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Enhancing Rabbit Welfare: The Role of Environmental Enrichment in New Zealand White breed

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Abstract:

In adherence to the Guide for the Care and Use of Laboratory Animals, this study investigated the effects of environmental enrichment on New Zealand White rabbits. Thirty male rabbits were divided into three age-specific phases: pre-enrichment (control) at 10 weeks, toy enrichment using tennis balls at 11 weeks, and post-enrichment at 12 weeks. Enrichment interactions, lasting three hours daily for three days each week, were videotaped in the rabbits' home cages to monitor their engagement with the enrichment objects and overall activity levels.

The results showed a significant increase in exploratory behaviors and a decrease in passive behaviors, such as lying, during the enrichment phase. Although ingestive behavior did not show marked improvement during the toy-enrichment phase, a positive trend in activity levels was observed in post-enrichment rabbits. These rabbits demonstrated increased dynamic activities, including frisky hopping, rearing, and exploratory behaviours, with a notable reduction in the frequency of lying compared to the pre-enrichment phase. This study underscores the beneficial impact of environmental enrichment on the behavioral parameters of caged rabbits, highlighting its significance in enhancing rabbit welfare.

Keywords: Environmental Enrichment, New Zealand White Rabbits, Behavior.

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1.Introduction

Rabbit farming plays a pivotal role in Egypt, significantly contributing to the nation's food supply, employment, and economic development. This sector, known for producing high-quality meat and fur, has gained both domestic and international recognition (FAOSTAT 2013).Contemporary practices in animal husbandry often involve rearing animals in densely populated conditions, potentially leading to environmental stress. Such stress can adversely affect productivity, cause physical and psychological distress, and in severe cases, lead to mortality (Feng et al. 2022). Key concerns in rabbit housing include group size, space allocation, and environmental quality (Zucca et al., 2012). The introduction of object enrichment has been noted to decrease aggressive behaviors and enhance animal welfare (Dalle Zotte et al. 2009).

Animal welfare, as defined by (Broom 1986) refers to an animal's well-being in relation to its ability to adapt to the environment. Improved welfare is anticipated to enhance productivity, provided that the animals' behavioral needs are adequately met

(Broom and Fraser 2010). The investigation of (Broom and Molento 2004) emphasized that unmet behavioral needs can significantly compromise an animal's welfare, an issue particularly relevant in commercial rabbit farming where interaction with the environment is often limited.

Environmental enrichment is a key aspect of animal welfare, particularly for small livestock like rabbits. It could be defined as the modification of the environment in which a domesticated animal lives in order to meet the species' behavioral needs (Poggiagliolmi,2011). Enrichment described as dynamic process aiming to expand behavioral choices for animals, encouraging species-specific behaviors and abilities, thereby improving welfare (Chamove 1990). The primary objective is to prevent abnormal behaviors by promoting natural behaviors and coping mechanisms.

The significance of animal welfare in production is highlighted by its role in alleviating environmental stress, reducing abnormal behaviors, and overall enhancing welfare (Bozicovich et al. 2016). Specifically in rabbit farming, Princz et al. (2007) observed that providing gnawing sticks to growing rabbits reduced stereotypical behaviors. Studies by (Ceballos et al. 2016, Trocino et al. 2019) demonstrated that structural enrichments and foraging opportunities can substantially improve welfare in caged rabbits. Furthermore, (Young 2013) also noted the positive impact of toys in fostering exploratory and locomotor behaviors, although their attraction is often short-lived unless they are related to food or nesting.

This study aims to evaluate the influence of environmental enrichment on normal and abnormal behavioral expressions in conventionally caged rabbits.

2.Materials and Methods

Animals and experimental design2.1. Ethical Declaration

This experiment received approval from the Ethics Committee for Scientific Research at the Faculty of Veterinary Medicine, Damanhour University, Egypt, and the Institutional Animal Care and Use Committee (IACUC) (Approval number: DMU/VetMed-2023/034). All procedures were designed to minimize animal discomfort and distress throughout the study.

٢.٢. Subjects and Housing Condition

The study was conducted on thirty 70-day-old New Zealand White (NZW) rabbits, each weighing approximately 737±17 g. The rabbits were individually identified with ear tags and randomly assigned to treatments in a completely randomized 2 × 3 factorial design .

The rabbits were housed in a semi-enclosed facility with natural ventilation augmented by automatically controlled exhaust fans. The building's concrete floor had a gentle slope to aid in the drainage of liquids and waste. Each rabbit was accommodated in standard-sized galvanized wire cages (60 x 35 x 35 cm), outfitted with galvanized steel feed hoppers and automatic nipple drinkers providing continuous access to fresh water.

Strict hygiene and environmental standards were maintained throughout the experimental period. The cages were regularly sanitized, and waste beneath the cages was cleared daily. The rabbits always had unrestricted access to fresh water.

Their diet consisted of a standard pelleted feed, meeting the nutritional requirements as per NRC (1977) guidelines, containing 2677 Kcal of digestible energy/kg, 17.9% crude protein, and 13.75% crude fiber as illustrated in Table 1. Feed was supplied twice daily, at 8 a.m. and 5 p.m.

2.4. Observational Procedure

Observations commenced when the rabbits reached 10 weeks of age, spanning two weeks at the end of April, and beginning of May. The thirty rabbits were divided into three stages: 1- Pre-enrichment (control), 2- Toy enrichment (tennis ball diameter as 6.54–6.86 cm (2.57–2.70 inches). Balls must have masses in the range 56.0–59.4 g (1.98–2.10 ounces). A tennis ball generally has 12 pounds per square inch (80 kPa; 0.8 atm) more of a nitrogen and oxygen mixture than the sea level ambient air pressure. Yellow and white are the only colors approved by the ITF. Most balls produced are a fluorescent color known as "optic yellow". Tennis ball felts comprise wool, nylon, and cotton in a mixture surrounding the rubber edge (https://www.networldsports.com/buyers-guides/tennis-balls-guide?) at 11 weeks, and 3- Post-enrichment at 12 weeks. There were 3 balls in each cage (ball/rabbit). Each rabbit allowed to interact with the enrichment toy for 3 hours daily (from 9 a.m. to 12 p.m.) over three consecutive days per week. The rabbits' interactions with the toys were recorded using cameras fitted in their home cages. Both the duration of interaction with the toy and the frequency of overall activity during

these sessions were documented. al., 2019)

Figure 1. explaining the rabbit engaged in toy enrichment in the study

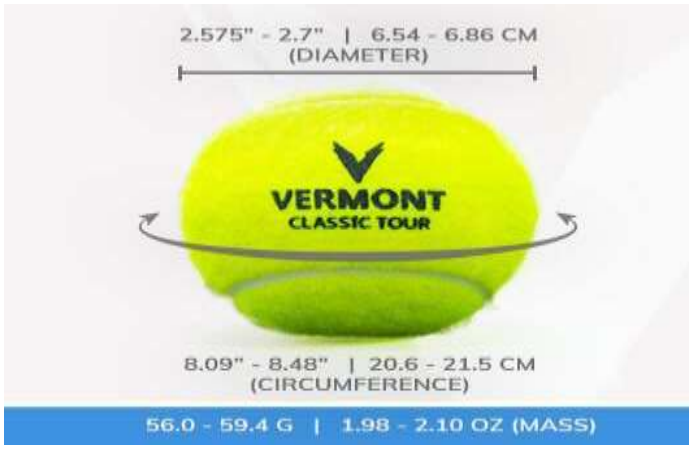

Figure 2. explaining the dimension of the tennis ball enrolled in the study

2.5Statistical Analysis

Following a normality check, the collected data were expressed as mean ± SEM and analyzed by one-way ANOVA analysis followed by Tukey's post hoc test using Graph Prism Software (V.6).

Means within the same row carrying different letters are significantly different at *P<0.05,**P<0.01,***P<0.001.

Results

Behavioral Responses to Enrichment in Caged Rabbits

The study observed a notable shift in the behavioral patterns of rabbits with the introduction of a tennis ball as an enrichment tool. There was a marked increase in exploratory activities, while periods of inactivity, specifically lying down, were reduced. While ingestive behaviors showed no significant enhancement during the enrichment phase, a tendency towards improvement was observed in the post-enrichment period. Notably, post-enrichment rabbits exhibited a greater propensity for dynamic activities, including lively hopping, upright rearing, and enhanced exploration. In contrast, the frequency of lying decreased in comparison to the pre-enrichment period. These findings suggest that the introduction of a tennis ball as an enrichment device positively impacts the behavior of rabbits under confinement conditions, as detailed in (Table 3 and 4).

Table 1. Experimental diet formulation (%)

Ingredients	**%**
Yellow corn	9.5
Soybean meal (44%)	15
Wheat bran	17
Barley	21.7
Barely hay	34.5
Dicalcium phosphate[1]	1.2
Ground limestone[2]	0.25
dl-methionine	0.05
Common salt	0.5
Vitamin + mineral premix[3]	0.3
Total	100
Chemical composition	
Dry matter	87.8
Moisture	12.2
Crude protein	17.9
Crude fiber	13.75
Ether extract	3.6
Nitrogen-free extract[4]	42.75
Ash	9.8
DE (kcal/kg)[5]	2677.97

[1] Dicalcium phosphate: 20% phosphorus and 25% calcium; [2] Limestone: 34% calcium. [3] Each 1 kg of ration contains vitamin A—12,000 and 900 IU of vitamins A and D3, respectively. While 2 mg each of the vitamins K3, B1, and B6, 50 mg vitamin E, 6 mg vitamin B2, 0.01 mg vitamin B12, 0.2 mg biotin, 20 mg pantothenic acid, 50 mg niacin, 5 mg folic acid, 8.5 mg manganese, 70 mg zinc, 75 mg iron, 5 mg copper, 0.75 mg iodine, 0.1 mg selenium. [4] Nitrogen-free extract was calculated by the difference 100 − (moisture % + CP% + EE% + CF% + Ash%). [5] Digestible energy (DE) was calculated according to values given in the feed composition tables of the **NRC (Council 1977).**

Discussion

Behavioral Adaptations to Enrichment

The study underscores the efficacy of environmental enrichment, specifically using

tennis ball toys, in modifying the behavior of laboratory rabbits. There was a notable decrease in passive behaviors such as sitting and lying during the enrichment period, compared to the pre- and post-enrichment phases. The interaction with the toy was markedly higher during its availability, with a consistent reduction in idle standing observed across all study phases. This aligns with findings by (Luzi et al. 2003) , who noted a decrease in time rabbits spent in a stretched position in enriched cages compared to controls, suggesting that varied enrichment forms reduce time spent in inactivity .

Concerning exploratory behaviors, although the differences were not statistically significant, there was an increased frequency of rearing up in the rabbits both before and after the enrichment period compared to during it. The frequency of sniffing was significantly higher during and post-enrichment tha n in the pre-enrichment phase, indicating that the enrichment possibly redirected the rabbits' attention to sniffing pen elements and the toy. The post-enrichment phase also saw a repositioning in self-grooming and allo-grooming behaviors, with the highest percentages observed in the post-enrichment period. This shift could be partly attributed to reduced inactivity owing to increased interaction with the enriched environment. Lidfors (1997) also noted a dependency of self-cleaning behavior expression on the type of environmental enrichment. Stereotypic behaviors, such as bar gnawing, progressively diminished during the enrichment phase. This corroborates (Siloto et al. 2009, Verga et al. 2005), indicating that environmental enrichment alleviates boredom and satisfies ethological needs by reducing abnormal behaviors. Increased engagement in feeding behaviors during and post-enrichment suggests an improvement in appetite possibly induced by the enriched environment. A progressive increase in drinking behavior was also noted throughout the study phases.

The study also detailed the day-to-day variations in rabbit behaviors across different phases. For example, the highest frequency of rearing and sniffing occurred on day 2 post-enrichment and day 1 during enrichment, respectively. In contrast, the lowest frequencies were noted on day 1 during enrichment and day 2 pre-enrichment, respectively. Grooming behaviors peaked on day 3 post-enrichment, while stereotypic gnawing was most pronounced on day 2 post-enrichment. These observations provide valuable insights into the temporal dynamics of rabbit behaviors in response to environmental enrichment.

Conclusion

In conclusion, this study demonstrates the significant influence of environmental enrichment on the behavior of New Zealand White rabbits. Post-enrichment rabbits exhibited enhanced activity, reduced lying time, and improved ingestive behavior. These findings underscore the importance of environmental enrichment in positively impacting the welfare of laboratory rabbits. Based on our results, we recommend the widespread adoption of environmental enrichment strategies in laboratory rabbit husbandry to promote their well-being . Regular monitoring of behavior and adherence to ethical standards are essential aspects of successful implementation. Researchers and animal caretakers should prioritize the incorporation of interactive toys and activities to improve the overall quality of life for laboratory rabbits.

Conflicts of Interest: “The authors declare no conflict of interest.”

Author Contributions:

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by SI, AA, and AA. The first draft of the manuscript was written by [SI] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Ethical approval

This work was performed in accordance with the guidelines of the Animal Care and Use Committee of Damanhour University.

Data availability

All data generated or analyzed during this study are included in this published paper.

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Table 2. Ethogram used for behavioral evaluation of White New Zealand rabbits with and without environmental enrichment: categories and their definitions.

Category	Behavior	Definition
Ingestive behavior	Feeding	Consumption of feed from the feeder, gnawing the pellet
	Drinking	Consuming water from nibble drinkers
Exploratory behavior	Rearing	Standing on the hind limb with tarsus perpendicular to the floor and the fore limb not touching floor
	Sniffing	standing or moving with fore legs while sniffing around
	Toy interaction	Playing or gnawing with cage enrichment material
Stereotype behavior	Gnawing	The rabbit is biting the bars or the floor
Locomotion behavior	Standing still	The rabbit spent still with its fore and hind legs unstretched and on the ground
	Frisky hop	Moving from one place to another pushing forward with hind limb followed by fore limb.
Resting behavior	Sitting	Animals with the forelimbs not folded beneath the body but being straight in a way that the thorax and abdomen were clear from the floor and visible
	Lying stretched	Lying in a stretched-out position, ventrally, dorsally, or laterally
Comfort behavior	Self-grooming	Licking, nibbling, scratching the own head or body or stroking the forelegs over it.
	Alleo-grooming	Licking or nibbling a pen mate

Table 3. Effect of enrichment toy on behaviors of rabbits during different phases.

Behaviours	**Enrichment**	**Mean± Std. Error**	**Behaviours**	**Enrichment**	**Mean± Std. Error**
Standing	Pre-enrichment	1.78 ± 0.40	Gnawing	Pre-enrichment	3.39 ± 0.98
	During enrichment	1.49 ± 0.59		During enrichment	2.78 ± 0.64
	Post enrichment	1 ± 0.24		Post enrichment	5.22 ± 0.71
Sitting	Pre-enrichment	1.94 ± 0.43	Frisky hop	Pre-enrichment	5.5 ± 0.81
	During enrichment	0.89 ± 0.32		During enrichment	5.28 ± 0.77
	Post enrichment	3.39 ± 0.73**		Post enrichment	6.22 ± 0.80
Lying	Pre-enrichment	6.5 ± 0.82**	Feeding	Pre-enrichment	7.00 ± 1.50
	During enrichment	3.22 ± 0.37		During enrichment	7.56 ± 1.68 *
	Post enrichment	4.83 ± 0.90		Post enrichment	13.22 ± 1.36*
Rearing	Pre-enrichment	2.78 ± 0.49	Drinking	Pre-enrichment	3.11 ± 0.91
	During enrichment	2.11 ± 0.49		During enrichment	3.56 ± 0.47
	Post enrichment	3.00 ± 0.57		Post enrichment	3.94 ± 0.66
Sniffing	Pre-enrichment	1.17 ± 0.36	**Toy interaction :**	Pre-enrichment	0.00 ± 0.00***
	During enrichment	5.22 ± 0.99**	A. Frequency	During enrichment	35.44 ± 5.66
	Post enrichment	5.78 ± 1.08**		Post enrichment	0.00 ± 0.00***
Self - Grooming	Pre-enrichment	19.28 ± 3.03	B. Time	Pre-enrichment	0.00 ± 0.00***
	During enrichment	15.83 ± 1.35		During enrichment	7.53 ± 0.93
	Post enrichment	25.5 ± 2.07**		Post enrichment	0.00 ± 0.00***
Allo-grooming	Pre-enrichment	4.28 ± 0.84**			
	During enrichment	3.61 ± 0.57			
	Post enrichment	8.11 ± 0.80***			

Means within the same row carrying different letters are significantly different at *P≤ 0.05, ** P≤ 0.01, *** P≤ 0.001.

Table 4. Least square means and their standard errors for the interaction effect of toy enrichment periods with days per period on behaviors of rabbits

Behaviors	**Pre-enrichment**			**During enrichment**			**Post-enrichment**		
	Day1	Day2	Day3	Day1	Day2	Day3	Day1	Day2	Day3
Standing	1.67±0.8	1.67±0.61	2.00±0.77	0.33±0.33	2.33±1.56	1.83±0.75	0.83±0.4	0.67±0.49	1.5±0.34
Sitting	2.5±1.09	1.83±0.75	1.5±0.22+	1.00±0.45	1.00±0.82	0.67±0.42+	1.17±0.54	2.17±0.6	6.83±1.08++
Lying	4.5±1.12	5.17±0.91#	9.83±1.19	3.33±0.95	2.83±0.84#	3.5±0.43b	5.33±0.67	6.83±2.36##	2.33±0.33
Rearing	2.67±0.67	1.83±0.87	3.83±0.91	1.17±0.83	2.83±0.87	2.33±1.56	1.67±0.92	4.17±1.14	3.17±0.7
Sniffing	1.17±0.6*	0.67±0.49#	1.67±0.8	7±1.77**	6.33±1.41##	2.33 ±1.56	5.67±0.56*	5.67±2.4##	6±2.39
Self - Grooming	17.33±3.37	15±3.3	25.5±7.71+	11.5±2.17	17.33±2.08	18.67±1.96+	19.33±3.62	23.67±1.33	33.5±2.81++
Allo - grooming	7.33±1.52*	2.17±1.14	3.33±0.76+	4.33±1.26*	4.17±0.95	2.33±0.79+	9.33±1.69**	5.33±0.61	9.67±1.02++
Gnawing	4.83±2.41	2.17±1.45#	3.17±1.11	3.33±0.76	4.33±1.43#	0.67±0.33	3.83±1.01	7.67±0.95##	4.17±1.19
Frisky hop	6.5+1.48	6.67±1.45	3.33±0.95	6 ± 1	5±1.9	4.83±1.11	8.33+1.23	4+1.03	6.33+1.45
Feeding	6.33±3.28	13.33±2.89	14.17±4.5	13.67±2.4	13.83±4.46	19.17±5.39	17.67 ±6.5	16.83±4.69	15.5±1.84
Drinking	2±1.1	2.67±1.15	4.67±2.28	3.5±0.96	3.5±0.62	3.67±0.99	4.17±1.49	4.83±1.11	2.83±0.79
Toy interaction									
Frequency	0.00±0.00*	0.00±0.00#	0.00±0.00+	30.33±4.99***	40.33±15.5###	35.67±7.19+++	0.00±0.00*	0.00±0.00#	0.00±0.00+
Time	0.00±0.00*	0.00±0.00#	0.00±0.00+	5.41±0.8***	8.83±2.01###	8.33±1.65+++	0.00±0.00*	0.00±0.00#	0.00±0.00+

Means within the same row carrying different letters are significantly different at *P≤ 0.05, ** P≤ 0.01, *** P≤ 0.001 (for the first day). #P≤ 0.05, ## P≤ 0.01, ### P≤ 0.001 (for the second day) and +P≤ 0.05, ++ P≤ 0.01, +++ P≤ 0.001 (for the third day).