

# **SUITABILITY OF BEHAVIOURAL TESTS FOR DETERMINING THE WAYS RABBITS FUNCTION IN THE ENVIRONMENT AND THEIR RELATIONSHIP WITH SOME PRODUCTIVE TRAITS**

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## **ABSTRACT**

This study aimed to obtain by selection two lines of New Zealand white rabbits differing in total motor activity and to compare them for fertility, prolificacy, mortality and weight gains of young rabbits from birth until weaning. All females in the groups were mated for the first time. The emotional states of animals such as fear were analysed with the behavioural tests (open field, tonic immobility) and stress-induced hyperthermia (SIH). The way in which animals react to different potentially dangerous situations is stable and reflects two fundamentally different, genetically determined strategies/ways of functioning in the environment, the active and passive strategies. Active individuals strive to avoid stress stimuli or try to manipulate them, while passive individuals show reduced reactivity to environmental stimuli. These tests were the criterion for assigning animals to two experimental groups: group I, 10 males and 20 females with the highest total motor activity (A); group II, 10 males and 20 females showing little or no motor activity (N). Important differences were found in male and female fertility during the first days of mating: during the first week, 80% of males mated in group I compared to only 20% in group II. Despite their normal breeding condition, passive males were timid and ponderous during mating, unlike active males which mated willingly and rapidly. During the first week, 45% of active females and 70% of passive females were mated. Fertility was high in both groups (87.5% and 94.7% in group I and II). Unlike females from group II, females from group I showed no tendency towards nest building. 40% of females from group I gave birth in different cage areas, while 90% of females from group II gave birth in nest boxes with down and straw bedding. Active females gave birth to a high percentage (22.2) of dead rabbits. There were highly significant differences in the mean number of rabbits reared per litter (5.2 in group I and 6.8 in group II). No differences were found between the active and passive lines for body weight at birth or at 35 days of age. Passive females were better mothers and showed greater maternal care. The high mortality in the active group was due to the trampling of the young rabbits, scattered all over the cage, by overactive mothers. In conclusion, the line of active females is likely less suitable to rearing in cages of standard dimensions, which are incompatible with the “active” type of behavioural strategy. Females from this group are much worse mothers. Passive animals preferred to remain in confined space, which is considered as an adaptive mechanism to cope with environmental conditions. Active females are more confident, attain breeding maturity earlier and begin mating more quickly, which is why they are recommended for further rearing.

**Key words:** Rabbits, Behavioural tests, Productive results.

## **INTRODUCTION**

Human and animal behaviour is mostly oriented towards the accomplishment of specific goals, in other words it is intentional. In the course of evolution, the biological mechanism of behaviour has evolved so as to survive and leave offspring in specific environmental conditions. Goal-oriented activities are accomplished in two ways: through inborn reflexes involving a reflex arc and through impulsive behaviour – by stimulating specific brain structures. Stimulation and satisfaction of impulses is a source of global behaviour, vegetative changes in the body and subjective processes

known as emotions. There are two aspects to emotions: subjective – associated with the presence and satisfaction of a certain biological need, and objective – covering behavioural changes (facial expression, body posture) and organ functioning (accelerated pulse, respiratory rate, etc). Long-term observations of farmed rabbits have shown that some animals are unable to completely adapt to rearing conditions provided. Rabbits are very skittish and susceptible to stress. The domestication of animals, which has proceeded over many centuries, reduces the reactivity of animals, while the selection by humans reduces the adaptive ability of animals. Breeding animals are exposed to many stress factors such as living in cramped conditions of the cage, environmental monotony, which favours the occurrence of stereotyped behaviours, high stocking density and contact with humans. As a consequence, these animals are affected by groups of stimuli to which their nervous systems are not suited, while being deprived of stimuli necessary for the implementation of their inborn needs. Another disadvantage is that animals are usually exposed not to one but to multiple simultaneous stressors at subthreshold levels. The emotional states of animals such as fear are analysed using common behavioural tests: the open field test, the tonic immobility test, the hand test, and the stress-induced hyperthermia (SIH) test. Research has shown that the way in which animals react to different potentially dangerous situations is stable and reflects two fundamentally different, genetically determined strategies/ways of functioning in the environment – the active and passive strategies. As a rule, active individuals strive to avoid stress stimuli or try to manipulate them, while passive individuals show reduced reactivity to environmental stimuli.

The aim of the study was to obtain, by selection, two lines of rabbits differing in total motor activity. The lines obtained were compared for traits such as fertility, prolificacy, mortality of young rabbits, and weight gains of young rabbits until they were separated from their mothers.

## MATERIALS AND METHODS

The experiment involved 182 New Zealand White rabbits (derived from 30 females and 10 males), from which 60 animals were selected for further studies. All rabbits were clinically healthy and were of the same age (the experiment was started at 2.5 months of age). Animals were kept in tiered cages, with 4 animals of the same sex per cage. Two weeks before the experiment, on several occasions rabbits were taken out of the cages into wicker baskets to familiarize the animals with the removal procedure.

The experimental rabbits were subjected to the following tests: open field, tonic immobility, and stress-induced hyperthermia (SIH), together with the measurement of respiratory rate. The open field was a rectangular area, 2.0 m long by 1.4 m wide. The field was divided into 20 rectangles of equal size, 0.4 m long by 0.35 m wide. The open field walls (1 m high) were made from white plywood. A wooden open-top start box with standard kindling box dimensions was placed in the corner of the field. Animals were brought to the field in wicker baskets, delicately taken out and put in the start box. The test lasted 5 minutes. Rabbits were observed by the experimenter sitting next to the field. At the end of the test, the box, floor and walls were wiped with a cloth moistened with a detergent to remove odour traces. The following behaviours were recorded during the test: the latency to leave the start box and enter the open field; the number of movements made in the start box before leaving for the open field (relocation of all legs counting as one movement); total locomotor activity in the open field (number of rectangles crossed ); and behaviour after leaving the start box – standing on hind legs, defecation, scent marking, grooming (licking, scratching), scratching open field walls, and attempts at jumping out of the area. Animals were tested twice between 8.00 am and noon at 10-day intervals, before feeding. The total score from two tests was used as the selection criterion. The tonic immobility test was performed twice after the open field observations ended. Tonic immobility was induced using the Gallup method by immobilizing a rabbit in the dorsal position in a U-shaped plastic trough. When tonic immobility occurred, i.e. the animal remained in the dorsal position, and the hands were withdrawn, the latency to spontaneously recover a normal body position was measured. The SIH test involved the measurement of body temperature (using an electronic thermometer with an accuracy of 0,01 °C ) before and after the animals were placed for 15 minutes in a closed wooden cage. In

addition, respiratory rate was measured during temperature measurements. These tests were used as the criterion for assigning individual animals to different groups. Because animals from group II did not leave the nest box, the number of movements performed during both nest box tests was used as an additional criterion.

Two experimental groups were created: group I – 10 males and 20 females with the highest total motor activity (A), group II – 10 males and 20 females showing little or no motor activity (N). Blood was sampled from all animals in the groups to analyse cortisol and progesterone. Progesterone concentration was determined as an additional test that could possibly explain very large differences in mating results. The hormones were determined using Spectria Cortisol RIA and Spectria Testosterone RIA kits (Orion Diagnostica). At the age of 5.5 months, all females in the groups were mated (according to the A♀xA♂, N♀xN♂ scheme) and the following data were gathered: conception rate, duration of pregnancy, litter size at birth and weaning, litter weight at 1 and 35 days of age, causes of young rabbit mortality until weaning. After mating, animals were moved individually to cages with a straw floor. The numerical data were analysed statistically using the SAS statistical package.

## RESULTS AND DISCUSSION

The first open field test made it possible to assign animals to two groups. Out of 182 rabbits tested, 63 were assigned to group I and 119 to group II. After the second test, group I was joined by 7 animals that left the nest box. Among animals that left the nest box and moved in the open field, the intensity of exploratory behaviour was the product of fear and curiosity in new surroundings. Rabbits crossed various numbers of fields, ranging from 8 to 78. In the first test, they moved mainly along the extreme fields around the walls, sniffing surroundings and climbing the open field walls, thus showing strong thigmotaxis (Węsierska and Turlejski, 2000). In the second test, rabbits changed their exploratory strategy and more often crossed the internal squares. During the first and second test, rabbits entering the open field were observed to mark territory with urine. This behaviour was significantly more frequent in males than in females. Table 1 shows the locomotor exploration of rabbits in the first and second test.

**Table 1:** Locomotor exploration of active rabbits in the open field test

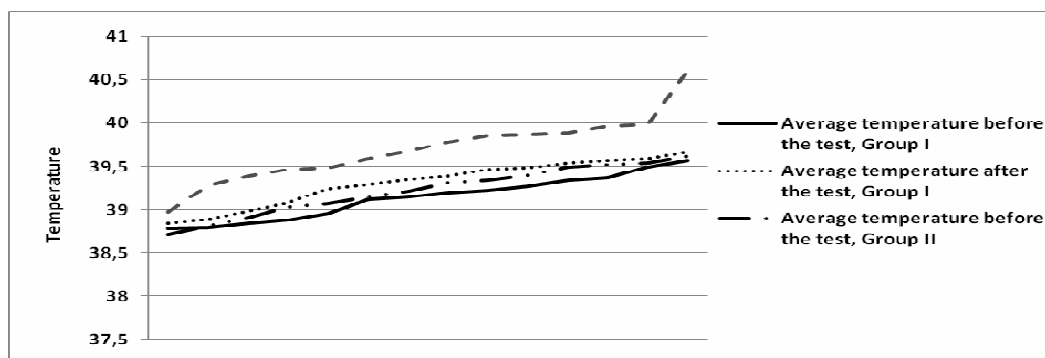
Item	Time spent in the nest box (minutes)		No. of movements in the nest box before leaving		No. of external squares crossed		No. of internal squares crossed	
	mean	se	mean	se	mean	se	mean	se
Test 1	1.3A	2.4	6.3A	12.5	42.4A	12.3	4.8A	3.6
Test 2	0.23B	0.9	3.7B	4.6	33.6B	9.8	26.4B	5.9

A,B – means with different letters differ significantly at  $P \leq 0.01$

Between the first and second test, highly significant differences were found in time spent in the nest box, number of movements in the nest box before leaving, and number of external and internal squares crossed. Analysis of the results obtained showed that fear drive was the strongest in the initial phase of exploration and the curiosity drive became predominant later on. Rabbits from group II showed little or no interest in surroundings and only moved inside the nest box. The mean number of movements in the nest box was considerably higher during the first test (6.3) compared to the second test (3.1), because animals were no longer curious of the environment they already got to know. The tonic immobility test revealed that more active animals in the open field test showed a shorter reaction of tonic immobility. The duration of immobility ranged from 1 to 34 seconds in group I and from 10 seconds to 4 minutes and 12 seconds in group II, with highly significant differences between groups.

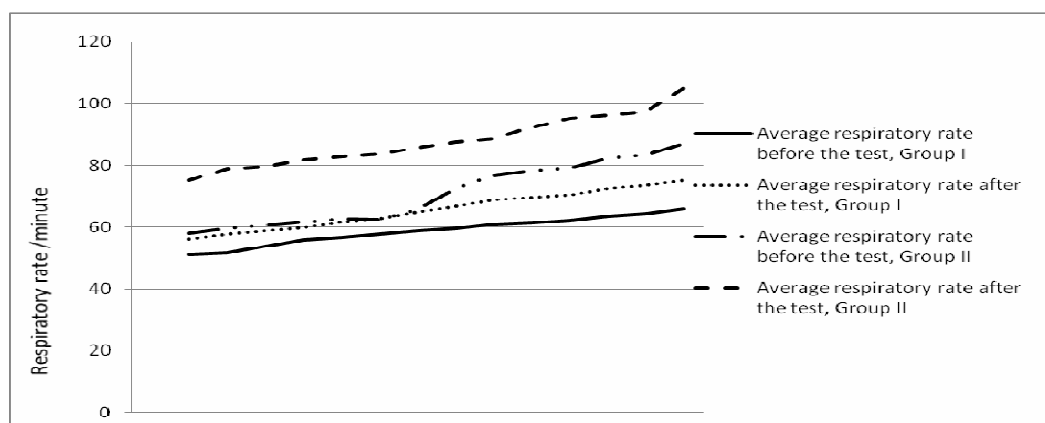
In rabbits, normal body temperature ranges from 38.5 to 39.5°C. Low fever is 40.5°C and high fever exceeds 41.5°C. In group I, the mean rectal temperature was 39.14°C at the start of the test and increased to 39.31°C after the application of the stress factor when animals were placed in a dark box for 15 minutes. In group II, the mean rectal temperature was 39.22°C at the start of the test and increased to 39.73°C at the end of the test. A highly significant difference was found between the groups in final body temperature. Among the most fearful rabbits, the difference between the initial

and final temperature increased by as much as 1.21°C (39.4°C–40.61°C). Figure 1 shows rectal temperature before and after the SIH test according to groups.



**Figure 1:** Rectal temperature before and after the SIH test (°C)

The normal respiratory rate in rabbits is 50-60 breaths/minute (Flecknell, 1993). It can reach 150 during stress. In group I, the mean respiratory rate was 58.8 before the test and 65.5 after the test. In group II, the mean respiratory rate was above normal – 70.8 before the test and 87.9 after the test. The differences obtained between the groups were highly significant. Figure 2 shows respiratory rate before and after the SIH test.



**Figure 2:** Respiratory rate before and after the SIH test

The mean cortisol level determined during the experiment was 11.6 nmol/l for males from group I and 9.0 nmol/l for males from group II. In females it was higher – 16.2 nmol/l in group I and 14.2 nmol/l in group II. Differences between the groups were highly significant (Table 2).

**Table 2:** Serum cortisol levels in rabbits (nmol/l)

Item	Males		Females	
	mean	se	mean	se
Group I	11.6A	3.7	16.2A	6.2
Group II	9.0B	4.2	14.2B	4.9

Table 3 presents reproductive results obtained during the first mating at 5.5 months of age. High percentage differences were found in male and female fertility between the groups during the first days of mating. During the first week, 80% of males mated in group I compared to only 20% in group II. Despite their normal breeding condition, passive males were timid and ponderous during mating, unlike active males which mated willingly and rapidly. During the first week, 45% of active females and 70% of passive females were mated. The testosterone level was additionally determined in both groups when analysing the results obtained. It showed highly significant differences ( $P \leq 0.01$ ) between the groups, averaging 5.41 nmol/l in group I and 3.26 nmol/l in group II. No differences were found in females, in which the testosterone level ranged from 0.20 to 0.21 nmol/l. Similar results of

testosterone determinations were obtained by Kasilima *et al.* (2004). The conception rate was high in both groups (87.5% in group I and 94.7% in group II).

**Table 3:** Reproductive results

	Group I		Group II	
	mean	se	mean	se
Percentage of males mating at:				
1 week	80.0		20.0	
2 week	0.0		10.0	
3 week	10.0		10.0	
4 week	0.00		0.00	
Together	90.0		40.0	
Percentage of females mating at:				
1 week	45.0		70.0	
2 week	25.0		20.0	
3 week	10.0		0.00	
4 week	0.00		0.00	
Together	80.0		90.0	
Percentage of females kindled	87.5		94.7	
Rabbits born:				
Live	99		129	
Stillborn	22		4	
Mean number at birth (head)	7.07	6.13	7.16	7.10
Mean number of rabbits reared to 35 days (head)	5.2A	3.20	6.8B	6.80
Mean weight of 1 rabbit after birth (g)	59.1	2.50	57.3	4.52
Mean weight of 1 rabbit at 35 days (g)	724	13.6	735	11.0

A,B – means with different letter differ significantly at  $P \leq 0.01$

Females from group I showed no nest building. 40% of females from group I gave birth in different cage areas, while 90% of females from group II gave birth in nest boxes with down and straw bedding. Highly significant differences ( $P \leq 0.01$ ) were found for this trait between the groups. There were highly significant differences in the mean number of rabbits reared per litter, but no differences were found between the active and passive lines for body weight at birth and at 35 days of age. The high mortality in the active group was due to the trampling of the young rabbits, scattered all over the cage, by overactive mothers.

Based on the results obtained it is hypothesized that the line of active females is less suited to rearing in cages of standard dimensions, which are incompatible with the “active” type of behavioural strategy. Females from this group are much worse mothers. The results obtained could be attributed to the fact that females had no prior maternal experience, but such poor reproductive results were not found in the extensive documentation available for this breed of rabbits. Passive animals preferred to remain in confined space, which is considered as an adaptive mechanism to cope with environmental conditions. Active females are more confident, attain breeding maturity earlier and begin mating more quickly, which is why they are recommended for further rearing. Motor activity in the open field and other ancillary tests could be included as an additional criterion for selection of breeding material on rabbit farms specializing in slaughter rabbit production and intended for specialist tests in laboratories.

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