International Transaction Journal of Engineering. Management. & Applied Sciences & Technologies

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PAPER ID: 11A14U

# INCREASE IN ADAPTIVE CAPACITY OF MICE VIA THE DISCHARGE METHOD

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**ARTICLEINFO**

*Article history*:
Received 30 March 2020
Received in revised form 04 August 2020
Accepted 18 August 2020
Available online 28 August 2020

*Keywords*:
Food unloading; Life sustainability; Rat adaptation; Hunger mice; Mice reproducibility; Mouse breeding; Mice progeny; Laboratory mice; Zooculture.

**ABSTRACT**

We studied the resistance of laboratory mice to complete food starvation and the possibility of using the unloading method in breeding laboratory animals. The use of one- and two-day unloading periods positively affected the duration of the reproductive period of starving mice. In mice with two unloading days, reproduction continued with an average for 22.3 months, that is, about two years. This is 5.5 times more than in the control group, where the duration of the reproductive period was only 4 months, that is, less than six months. Positive changes were also obtained in terms of the life expectancy of experimental animals. Individual animals in the group with two hungry days a week lived more than 1050 days and on average 28.9 months. The life expectancy of mice of the control group in females, on average, was 563, and in males 604 days, which is 50% less than the life expectancy of mice in the experimental group. The use of one and two fasting days per week violates the reproductive cycle of females and the insemination ability of males.

**Disciplinary**: Biology, Bioscience, Animal Sciences, Veterinary Science.



## 1 INTRODUCTION

It is known that the food factor is one of the fundamental in living nature. The availability of food resources to a large extent ensures the maintenance of a high number of animals in natural conditions. The resistance of animals to starvation is a reflection of the ecology of the species, its important biological characteristic (Pavlinin, 1951). In food adaptations, all the animal's vital needs for energy and structural nutrients that determine their vital activity are biologically balanced.

In the process of the evolutionary development of living organisms, specific adaptations to the natural rhythmic change in the nutritional factor were formed. Wildlife in its evolutionary development has adapted various organisms to the seasonality of natural phenomena and to periodic changes in food conditions (Slonim, 1979; Saraev, 1974; Zabolotskih, 1997; 2000).

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The most important characteristic of the level of ecological plasticity of animals of different species is their adaptability to poor nutrition or the periodic lack of feed. The problem of the resilience of animals of different species to temporary starvation is not only scientific but also practical, as it allows to rationalize the production process, significantly reduce the cost of maintaining and feeding farm animals (Arakeljan, 1973; Saraev, 1974; Zabolotskikh, 1997; 2000).

In modern zooculture, the unloading method has been developed and is beginning to be used as a zootechnical technique to maintain factory fatness in the breeding stock. For example, in fur farming, food unloading in the form of one or two "hungry days" per week is quite effective. Besides, taking into account the biological characteristics of animals, to increase reproducibility, it is possible to use periodic, longer food unloadings (Saraev, 1974; Zabolotskih, 1987; 2004).

Along with farm animals, a significant place in terms of mass is occupied by various laboratory animals. Today there is a whole industry for growing laboratory mice. In the laboratories of the world, white mice and forms of other colors obtained from a house mouse are most often contained. For many studies, these animals are completely indispensable; keep and breed them is easy. Laboratory mice achieved a high degree of domestication, lost their natural wildness, and were easy to handle. Mice are unpretentious, they are easy to feed, they are small in size (Zapadnjuk, 1983).

The mouse is a good model of biological processes in humans and animals. For works that do not require absolutely identical animals, mice that do not belong to clean lines, the so-called "non-linear mice," are raised. The speed of reproduction is one of the many advantages of the mouse as an experimental animal, and obtaining offspring is an integral part of many experiments (Sidorchuk, 2009).

The duration of pregnancy in a laboratory mouse is 20-25 days. Muscles are born naked and blind and weigh only 1-2 grams. However, they are developing rapidly. From the ninth day, the mice begin to become woolly, and on the 9-14th day, their eyes already open. The mother feeds the babies with milk for about 20-25 days, but by the end of this time, they eat everything that adult animals eat. At two to three months of age, the mice become quite adults (Zapadnjuk, 1983). The weight of an adult mouse is 20-35 grams. Life expectancy is one and a half to two years. But they breed very quickly: in one litter, on average, there are from 5-8 cubs, and in a year a female can give birth from four to nine times (Zapadnjuk, 1983; Sidorchuk, 2009).

Finding experimental animals in the vivarium involves certain costs associated with the care and feeding. Daily feed consumption rates are differentiated by weight and age groups of animals. For example, in young mice weighing from 8-14 grams, the daily rate of consumption of the grain mixture is 4 grams; weighing from 14-22 grams - 5 grams of the mixture; in adults 11 grams of grain mixture. The main thing for the normal existence of animals is the variety of feeds. Feed must be fed to the feeders in small quantities. Otherwise, animals quickly contaminate their cage with surplus feed, which are not used further, because animals get used to using fresh food. Thus, part of the feed is simply thrown away, increasing the cost of maintaining animals (Zapadnjuk, 1983; Glukhova, 2014).

It was found that excessive feeding of animals of the maternal herd adversely affects reproduction. Therefore, in recent years, the unloading method in the form of one or two "hungry" days per week has gained increasing relevance (Zabolotskih, 2004; Glukhova, 2014).

## 2 STUDY DETAILS

This research aimed to study the level of resistance of laboratory mice to complete food starvation and the possibility of using the discharge method for breeding laboratory animals.

The experiment was conducted in the vivarium of the Vyatka State Agricultural Academy and lasted about three years. The test was carried out on clinically healthy white mice, the same age of 45-50 days, which had not previously been tested. The average mass of males was $20.7 \pm 0.58$ grams; the average weight of the females is $18.0 \pm 0.65$ grams. Before the experiment, the animals were quarantined for 10 days (Figure 1).

**Figure 1**: Animals in quarantine.

To identify the effect of fasting days on the reproductive function of white mice, based on analogues, three groups of 10 individuals were formed. Of the males and females, they were not related pairs and were transplanted into separate cells (Figure 2). Mice were fed daily with a mixed-type diet. Drinking water was not limited. The first group was used for control. In the second group, one fasting day per week was arranged for mice, and in the third group, two fasting days per week. On fasting days, the animals did not receive food at all, and the water remained plenty.

**Figure 2**. Allocation in pairs.

The second part of the experiment was to determine the level of resistance of animals to complete starvation. The experiments were carried out on white mice of different sexes and ages in the vivarium of the Vyatka State Agricultural Academy. The air temperature in the room was in the range of 20-25°C. Of the 40 experimental white mice, two groups of 20 mice each were created. Each group included young animals aged 30-35 days, middle-aged 45-50 days, and adult animals 75-80 days, which were already involved in reproduction. All animals were seated in individual cages. The animals of the first group were completely devoid of food, and access to water was not limited (complete starvation). Animals of the second group were deprived of food and water (absolute starvation).

## 3 DISCUSSION

Mouse-like rodents are multiple-breeding animals that reach puberty early with short, multiple repetition cycles. As you know, the animal reproductive system is very sensitive to various negative factors (Zapadnjuk, 1983; Sidorchuk, 2009).

Reproduction of mice in all groups began at one time, one and a half months after the formation of pairs. In the first reproductive cycle in the control group, litter was obtained in only one pair of five. In the first experimental group with one fasting day, the offspring appeared in all couples, and in the group with two fasting days in the first breeding cycle, 75% of the females were born. The number of newborn babies in one litter varied from 5-11 mice per female (Figure 3).

**Figure 3**. Grown young animals.

In all groups of experimental mice, the average body weight at the beginning of the experiment for young females was at the level of 17.5-18.0 grams, for males 19.5-20.0 grams. By the beginning of breeding, the indicator of average body weight in males in all groups increased to 31.5 grams, in females, body weight also increased and was within 30-31 grams. Pregnant females, depending on the number of gestating fetuses and the stage of pregnancy, reached a weight of 45-53 grams. Fasting days changed the rhythmic growth rate of young animals but did not adversely affect the weight and linear parameters of mice that received fasting days once or twice a week. The body length of females and males in all groups averaged 8.6-8.7 cm. One year after the start of the breeding season, the bodyweight of mice increased slightly. The average weight of males in the experimental groups was 34,8 grams, and in the control, the weight of males was 33.7 grams, i.e. 1 gram less. Somewhat large differences in body mass indices in females of different groups. In the experimental groups, adult females, despite the presence of fasting days, weighed, on average, 34 grams, and in females in the control, the average body weight was 3 grams less. Consequently, fasting days did not adversely affect the dimensional performance of experimental animals. On the contrary, there was a positive trend in increasing body mass in both females and males.

The number of litters in different females of the control group ranged from 1 to 5 and, on average, was 2.5 litters per female for the entire breeding period. In mice starving 1 day per week, the number of litters ranged from 3-8 and averaged 6.3 litters per female. In mice of the second experimental group, with two fasting days, the reproductive activity of females was the highest. In individual females, the number of litters reached 10-11 and, on average, amounted to 6.8 litters per female. This is 2.4 times more than in the control group (Table 1).

**Table 1**: The results of the propagation of laboratory mice during food unloading

| Groups animals | Number of pairs in Group | Number litters on average on the female | Number born mice average on the female | Number well-fed mice average on the female | Survivors mice (%) at the age of 1 month | Death mice (%) before weaning |
|---|---|---|---|---|---|---|
| 1 Unloading day in a week | 5 | 6.3±0.13 | 37.8±0.61 | 25.8±0.45 | 68.2 | 31.8 |
| 2 Unloading days in a week | 5 | 6.8±0.70 | 47.0±0.13 | 41.0±0.38 | 87.2 | 12.8 |
| The control | 5 | 2.5±0.63 | 15.0±0.28 | 7.3±0.46 | 48.7 | 48.7 |

During the entire breeding period in the group with one fasting day, the fecundity of mice averaged 6,3 cubs per female. The highest fecundity was in the group with two fasting days. In the first year, an average of 10 mice per female. In the second year of life, the fertility of females of this group decreased slightly and the average fertility for the entire period was 6.9 mice per female. The control group showed the lowest fecundity for the entire breeding period of 5.5 cubs per female.

The number of mice born is also significantly different in different groups. So in the experimental group with one day of fasting, 37.8 mice were born per female. Starving people twice a week have 47 mice, which is three times more than in the control group, where, over the entire observation period, on average, only 15 cubs were born per female, of which 7.3 calves survived and were bred the female. Therefore, the departure of young animals in the postembryonic period in the control group amounted to more than 50% (Figure 1).

The bodyweight of newborn mice in groups in different reproductive cycles had certain differences. In the first reproductive period, there was a slight excess in body weight in newborns of the control group. The average weight of the newborn was 1.48 grams. In the experimental group with one fasting day, the weight of the newborn mouse was 1.34 grams, and in the group, with two fasting days, this figure was 1.35 grams. The lower body weight in newborn mice in the experimental groups is objectively associated with a large number of offspring in offspring in starving females, and not with embryogenesis pathology. In subsequent reproductive cycles in the experimental groups, the average body weight in newborns increased to 1.8 grams in the group with one fasting day and up to 1.5 grams in the group with two fasting days, despite the steadily increased fertility. The body weight in newborn babies in the control group in subsequent reproductive cycles remained unchanged. Indicators of the safety of cubs in the post-embryonic period indicate the usefulness of the young obtained from starving mice. The viability level of mice obtained in different groups had significant differences (Table 1). The mice from the first offspring obtained in the control group had low viability and all died during the lactation period. In the future, the safety of young animals in this group improved significantly and amounted to 48.7%, but remained significantly lower than in the experimental groups. In the first experimental group with one fasting day, 68.2% of the mice that were born were preserved and reared before precipitation. In the group with two days of fasting, 87,2% of the mice of the total number of newborns in this group were raised to deposit.

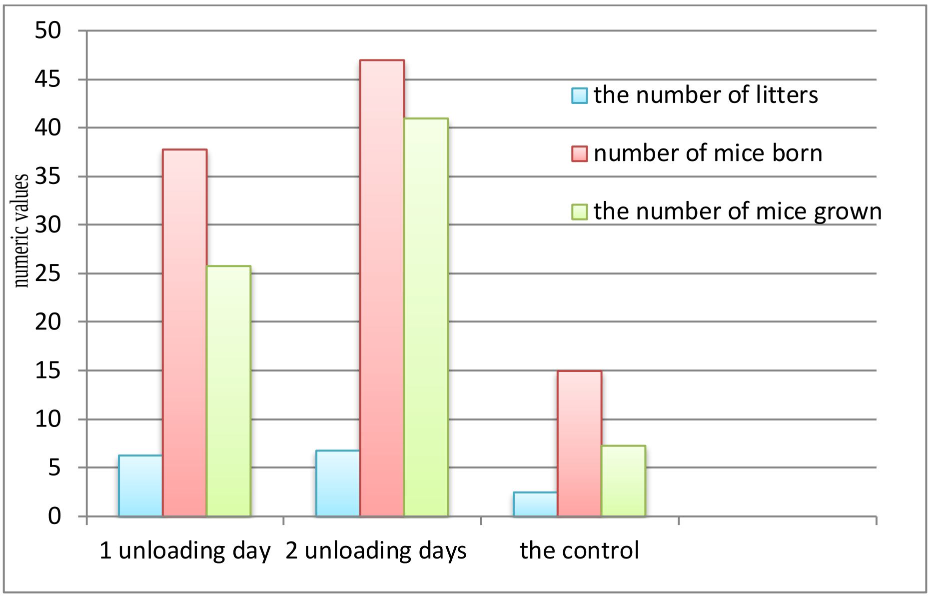


**Figure 1**: Results of breeding mice with one and two fasting days per week and in control.

The safety of young animals is an indicator not only of the increased viability of young animals obtained from “starving” females, but also of the high milk production of females. Milkness in female mice of all groups was determined in the first reproductive period. We used the technique used in rabbit breeding (Gogelija, 1966; Zabolotskih, 2005; Nigmatullin, 2011), with a milk ratio of 2. The calculations included only a 20-day period of milk feeding before the use of young vegetable feed. During the lactation period, the fasting days were canceled, and after the young were deposited, they were resumed and carried out weekly according to the previously outlined scheme. According to the calculations made over a twenty-day lactation period in the first experimental group with one fasting day, the milk yield indicator was within 83 grams of milk per female. In the second experimental group, with two days of fasting and fertility of up to 10 calves, the milk yield in mice reached 118 grams of milk per female, which is two times more than in females of the control group, in which milk averaged 58 grams of milk per female.

The use of one- and two-day unloading periods had a positive effect on the duration of the reproductive period of starving mice (Table 2).

**Table 2**: Duration of reproductive period and life of laboratory mice with food unloading

| Groups animals | Number of pairs in Group | Duration reproductive period (months) | Duration all life (months) | Attitude period breeding mice to duration life in % |
|---|---|---|---|---|
| 1 Unloading day in a week | 5 | 12.0 | 23.6 | 50.8 |
| 2 Unloading days in a week | 5 | 22.3 | 28.9 | 77.2 |
| The control | 5 | 4.0 | 19.4 | 20.6 |

In the group with one fasting day, the reproductive period lasted 12 months, and in mice with two fasting days, reproduction lasted an average of 22.3 months, that is, about two years. This is 5,5 times more than in the control group, where the duration of the reproductive period was only 4 months, that is, less than six months.

Positive changes were also obtained in terms of the life expectancy of experimental animals. In the first experimental group with one fasting day, females, on average, lived 733, and males 685 days. An even more significant increase in life expectancy was observed in the group with two fasting days per week. Some animals in this group lived more than 1050 days and on average 28.9 months. The life expectancy of mice of the control group in females averaged 563 days and in males 604 days, which is 21-22% less than in the first experimental group and 50% less compared to the life expectancy of mice in the second experimental group.

The observed ratio of life expectancy between females and males in starving mice is in good agreement with the well-known general biological pattern, characterized by increased life stability of females compared to males (Zapadnjuk, 1983; Sidorchuk, 2009). In both experimental groups, the life expectancy of females is 1-1.5 months longer than that of males. In the control group, females lived on average 40 days less than males. Such a mismatch in the ratio of the life expectancy of females and males in the control group can be explained by the imperfection of the technology for keeping and feeding animals adopted in zooculture. A peculiar violation in energy metabolism leads to a decrease in the overall life stability of animals and a decrease in life expectancy.

In the complete absence of feed, life expectancy in mice of different sex and age ranged from 2 to 10 days. The most resistant to complete starvation were adult females. Before fasting, the bodyweight of females averaged 31.2 grams. The fatness index (the ratio of body weight to body length) ranged from 3.2-4.5. During the starvation of mice, a gradual decrease in body weight was observed. In individuals, the final weight parameters decreased by 23-53% of the initial weight of the animal.

The maximum life expectancy without feed was observed in the female who had the largest body weight (35.6 grams) and the highest fatness index (4.5). But from the results of the experiments, it can be seen that the other female, who had the smallest body mass among adult females and a low fatness index, also lived without food close to the deadline of 9 days. While one of the mice, which had a relatively high fatness index, died without food after three days, that is, a week earlier than the first. Not only body weight and fatness index affect the level of animal resilience to prolonged fasting. Of great importance is the intensity of metabolic processes, which largely determine the rate of weight loss during fasting. For example, in an adult 80-day old female, during the first three days of fasting, body weight decreased by only 18.5%, and in a female who died three days after the start of fasting, the weight loss was 23.3%, i.e. almost 5% more. Probably, in this animal, the adaptation mechanisms turned out to be less perfect, which led to an accelerated consumption of internal energy resources and an intensive decrease in body weight. A similar pattern was observed in starving males. Adult males of white mice were not inferior in size to females. The body length parameters of the males were 0.6 cm, the masses were 6.7 grams more than the females, and the fatness index was 0.6. Males were less resistant to starvation, in contrast to females. However, there are observations when males with a relatively small mass and a low level of fatness lived without food for a long enough time, while others, on the contrary, having a high level of fatness died quickly.

The presence or absence of drinking water in white mice did not significantly affect the resistance of males to starvation. Life expectancy at absolute fasting without water in adult males ranged from 3-6 days, and during fasting with water was observed in the range from 2-7 days. Loss of body weight in males, with absolute starvation, ranged from 26-44% and averaged 35%. With complete starvation, male indicators of weight loss were in the range from 22-38%, on average, a decrease in mass occurred by 32% from the initial level.

Of great importance in resistance to starvation is the age factor. In adult females, the average life expectancy during complete fasting was almost 8 days, and in adult males about 4.5 days. The decrease in body weight in starving females at the age of 80 days was within 35-41% and in adult males 32.2-35.0%. At the age of 45 days, the average life expectancy in males reached 3.6, and in females up to 3.8 days. The decrease in body weight in males was 33.9%. and in females 34.5%. Males aged 30-35 days, died within 3 days. The average decrease in body weight during the starvation of young males was 24.6%, which is 7-10% less than in adults. In this experiment, the males were not inferior to the females in linear dimensions, and in terms of fatness and body weight were 5-6% superior to females of the same age. Despite this, the life expectancy of males was somewhat less than that of females.

## 4 CONCLUSION

Based on the obtained experimental results, the following conclusions can be drawn: the use of one and two fasting days per week does not violate the reproductive cycle of females and the insemination ability of males; lengthens the reproductive period and the total life expectancy of animals; the use of fasting days increases milk production in lactating females and ensures good viability of young animals; female mice are more resistant to nutritional deficiencies than males; the limits of adaptive capabilities and resistance to starvation in mice significantly depend on age and to a lesser extent on the level of fatness.

Food unloading is biologically and scientifically substantiated, it is an important structural link in any biological system, contributing to the realization of the reproductive potential and vitality of animals of different taxonomic groups. The development and use of the discharge method in zooculture are technologically feasible and cost-effective (Kerimbekov 1989; Zabolotskih 2000; 2004; 2005). The unloading method should take its rightful place in progressive zootechnology and, as an innovative element, be more actively used in the national economy.

## 5 ACKNOWLEDGMENTS

I express my deep gratitude to my colleague and mentor, Yuri Stepanovich, Zabolotsky. His help, support, and extraordinary performance have become decisive in carrying out the work to which this article is devoted.

## 6 AVAILABILITY OF DATA AND MATERIAL

Information can be made available by contacting the corresponding author.

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**Note**: The original version of this article has been reviewed, accepted, and presented at the International Scientific and Practical Conference “From Inertia to Development: Scientific and Innovative Support for Agriculture” (IDSISA2020) at the Ural State Agrarian University, Ural, Russia, during 19-20 February 2020..