of the experimental animals. The effects observed include non-specific symptoms like weight loss, feed refusal.

Over all means indicated that treatment with zearalenone caused significant (P<0.05) increase in feed intake (FI) and drinking water compared to control animals in dose dependent

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manner, no changes in body weight (BW) wereobserved. In agreement with the present results, Vargas et al., [28] found that No differences between treated animals and controls were seen in body weight gain.

In a study of the rabbits which were given zearalenone in the diet at concentrations of 0, 0.15, or 0.03 mg/kg bw per day for 18 days. The four month old rabbits showed a treatment related increase in body weight gain, food and water consumption. [29]
The present study revealed a statistically significant increase ($p < 0.01$) in feed intake and water intake.

The similar growth rate, feed intake, and feed efficiency of the piglets among all the treatment groups indicated that dietary ZEA concentration in the range of 1.1 to 3.2 mg/kg diet had no negative effects on growth performance of the gilts fed a corn meal- and soybean meal-based diets. This combined with the observation that gilts did not sort the diet due to the ZEA supplementation (data not shown) suggests that gilts within a treatment group likely consumed a similar amount of ZEA, and that differences obtained among treatment groups were likely attributable to the different concentrations of ZEA in the diet[30].Speranda et al, [31] found that, even with ingestion of equal and greater amounts ZEA, as high as 2mg/kg, there were no changes in animal performance. This reinforces the action of ZEA mainly in the reproductive tract, without changing the zoo technical performance parameters significantly. [32]

Effect of zearalenone on semen characteristics

In the present study a dose dependent reduction of sperm count and increase in the number of abnormal spermatozoa occurred. Semen quality of rabbits was also impaired after a treatment with different doses of zearalenone for about 12 weeks, with poor progressive motility and poor morphology of spermatozoa being observed.

Evaluation of sperm motility represents an important parameter because it is significantly correlated with the total number of off spring born, for example, fertility[33-35].

It is an accepted fact that normal ejaculates do contain a small percentage of spermatozoa that are abnormal in morphology. A semen sample containing a high percentage of abnormal sperm is indicative of impaired fertility. [35-37]

In the present work, a high concentration of zearalenone resulted in decreased motility and an increase in the ratio of morphologically abnormal cells, after the exposure. A direct toxicity (cytotoxic effect) of zearalenone toxin on the spermatogenic compartment of testis may be considered as one of the mechanisms of action of zearalenone toxin in producing the abnormal spermatozoae.

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Cytotoxicity has been associated with either the impairment of protein synthesis by the binding of compounds to the ribosomes of eukaryotic cells, or the dysfunction of cellular membranes[38].

Decreased motility in the toxin treated group can be the result of the impaired epididymal function as well, that is, maturation of spermatozoa, leading to an impairment of sperm motility [35,39]. As a general principle, a decrease in the concentration of the biochemical components of the seminal plasma produced by the accessory male organs indicates a dysfunction of the secreting organs. [39,40]

Zearalenone and α-zearalenol reduce spermatozoa viability in vitro, but the report emphasizes that the effect of the toxin depends on the time period and dose. [41]
The effect of zearalenone applied to rabbits in different doses was manifested by decreased sperm motility,[42] increased number of spermatozoa with morphological abnormalities, decrease in concentration of fructose in seminal plasma, in addition to taking reproductive disorders into consideration from an animal production prospectives, studying effects in rabbits can be of special interest, as this animal species is known to be a good model of human reproductive toxicology. Yang et al. [43] shows that the treatment with ZEA or α-ZOL at 0, 25, 50 and 75 mg/kg i.p. once a day for 7 consecutive days, in Kunming male mice decreased the number of live spermatozoa, and increased the number of abnormal spermatozoa.

Using the rabbit in reproductive toxicological studies has several advantages, e.g. the male rabbit is the smallest, least expensive animal that can be ejaculated with an artificial vagina, permitting longitudinal evaluation of semen characteristics [44].

Testosterone supports spermatogenesis, sperm maturation, seminal plasma production and sexual functions [45]. The disruption of testosterone biosynthesis in Leydig cells can adversely affect male fertility. [35]

The recognition of the importance of sperm morphology measurements in men exposed to various agents, and the repeatability of morph metric measurements of rabbit sperm add to the value of the male rabbit as an especially useful model [46].

Precocious separation of X-Y chromosome has been discussed as a mechanism of male sterility [47] however; the effect of ZEN on germ cells may result in the reduction of fertility. El-Makawy et al.[48] reported that ZEN was shown to increase chromosome breaks in mouse spermatocytes at a dose of 10µg/kg. Moreover, ZEN has been reported to disturb spermatogenesis and decrease fertility in male rats fed naturally contaminated corn[49]. Also ZEN- induced a stressful effect on the testicular function included, increase in chromosome aberration and sperm abnormality, decrease in sperm counts and motility.

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In rabbit bucks, ZEN impairs spermatogenesis and decreases libido, although only at high doses (117.3mg/kg feed). [50]

ZEA was found to have hepatonephrotoxicity and could disturb the enzymatic and hematological parameters of mice within 48 h taken orally. [51]
In boars high concentration of zearalenone in feed may cause reduced libido.[52]

Zearalenone also leads to disorders of spermatogenesis[53]. A study in pre pubertal boars found the zearalenone administration and a subsequent reduction in libido.[54] Young boars may have reduced libido. [55, 56]

In toxicological experiments the effects of ZON on reproductive performance have been observed in boars such as lowered testicular weight and decreased motility of spermatozoa.

A reduced libido, associated with a decreased testosterone concentration in plasma. Moreover, exposure of boar semen to ZON or α-ZOL at concentrations of 40 to 80 μg mL−1 of diluted semen induced significant reductions in sperm motility, viability and binding ability to zone pellucid. [57] β-ZOL exclusively affected motility parameters [58].

Zearalenone is metabolized in the liver with production of alpha-zearalenol (α-ZEN) and beta-zearalenol (β-ZEN) as essential metabolites. First data were reported on the capability of ZEN to induce liver lesions with subsequent development of hepatocarcinoma and pituitary carcinomas [59]. The low dose of ZEN (2.7 mg/kg b.w.) relative to LD50 (500 mg/kg b.w.) was chosen in this study to examine its effect by single and oral repeated administration in Balb/c micee as a sensitive laboratory model. In the present research, a significant increase in liver enzymes ALT, AST and ALP was observed after 48 hours of oral administration of ZEN. Similar observation was found in the group administered the toxin for two weeks. No significant changes in AST and ALP in animals treated for one week while ALT was found increased.
[60]

Clinically, measuring transaminases is very important and useful in diagnosis of liver function disorders[61] this increase in liver enzymes reflected hepatocellular damage. Similar observation was described by Maaroufi et al.[62] and Čonková et al.[63]
In gilts exposed to 3mg/kg of ZEA the levels of increase of AST,GGT and lactate dehydrogenase were increased. [31]

The result of enzymatic parameters changes in the present study may be attributed to estrogen like effect of ZEN, because estrogens can interact with specific receptors called estrogen receptors (ER) in the liver and exerting a number of toxic effects, including liver adenomas and hepatocarcinomas [64,65].

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Biochemical results in the present study showed alteration in ALT, AST and ALP enzymes which pointed to some sort of hepatocytes necrosis observed herein. Similar association between increased level of hepatic enzymes and cell necrosis were described by many investigators especially with mycotoxin hepatotoxicity. (66)

Effects of low (10 µg kg-1 b.w.) and high (100 µg kg-1 b.w.) oral doses of ZEN on some blood serum enzyme activities of AST, ALT, ALP, and total LDH of rabbits were studied by Čonková et al.(63) Low doses resulted significant increase in ALP activity, while significant increases in activities of AST, ALT, AP, GGT, and LDH were observed, indicating possible liver toxicity due to chronic effects of the toxin.

In rabbit bucks, ZEN impairs spermatogenesis and decreases libido, however only at extremely high dose levels.(50)

In the current study, measurements of ALT in pigs fed the 2- and 3.2-mg/kg ZEA diets were beyond a normal range of ALT (31 to 58 U/L).(67) It has been reported that elevated serum ALT may result from acute hepatic necrosis. (68,69) The alterations of ALT activities and urea concentrations observed in pigs fed 2 and 3.2 mg/kg ZEA contaminated diets suggests possible ZEA induced hepatic damage. Also `the impaired hepatic function caused after administration of ZEN that might be a reason for activation of hepatocytes led to increase hepatic marker enzymes, as seen in the present work.

The amounts of alanine aminotransferase in the experimental group's boar blood serum were statistically significantly higher. This implies that mycotoxin zearalenone might have an effect on boar organism ($p<0.05$). Reports of experiments with rats also mention the increased levels of alanine aminotransferase and aspartate aminotransferase in the blood serum of animals, directly injected with synthetic mycotoxin zearalenone (62).

Increased total LDH activity may be caused to skeletal muscles, myocardium, liver parenchyma, in tissue malignancy, as well as in various anaemias. (70) Its increased values in the group treated with 10µg of zearalenone also indicate the possible damage to liver tissues. (63)

Stadinlk et al. (71) clearly indicate active free radical reactions under the influence of ZEA in the liver. It may lead to damage of lipids, proteins, and other cell components under the influence of free radicals. This and oxidative stress may be partially responsible for cytotoxicity of ZEA.

Intracellular accumulation of reactive oxygen species can arise from toxic insults and can perturb the cell's natural antioxidant defense system resulting in damage to all major classes of biological macromolecules. During the last decades, the oxidative stress has been pointed out as major component of several biological and pathological processes like aging,

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inflammation, carcinogenesis and several other diseases [72]. Additionally, some reports suggest that oxidative stress is a key determinant of ZEA-induced toxicity in vivo and in vitro. [73-77]

Glutathione S-transferase plays an important role in the biotransformation and detoxification of many xenobiotics, and semen contains significant amount of glutathione S- transferase; GST, important for sperm protection against oxidative stress [78]. Reduced activity of GST and increased reactive oxygen species (ROS) levels lead to sperm membrane damage. [79]

On the other hand, orally treated male mice treatment for 28 days with ZEA (40 mg/kg, i.p.) decreased glutathione peroxidase, superoxide dismutase SOD and, catalase activities in testes. [77] These apparent conflicting data can be explained by the differences in animal species, strain, sex as well as routes, schedules and doses of ZEA used. Regarding this point, Malekinejad et al.[80] has reported differences between species in hepatic biotransformation of ZEA in pigs, sheep, cattle, chicken and rats.

Recently, Wang et al. [81] reported that dietary ZEN resulted in decreased nutrient digestibility, increased oxidative stress, and reduced growth rate of pigs. Also Abid-Essefi et al. [74] found that ZEN induced several toxic effects and significant alterations mediated by oxidative stress mechanism.

However, mycotoxins can affect immune responses of animals in different ways, from changes in the production of enzymes and cytokines to an increased production of free radicals that indirectly affect these parameters. [82]

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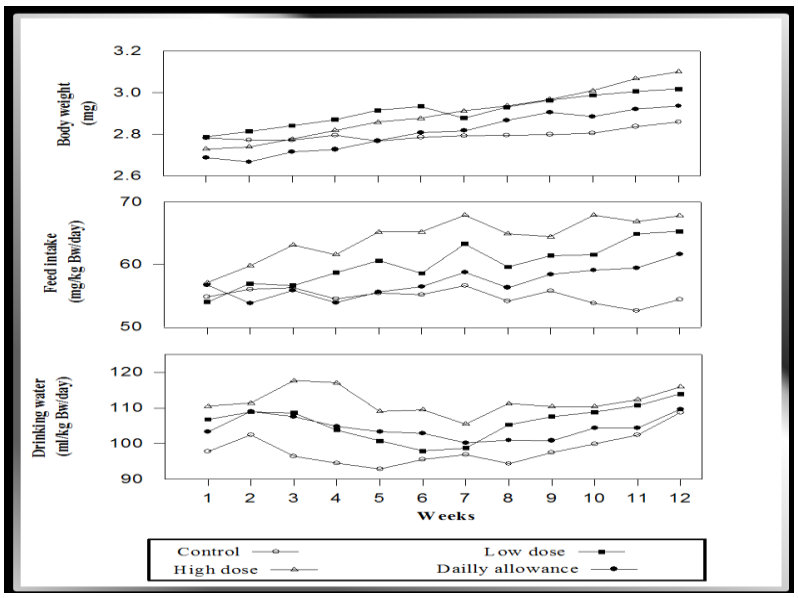

Figure (1): Changes in body weight, feed intake and drinking water during treatment of male rabbits with low, high doses and daily allowance of zearalenone.

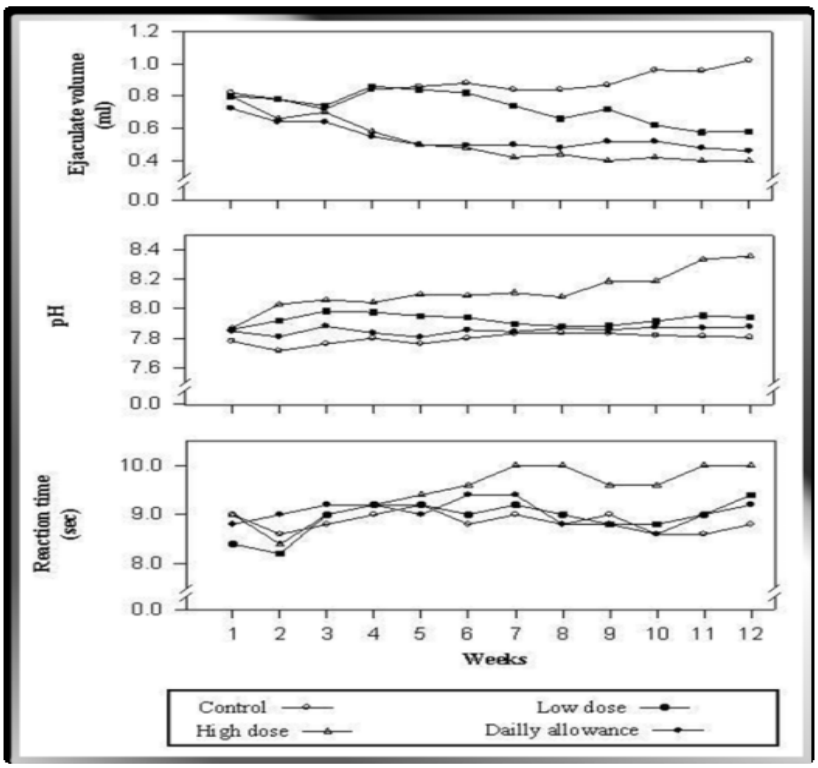

Figure (2): Changes in ejaculate volume, pH and reaction time during treatment of male rabbits with low, high doses and daily allowance of zearalenone.

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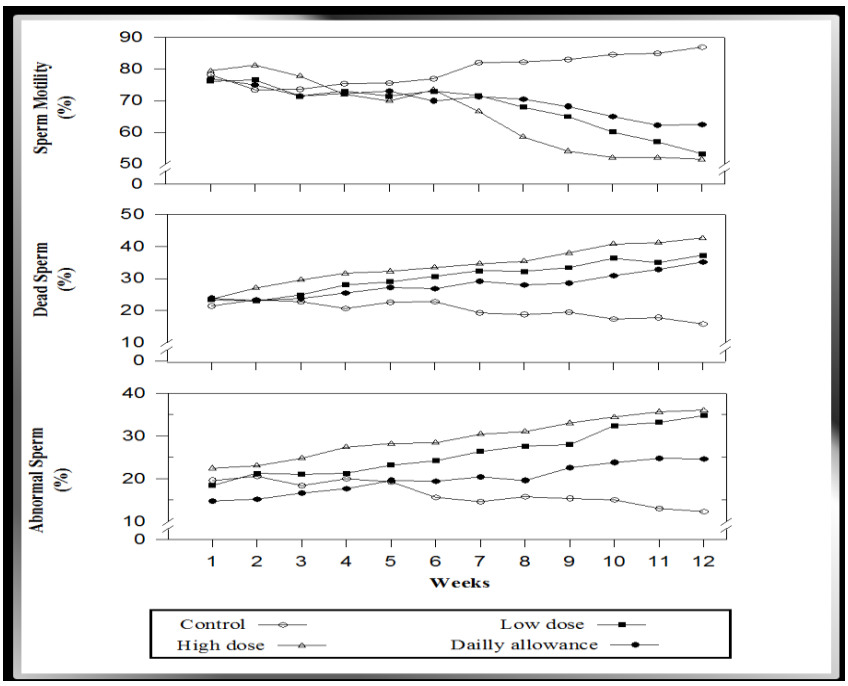

Figure (3): Changes in sperm motility, dead sperms and abnormal sperms during treatment

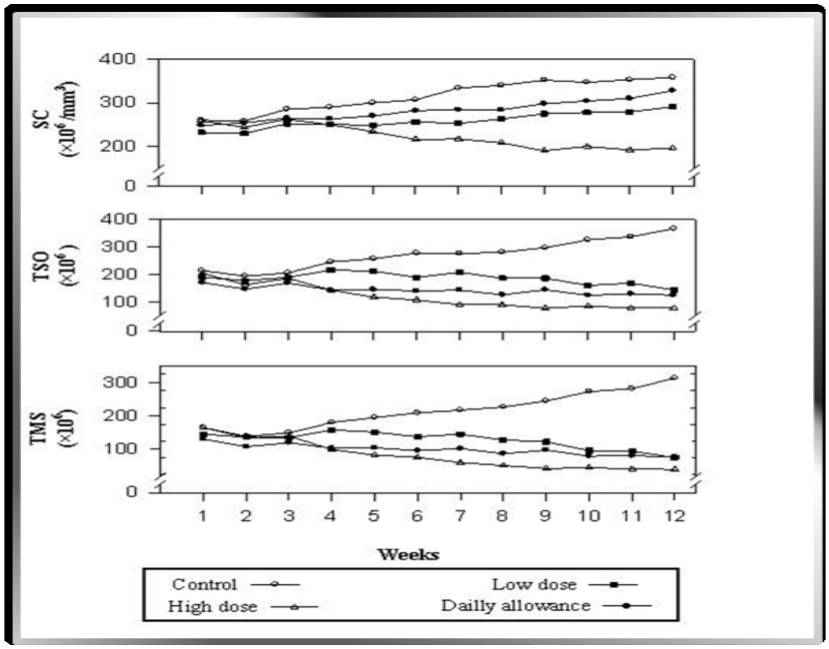

Figure (4): Changes in sperm concentration (SC), total sperms output (TSO) and total motile sperm (TMS) during treatment of male rabbits with low, high doses and daily allowance of zearalenone.

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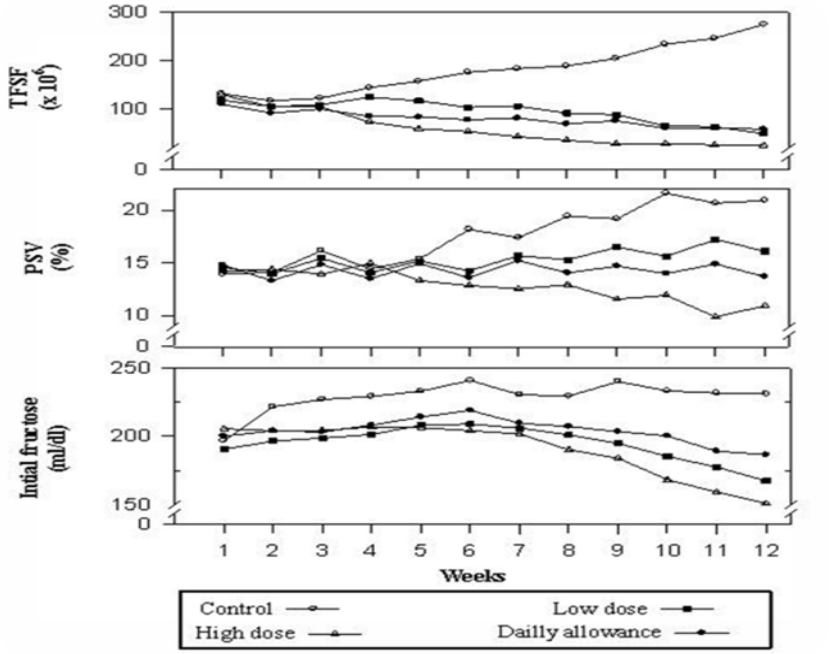

Figure (5): Changes in total functional sperm fraction (TFSF), packed sperm volume and initial fructose during treatment of male rabbits with low, high doses and daily allowance of zearalenone.

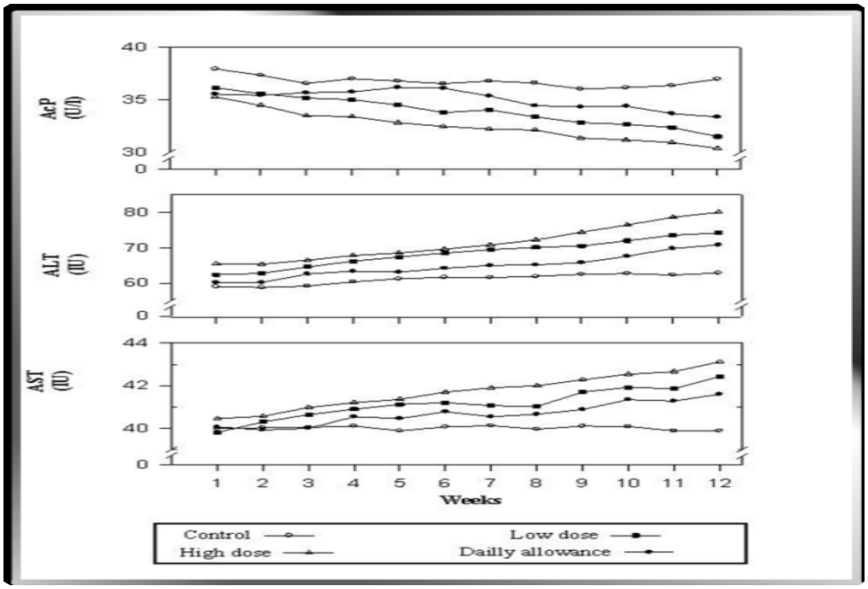

Figure (6): Changes in` seminal plasma acid phosphatase (ACP), alanine aminotransferase (ALT) and aspartate amino transferase (AST) during treatment of male rabbits with low, high doses and daily allowance of zearalenone