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Research Article

Impact of Dietary Exposure to Heavy Metal Contaminated Soil on The Growth Performance and Hematological Indices of Growing Rabbits

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Abstract

This study was conducted to evaluate the impact of dietary exposure to heavy metal contaminated soil on the growth performance and haematological indices of growing rabbits. A total of 60 rabbits were randomly assigned to four dietary treatments (T1, T2, T3 and T4) in a completely randomized design with each treatment consisting of 15 rabbits. The basal diet was formulated to meet the nutritional requirements of growing rabbits according to NRC (1977) standards. T1 served as the control (basal diet alone), while T2, T3 and T4 were supplemented with contaminated soil at 50 g, 100 g and 150 g/kg diet respectively. The contaminated soil contained lead, cadmium, chromium, mercury, tin, nickel and silver exceeding tolerable safety limits. The experiment lasted for 12 weeks which included a 2 weeks quarantine period. Feeding was provided ad libitum and clean water was available at all times. Results obtained showed that rabbits in T1 exhibited the highest average daily weight gain, superior average daily feed consumption and most efficient feed conversion ratio, while these values decreased progressively from T2 to T4. Haematological parameters (haemoglobin, pack cell volume, red blood cell and white blood cell count) remained within the normal physiological ranges for healthy rabbits. Animals in T2 to T4 showed a significant reduction in haemoglobin, pack cell volume, red blood cell and white blood cell count. It is concluded that even at 50 g/kg diet (low inclusion level) contaminated soil with heavy metals severely compromised rabbits performance and health status.

Keywords: Rabbits, Heavy metals, Contaminated soil, Performance, Haematology

Introduction

As the global demand for lean meat increases, rabbit production has emerged as a sustainable solution [1,2]. However, environmental pollution particularly from heavy metals threatens this potential [3,4] Rabbits which often consume forage are susceptible to environmental contaminants, most notably heavy metals found in soil [5,6]. Soil act as a primary sink for heavy metals resulting from mining/ industrial discharge, excessive use of agrochemicals amongst others [7,8]. Unlike organic pollutants, heavy metals are non-biodegradable [9,10]. They enter the food chain via contaminated feedstuffs or direct ingestion [11-13]. Heavy metals such as cadmium, lead, mercury, chromium, nickel, tin, arsenic and silver are of particular concern due to their high toxicity even at a lower concentrations [14-17].

Previous studies by notable researchers [18-20] Erdogan et al., 2025) have shown that a high dietary concentrations of heavy metals have been shown to reduce activity of pancreatic enzymes, over production of reactive oxygen species which deplete cellular proteins and cause lipid peroxidation in the livers and kidneys, disrupts body weight gain, meta-

bolic rate often leading to a high feed conversion ratio and promotes microcytic anemia.
Despite the known risk of heavy metals, there is a gap in literature regarding the specific impact of ingested contaminated soil as a complex mixture of multiple metallic toxins [4]. Most studies focuses on single element exposure in water. This experiment, therefore utilizes a dose response design (50 g, 100 g and 150 g/kg diet) by adhering to the [21] standard for growing rabbits. This studies ensures that any observed decline in performance is strictly attributable to the contaminated soil rather than nutritional deficiencies. This research will further help to promote animal sustainability and food safety.

Materials and Methods

Location of the study

The experiment was carried out at the Rabbit unit of the Gandhi College of Agriculture, Rajasthan, India. The study site lies between latitude 23° 03'N and 30° 12'N and longitude 69° 30'E and 78° 17'E. The mean annual rainfall and temperature range from 1100 to 1650 mm and 25.8 to 35.1°C, respectively. Relative humidity is about 80 % during the raining season and 30 % during the dry season.

Collection of contaminated soil samples and analysis

Top soil samples of 500 g each were collected ten from various points at Zawar mines (Udaipur) Rajasthan, India. These individual samples were pooled and thoroughly homogenized; from this mixture, a 300 g representative portion was sent for laboratory analysis, while the remainder was stored in a sealed labeled polythene bag and kept under room temperature prior to the formulation of feed. Composition of heavy metals in contaminated soil were analyzed using Septra Atomic Absorption Spectrometer (AAS 3400C, China). To ensure precision in results, manufacturers' instruction was strictly adhered to (Table 2).

Animals and treatments

Sixty clinically healthy male New Zealand white weaned rabbits, at five weeks of age with 610 ± 0.21 g mean initial body weight (BW) were used for the study. The animals were stratified by BW, such that the a`nimals in each treatment group had similar average initial BW, and randomly allocated to one of four experimental treatment groups (n = 15) in a completely randomized design for a period of 12 weeks including two weeks acclimatization period. Surrounding environment were thoroughly cleaned and disinfected two weeks before the commencement of the experiment. During the adjustment period, animals were dewormed against intestinal parasites using Albendazole tablet (Arrand Pharmaceutical, Rajasthan, India). Rabbits were individually housed in an all wired cages measuring 65 cm length by 40 cm width by 50 cm high and treated in adherence to the accepted procedures for the humane treatment of animals. Feed and clean drinking water were offered at all times. The dietary treatments were: (i) basal diet without contaminated soil (ii) basal diet + 50 g contaminated soil/kg diet (iii) basal diet + 100 g contaminated soil/kg diet and (iv) basal diet + 150 g contaminated soil/kg diet. Basal diet was formulated according to the nutrient requirements for growing rabbits according to [21] as presented in Table 1. A completely randomized experimental design was adopted and the trial lasted for 10 weeks including the 2 weeks adjustment period. Proximate composition of experimental diet was carried using Near Infra-Red feed analyzer NIRS DS396F, Denmark. The kit has the following technical specifications: wavelength (400 – 2500 nm), spectral resolution (0.5 nm to 8.0 nm), wavelength accuracy (<0.05 nm) and analysis time of 6 to 60 seconds.

Performance parameters

Feed intake was estimated as the difference between the amounts of feed rejected and the feed offered. Live body weight of rabbits were recorded at weekly intervals using digital sensitive scale while final body weight and total feed intake were recorded at the end of the experiment. Body weight gain was estimated as the difference between live body weight and the final body weight. Average daily weight gain and average daily feed consumption was calculated by dividing the body weight gain and total feed consumption by the experimental period in days. Feed conversion ratio (FCR) was calculated using the formula below:

$$FCR = \frac{\text{Feed consumption}}{\text{Body weight gain}} \times 100$$

Blood sample collection and analysis

On the last day of the trial, blood samples were taken from the five rabbits in each treatment via marginal vein using a 5ml syringe. 3 ml blood sample was collected into labelled sterile bottles containing ethylene diamine tetra acetic acid (EDTA) as anticoagulant for the determination of hematological parameters. Collected samples were placed in an ice pack, sent to the laboratory and analyzed using Sysmex Auto Haemo-Analyzer (BS3400 C, China). Haematological samples were analyzed for concentrations of red blood cell, haemoglobin, pack cell volume and white blood cells adhering strictly to manufacturer instructions on operation of kits.

Data analysis

Data obtained on growth performance and haematological studies were subjected to analysis of variance for a complete randomized design using Statistical Package for the Social Sciences (SPSS version 27). When the ANOVA was significant, means were separated using Duncan's multiple range test at the level of $P \leq 0.05$.

Results

Ingredient and chemical composition of the basal diet (% DM) is presented in Table 1. The feed contained a dry matter of 91.24 %, crude protein (17.42 %), organic matter (90.67 %), ether extract (4.38 %), crude fibre (13.49 %), ash (9.33 %) and energy (2705.8 Kcal/kg).

Ingredients	Percentage DM
Maize	38.73
Wheat bran	14.01
Soymeal	20.06
Palm kernel meal	20.00
Mono Calcium Phosphate (MCP)	4.00
Calcium bicarbonate	2.00
Lysine	0.20
Methionine	0.20
Premix (Vitamin and Mineral)	0.25
Salt	0.35
Toxin binder	0.20
Total	100.00
Chemical composition	
Crude protein	17.42
Dry matter	91.24
Organic matter	90.67
Ether extract	4.38
Crude fibre	13.49
Ash	9.33
ME (kcal/kg)	2705.8

Table 1: Ingredient and chemical composition of the basal diet (% DM)

Each 2.5 kg consists of: Vit A 10,000, 000 IU; Vit D3, 6000, 000 IU; Vit. E. 10g; Vit k3 2 g; Vit B1, 1000 mg ; Vit B2, 49g ; Vit B6, 105 g; Vit B12, 10 mg; Pantothenic acid, 10 g; Niacin, 20 g , Folic acid , 1000 mg ; Biotin, 50 g; Choline Chloride, 500 mg, Fe, 30 g; Mn, 40 g; Cu, 3 g; Co, 200 mg; Si, 100 mg and Zn , 45 g

Chemical analysis of contaminated soil with heavy metals is presented in Table 2. Chromium had the highest concentration of 289.1 mg/kg followed by nickel (188.6 mg/kg), tin (56.74 mg/kg), lead (22.61 mg/kg), silver (4.74 mg/kg), arsenic (2.86 mg/kg), cadmium (1.80 mg/kg) and mercury (0.38 mg/kg) respectively.

Constituents	Composition (mg/g)	*Maximum tolerable levels (mg/kg)
Lead	22.61	10.00
Cadmium	1.80	1.00
Mercury	0.38	0.10
Arsenic	2.86	2.00
Chromium	289.1	500
Silver	4.74	5.00
Nickel	188.6	250
Tin	56.74	150

Table 2: Chemical analysis of contaminated soil with heavy metals

*NRC (2005); FDA (2024); Codex (2025); AAFCO (2025).

Effect of dietary addition of contaminated soil with heavy metals on the growth performance of growing rabbits is presented in Table 3. Average daily weight gain of rabbits in treatment 2 [T2] (899.31 g) and T3 (887.29 g) were similar ($p>0.05$) those in T4 (870.9 g) but significantly lower ($p<0.05$) than T1 (1297.2 g). Average daily feed consumption was higher ($p<0.05$) in T1 (135.0 g) than T2 (124.9 g), T3 (124.8 g) and T4 (124.7 g). Feed conversion ratio and mortality were ($p<0.05$) influenced by the treatment. Feed conversion followed this rank order: T4 > T3 > T2 > T1 ($p<0.05$). Higher mortality was recorded T4 (3.50 %) followed by T3 (3.00 %), T2 (2.50 %) none was recorded in T1 ($p<0.05$).

Parameters	Treatment 1	Treatment 2	Treatment 3	Treatment 4	SEM
Number of animals per treatment	15.00	15.00	15.00	15.00	
Initial body weight (g)	610.00	609.79	610.21	609.7	0.07
Final body weight (g)	1907.2^{a}	1509.1^{b}	1497.5^{b}	1480.6^{b}	0.03
Body weight gain (g)	1297.2^{a}	899.31^{b}	887.29^{b}	870.9^{b}	0.02
Average daily weight gain (g)	23.16^{a}	16.05^{b}	15.84^{b}	15.55^{b}	0.01
Feed consumption (g)	7560.0^{a}	6998.2^{b}	6990.2^{b}	6982.5^{b}	0.02
Average daily feed consumption (g)	135.0^{a}	124.9^{b}	124.8^{b}	124.7^{b}	0.01
Feed conversion ratio	5.82^{b}	7.78^{a}	7.87^{a}	8.01^{a}	0.04
Mortality (%)	-	2.50^{b}	3.00^{a}	3.00^{a}	

Table 3: effect of dietary addition of contaminated soil with heavy metals on the growth performance of growing rabbits
Means within a row with different letters and significantly different ($p< 0.05$); SEM Standard error; T1: Experimental diet without contam-

inated soil (control); T2: experimental diet + 50 g contaminated soil /kg diet; T3: experimental diet + 100 g contaminated soil /kg diet; T4: experimental diet + 150 g contaminated soil /kg diet

Effect of dietary addition of contaminated soil with heavy metals on the haematological indices of growing rabbits (Table 4). Pack cell volume was higher in T1 (32.81 %) than T2 (24.06 %), T3 (23.97 %) and T4 (23.61 %) ($p<0.05$). Haemoglobin concentration was lower in T4 [(7.58 g/dL)], T3 [(7.94 g/dL)], T2 [(8.09 g/dL)] than T1 [(12.68 g/dL)] ($p<0.05$). Red blood cell and white blood cell count varied from 9.90 – 15.11 (1012/L) and 7.07 – 12.54 (109/L) respectively.

Parameters	Treatment 1	Treatment 2	Treatment 3	Treatment 4	SEM
Pack cell volume (%)	32.81^{a}	24.06^{b}	23.97^{b}	23.61^{b}	0.04
Haemoglobin (g/dL)	12.68^{a}	8.09^{b}	7.94^{b}	7.58^{b}	0.02
Red blood cell (1012/L)	15.11^{a}	10.22^{b}	9.97^{c}	9.90^{c}	0.01
White blood cell (109/L)	12.54^{a}	7.33^{b}	7.21^{b}	7.07^{b}	0.02

Table 4: effect of dietary addition of contaminated soil with heavy metals on the haematological indices of growing rabbits

Means within a row with different letters and significantly different ($p< 0.05$); SEM Standard error; T1: Experimental diet without contaminated soil (control); T2: experimental diet + 50 g contaminated soil /kg diet; T3: experimental diet + 100 g contaminated soil /kg diet; T4: experimental diet + 150 g contaminated soil /kg diet

Discussion

The concentrations of heavy metals identified in this study were above the safety limits in animal feed [22]. These cocktailed heavy metals (lead, cadmium, mercury, arsenic, chromium, nickel, silver and tin) may have deleterious effect on the performance of rabbits. A significant decrease in average daily weight of rabbits fed T2 (50 g contaminated soil/kg diet), T3 (100 g contaminated soil/kg diet) and T4 (150 g contaminated soil/kg diet) suggest a disruption in nutrient absorption in the gastro intestinal tract [23,24]. In T1 (control) the metabolic energy is primarily used for tissue synthesis whereas in T2, T3 and T4, a portion of the energy is diverted toward the synthesis of metallothioneins- a specialized proteins used to sequester metals like cadmium and mercury, thus leading to a significant decline in weight gain among animals (Khan et al., 2019; [25]. The presence of lead in high concentration could also inhibit the activities of digestive enzymes and cause the production of reactive oxygen species which causes a breakdown in the normal function of the body [18]. According to [25], high concentration of mercury and silver in the diet of animals can damage the mucosal lining of the small intestine reducing the available area for nutrient update. This result is in agreement with the report of [18] who recorded a significant reduction in the body weight of body weight of weaner rabbits fed high concentration of heavy metals. The decrease in feed intake in T2, T3 and T4 indicates that the contamination levels triggered sensory and metabolic signals to limit consumption. Addition of 50 g to 150 g of contaminated soil in the diet of animals altered the taste of the diet [26,27]. The presence of lead, mercury, cadmium and chromium may cause gastrointestinal irritation leading to reduced appetite and high mortality among rabbits [27]. Treatment 1 had the best feed conversion ratio compared to other treatments, this suggests that non-interference with the nutrients in basal diet. However, 50 g to 150 g of contaminated soil dilutes the nutrient density of the basal diet causing a rapid decline in performance of rabbits. The results obtained aligns with the report of [2] when battery waste added to the diet of broiler chickens at 5 %.

Haematological indices are indexes used to evaluate nutrient deficiencies, toxicity as well as diseases [28]. In the present study, the experimental rabbits, particularly those fed 50 g to 150 g of contaminated soil had a decrease in red blood cell, haemoglobin and pack cell volume suggesting that animals were anemic. This is a hallmark of heavy metal poisoning particularly involving lead mercury and cadmium. High concentration of lead can interfere with iron synthesis leading to a drop in haemoglobin levels Alagbe, 2017 [18]. Similarly, higher dietary concentrations of mercury, chromium and silver can increase the fragility of the red blood cell causing oxidative damage, this leads to a shorter lifespan of the red blood cell and their premature destruction in the spleen, resulting in lower red blood cell and pack cell volume values [29]. The red blood cell, pack cell volume and haemoglobin level of rabbits in T1 were within the normal physiological ranges for growing rabbits [26]. A decline in white blood cell count among rabbits in T2, T3 and T4 suggests that the contaminated soil has an immune-suppressive effect on the animals. This result is in consonance with the reports of Khan et al. (2019) who discovered a significant decline in white blood cell counts in rabbits exposed to lead and cadmium, attributing it to the direct cytotoxic effects of these metals on the lymphoid organs.

Conclusion

In conclusion, the addition of contaminated soil in the diets of rabbits exerts a negative effect on growth performance and haematological indices. The study demonstrates that as the level of contaminated soil increased from 50 g to 150 g there was a significant decline in average daily weight gain and feed consumption. It also resulted in higher mortality from 2.50 to 3.00 %. These deterioration is attributed to the synergistic toxicity of heavy metals which exceeded the maximum tolerable levels for rabbits. This contaminants triggers irritation in the gastrointestinal tract and metabolic failures, anemia and oxidative destruction of erythrocyte membrane. Even at lowest inclusion level (50 g) the presence of these heavy metals compromises the animal's physiological status.

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