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Gnawing Sticks and Sibling Ameliorative Effects on Growing Rabbits' Behavior, Production, and Stress Level under Barren Environment

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ABSTRACT

Environmental enrichment could lower stress in farm animals; however, inadequate data is offered on the level of productivity and welfare indicators in rabbits reared with or without environmental enrichment. 84 young White New Zealand rabbits were used to identify two examples of environmental enrichment (gnawing sticks and sibling) influences on the behavior, production, and stress level of growing rabbits. Two experiments were done; In experiment one; 36 young rabbits were used to test the effect of sex and sibling relationship on the behaviour and weight gain of the growing rabbits, four groups G1 male sibling, G2 male unsibling, G3 female sibling, and G4 female unsibling. In experiment two; 48 young rabbits were used in two steps; in the first step of the trial the preference test of gnawing sticks tree species was performed by using visual evaluation and the second step was to test the influence of the prefered gnawing sticks tree species in the 1st step on the behaviour, performance, and stress level of growing rabbits. They were classified in to two groups G (1) with the prefered G.S and G (2) rabbits without any gnawing sticks. The behaviour of the rabbit groups was recorded at 4 week old till 11 weeks old using focal sampling technique. Weekly body weight and serum cortisole level at 6 and 10 weeks in both experiment was recorded. Gnawing sticks and sibling housing during the growing period result in improvement in growing rabbits behavior (feeding, self grooming, and social smelling), lowering biting wire and feeder, and decrease cortisole hormone level. So that this study recommends to use them for growing rabbit cages during the growing periods to override the barren environment adverse effects.

1. INTRODUCTION

Rabbits behaviour were influenced by the external environment, which affects their welfare and reflected on their production under such variables. When the animals are unfulfilled, they fail to show their normal behaviour such as decreasing the occurrence of grooming and increasing the occurrence of licking others or resting in certain abnormal posture which are indicators of poor welfare (Broom, 2021). Rabbits in Egypt are currently far from achieving the limits of their capability for competent production. In large rabbit farms, cage housing complaints is the barren environment as rabbit was isolated from contact with others, lodged in single cage made almost from wire net.

The barren environment leaded to abnormal behaviour such as stereotypies, i.e. biting, chewing or licking the bars of the cage, aggression and/or apathy (Jordan et al., 2006). It might be due to a lack of environmental stimuli and to a lack of control over the environment, although pawing on the cage floor or gnawing also considered 'normal' behaviours in an inadequate environmental context (Jaén-Téllez et al., 2021).To avoid these problems several cage enrichment forms were studied with regard to growing rabbits productive performance, behaviour and welfare. One of the most common enrichments is the placement of wooden sticks of

different species, size and position in the cages (Del Bosco et al., 2002; Jordan et al., 2006). The gnawing sticks (G.S) were made of soft trees (linden, willow) their consumption was higher compared to hard gnawing sticks (locust) and it could be better used as environmental enrichment (Princz et al., 2006 b). The mechanical and chemical characteristics of the tree species (hardness, smell and taste) might determine which of the investigated species was suitable for gnawing stick. Little-leaf linden is better than White buckeye and White willow according to amount of consumption (Szendro et al., 2007).

The application of G.S in cage systems of rabbit housing would affect the behaviour and performance of fattening rabbits as the feeding and caecotrophy were increased (Verga et al., 2004; Luzi et al., 2003a; Princz et al., 2009: Dalmau et al., 2020) also, slaughter weight was higher in enriched cages (Princz et al., 2005a). It incraeses rabbits' total lying time and lying stretch (Hansen and Berthelsen, 2000) also, it reduced aggressiveness and related injuries on the bodies of rabbits as rabbits with G.S exhibit abnormal and aggressive behaviour forms at lower frequencies (Johnson et al., 2003; Verga et al., 2004; Princz et al., 2005 b; Szendro et al., 2007; Szendro et al., (2008) in addition, it decreased the stress of an aggressive animal in a larger group by social facilitation aids (Princz et al., 2008). It reduced stereotypies; bar, wire or feeder biting (Szendro et al., 2007). It increased the social activities, such as sniffing the other rabbit and allo-grooming (Szendro et al., 2007) while, the occurrence of self-grooming lessened (Jordan et al., 2003). It affected cage preference as rabbits spent more time in cages supplied with G.S (Szendro et al., 2008).

On the other hand, Szendro et al., (2007) and Hansen and Bertheisen (2000) concluded that addition of gnawing sticks had no significant influence on rabbits' behaviour except for duration (min) of consumption (eating pellets, drinking or coprophagy) and gnawing wooden stick time. Rabbits with G.S spent less time eating, less time resting, higher frequency of hopping and sniffing another rabbits and the proportion of locomotion and comfort behaviour increased (Verga et al., 2004). Also, Johnson et al., (2003) did not find any difference in feed intake of rabbits reared with or without environmental enrichment. Besides, Princz et al., (2009) reported that gnawing stick consumption had no influence on feed intake or feed conversion. In addition, Luzi et al., (2003b) concluded that the environmental enrichment has no effect on the production of the rabbits as the growth and carcass traits were not affected. Occupation of rabbits with environmental enrichment had no any significant decrement of biting wire and feeder, which was one of the most common vices in cage systems (Matzek et al., 2021).

The second enrichment is social buffering which is the attendance of encouraging social interactions with bonding partners, siblings, and close kin as they can effectively increase an individual's ability to cope with challenging situations and it has the potential to reduce (chronic) stress, including its negative consequences on health and wellbeing (DeVries et al., 2003). Siblings might obtain benefits from each other's presence such as a more favorable thermal environment (Sokoloff and Blumberg, 2001). The presence of litter siblings increases levels of encouraging social interactions with litter siblings and it is also associated with lesser stress hormone (corticosterone) levels and with a healthier status in terms of lower loads with an intestinal nematode (Rödel, 2022). Also, mammals' siblings could affect each other's growth (Lopez-Tello et al., 2023), and it could influence individual memories, morphological, and behavioral phenotypes advancement (Hudson and Trillmich, 2008). It was difficult to differentiate between sibling and maternal influences on development and to experimentally manipulate sibling interactions without disturbing the normal mother-young relationship (Gonzalez and Poindron, 2002).

Furthermore, the quantification of cortisol levels or its metabolites by ELISA in serum or other biological samples is a physiological indicator for evaluating stress (Manteca, 2009). IL-6 is a pleiotropic cytokine that activates the JAK/STAT signaling pathway and exerts both pro- and anti-inflammatory action (Ahmed and Ivashkiv, 2000). Previous studies recorded some effects of using G.S in growing rabbit cages but there was no clear assumption of its species also, there were no previous studies identifying sibling effects as environmental enrichment. Thus, this study designed to categorize gnawing sticks and sibling ameliorative effects on growing rabbits' behavior, production, and stress level under barren environment.

2. MATERIALS AND METHODS

2.1. Rabbits, housing and environmental conditions

Two farms were used in this experiment, the faculty of Agriculture farm and the faculty of veterinary Medicine farm, Suez Canal University. Temperature and relative humidity were within the range of comfort zone (21 °C, 65%). Each cage was equipped with nipple drinker and feeders inside the cage, they were fed on pelleted diet ad-libitum (Mbanya et al., 2004). Fresh water was continuously available through water nipples.

In experiment one; 36 young White New Zealand rabbits (18 males and 18 females) were obtained from rabbit farm of the Faculty of Veterinary Medicine, Suez Canal University, Egypt. They were used to test the effect of sex and sibling relationship on the behaviour and weight gain of the growing rabbits, and they were classified into four groups (three replicates for each). The first group was male sibling (G1-MS), the second group was male unsibling (G2-MUS), the third group was female sibling (G3-FS), and the fourth group was female unsibling (G4-FUS). The experiment started after weaning of all young rabbits at four weeks old. These weanling rabbits were randomly assigned to one of the four following treatments according to sex (male or female) and the relationship between them (sibling or unsibling). Siblings are animals which share at least one parent. A male sibling is called a brother; and a female sibling is called a sister. In most animal societies throughout the world, siblings usually grow up together and spend a good deal of their bunnyhood socializing with one another.

In experiment two; 48 young White New Zealand rabbits were used in two steps; in the first step of the trial the preference test of G.S tree species was performed and the second step was to test the influence of the prefered gnawing sticks tree species in the 1st step on the behaviour, performance, and stress level of growing rabbits.

During the first step the preference of rabbits for four different tree species of G.S (ingested vs. rejected species) was monitored to choice the main accepted one (Preference test). Young rabbits (n=24) were weaned at 4 weeks old and randomly allocated into four cages (6 rabbits / cage). They were provided with the different gnawing sticks tree species White willow (W), Mango (M), Guava (G), and Mulberry (MU) respectively. These gnawing sticks were placed horizontally 20 cm high on the wall of the adjacent cage. Preference test was determined on the basis of five grades of visual evaluation as following: Grade 1: Visible marks of teeth or completely intact, Grade 2: Slightly gnawed, Grade 3: Moderately gnawed, Grade 4: Severely gnawed, Grade 5: Extremely gnawed. These five grades adopted according to Szendro et al., (2007). These visual evaluation used instead of weighing the wooden stick due to the relative humidity of the wood was changing with regard to relative humidity of the air in the rabbitry, which caused oscillation in the weight of wooden sticks. For this cause it was difficult to verify the quantity of gnawed wood from the weight of the wooden stick.

The second step was to examine the effect of the most preferred gnawing sticks on growing rabbit's behaviour and performance. 48 weaned rabbits (White New Zealand) were used and classified in to two groups according to the presence or absence of gnawing sticks. Group (1) with G.S (only the main accepted G.S tree species) the old 24 rabbit that used in 1st step and remove the other three G.S tree species. Group (2) 24 rabbit (6 rabbits / cage) four cages without any gnawing sticks.

2.2. Observations and records

The behaviour of the four groups in the first experiment was recorded by a Panasonic WV Ns202ae network camera in one period (60 min) during the light period (9:00 am- 10:00 am) in an interval of day after day (7 weeks of behavioural recording). The behavioural observations started at the beginning of the experiment at 4 week old till 11 weeks old using focal sampling technique by marking one individual of each group. The frequency of all behavioural activities were recorded during the duration of observations after 2 minute of adaptation. All observations for each group were recorded according to Abdelfattah et al. (2013). The recorded behaviours were as follows: Ingestion behaviour (Consumption and Gnawing) frequency and duration (min), Drinking behaviour, Rest and sleep duration (min), Comfort behaviour (Mcfarland, 1981), Social behaviour (Broom, 2021), Investigatory behaviour, Locomotory behaviour (McFarland, 1981), Eliminative behaviour (defecation, urination), and Caecotrophy (Broom, 2021).

Rabbits behaviour was recorded in the second experiment for 24 hour by a Panasonic WV Ns202ae network camera in the first and the last experimental week (6 and 10 weeks) and the frequency of the behavioural elements was continually recorded according to **Szendro et al., (2007)**. The observed behavioural elements were (sitting, eating feed, drinking, caecotrophy, biting wire and feeder, wood gnawing, grooming, sniffing, hopping, stretching, inactivity, and contact with the

neighbor rabbit were observed). The important behaviours result from the rabbits welfare point of view were inactivity duration, biting wire and feeder frequency.

Weekly body weight for individuals in both experiment of each group throughout the duration of the experiment was recorded.

The experimental protocol was approved by the Faculty of Veterinary medicine Animal Ethics Committee (SCU-VET 2024031). All rules applying to animal safety and care were observed.

2.3. Blood sampling for cortisol measuring

Twenty rabbits per trial were randomly selected for measuring the blood cortisol levels taken at 8:00 am before the rabbits received their first daily meal in both trials. Rabbit blood samples were collected from auricular arterial blood via venipuncture, and it was collected as gently as possible to avoid stress. Blood specimens were placed within serum-separating tubes. To better assist serum separation, the specimens were placed in a water-bath (38 °C) for 30 min, followed by centrifuging (10 min / 3000 × g) and immediate serum collection/analysis. The serum-hormonal levels were determined using a rabbit cortisol hormone ELISA Kit (HePengBio, Shanghai, China).

2.4. Statistical analysis

Data were represented as mean ± SE, and significance was defined as $P < 0.05$ with a rejection-criterion of 0.05 was set for all statistical tests. Data were analyzed using suitable statistical test; Independent Samples t-test for the second trial or one-way analysis of variance (ANOVA) for the first trial, followed by Duncan's were used for comparing the means value obtained in the different groups by using (IMB-SPSS version 28.0 for Mac OS) according to Knapp, (2017).

3. RESULTS

3.1. Experiment One: Our results verified that there was a moderate improving effect of sex and sibling relationship on the behaviour, weight gain, and stress level of the growing rabbits during the growing period.

Concerning the measured behavioral parameters in the four groups of growing rabbits Fig (1). Some behaviors showed a highly significant at $P \leq 0.01$ such as; eating, body care rubbing, and social smelling as the second group male unsibling (MUS) recorded the highest duration (min) when compared to other groups. on the other hand, there was no significant difference (NS) at $P>0.05$ between groups for the other measured behaviors (Gnawing of food duration, drinking duration, Sleeping, Lying, Sitting, Body care licking, licking each other, smelling things, rubbing things licking things, walking, Stretching, Standing, Defecation, Urination and Ceacotrophy).

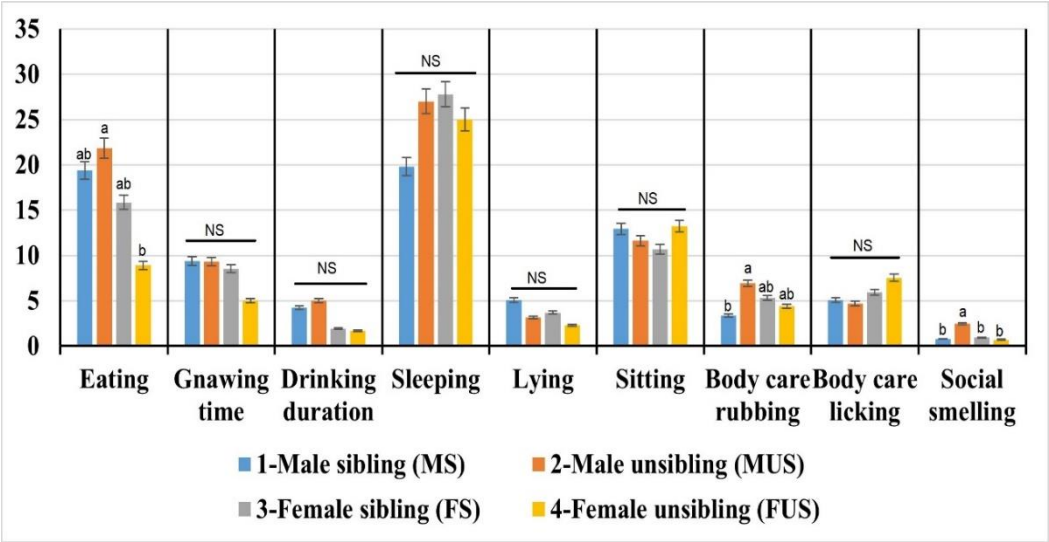


Figure (1): Measured behavioral parameters in the four groups of growing rabbits. Error bars represent the 95% confidence limits. Superscripts with different letters (a, b) differ highly significantly at $P \leq 0.01$. There was no significant difference (NS) at $P>0.05$.

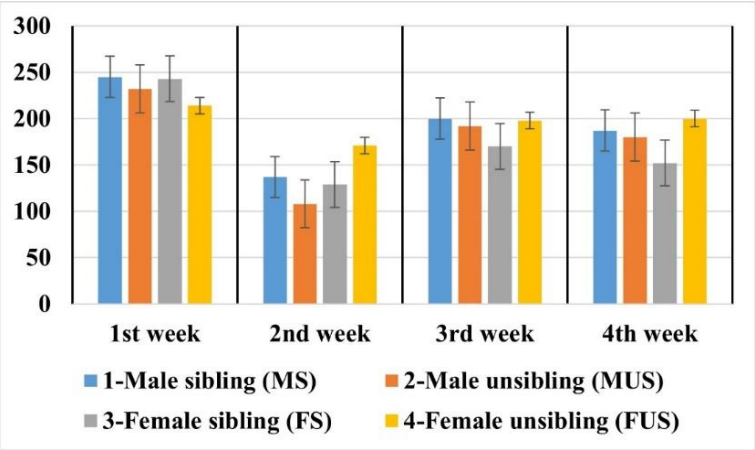


Figure (2): Weekly weight gain (g) of the four growing rabbit groups during the growing period.

Figure (2) showed that there was no significant difference at P>0.05 in weekly weight gain (g) among the four groups of the growing rabbits during the growing period. Male sibling group showed a higher weekly weight gain (g) when compared to male unsibling group in the four weeks. In contrast, Female unsibling group showed a higher weekly weight gain (g) when compared to female sibling ones. The first week recorded the highest weight gain (g) among the growing period while, the second week recorded the lowest weight gain (g) among the growing period.

Concerning the stress level of growing rabbits under the effect of sex or sibling of growing rabbits at 6 and 10 weeks. Figure (3) showed that there was a highly significant difference at P ≤0. 01 between growing rabbits' groups. Group two male unsibling recorded the highest level of cortisol hormone levels (ng/ml) when compared to other groups at the 6^{th} or 10^{th} week.

3.2. Experiment Two: Our results verified that white willow Gnawing sticks tree species have the power to improve the behaviour, weight gain, and stress level of the growing rabbits during the growing period.

Results in Figure (4) showed that after performing the preference test white willow tree species of gnawing stick recorded the highest degrees of visual evaluation and was the most gnawed species over the other tree species of gnawing sticks in rabbit cages and followed by mango and mulberry then guava.

Concerning the effect of gnawing sticks on growing rabbit behavior Fig (5) showed that there was a highly significant at P ≤0.01 reduction in biting wire and feeder at the 6-week Fig (5A) and at the 10 week Fig (5B). There was no significant difference between groups in the other recorded behaviors in activity or eating feeders.

Results in Fig (6) showed that there was no significant at P>0.05 difference in weekly weight gain (g) in both cages of growing rabbits' groups supplied with gnawing sticks or not supplied from 5^{th} weeks till 10^{th} weeks old.

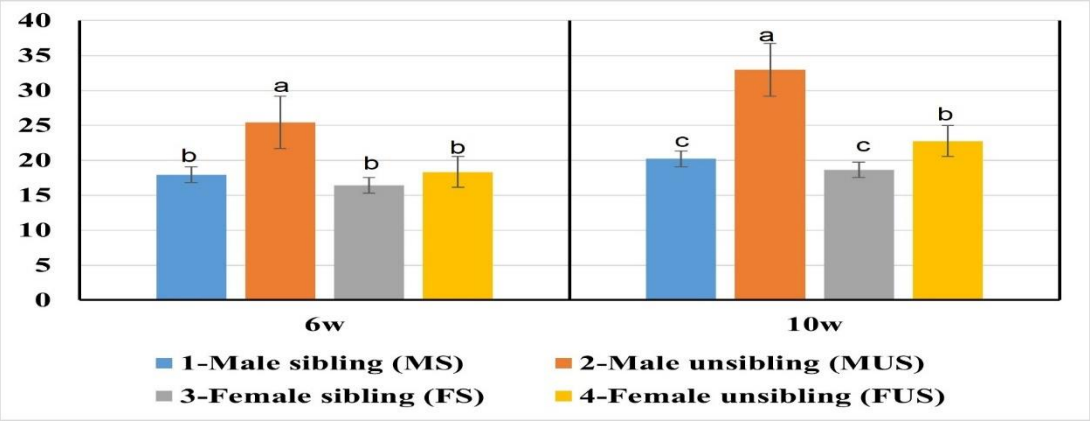


Figure (3): Cortisol hormone levels (ng/ml) in the four groups of growing rabbits at 6 and 10 weeks. Superscripts with different letters (a, b) differ highly significantly at P ≤0. 01.

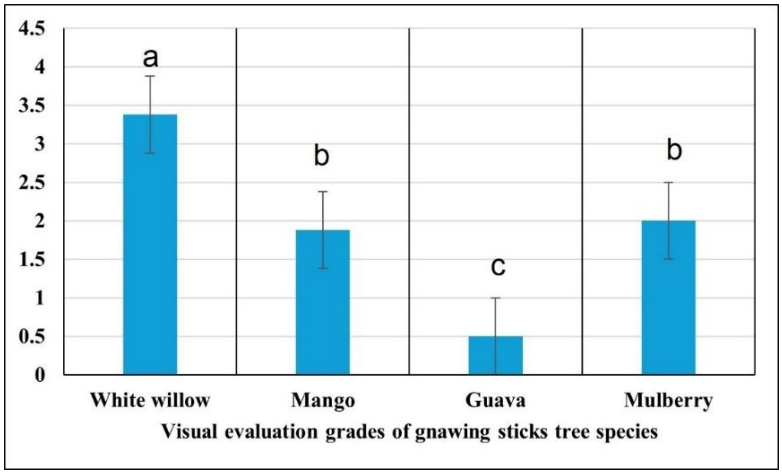
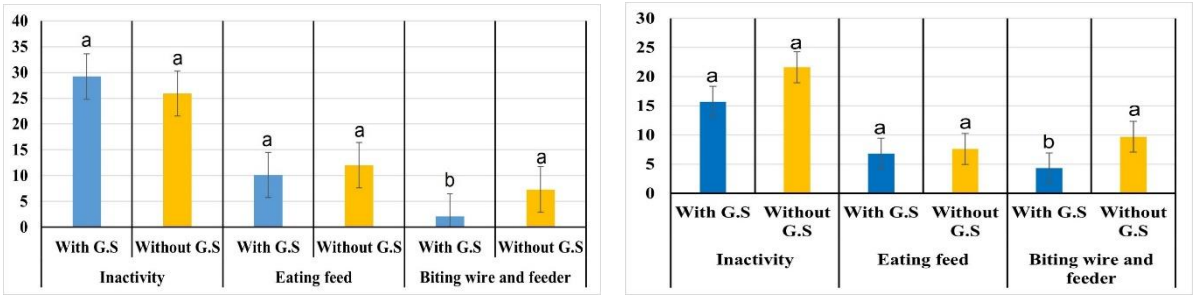


Figure (4): Visual evaluation grades of preference test for different types of gnawing sticks tree species inside growing rabbit cages (Mean ±S.E); Superscripts with different letters (a, b) differ highly significantly at P ≤0.01.



A (at 6 weeks)	B (at 10 weeks)

Figure (5): Duration (min) of measured behaviors in both cages of growing rabbits' groups with and without gnawing sticks (G.S). Superscripts with different letters (a, b) differ highly significantly at P ≤0. 01.

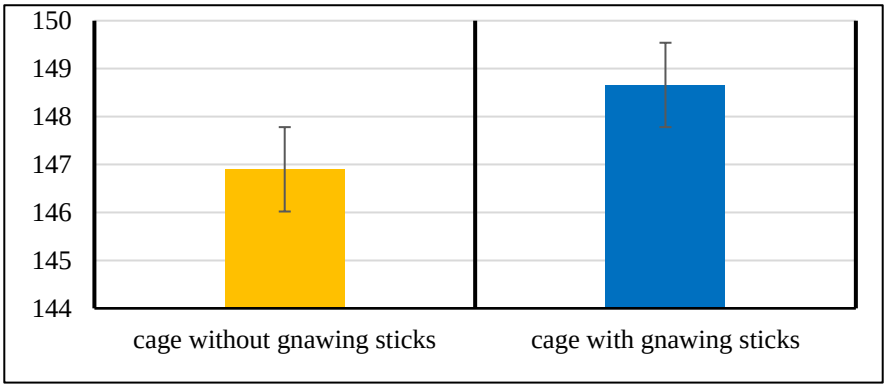


Figure (6): Weekly weight gain (g) in both cages of growing rabbits' groups with and without gnawing sticks from 5th weeks till 10th weeks old. There was no significant difference at P>0.05.

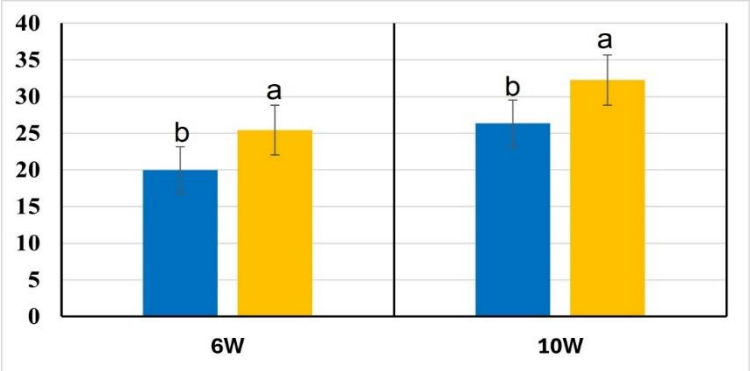


Figure (7): Cortisol hormone levels (ng/ml) in both cages of growing rabbits' groups with and without gnawing sticks (G.S) at 6 and 10 weeks. Error bars represent the 95% confidence limits. Superscripts with different letters (a, b) differ highly significantly at P ≤0. 01.

Concerning the stress level of growing rabbits under the effect of gnawing sticks supplement at 6 and 10 weeks. Figure (7) showed that there was a highly significant difference at P ≤0. 01 between growing rabbits' groups supplied with gnawing sticks and the other group. Supplied group with gnawing sticks recorded the lowest level of cortisol hormone (ng/ml) when compared to non-supplied group at the 6^{th} or 10^{th} week.

4. DISCUSSION

The barren environment has many behavioral and welfare problems that affect the development and productivity of growing rabbits under cage housing systems such as bare biting, chewing and aggression. In this study two proposed environmental enrichment (sibling housing and gnawing sticks application) was discussed to detect their alleviative effect for that bad effect of the barren environment.

Early sibling interactions were generally difficult to study in mammals because they are nocturnal, their offspring hide in burrows, nests. Added to, most mammals live with the same or different age siblings that might affect individual physical and behavioral development, survival, and fitness. In contrast, they might fight for limited resources such as food, shelter, and parental attention (Mock and Parker, 1997).

Results in figure (1) show that there was a significant difference between means of food consumption frequency at P≤0.01 of the male unsibling and female unsibling groups of growing rabbit while, there was no significant difference between other groups. The previous finding agrees with Hudson and Trillmich (2008). This may be attributed to the competition between group members of growing rabbits for limited resources such as food. This explanation is consistent with Mock and Parker (1997).

Male unsibling and female sibling groups of growing rabbits have a significantly higher frequency of rubbing the body as a mean of self-grooming behaviour at P≤0.01 than the male sibling and female unsibling groups (figure 1). This finding might be attributed to self-grooming behaviour is directed at unfamiliar opposite-sex sibling as the cage of male unsibling groups was beside the cage of female sibling groups while the cage of male sibling and female unsibling was beside the cage of the same sex siblings. This finding and explanation nearly agrees with Guillermo et al. (2002).

Data from figure (1) showed that the male unsibling groups of growing rabbits have a significantly higher frequency of smelling each other's a mean of social behavior in rabbits (2.46±0.65) than the male sibling groups of growing rabbits (0.77±0.34). This might be due to there was no previous interaction between unsibling rabbits as they were from different mothers' litter. This finding agrees with what was mentioned by Susan (2006). There was no significant difference between the four groups of growing rabbit in the other recorded behavioral parameter (Gnawing of food duration, drinking duration, Sleeping, Lying, Sitting, Body care licking, licking each other, smelling things, rubbing things licking things, walking, Stretching, Standing, Defecation, Urination and Ceacotrophy). The obtained findings agree with what was mentioned by Mbanya et al. (2004).

From figure (2) there was no significant difference between means at P≥0.05 of the four groups of growing rabbits in the weekly weight gain in the whole period of the experiment which is one month after weaning. This may be attributed to absence of conflicts among the group members of growing rabbits. The obtained finding agrees with what was mentioned by Jaén-Téllez et al. (2021); Drummond et al. (2000) and Mbanya et al. (2004). The growing rabbits used in this experiment were from the same farm and the same breed so that, being sibling or unsbling (parents even same or different) may not have a notable effect on the weekly weight gain of their litters. From the above finding, it is recommended to group the growing rabbit during the fattening periods without any attention to sex or sibling state. Weekly weight gain varied significantly from the 1st to 4th week as there was highly significant difference between the 1st, 2nd, 3rd weeks and 1st, 2nd and 4th weeks but there was no significant difference between the 3rd and 4th weeks.

Concerning the stress level of growing rabbits under the effect of sex or sibling of growing rabbits at 6 and 10 weeks. Figure (3) showed that there was a highly significant difference at P ≤0. 01 between growing rabbits' groups. Group two male unsibling recorded the highest level of cortisol hormone levels (ng/ml) when compared to other groups at the 6th or 10th week. In addition, Sibling groups have lower cortisol hormone levels (ng/ml) when compared to unsibling groups. This finding confirms a lower level of stress in sibling growing rabbits groups. This finding may be due to the spirit of litter siblings increasing levels of convinced social interactions with litter siblings and it is also connected with minor stress hormone (corticosterone) levels (Rodel, 2022).

From figure (4) it can be stated that there was a highly significant difference between means at P≤0.01 of gnawing stick tree species (White willow, Mango, Guava and Mulberry) visual evaluation grades. White willow gnawing stick was the most preferred tree species. So that, it will be a suitable environmental enrichment to be applied in rabbit cages. While guava species were rejected, no ingestion of these species could be observed. White willow is a soft tree so their consumption was higher compared to other trees. This finding and explanation is nearly similar to what was mentioned by Kovacs (1979); Princz et al., (2006 b) and Szendro et al., (2007).

From figure (5 A&B) it can be stated that there was no significant difference at P≥0.05 in both cages of growing rabbits' groups with and without gnawing sticks at 6 weeks mean duration (min.) of inactivity and also, at 10 weeks. The above findings agree with what mentioned by Jordan et al., (2006) and Szendro et al., (2007). Meanwhile, this finding disagrees with Verga et al., (2004).

There was a non-significant decrease in feeding duration (min.) in cages of growing rabbits group enriched with gnawing sticks at 6 weeks (figure 5A) and at 10 weeks (figure 5B). This is may be because of longer time spent in gnawing of wooden stick. This finding and explanation agrees with Johnson et al., (2003); Verga et al., (2004) and Princz et al., (2009). Meanwhile, this finding disagrees with Szendro et al., (2007).

The mean duration (min.) of biting wire and feeder decreased significantly at P≤0.01 in cages of growing rabbits group enriched with gnawing sticks at 6 weeks (figure 5A) and at 10 weeks (figure 5B). This result from the absence of environmental stimuli and to the absence of manipulation over the environment would allow rabbits to display abnormal behaviours. The wasted time in biting wire and feeder representing abnormal behaviours replaced by biting and gnawing in the wooden sticks applied in rabbit cages as environmental enrichment which reduces any abnormal behaviour in rabbit cages. This finding and explanation is consistent with Johnson et al. (2003); Jordan et al. (2003): Szendro et al. (2007) and Jaén-Téllez et al. (2021). Meanwhile, this finding disagrees with Matzek et al. (2021). Gnawing stick application had a favorable effect on the rabbits' welfare as it might decease the time lost in biting wire and feeder. This finding agrees with what was stated by Luzi et al., (2003a).

Data from figure (6) reveal that there was no significant difference at P≥0.05 of mean weekly weight gain in both cages of growing rabbits with and without gnawing sticks from 5 weeks till 10 weeks old. This finding might be due to the gnawing sticks having no influence on feed intake or feed conversion. The later finding and explanation are consistent to what mentioned by Jordan et al., (2003); Johnson et al. (2003) and Verga et al., (2004). This finding disagrees with Luzi et al., (2003a & b) and Princz et al., (2009).

Concerning the stress level of growing rabbits under the effect of gnawing sticks supplement at 6 and 10 weeks. Figure (7) showed that there was a highly significant difference at P ≤0. 01 between growing rabbits' groups supplied with gnawing sticks and the other group. Supplied group with gnawing sticks recorded the lowest level of cortisol hormone (ng/ml) when compared to non-supplied group at the 6th or 10th week. This finding

agreed with Princz et al. (2008) who stated that gnawing sticks application decreased the stress of an aggressive animal in a larger group by social facilitation aids.

5. CONCLUSION

In conclusion, the present study verified that application of environmental enrichement examples as sibling housing and gnawing sticks inside growing rabbit cages during the growing periods result in several improvement in growing rabbits behavior such as feeding, self grooming, social smelling and lowering biting wire and feeder.In addition to decreasing the stress levels by the barren environment. So that this study recommends to use this two examples of environmental enrichement inside growing rabbit cages during the growing periods to override the barren environment adverse effects.

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6. Authors' declarations

Publication consent

Each author has demonstrated their consent for the publication of the current manuscript.

Data and material availability:

All data of this study is provided.

Conflict of interests

All authors have stated the absence of any conflicts of interest.

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Authors' Contributions

AAA designed the experiment, conceptualization, participated in preparation, executing the experiment, data collection, formal Analysis, and in writing the manuscript.

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