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Impact of Lead and Cadmium-Contaminated Soil on the Reproductive Physiology and Systemic Oxidative Status of Pre-Pubertal Rabbits

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Abstract

This study investigated the effect of contaminated soil on semen quality, hormonal profile and oxidative stress indices of growing rabbits. 40 growing Chinchilla × New Zealand white crossbred rabbits of 8-10 weeks of age were used for this trial. Rabbits were randomly assigned to the four dietary groups (3 bucks and 7 does /group) in a feeding trial that lasted 20 weeks including 2 weeks adjustment period. Treatments include; Treatment 1 (control): basal diet alone without contaminated soil, T2 through T4 received basal diet with contaminated soil at 5.00 g, 10.00 g and 15.00 g/kg diet. Fresh clean water and feed was unlimitedly offered. A completely randomized experimental design was adopted. Basal diet formulated according to the requirement of rabbits. Contaminated soil contained: lead (109.4 mg/kg), arsenic (88.62 mg/kg), chromium (76.52 mg/kg), nickel (60.07 mg/kg), cadmium (22.09 mg/kg) and mercury (10.06 mg/kg). Results revealed a decline ($p<0.05$) in hormonal parameters: Follicle stimulating hormone dropped from 3.82 – 1.87 (IU/ml), luteinizing hormone (2.41 – 0.71 IU/ml) and testosterone (4.77 – 1.62 ng/m). Catalase, total antioxidant activity, superoxide dismutase and glutathione peroxidase followed similar download trend ($p<0.05$). Sperm volume, sperm motility, sperm concentrations, live sperm cells were significantly ($p<0.05$) affected except semen colour ($p>0.05$). It was concluded that the progressive addition of contaminated soil from 5-15 g/kg diet exert a negative effect on the reproductive health and performance of growing rabbits.

Keywords: heavy metals; semen; lead toxicity; cadmium; sperm motility; reproductive health; hormone

Introduction

The reproductive health of male animals is increasingly compromised by the pervasive presence of heavy metals in the environment [1]. In rabbits, heavy metals such as cadmium, lead, mercury and arsenic are recognized as significant environmental pollutants that originate from industrial discharge, agricultural pesticides, contaminated water and soil sources and mining activities. Because rabbits are often raised in proximity to agricultural and industrial zones, they serve as critical sentinel species – early indicators of environmental hazards that may eventually affect human health [2, 3]. Heavy metals also enters a rabbit's body primarily via ingestion of contaminated forage and water. The rapid expansion of industrialization has led to a significant release of heavy metals [4, 5]. These toxic elements contaminate soil, water and forage consumed by livestock's including rabbits. Once absorbed, these metals exhibits a high affinity for the testis and epididymis due to the presence of specific metal-binding proteins and the high metabolic activity of these tissues [6]. Unlike other toxins that the body can readily metabolize and excrete, heavy metals are characterized for their long biological half lives and their tendency to bioaccumulate [7, 8]. This accumulation disrupts the blood testis barrier, a physical and physiological gate keeper that normally protects developing sperm cells from harmful substances [9].

Previous studies have shown that exposure of cadmium and lead at 3 mg/kg in the diet of rabbits resulted in retarded growth, impaired reproductive efficiency, significant decline in sperm production, reduce libido and suppression of body's antioxidant defense system making them susceptible to diseases and infection [10, 11]. A study by [12] revealed that addition of chromium at 5 mg/kg lead to a disruption in hormonal profile and promotion of lipid peroxidation. Despite the widespread recognition of heavy metals as reproductive toxicants, there is a persistent challenge in diagnosing and mitigating their sub-lethal effects on male fertility [13]. In rabbits, the exposure does not result in immediate physical illness but manifests as silent reproductive failure. Chronic exposure disrupts critical biological barriers, such as blood testis barrier, and induces systemic oxidative stress that damages sperm DNA beyond repairs [14]. There is critical need to clearly define the extent of these damages in rabbits to safeguard their role as both

a livestock resource and their reliable sentinel for human environmental health risks.

Materials and methods

Ethical approval and experimental sites

The Rabbitary at Gandhi Research Teaching Unit located between latitude 23° 03 N to 30° 012 N and longitude 69° 30 E to 78° 17 E was used for the experiment. The area has a mean annual rainfall and humidity of 575 mm and 55 % respectively. All experimental procedures were approved by the Institutional Animal Care and Ethics Committee (AGN/30221/20261) ensuring adherence to compliance with standard animal welfare standards over the 20 weeks study.

Soil sample collection, preparation and analysis

The soil sample collection was carried out at a mining site in Rajasthan India. Samples were strategically collected from multiple points across the site to ensure a representative distribution of potential contaminants. At each point, soil was excavated from a consistent depth of 10 cm. To ensure representative analysis of sites overall contamination level, all individual samples were thoroughly pooled together and mixed to create a single homogenous sample. From this stabilized sample, 250 g sub-sample was collected and sent to the laboratory for analysis. Analysis was carried out using Aligent 2499AA Atomic Absorption Spectrometer operated according to the manufacturer's recommendation.

Animal Care, experimental diet and design

40 growing Chinchilla × New Zealand white crossbred rabbits of 8-10 weeks of age were used for the trial. Before the arrival of animals, metallic cages, feeding and watering troughs were properly cleaned and disinfected. Upon arrival, rabbits were placed on a two weeks acclimatization period and fed basal diet formulated according to the requirement of rabbits by NRC [15]. Animals were given Ivermectin Plus® injection against internal and external parasites. Animals were balanced for weight using a digital sensitive scale the rabbits were randomly assigned to the four dietary groups (3 bucks and 7 does /group) in a feeding trial that lasted 20 weeks including 2 weeks adjustment period. Treatments include; Treatment 1 (control): basal diet alone without contaminated soil, T2 through T4 received basal diet with contaminated soil at 5.00 g, 10.00 g and 15.00 g/kg diet. Fresh clean water and feed was unlimitedly offered. A completely randomized experimental design was adopted, all management practices were thoroughly observed throughout the trial period. Proximate analysis of basal diet was carried out using Foss NIRS (DS24000C) equipped with high performance silicon (400 – 1100 nm) and lead sulfide (1100 – 2500 nm). After calibration, kit was adjusted to a wavelength of 400 – 2500 nm and resolution of 0.5 nm to ensure precision in results.

Hormonal assay

On the last day of the trial, blood sample (5 ml) were collected from the marginal ear vein of 5 randomly selected rabbit per treatment into non-coagulant sample bottles and sent immediately to the laboratory for further analysis. BioTech Automated Elisa Kit (Model XC3201, Japan) was used for analysis. After calibration, kit was kept at a detection range of 156 – 1000 m lU/ml, sensitivity (0.94 m IU/ml), sample volume of 100 µL and precision (CV < 1.0 %). Parameters analyzed include follicle stimulating hormone, luteinizing hormone and testosterone.

Evaluation of oxidative stress indices

At the end of the trial, blood samples collected for hormonal assay was also used to determine oxidative stress parameters (Total antioxidant capacity, catalase, superoxide dismutase, glutathione peroxidase). Samples were first diluted with 0.2 ml DNPH solution and analyzed using Oxy IHC Oxidative stress detection kit (model S7450, USA) which operates via colorimetric innunohisto-chemistry to ensure precision in results. Semen collection and evaluation A 4-week period was used to train the bucks for semen collection. Semen was finally collected from the buck using the artificial vagina. Prior to semen collection, the artificial vagina was warmed for a few minutes in warm water at a temperature slightly above body temperature and thereafter drained. Semen collection was done very early in the morning to ensure that optimum quality semen were obtained. The semen was promptly assessed for semen quality parameters such as semen colour, semen volume, sperm motility, sperm concentration and percentage live sperm. The volume of semen collected was measured using the graduated collection tube. The semen colour was noted and recorded immediately after collection. For mass activity, a drop of undiluted fresh semen was placed on a sterile glass slide and observed under the microscope with an objective lens at a magnification of ×10. To determine the motility of the sperm cells, a drop of undiluted semen mixed with a drop of slightly warmed diluents (sodium citrate) was placed on a sterile slide, covered with a cover slip and observed under the microscope at (Magnification of ×400) and scored within a rating of 0 - 100 %. Live sperm cells determination was done by placing a drop of semen mixed with one drop of eosin nigrosine stain on a slide and observed under the microscope. The unstained cells represented the live cells while the stained cells are the dead ones. Sperm concentration/ejaculate was calculated as: sperm concentration per ml × volume of ejaculate. Sperm concentration per ml of semen was evaluated using a visual count under the microscope using improved Neubauer haemocytometer.

Statistical analysis

Data obtained on hormonal assay, oxidative stress and semen quality were examined using Analysis of variance (ANOVA) for Completely Randomized Design (CRD) according to statistical analysis system (SAS, 2003) at $p< 0.05$. Differences between means were separated by the Duncan's Multiple Range Test (DMRT) of the same software.

Results And Discussion

Ingredient and chemical composition of experimental diet revealed that it contained crude protein at 16.94 %, crude fibre, ether extract, ash and energy at 13.88 %, 3.02 %, 9.47 % and 2750.5 kcal/kg respectively. The experimental diet meets the nutritional standards according to NRC [17] standards as presented in Table 1. In Table 2, concentrations of heavy metals in contaminated soil revealed that lead had the highest concentration of 109.4 mg/kg while cadmium had the lowest concentration of 22.09 mg/kg. For the other compounds, arsenic (88.62 mg/kg), mercury (70.06 mg/kg), chromium (46.52 mg/kg) and nickel (60.07 mg/kg). Results obtained in this study suggests that concentrations of heavy metals in contaminated soil were above the safe recommended levels for rabbits. Therefore, they are bio accumulate quickly leading to a systemic organ failure [18]. Hormonal profile of rabbits fed varied levels of contaminated soil with heavy metals is presented in Table 3. Follicle stimulating hormone (FSHx) was lower in T2 (2.06 IU/ml), T3 (2.00 IU/ml), T4 (1.87 IU/ml) than T1 (3.82 IU/ml) ($p<0.001$). Luteinizing hormone (LHx) and testosterone also follow similar trend, values obtained varied from 0.71 – 2.41 IU/ml and 1.62 – 4.77 ng/m and were higher in T1, intermediate in T2, lower in T3 and T4 ($p<0.001$). The significant drop in LHx values obtained suggests that the pituitary gland of the birds have been affected by synergy between heavy metals in the contaminated soil. Similarly, the presence of lead in higher concentration poses serious deleterious effect Leygid cells making it difficult to produce testosterone [19]. A reduction in FSHx in T2 – T4 indicates a failure in the activities of

sertoli cells, this could lead to a decline in spermatogenesis [20]. A lower testosterone values among rabbits in T2-T4 is an evidence of rapid accumulation of heavy metals at the Leygid cells which can lead to a serious damage affecting sperm count in bucks and reduction in libido [21]. Previous report by [23] have shown that heavy metals have different predilection sites, for instance lead alters the central nervous system, arsenic (liver), cadmium (kidney), chromium (kidney), mercury (skin) amongst others. The result obtained in this study is in consonance with the reports of [24, 39,40] who observed a serious damage in the sperm cells when rabbits were fed diet that contains chromium at higher concentration. Oxidative stress indices of rabbits fed varied levels of contaminated soil with heavy metals is revealed in Table 4. Total antioxidant capacity, catalase, superoxide dismutase and glutathione peroxidase values varied from 16.35 – 29.13 (U/mg), 27.18 – 48.67 (U/mg), 17.32 – 36.90 (U/mg) and 10.99 – 26.72 (U/mg). It is worthy to note that the concentrations of these values significantly declined as the level of contaminated soil increases across the treatment ($p<0.05$). This is a clear sign that the activities of antioxidant enzymes have been compromised due to heavy metal stress. Exposure to heavy metals such as lead, mercury and cadmium have been reported to structurally deform enzymes making animals susceptible to infection or death [25, 38, 41]. The significant higher concentrations of total antioxidant capacity, catalase, superoxide dismutase and glutathione peroxidase in T1suggests that rabbits were in a state of homeostasis and their natural antioxidant system has not been negatively affected [26, 36, 37]. The result obtained in this experiment aligns with the reports of [27, 28, 35, 42]. Semen characteristics of rabbit bucks fed varied levels of contaminated soil with heavy metals (Table 5). The semen has a milky colour throughout the treatment ($p>0.05$), sperm volume was lower in T2 (0.61 ml), T3 (0.55 ml) and T4 (0.50 ml) than T1 (1.00 ml) (<0.001). Live sperm cell, sperm concentration and sperm motility values which varied from 59.00 – 84.00 %, 45.12 – 89.35 (× 107/ml) and 58.29 – 86.12 % respectively were significantly influenced ($p<0.05$) by the treatments. In this experiment, their outcome follow similar trend as values were greater in T1, moderate in T2, T3 and smaller in T4. The significant lower values in semen volume, semen motility, sperm concentration and percentage live sperm cells indicates that the heavy metals in the contaminated soil negatively altered the reproductive health of bucks by inducing oxidative stress making the animal susceptible to disease. Animals in T1 (basal diet only) had an enhanced antioxidant defense system. According to Massanyi [29, 30, 43], exposure to rabbit bucks to cadmium at 1 mg per kg diet for 12 weeks significantly reduces their sperm count. Dietary addition of cadmium in the diet of rabbits caused a serious decline in sperm production and reduced libido [31, 32, 33, 34].

Ingredients	Inclusion level
Maize	38.00
Wheat bran	14.50
Palm kernel meal	22.00
Soy bean meal	20.00
Limestone	1.50
Bone meal	3.00
Mineral-Vitamin Premix	0.25
Methionine	0.20
Lysine	0.20
Salt	0.35
Total	100.0
Analyzed values (%)	
Crude protein	16.94
Crude fibre	13.88
Ether extract	3.02
Ash	9.47
Energy	2750.5

Table 1: Ingredient and chemical composition of experimental diet (% DM).

Constituents	Composition (mg/kg)	**Typical toxic threshold
Arsenic	88.62	> 50 mg/kg
Mercury	10.06	> 2.0 mg/kg
Lead	109.4	> 30 mg/kg
Cadmium	22.09	> 5.0 mg/kg
Chromium	76.52	> 30 mg/kg
Nickel	60.07	> 45 mg/kg

Table 2: Concentration of heavy metals in contaminated soil.

Parameters	T1	T2	T3	T4	SEM	p-values
Level of Inclusion (g/kg diet)	5.00	10.00	15.00	20.00	-	-
Number of animals	10.00	10.00	10.00	10.00	-	-
Duration	60.00	60.00	60.00	60.00	-	-
Follicle stimulating Hormone (IU/ml)	3.82^{a}	2.06^{b}	2.00^{c}	1.87^{c}	0.24	<0.001
Luteinizing Hormone (IU/ml)	2.41^{a}	1.22^{b}	0.85^{c}	0.71^{c}	0.16	<0.001
Testosterone (ng/m)	4.77^{a}	2.08^{b}	1.77^{c}	1.62^{c}	0.01	<0.001

Table 3: Hormonal profile of rabbits fed varied levels of contaminated soil with heavy metals.

a,b,c Means within the same row with different superscript differ significantly ($p<0.05$).

Parameters	T1	T2	T3	T4	SEM	p-values
Level of Inclusion (g/kg diet)	5.00	10.00	15.00	20.00	-	-
Number of animals	10.00	10.00	10.00	10.00	-	-
Duration	60.00	60.00	60.00	60.00	-	-
Catalase (U/mg)	48.67^{a}	38.61^{b}	30.88^{c}	27.18^{d}	1.20	<0.001
Superoxide dismutase (U/mg)	36.90^{a}	29.40^{b}	20.64^{c}	17.32^{d}	1.05	<0.001
Glutathione Peroxidase (U/mg)	26.72^{a}	19.83^{b}	11.63^{c}	10.99^{c}	0.03	<0.001
Total Antioxidant Capacity (U/mg)	29.13^{a}	17.44^{b}	16.39^{b}	16.35^{b}	0.04	<0.001

Table 4: Oxidative stress indices of rabbits fed varied levels of contaminated soil with heavy metals.

a,b,c,d Means within the same row with different superscript differ significantly ($p<0.05$).

Parameters	T1	T2	T3	T4	SEM	p-values
Level of Inclusion (g/kg diet)	5.00	10.00	15.00	20.00	-	-
Number of animals	10.00	10.00	10.00	10.00	-	-
Duration	60.00	60.00	60.00	60.00	-	-
Semen colour	Milky	Milky	Milky	Milky	-	-
Semen volume (ml)	1.00^{a}	0.61^{b}	0.55^{b}	0.50^{b}	0.02	<0.001
Sperm motility (%)	86.12^{a}	69.06^{b}	68.11^{b}	58.29^{c}	0.13	<0.001
Sperm concentration ($\times 10^{7}$/ml)	89.35^{a}	47.18^{b}	45.60^{b}	45.12^{b}	0.18	<0.001
Live sperm cells (%)	84.00^{a}	60.02^{b}	59.16^{b}	59.00^{b}	0.22	0.001

Table 5: Semen characteristics of rabbit bucks fed varied levels of contaminated soil with heavy metals.

a,b,c Means within the same row with different superscript differ significantly ($p<0.05$)

Conclusion

The outcome of this trial clearly shows that the dietary addition of contaminated soil exert a deleterious effect on the reproductive health of rabbits. While T1 (control) maintained physiological normalcy. The progressive addition of contaminated soil from 5g to 15 g/kg diet in T2 – T4 caused a decline in semen quality and disruption in hormonal profile leading to testicular dysfunction, overpopulation reactive oxygen species which overwhelms the rabbit's antioxidant defense making them susceptible to infection.

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