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Effects of different levels of dietary zinc supplementation on the testis parameters of the Japanese quail males

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ABSTRACT

This study was conducted in poultry farm of Agriculture College, University of Anbar, Iraq to assess the effects of different levels of zinc as dietary supplementation in volume density and relative weight of Japanese quail, *Coturnix japonica* testis contents. Sixty males of 35 day-olds were used in this study. Quails distributed randomly in four treatments and three replicates, each including 5 males. Birds fed balanced diets contain four levels of metal zinc as: T_1: control diet without any addition of zinc; T_2: diet by adding 25 mg kg^{-1}; T_3: 50 mg kg^{-1}; and T_4: 75 mg kg^{-1}. Males were slaughtered at 147^{th} day-old then testes weight, testes relative weight, seminiferous tubules, interstitial cells volume density and relative weights (mg g^{-1}) as well as diameters of seminiferous tubules were measured. Results exhibited no significant differences in testis weight and relative weights in all treatments compared to control group. Significant elevation ($p < 0.0001$) in volume density of spermatogonia was observed in T_3 and T_4. Significant differences ($p < 0.0001$) was found in volume density of spermatocytes in all treatments. There was also significant increasing ($p < 0.1433$) in total sperm contents in T_4. Data refers to significant rise ($p < 0.0024$) in relative weight of spermatogonia in T_3, and also significant elevation ($p < 0.0453$) in volume density of spermatocytes in T_3 and T_4.

Keywords: Zinc, Japanese quail, Testis tissues.
Article type: Research Article.

INTRODUCTION

Zinc is an essential mineral for growing and development of all most life because of important role as co-enzymes for more than 300 enzymes and protein synthetic (Sahraei *et al.* 2014). Zinc also essential for metabolic activity of carbohydrate, proteins, RNA and DNA induction (Feng *et al.* 2010), enhancing different biological functions, reproduction and immunity. It is a powerful antioxidant in cells life due to its role in stimulating carbonic anhydrase enzyme to get rid of free radicals (Babaei 2007). Wherefore, dietary addition of zinc makes reduction in cells oxidation damage caused by free radicals (Tupe *et al.* 2010). On the other hand, zinc is a fortified for some hormones activities such as glucagon, insulin and sex hormones (Chand *et al.* 2014). Zinc plays an important role in enhancing fertility by preventing sperms nuclei DNA chromatin from damage by free radicals (Babaei 2007; Kothari *et al.* 2016). Also it elevates sperm production and motility in semen by role of working as antibacterial and prevents sperm cells from damage (Kothari 2016). Zinc also plays important role in preserving perfect blood testosterone levels by enhancing testes growing and work (Egwurugwu *et al.* 2013). In addition, it plays an important role in insulin-like growth factor (IGF-1) secretion (Starks *et al.* 2006). Reports show that (IGF-1) controls Sertoli cells in pre-puberty period (Pitetti *et al.* 2013). Also IGF-1 enhances testosterone secretion from Leyedic cells (Yoon & Rooser 2010). So, zinc makes to develop puberty for avian males fed with zinc-supplemented diets by stimulating IGF-1 gene expression in testes (Khoobbakht *et al.* 2020). There are also some reports about other element supplementation in the world (Zahmatkesh, *et al.* 2020; Roitman *et al.* 2021; Mir Rasekhian *et al.* 2022). This study was conducted to assess the effects of different levels of dietary zinc in the testis tissues of the Japanese quail males, as well as the seminiferous tubules and interstitial cells contents traits.

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MATERIAL AND METHODS

This study was performed in a poultry farm, Department of Animal Production, College of Agriculture, University of Anbar Iraq to assess the effects of different dietary zinc supplementation levels on the Japanese quail testis tissues. Sixty Japanese quail males were used in this study (45-days age) dividing into three treatments, with three replicates for each (each replicate consists of 5 males). Birds fed balanced diets containing four levels of metal zinc as T_1: control diet without any addition of zinc; T_2: diet by adding 25 mg kg^{-1}; T_3: 50 mg kg^{-1}; and T_4: 75 mg kg^{-1}. Males were slaughtered at 147^{th} days old, then males and testes were weighed and dissected (one male from each replicate). Thereafter, testes were removed and packed in plastic containers with 10% formalin. Then testes processed according to Uni *et al.* (1998) and Tako *et al.* (2004). Testes weighed in sensitive scale and relative weight was calculated as below:

$$\text{Testes relative weight} = \frac{\text{Testes weight}}{\text{Life body weight}} \text{ X } 100$$

Morphometric analyses was performed for estimated seminiferous tubules, volume density (%) of interstitial cells, volume density (%), relative weight (g kg^{-1}) and diameter contents according to Weible (1979).

Volume density = Relative weight × testes relative weight

The parameters of seminiferous tubule contents were assayed including spermatogonia, spermatocytes, speamatides, sperms, spermatogenic cells, Sertoli cells, lumen, vacuoles, basement membrane and total seminiferous tubules contents. The parameters of interstitial cell contents examined were myoid cells, Leydig cells, blood vessels, Interstitial spaces, total intestinal contents and ratio of total seminiferous tubule contents to total intestinal contents. Parameters were calculated as a mean of three sections for each slide. In this experiment, complete random design (CRD) within three treatments, and three replicates was used. Data were analysed using GLM model procedure of SAS (Statistical analysis system; SAS 2001) including concentrations of zinc in diets. Means for treatments compared by Duncan's polynomial using different significant levels to determine significant differences between the averages (Duncan 1955).

RESULTS AND DISCUSSION

Results in Table 1 depict a significant elevation ($p < 0.0579$) in seminiferous tubule diameters in T_3, while there was no significant differences in testis weight and relative weights, lumen diameter and mean of germ cell diameters in all treatments compared to control group. Results in Table 2 present a significant increasing ($p > 0.0001$) in volume density of spermatogonia in T_3 and T_4. Significant differences ($p < 0.0001$) were also found in volume density of spermatocytes in all treatments. It was also true for the total sperm contents in T_4 ($p < 0.1433$), while, there was no differences in volume density for other contents of seminiferous tubules and interstitial cells.

Table 1. Effects of the dietary zinc supplementation on testes weight and the seminiferous tubules content parameters in Japanese quail males.

Traits	Treatments				Mean	SEM*	Significant level
	Zn (0 mg kg^{-1})	**Zn (25 mg kg^{-1})**	**Zn (50 mg kg^{-1})**	**Zn (75 mg kg^{-1})**			
Testes weight mean	$2/60^{a}$	$2/40^{a}$	$2/30^{a}$	$2/36^{a}$	2/41	0/193	NS
Testes relative weight	$1/40^{a}$	$1/32^{a}$	$1/31^{a}$	$1/18^{a}$	1/30	0/090	NS
Seminiferous tubules diameter	$26/4^{ab}$	$24/3^{b}$	$28/6^{a}$	$24/7^{b}$	26/04	0/640	0/0597
Lumen diameter	$10/7^{a}$	$12/6^{a}$	$13/1^{a}$	$12/4^{a}$	12/25	0/785	NS
Mean of germ cells diameter	$5/55^{a}$	$5/94^{a}$	$6/72^{a}$	$6/00^{a}$	6/055	0/258	NS

Note: Letters refer to differences between columns; *SEM: standard error of means; **NS: No significant differences between columns.

Data refers to significant increasing ($p < 0.0024$) in relative weight of spermatogonia in T_3 and also significant elevation ($p < 0.0453$) in volume density of spermatocytes in T_3 and T_4, while significant decline ($p < 0.0011$) in relative weights of sperms in T_3 and T_4 and also significant drop ($p < 0.0483$) in relative weights of basement membrane in T_4 compared to control group. However, there was no difference in relative weight in other contents of seminiferous tubules and interstitial cells.

Table 2. Effects of the dietary zinc supplementation on seminiferous tubules and the interstitial cells contents and volume density percentage (%) in Japanese quail males.

Traits	Treatments				Mean	SEM*	Significant level
	Zn (0 mg kg^{-1})	Zn (25 mg kg^{-1})	Zn (50 mg kg^{-1})	Zn (75 mg kg^{-1})			
Spermatogonia	2.97^{b}	2.97 ^{b}	6.67^{a}	5.50^{a}	4.69	0.351	0.0001
Spermatocytes	5.55^{c}	7.65^{b}	8.77^{ab}	10.57^{a}	8.13	0.436	0.0001
Spermatids	7.50^{a}	6.23^{a}	5.79^{a}	7.11^{a}	6.66	0.302	NS
Sperms	8.23^{a}	7.60^{a}	4.28^{b}	5.01^{b}	6.28	0.399	0.0001
Spermatogenic cells	24.2^{b}	25.0^{ab}	25.5^{ab}	28.2^{a}	25.7	0.638	0.1443
Sertoli cells	1.31^{a}	1.17^{a}	1.41^{a}	1.02^{a}	1.23	0.079	NS
Vacuoles	3.55^{a}	5.01^{a}	4.18^{a}	3.70^{a}	4.11	0.405	NS
Lumens	4.19^{a}	4.77^{a}	3.06^{a}	4.14^{a}	4.04	0.350	NS
Basement membrane	3.50^{a}	2.77^{a}	2.68^{ab}	1.80^{b}	2.69	0.216	0.0417
Total seminiferous tubules contents	39.863^{a}	40.35^{a}	39.23^{a}	41.18^{a}	40.15	0.58	NS
Interstitial cells							
Myoid cells	2.77$_{a}$	2.63$_{a}$	3.65$_{a}$	2.58$_{a}$	2.91	0.181	NS
Leydig cells	0.97$_{a}$	0.87$_{a}$	0.83^{a}	0.83$_{a}$	0.87	0.057	NS
Blood vessels	1.31$_{a}$	1.02$_{a}$	0.97$_{a}$	1.02$_{a}$	1.08	0.066	NS
Interstitial spaces	3.55$_{a}$	5.01$_{a}$	4.18$_{a}$	3.70$_{a}$	4.11	0.405	NS
Total intestinal contents	8.62$_{a}$	9.54$_{a}$	9.65$_{a}$	8.13$_{a}$	8.99	0.40	NS
Ratio of total seminiferous tubules contents to total intestinal contents	5.10$_{a}$	4.81$_{a}$	4.21$_{a}$	5.19^{a}	4.83	0.26	NS

Note: Letters refer to differences between columns; *SEM: standard error of means; **N S: No significant differences between columns.

Table 3. Effects of the dietary zinc supplementation on seminiferous tubules, interstitial cell contents and relative weight in the Japanese quail males.

Traits	Treatments				Mean	SEM*	Significant level
	Zn (0 mg kg^{-1})	Zn (25 mg kg^{-1})	Zn (50 mg kg^{-1})	Zn (75 mg kg^{-1})			
Spermatogonia	4.03^{b}	4.79^{B}	9.26^{a}	6.54ab	6.15	0.566	0.0024
Spermatocytes	7.64^{b}	10.13^{Ab}	11.70^{a}	12.49^{A}	10.49	0.668	0.0453
Spermatids	10.45^{a}	8.19^{a}	7.61^{a}	8.40^{A}	8.66	0.540	NS
Sperms	11.93^{a}	9.98^{a}	5.58^{b}	5.85^{B}	8.33	0.732	0.0011
Spermatogenic cells	34.06^{a}	33.10^{a}	34.17^{a}	33.29^{A}	33.65	1.622	NS
Sertoli cells	1.99^{a}	1.54^{a}	1.86^{a}	1.23^{A}	1.65	0.151	NS
Vacuoles	4.45^{a}	6.70^{a}	5.38^{a}	4.30^{A}	5.21	0.552	NS
Lumens	6.02^{ab}	6.36^{a}	3.73^{b}	4.29^{ab}	5.10	0.438	0.0839
Basement membrane	5.10^{a}	3.66^{ab}	3.50^{ab}	2.21^{B}	3.62	0.372	0.0483
Total seminiferous tubules contents	30.84^{a}	30.73^{a}	31.40^{a}	37.01^{A}	32.49	1.41	NS
Interstitial cells							
Myoid cells	3.83^{a}	3.42^{a}	4.82^{a}	3.23^{A}	3.83	0.290	NS
Leydig cells	1.32^{a}	1.14^{a}	1.07^{a}	0.99^{A}	1.13	0.082	NS
Blood vessels	1.81^{a}	1.36^{a}	1.26^{A}	1.23^{A}	1.41	0.104	NS
Interstitial spaces	4.45^{a}	6.70^{a}	6.70^{a}	4.30^{A}	5.21	0.552	NS
Total intestinal contents	7.05^{a}	7.25^{a}	7.82^{a}	7.10^{A}	7.31	0.43	NS
Ratio of total seminiferous tubules contents to total intestinal contents	3.72^{a}	3.66^{a}	3.31^{a}	4.77^{A}	3.86	0.24	NS

Note: Letters refer to differences between columns; *SEM: standard error of means; **N S: No significant differences between columns.

The improvement in volume density and relative weights of some testis compounds may be due to role of zinc as a regulator and enhancement in cells, since zinc play an important role in IGF-1 regulation (unpublished result), Khoobbakh (2020) reported that zinc methionine dietary addition leads to enhancing mRNA production of IGF-1 then improvement of IGF-1 production. Result of high production of IGF-1 play main role in male reproduction by enhancing spermatogenesis significantly as a result of directly stimulation of gonadotropin releasing hormone (GnRH), then FSH and LH production (Barb 1991; Al-Bayar *et al.* 2020). In addition, Ruiz-Cortiz (2012) reported that using ZnO and Zn-Met as a feed additives stimulates the Japanese quail males testis growth before puberty by elevating the steroids hormones level in plasma. Also declining zinc ratio in the broiler breeders diets leads to delay puberty (Kumar 2003). Zinc plays a major role in morphological development of the Japanese quail testes tissues (Fu *et al.* 2001; Roser & Yoon 2010; Salwan *et al.* 2021). On the other hand, IGF-1 participate in Sertoli cells work in pre puberty period and plays an important role by enhancing males fertility (Pitetti *et al.* 2013). In conclusion, in this study, dietary supplementation of zinc oxide for Japanese quail males led to elevation in volume density and also relative weight of spermatogonia and spermatocytes, while declining in the basement membrane and sperms.

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