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Original Article

Weaning induces changes in behavior and stress indicators in young New Zealand rabbits

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ABSTRACT

Objective: The present study was carried out to investigate the influence of weaning on the abnormal behavior, behavioral problems and some stress indicators in young rabbits.

Materials and methods: The study was conducted on seventy two New Zealand rabbit kits which were divided into two groups, one reared with their dams (unweaned rabbits) and the other group reared without their dams (weaned rabbits). Rabbits are reared on traditional wire net batteries. A scan sample technique was used during the observation period of the recorded traits. Rabbits behavior was observed three times per day at (from 8.00 to 9.00 h), (from 12.00 to 13.00 h) and (from 15.00 : 16.00 h) for three days weekly to each group during the experimental period (6 weeks). The observed activities were recorded at 10 min interval for rabbits in each subgroup (6 subgroups for each group). Two blood samplings were taken after weaning process for monitoring some stress indicators.

Results: The results showed that the bare biting and lixite bite were significantly affected by weaning which were increased in the weaned group than the unweaned group. Other abnormal behavioral traits and behavioral problems were not influenced by weaning process. The results found that cortisol, growth hormone levels, superoxide dismutase and catalase enzyme activities were significantly increased in serum after weaning.

Conclusion: The weaning process has negative effect on some behavioral and physiological responses. So, rearing the young rabbits with their mothers is advantageous from the viewpoint of animal welfare. The findings of this study may help to find out suitable strategies to minimize post weaning stress in both human beings and domestic animals.

KEYWORDS

Abnormal behavior; Behavioral problems; Enzymes; Hormones; Rabbits

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INTRODUCTION

Understanding of rabbit behavior is essential for knowledge species requirements and the consequent adaptation of intensive rearing systems. Exhibition of normal behavior is among five basic freedoms forming animal welfare specified by Farm Animal Welfare Council (FAWC, 2001). Maintaining animals in improper method which includes some specific dispossession that may cause abnormal behavior called stereotypies (Lawrence and Rushen, 1993). Well benefit implies that careful attention for animals while bad welfare leads to anomalous behavior that affects negatively on efficiency of the animal and indicates poor psychological well-being in these animals. Physiological parameters changes are not every time related to stereotypies and guide to pain. Weaning is considered a stressful situation for young rabbits which may result in change in their normal behavior, for example, Chewing is a normal behavior for rabbits, and they chew to take food and to investigate and change their environment. When chewing becomes repetitive and purposeless, it is considered a stereotypical behavior. Performing stereotypical behavior as pacing the cage, chewing the bars, over grooming, or rattling their water bottle, it is an indication that animals are bored and stressed (Mills and Marchant-Forde, 2010).

The information relating to characteristics of behavioral interactions of female rabbits with their kits and to the behavior of the young in the prepubertal period are not plentiful. When young rabbits (without mothers) are kept together in cages with high crowding (more than 5) several wounds were found on animals' bodies which may lead to death of some of them due to aggressive interactions. Such injuries are not always the result of biting or attacking by paws but they may be due to hit into the cage walls as result of fear during catching or aggression. However, this was not observed during long term of offspring leaving for 3 months with parent (Fedosov et al., 2015).

For appropriate assessment of rabbit welfare, multiple indicators other than behavior should be considered as physiology, injury, disease and performance (Trocino and Xiccato, 2006). In animal industry, the stress is an important factor as it has a direct relation to animal welfare, performance, meat quality and disease susceptibility (Yamane et al., 2009). Moreover, there is a strong link between behavior, stress and neurocrine system (Mann, 2003).

As a result of stress, the secretion of some hormones is enhanced as glucocorticoids and growth hormone to increase mobilization of energy sources and facilitate adaptation to the new circumstance. Weaning is generally regarded as a significant source of psychological and physiological stress. Weaning stress has been found to increase the concentration of cortisol (Hickey et al., 2003; Kim et al., 2011). A stressful stimulus causes a release of corticosteroids from the adrenal cortex as a result of activation of the hypothalamic-pituitary-adrenal axis (Hau et al., 2011). In domestic animals, cortisol level in blood has been used as a marker of stress. Growth hormone also is a stress hormone that increases the blood glucose and free fatty acid levels (Ranabir and Reetu, 2011).

A recent study indicated that weaning disrupted the physiologic equilibrium of oxidant and antioxidant and led to oxidative stress (Zhu et al., 2012). The classical enzymatic antioxidants represented a first line of defense against reactive oxygen species (ROS) by detoxifying them; antioxidants can remove ROS rapidly and efficiently from the intracellular environment (Zhang et al., 2015). The result of overproduction of reactive-oxygen species and reactive-nitrogen species is incidence of oxidative stress (Valko et al., 2007).

In addition, the antioxidant defense systems, including catalase, superoxide dismutase (SOD), and glutathione peroxidase are changed (Han et al., 2011). Catalase was the first antioxidant enzyme to be characterized and catalyses the two stage destruct hydrogen peroxide to water and oxygen and sharing this function with Glutathione peroxidase (Sidhu et al., 2004). Superoxide dismutase induces the formation of H_2O_2 due to efficient dismutation of O^{2-} then H_2O_2 is removed by catalase and glutathione peroxidase (Rodriguez et al., 2004). Oxidative stress ensues in the absence of an appropriate compensatory response from the endogenous antioxidant network. The objective of this research is to investigate the effect of weaning on appearance of some abnormal behavioral patterns and behavioral problems and studying the level of some stress indicators in relation to weaning in young rabbits.

MATERIALS AND METHODS

Ethical approval: This experiment was carried out at laboratory animal experimental unit belonging to Faculty of Veterinary Medicine; Zagazig University. The research protocol of this work was approved by the institutional animal care and use committee, Zagazig University (approval no. ZU-IACUC/2/F/2/2018).

Table 1. Abnormal behavior and behavioral problems studied:

Observed traits	Definition
(A) Abnormal behavior (vices): any activity judged to be outside the normal behavior pattern for rabbits which is a repetitive non functional activity (Lawrence and Rushen, 1993) such as:	
1-Bar bite	Biting the bars of the cage.
2-Floor chewing	Repetitive gnawing the floor grid of the cage.
3- Drinker pressing	Pressing on the drinker without drinking.
4- Licking objects	Licking of the floor, bars, hopper, and wall.
5-Lixit biting	Biting of its own fingers.
6-Head swaying	Presented frequent movement with the head in the longitudinal axis, from side to side.
7-Nose rubbing	Rubbing the nose in the around objects or cage.
8-Ear licking	Licking the ears of the others kits in the cage.
9-Hair licking	Licking the hair of other cage mates.
10- Eat fur	Nibbling of the fur, most commonly around the neck. The kit repeatedly passes the same fur through its mouth and also the fur on roof of own cage.
11-Sniffing back	Sniffing the others back.
(B) Behavioral problems: (Gunn and Morton, 1995):	
1-Digging	Digging the cage with its paws
2-Frisky hop	A very rapid circling around the cage.
3-Flipping food	Flipping or disperse the food from hopper without eating.
4-Pulling objects	Pulling any around objects.
5-Bad litter box habit	Doing excretion inside litter box.
6-Burrowing fur	Burrow in fur of its own mother or on other kits.

Animal used and management: A total number of seventy two New Zealand rabbit kits were used in this work which were divided into two groups, one are reared with their dams (Unweaned rabbits) and the other reared without their dams (Weaned rabbits). All animals were housed on wire net cage floor throughout the experiment. The average environmental temperature and relative humidity % during the experimental period were 24±0.56ºC and 51±1.30% respectively, and were recorded daily.

During the experimental period, a 16L: 8D lighting schedule was applied and obtained by the natural day light and artificial light by fluorescent lamb hanged centrally from the roof. Water and commercial balanced pelleted ration were available *ad libitum*. Good ventilation and fresh air were admitted to reduce ammonia concentration in the house in which the natural ventilation without thermal insulation or cooling system has been used. The hygienic disposal of wastes and floor disinfection were performed**.**

Observation technique and data collection: A scan sample technique was used during the observation period. The abnormal behavioral activities and problems were recorded during the daylight hours during experimental period. Animals were observed three times daily (from 8.00 to 9.00 h.), (from 12.00 to 13.00 h) and (after 15.00 h) for three days weekly for each group during the experimental period. The observed activities were recorded at 10 min interval for rabbits in each subgroup (6 subgroups for each group) and after 5 min of their adaptation to the observer. Results were expressed as the percentage of rabbits engaged the behavior/total observed rabbits (Reiter and Bessei, 2000). A detailed description of the parameters observed (abnormal behavioral traits and behavioral problems) is presented in **Table 1**.

Blood sampling: Two blood samplings were taken, the 1st blood sampling was in the second day post-weaning and the 2nd blood sampling was 2 weeks post-weaning. Five young rabbits from weaned and unweaned group (in each sampling) were slaughtered at the same time in the morning. Blood samples were taken in test tubes without anticoagulant and were left sloped for half an hour at room temperature for coagulation then centrifugation at 3000 rpm for 15 min to obtain serum was done. Serum samples were stored at -20ºC until analysis. Cortisol, growth hormone, superoxide dismutase and catalase level in serum were measured. Cortisol was measured by using Rabbit Cortisol ELISA Kit according to (Dyavolova et al., 2014) and growth hormone was quantified by using Rabbit growth hormone ELISA kit (Sandwich ELISA)

(Lifespan Biosciences) according to Reeds et al. (1971). Superoxide dismutase activity was measured according

Table 2. Mean±SE of some abnormal behavioral patterns in young rabbits in relation to weaning.

Behavior / Group	Bare bite	Floor chew	Drinker pressing	Licking objects	Lixit bite	Head swaying	Nose rubbing	Ear licking	Hair licking	Eat fur	Sniffing back
Weaned young rabbits	15.55 ±1.59	3.22 ±1.15	0.66 ±0.37	0.88 ±0.30	2.44 ±0.66	1.0 ±0.40	0.33 ±0.23	2.22 ±0.74	2.66 ±0.97	2.66 ±0.55	2.33 ±0.52
Unweaned young rabbits	5.11 ±0.87	7.22 ±2.76	0.77 ±0.36	1.55 ±0.5	0.55 ±0.242	11.1 ±0.3	0.55 ±0.33	1.77 ±0.57	2.33 ±0.57	1.44 ±0.52	1.22 ±0.14
P-value	0.00	0.20	0.834	0.276	0.017	0.831	0.59	0.641	0.772	0.130	0.059

Table 3. Mean±SE of some behavioral problems in young rabbits in relation to weaning.

Behavior / Group	Digging	Frisky hop	Flipping food	Pulling object	Bad litter box habit	Barrowing fur
Weaned young rabbits	0.33±0.23	4.55±0.72	1.88±0.45	1.11±0.38	0.22±0.22	1.44±0.37
Unweaned young rabbits	0.50±0.22	4.3±1.10	2.70±0.42	0.50±0.22	070±0.39	2.4±0.33
P-value	0.615	0.853	0.208	0.180	0.322	0.076

to Kakkar and Das (1984) and catalase activity was measured according to Aebi (1984).

Data analysis: Statistical analysis was performed by mean of SPSS (2013) software package. All results obtained were expressed as means± standard error and significance was declared at ($P \leq 0.05$).

RESUTS AND DISCUSSION

There is a significant effect of adult female behavior on the young (Fedosov et al., 2015). The data in **Table 2** revealed that there was a highly significant increase in the frequency of bare biting and lixite bite in weaned young rabbits (15.55±1.59 and 2.44±0.66, respectively) than that in unweaned ones (5.11±0.87 and 0.55±0.242, respectively). These results are coincided with that recorded in foals by Waran et al. (2008) who illustrated that before separation from mother the anomalous in young appear and curve increases after separation. The increase of some abnormal behavior in weaned rabbits may be explained by increasing stress and frustration due to weaning resulting in repetitive nonfunctional behavior .Weaning is a stressful process so emotional anxiety and physiological changes are induced by abrupt weaning many domestic species (Apter and Householder 1996; Day and Webster 1999). So that the rearing of the young with their mother had advantage to some extent for decreasing these abnormal behaviors. There was non significant increase in some abnormal behavior as ear and hair licking, eating fur and sniffing back in young rabbits that were weaned than other kept with their mothers. Our result was in harmony with the findings of Würbel and Stauffacher (1997) who mentioned that a major cause of stereotypic behavior in many animals including rodents is the practice of early weaning. Kotenkova et al. (2011) reviewed that the active maternal influence on behavioral interactions of young which can substantially decrease stress level among the young rabbits by preventing agonistic interrelations between them. Stereotypic behavior is more frequent at night when rabbits are more active. Animals that appeared more active may be more frustrated and perform more stereotypic behavior (Gunn and Morton 1995). One of the positive aspects of maternal influence on young rabbits not separated from their mother for a long time was that it defines the youngs' behavior and character. For instance, when the mother approached her young (or other forms of directive behavior) it could change their behavior from sitting to more active behavioral forms. Attracting the attention of the young rabbits, the doe could prevent them from doing certain things, including acting aggressively. In some cases the mother took an active part in the conflicts of young rabbits and protected the one who was being treated aggressively (Fedosov, 2007).

Some other vices (as floor chewing, drinker pressing, licking objects, nose rubbing and head swaying) studied and appeared to be slightly higher in un weaned rabbits than other group which were weaned. Tölü et al. (2016) observed that abnormal oral behavior (included licking, biting on paddock installations and/or hairs of other kids) was significantly higher in the structured group (enriched with different objects) before weaning and lower after weaning. Cooper et al. (2005) suggested that high level of meal taken availed for decrease the frequency of oral anomalous but not locomotory anomalous in foals.

In the present study, the data in **Table 3** show that the behavioral problems were not affected by the weaning process. From data in **Table 4**, it was noticed that the

Table 4. Mean±SE of some physiological parameters in young rabbits in relation to weaning.

Groups	1st sampling (2nd day after weaning)		*P*-value	2nd sampling (2 weeks after weaning)		*P*-value
	Weaned group	Unweaned group		Weaned group	Unweaned group	
Cortisol (ng/mL)	73.33±1.20	61.33±0.88	0.001	91.33±0.88	86.00±0.57	0.007
Growth hormone (ng/mL)	0.006±0.0005	0.002±0.0003	0.005	0.004±0.0003	0.01±0.0008	0.003

Table 5. Mean±SE of some biochemical parameters in young rabbits in relation to weaning.

Groups	1st sampling (2nd day after weaning)		*P*-value	2nd sampling (2 weeks after weaning)		*P*-value
	Weaned group	Unweaned group		Weaned group	Unweaned group	
Superoxide Dismutase (ng/mL)	0.13±0.00	0.07±0.01	0.01	0.14±0.03	0.07±0.01	0.01
Catalase (ng/mL)	8.56±46.86	4.99±66.61	0.01	9.09±254.7	5.49±46.77	0.03

level of cortisol was significantly higher in the weaned group in both 1st and 2nd sampling. This result is in agreement with that of Hickey et al. (2003) and Kim et al. (2011) which found that weaning stress enhance cortisol level in calves. Also the result of McCall et al. (1987) in foals confirm the high increase in cortisol with total separation at weaning and indicating they were stressed. The increase in cortisol hormone may be explained by stimulation of the hypothalamic-pituitary-adrenal axis by weaning stress resulting in release of glucocorticoids (Kim et al., 2011). So weaning represents a stressful event which can elevate the level of the stress hormone along with inducing behavioral changes.

Concerning growth hormone, a significant increase was observed in the weaned group at the first sampling only (**Table 4**). There is a lack of clear information concerning growth hormone relation to weaning stress but some studies in human reported a significant increase in growth hormone under stress conditions such as the study of Schalch (1967). In addition, Tamai et al., (1986) reported that a significant increase in growth hormone observed only when thyrotropin releasing hormone and psychological stress were combined as stimuli. The increase in growth hormone just after weaning may be explained by the high cortisol level as in the acute setting of stress, growth hormone genes are stimulated by glucocorticoids resulting in enhanced growth hormone secretion (Casanueva et al., 1990). However, growth hormone release is suppressed with more prolonged stress by CRH-induced elevation in somatostatin levels (Raza et al., 1998). On the other side, glucocorticoids can inhibit the effect of growth hormone through its inhibitory effect on insulin-like growth factor-1 and other growth factors (Unterman and Phillips, 1985).

In **Table 5**, it was observed that the level of superoxide dismutase and catalase in serum is significantly increased in the weaned group in both 1st and 2nd sampling indicating exposure to oxidative stress resulting from weaning. The elevated level of antioxidant enzymes is a way to overcome the produced ROS. Environmental stress induces the accumulation of ROS in the cells resulting in severe oxidative damage. The redox homeostasis is commonly known as the balance between the production of ROS and its scavenging. Many studies have reported that after exposure to a stress, an increase in the concentration of hydrogen peroxide (H_2O_2) occurred and its production depends on the duration and intensity of the stress. Moreover, the H_2O_2 level varies between different cellular compartments and is related to the kind of stress (Slesak et al., 2007).

CONCLUSION

Keeping young rabbits in cages near their mother may decrease stress of weaning, and the young rabbits may be kept in this condition till mating of their mother.

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CONFLICT OF INTEREST

The authors declare that there is no conflicting interest with regards to the publication of this manuscript.

AUTHORS' CONTRIBUTION

AF designed the plan of work. All authors shared in fulfillment of the work and were involved in writing up of the manuscript as well as refined English of the draft. Finally, the manuscript was read and commissioned by all authors.

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