Vol. 11. No.4. 2022.

DOI:10.13140/RG.2.2.15772.36483

Contents available at:
http://www.crdeepjournal.org

International Journal of Life Sciences (ISSN: 2277-193x) CIF: 5.411; SJIF: 6.431
A Peer Reviewed Journal

Full Length Research Paper

Semen Characteristics and Sperm Production Potentials of Rabbits Fed Di(2-ethylhexyl) Phthalate

Olatundun, B. E* and Ogunlade, J. T**
**Department of Agricultural Education, Osun State College of Education, Ilesa, Nigeria*
***Department of Animal Science, Ekiti State University, Ado-Ekiti, Nigeria*

ARTICLE INFORMATION

Corresponding Author:
Olatundun, B. E

Article history:
Received: 10-10-2022
Revised: 12-10-2022
Accepted: 30-10-2022
Published: 07-11-2022

Key words:
Di(2-ethylhexyl) phthalate, sperm production, sperm reserves, semen characteristics, rabbit bucks.

ABSTRACT

Phthalates are used primarily in the plastics industry as plasticizers and have become ubiquitous environmental contaminants due to volatilisation and leaching from their widespread applications, and they have been detected in the environment and in foods. Limited information is available on their effects on semen characteristics and sperm production ability of rabbit bucks hence the aim of this study. In a 75-day feeding trial, a study was conducted with forty-five weaned crossbred rabbit bucks with an average of initial live weight of 1.37 kg to evaluate the effects of di(2-ethylhexyl) phthalate (DEHP) on semen characteristics and sperm production potentials of the rabbits. The rabbits were randomly allotted to 5 dietary treatments and were housed individually. Control diet (T_1) contained no DEHP while T_2, T_3, T_4 and T_5 contained DEHP at 100, 200, 300 and 400 ppm inclusion levels respectively. It was revealed in the results that the semen volume was statistically similar in all the treatments. Sperm motility and viability were significantly ($p<0.05$) reduced in rabbits fed diets containing DEHP compared with the rabbits on control diet. There were no significant differences ($p>0.05$) in the weights of left testis, right testis and paired testes of the rabbits across the dietary treatments, however, the testicular sperm reserves of the rabbit bucks decreased significantly ($p<0.05$) with increasing level of dietary DEHP. The weights of the left, right and paired epididymides were not significantly ($p>0.05$) influenced by DEHP, however, epididymal sperm reserves in the bucks were significantly ($p<0.05$) depressed as the DEHP level increased in the diets. In addition, dietary DEHP significantly ($p<0.05$) reduced the daily sperm production and sperm production efficiency of the rabbits. This study revealed that DEHP at the various inclusion levels caused significant reductions in sperm motility, viability as well as sperm production ability of the rabbits.

Introduction

Di(2-ethylhexyl) phthalate (DEHP) is an industrial chemical found in many products used by humans, such as soap, shampoo, cosmetics and hairspray. Primarily, DEHP is used in the plastics industry as plasticizer and has become ubiquitous environmental contaminant due to volatilization and leaching from its widespread applications. Their presence in water, soil and animal feedstuffs has been reported (Rudel, & Perovich 2003; Jarosova, 2006). DEHP is not chemically bound in the polymers, therefore migration or emission of the phthalate from the product to the environment is likely to occur (Koo et al. 2002; GreenFacts 2018).

Developmental and reproductive toxicity of phthalates has been reported. Dibutyl phthalate (DBP) is an endocrine disruptor and known to increase sperm abnormality rate in rabbits at 250, 500 and 750 mg/kg/day for 4 weeks (Rihani et al. 2015). Agarwal et al. (1986) also reported that dietary exposure of adult male rats to 0, 320, 1250, 5000 and 20000 ppm DEHP for 60 days resulted in decreased testicular zinc content, reduced epididymal sperm density and motility, and increased occurrence of abnormal sperm at 20,000 ppm. Abd-Ellah et al. (2015) reported significant decrease in sperm motility, sperm count and daily sperm production in rats given 0, 300, 600 and 900 mg/kg/day DEHP for 15 consecutive days. There is paucity of information on the effects of DEHP on semen characteristics of adult rabbits, therefore, the objective of this study was to determine whether exposure to DEHP at the dose levels would alter adult rabbits' semen quality, production potentials and fertility.

Materials and methods

The experiment was carried out at the rabbitry unit of the Teaching and Research Farm, Ekiti State University, Ado Ekiti, Nigeria. The site is located on Latitude $7^{o}37^{1}15^{11}$N and Longitude $05^{o}13^{o}28^{o}$E with the temperature range of 21 to 28^{o}C.The DEHP was purchased from Sigma-Aldrich, USA through Bristol Scientific Company, Lagos, Nigeria. Five diets were formulated including the control (diet 1) with crude protein of 17.99 %, crude fibre of 11.33 % and digestible energy of 2789 kcal/kg. All diets were iso-nitrogenous and iso-caloric. Diets 2, 3, 4 and 5 contained the same ingredients as the control diet but also contained DEHP at inclusion levels of 100, 200, 300 and 400 ppm respectively. In the experiment that lasted for 12 weeks, a total of forty five weaned rabbits with an initial average weight of 1.37kgwere randomly assigned to the five dietary treatments. Each treatment had three replicates of three rabbits each which were housed individually in vermin-proof cages in a completely randomized design. Feed and water were provided *ad libitum* for the rabbit bucks. At the end of the feeding trial, three rabbits were randomly selected from each treatment for semen collection and sperm production evaluation. Semen collection was done in the morning using an artificial vagina, followed by semen analysis using the method described by Ogunlade et al. (2006). The rabbits were later sacrificed and their reproductive tract dissected. Gonadal and epididymal sperm reserves were determined according to the method described by Rekwot et al. (1994) and Ogunlade et al. (2006). The testes and epididymides were carefully extracted, weighed and homogenized separately in 0.9 % NaCl solution. About 5 ml of the homogenate was transferred to a conical flask and further dilution was done with 40 ml of saline, after which the homogenate was stored overnight at 5^{0}Cto allow sperm cells to ooze out of the tissues and the sperm concentration therein was determined by direct haemocytometric counts.

The daily sperm production (DSP) was estimated from the testicular sperm reserves and was calculated using the following formula proposed by Amann (1970):

$$DSP = \frac{\text{Testicular sperm count}}{Time\ divisor\ (3.43)}$$

Data Analysis

The data obtained were subjected to statistical analysis using the analysis of variance (ANOVA) procedures of Minitab statistical software version 16. The treatment means were presented with group standard errors of means and where significant, they were compared using the Duncan Multiple Range Test of the same software.

Results and discussion

The semen characteristics of rabbits fed DEHP contaminated diets are shown in Table 1. There were no significant differences ($p>0.05$) in the semen volume, sperm concentration and morphology of the rabbit bucks. However, sperm progressive motility and viability values were reduced significantly ($p<0.05$) with increasing levels of dietary DEHP. The reduction in sperm motility could be attributed to effect of DEHP as a result of consequent reduction in the testosterone level of the animals (Abd-Ellah et al. 2016) or activation of DNA damage in the sperm resulting in suppressed testicular ATP levels (Li et al., 2014).Spermatozoa membranes are rich in polyunsaturated fatty acids, so they are susceptible to reactive oxidative stress attack and lipid peroxidation (LPO) caused by DEHP. LPO causes membrane damage that leads to a decrease in sperm motility, presumably by a rapid loss of intracellular ATP, and an increase in sperm morphology defects (Guthrie & Welch, 2012). Testicular and epididymal characteristics of the rabbits are shown in Table 2. Testicular andepididymal weights of the rabbit bucks were not significantly influenced ($p>0.05$) by the dietary DEHP levels. Testicular (gonadal) and epididymal (extragonadal)sperm reserves were significantly ($p<0.05$) reduced with the increasing levels of dietary DEHP while daily sperm production and sperm production efficiency followed a similar trend of significant reduction ($p<0.05$) across the treatments. The testicular sperm reserves might have been reduced as a result of progressively depressed spermatocytogenesis and by extension, sperm storage in the testis (Ogunlade et al., 2006).

The observed decrease in the epididymal sperm reserves may indicate that DEHP affects the early stages of spermiogenesis, which is well supported by the observed decrease in daily spermatozoa production (Abd-Ellah et al., 2016). DEHP could cause injury of spermatogonia and spermatids, and can lower spermatogenesis by adversely affecting spermatogonia followed by depletion of spermatids and spermatozoa (Abd-Ellah et al., 2016). Also, changes in enzymes activity may induce destruction of seminiferous epithelium and loss of germinal elements, resulting in decrease in the number of spermatids associated with decrease in the DSP in the testes (Hodgen & Sherins, 1973).

Table 1: Semen characteristics of rabbits bucks fed varied levels of DEHP.

	Dietary Treatments					
	T1	**T2**	**T3**	**T4**	**T5**	
Parameters	**Control**	**100 ppm**	**200 ppm**	**300 ppm**	**400**	**ppm**
SEM						
Semen Vol. (ml)	0.50	0.49	0.49	0.47	0.47	0.03

Sperm Motility (%)	86.67[a]	71.67[ab]	51.67[abc]	45.00[bc]	30.00[c]	1.48
Sperm Conc. ($x10^6$/ml)	90.53	90.60	89.60	85.32	86.53	2.77
Sperm Viability (%)	93.00[a]	57.67[ab]	62.33[ab]	49.00[b]	35.00[c]	3.00
Morphologically Normal Sperm (%)	79.00	77.67	80.67	78.33	76.00	0.66

SEM = Standard Error of Mean, ppm = parts per million (equivalent of mg/kg)

Table 2: Testicular and epididymal characteristics and DSP of rabbits bucks fed varied levels of DEHP.

Dietary Treatments	T1	T2	T3	T4	T5	
Parameters	**Control**	**100 ppm**	**200 ppm**	**300 ppm**	**400**	**ppm**
SEM						
Testicular weights (g)						
Right Testis	1.93	1.91	1.87	1.84	1.83	0.03
Left Testis	2.05	1.96	2.05	1.94	1.91	0.04
Paired Testes	3.98	3.87	3.92	3.78	3.74	0.07
TSR (x!0^6)	26.30[a]	25.00[ab]	23.93[b]	22.62[b]	20.00[c]	0.81
Epididymal weights (g)						
Right Epididymis	0.35	0.47	0.61	0.39	0.42	0.01
Left Epididymis	0.36	0.48	0.50	0.34	1.57	0.07
Paired Epididymis	0.71	0.95	1.10	0.73	1.99	0.02
ESR ($x10^6$)	84.40[a]	82.60[ab]	81.53[ab]	62.00[b]	54.30[c]	1.38
DSP (per testis x 10^6)	7.67[a]	7.23[ab]	6.98[b]	6.59[b]	5.83[c]	0.21
SPE (DSP/g testis x 10^6)	1.93[a]	1.87[ab]	1.78[b]	1.74[b]	1.56[c]	0.05

SEM= Standard Error of Mean, TSR = Testicular Sperm Reserve, ESR = Epididymal Sperm Reserve, DSP = Daily Sperm Production, SPE = Sperm Production Efficiency, ppm = parts per million(equivalent of mg/kg).

Conclusions

The results obtained revealed that DEHP, at the various inclusion levels used in this study, has deleterious effects on sperm motility, viability, gonadal and extragonadal sperm reserves. Therefore, rabbit bucks intended to be used for breeding purpose should not be exposed to diets containing DEHP up to 100ppm.

References

Abd-Ellah, M., Aly, H., Mokhlis, H., & Abdel-Aziz, A (2016). Quercetin attenuates di-(2-ethylhexyl) phthalate-induced testicular toxicity in adult rats. *Human & Experimental* Toxicology, 35(3), 232–243.

Agarwal, D.K., Eustis, S., James C. Lamb, J.C., Jerry R. Reel, J.R. and Kluwe, W.M (1986). Effects of Di(2-ethylhexyl) Phthalate on the Gonadal Pathophysiology, Sperm Morphology, and Reproductive Performance of Male Rats. *Environmental Health* Perspectives. Vol. 65, pp. 343-350.

Amann, R.P (1970). The male rabbit IV. Quantitative testicular histology and comparison between daily sperm production as determined histologically and daily sperm output. Fertil. Steril. 21: 662 – 672.

GreenFacts, (2018). Phthalate Di-butyl phthalate. Retrieved May 25, 2018 from https://wwwgreenfacts.org

Guthrie, H.D and Welch, G.R (2012). Effects of reactive oxygen species on sperm function. Theriogenology, 78: 1700–1708.

Hodgen, G.D and Sherins, R.J (1973). Enzymes as markers of testicular growth and development in the rat. Endocrinol, 93: 985–989.

Jarošová A. (2006): Phthalic acid esters (PAEs) in the food chain. Czech J. Food Sci., 24: 223–231.

Koo, J., Parham F., Kohn M., Masten S., Brock J., Needham L., Portier C (2002). The association between biomarker-based exposure estimates for phthalates and demographic factors in a human reference population. Environ Health Perspect., 110(4), 405-410.

Li, X., Fang, E. F., Scheibye-Knudsen, M., Cui, H., Qiu, L., Li, J. and Guo, H (2014). *Di-(2-ethylhexyl) phthalate inhibits DNA replication leading to hyperPARylation, SIRT1 attenuation, and mitochondrial dysfunction in the testis.* Scientific Reports, 4(1).

Ogunlade, J. T., Ewuola, E. O., Gbore, F. A., Bandyopadhyay, R., Niezen, J. and Egbunike, G. N (2006). Testicular and epididymal sperm reserves of rabbits fed fumonisin contaminated diets. World Appl. Sci. J., 1: 35 – 38.

Rekwot, P. J., Oyedipe, E. O and Ehoche, G.W (1994). The effects of feed restriction and regimentation on the growth and reproductive function of Bokoloji bulls. Theriogenology. 42:287 – 295

Rihani, L., Loukil, B. and Khelili, K (2015). Effect of Dibutyl Phthalate on Sperm Quality and Liver Injury in Adult Male Rabbits. American-Eurasian Journal of Toxicological Sciences, 7 (1): 34-38.

Rudel, R.A. and Perovich, L. J (2003). Phthalates, alkylphenols, pesticides, polybrominateddiphenyl ethers, and other endocrine-disrupting compounds in indoor air and dust. Envi Sci & Tech., 37, 4543–4553.