Original Article

BREEDING PERFORMANCE OF NEWLY ACQUIRED NEW ZEALAND WHITE RABBITS IN LABORATORY ANIMAL RESOURCE UNIT (LARU), INSTITUTE FOR MEDICAL RESEARCH (IMR) MALAYSIA

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ABSTRACT

The rabbit breeding program conducted in 2021 at the Rabbit Breeding Unit of the Laboratory Animal Resource Unit (LARU), Institute for Medical Research (IMR), was designed to evaluate the reproductive performance of newly arrived New Zealand White rabbits within a structured breeding and management system. The key aspects of the study included random pairing and mating of rabbits every three weeks. All six rabbits in the program were housed under natural conditions, with ambient temperature and a uniform feeding regimen. The findings revealed that does with larger litters exhibited higher pre-weaning mortality rates, indicating a potential challenge associated with litter size. Interestingly, factors such as ambient temperature did not significantly impact gestation length, conception rate, kindling rate, or parental mortality rates. This suggests that the breeding success may be more closely linked to husbandry practices and overall management rather than external environmental conditions. Despite this being the first breeding cycle for all rabbits, the program's success underscores the importance of effective husbandry, management, and dedicated care in achieving positive reproductive outcomes. This study emphasised the importance of proper husbandry and management practices in ensuring breeding success while minimising stress on the animals.

KEYWORDS: rabbits, reproductive performance, comprehensive breeding and management, ambient temperature, breeding cycle

INTRODUCTION

The New Zealand White (NZW) rabbit (*Oryctolagus cuniculus*) is one of the most commonly used rabbit breeds in biomedical research. This popularity is attributed to their less aggressive nature, fewer health complications compared to other breeds, and high reproductive rate when compared to other livestock species (1). Female rabbits breed at five months old for smaller breeds, 6 to 7 months for medium-sized breeds, and up to 9 to 12 months for larger breeds, or when they reach approximately 75% of their adult weight (2). Male rabbits typically mature one month later than females (3). Unlike other mammals, rabbits do not have a specific oestrous cycle. Similar to humans, the release of an ovum in rabbits is triggered by a cycle of hormones; however, in rabbits, sexual intercourse itself stimulates the release of the ovum. (3) Instead, they are generally receptive to mating for 14 days in a 15-day cycle. Signs of receptiveness in females, or does, include a change in vulva colour and moisture from a pink, dry appearance to a red and moist one (4). In our program, a 1:1 mating ratio was practised, which differs from other breeding programs that use 2 to 5 females, or up to 10 females, with one buck (2,5).

Rabbit breeding can be done using four primary methods: natural mating, forced mating, confined mating, and artificial insemination. Natural mating is the most commonly used method and has been shown to achieve high conception rates, often exceeding 90% when the doe is in heat (6). In this method, the doe is placed in the buck's cage for mating. Given that rabbits are territorial, female rabbits often exhibit aggression to protect their space if males are introduced into their cages (2). In some breeding programs, a buck can be paired with two to five does, and in group breeding systems, one buck with two does in the same pen can also be effective (5).

Male rabbits can mate with up to 10 females, but it is important to limit this to no more than two or three times a week to avoid overbreeding (2). The gestation period in rabbits is relatively short, averaging 28 to 35 days. The risk of stillbirth increases if the pregnancy extends beyond day 32 (4). Does can be rebred immediately after kindling or giving birth, though lactation reduces their receptiveness until after the weaning period (4,7). Rabbits are known for producing large litters, typically ranging from 4 to 12 offspring per birth. In commercial rabbit farming, does can produce up to 60 weaned kits per year under intensive breeding programs (7). Does nurse their young twice daily, with each nursing session lasting approximately 5 to 10 minutes, depending on the doe's cooperation.

This study aimed to provide a deeper understanding of the breeding success and reproductive performance of NZW rabbits in open-house laboratory settings. By examining the effects of comprehensive husbandry practices and efficient laboratory management systems, this research aimed to optimise breeding outcomes, improve animal welfare, and support the development of best practices in rabbit breeding for biomedical research. Understanding these factors is essential for increasing breeding efficiency, reducing the risk of reproductive failures, and ensuring the health and productivity of rabbits used in scientific studies, ultimately contributing to more reliable and ethical research practices. By studying breeding management systems, researchers can identify optimal breeding practices, leading to the production of higher-quality offspring and improved overall breeding outcomes. Furthermore, this study will serve as a resource for new researchers at the Institute for Medical Research (IMR), emphasising the importance of proper animal care that aligns with effective research practices. Thus, it will ensure that animals are well cared for while being effectively utilised in scientific experiments.

MATERIALS AND METHODS

Subject of Study: NZW Rabbits

In November 2020, the Laboratory Animal Resource Unit **(**LARU) received three pairs of NZW rabbits for breeding purposes. Upon arrival, all six rabbits were approximately 3 months old. A thorough physical examination revealed that all rabbits were bright, alert, and responsive, with no abnormalities detected.

The rabbits were individually identified for tracking and monitoring:

- Male Rabbits: H01, H02, and H03
- Female Rabbits: H04, H05, and H06

These rabbits were monitored for health, behaviour, and reproductive outcomes during the breeding program.

Animal housing

These newly arrived rabbits underwent a quarantine period of two weeks to ensure they were free from infectious diseases. They were kept in conventional standard individual stainless-steel cages, which are commonly used in laboratory animal housing due to their ease of cleaning and maintenance. The cages were equipped with corncob bedding in the tray beneath the cage, which was replaced and cleaned every 5 days to maintain a hygienic environment. The lighting schedule was set up to 8-9 hours of light and 14-15 hours of darkness.

The temperature within the breeding room was carefully maintained between 24°C and 34°C to ensure the rabbits' well-being, as extreme temperatures can affect breeding success and animal health. All rabbits were provided with a balanced diet consisting of a variety of fresh vegetables such as cabbage, cauliflower, broccoli, lettuce, carrots, pak choi, mustard, and spinach. In addition to the vegetables, they were fed a commercial pelleted diet daily, with the ingredient composition detailed in Table 1, to meet their nutritional requirements. Clean tap water was available ad libitum to ensure proper hydration.

Table 1: Commercial pelleted ingredients' percentage

Ingredients	%
Crude protein	17.5
Crude fat	4.0
Crude fibres	14.5

Breeding program

The breeding program began in February 2021, two months after the rabbits' arrival, following the timeline outlined in Table 2. The rabbits were divided into three male-female pairs, labelled H01, H02, and H03 for the males, and H04, H05, and H06 for the females. A natural mating method was used in the breeding program, where the does (females) were introduced into the bucks' (males) cages between 0800 and 1000 hours for three consecutive days. During this time, mating behaviour was observed, with successful mating occurring when the male rabbit mounted and grasped the female.

To manage the breeding process efficiently, mating occurred at one-week intervals, providing adequate time for the caretaker to focus on other essential tasks such as feeding, cleaning, and monitoring the rabbits' health without being overwhelmed by constant breeding cycles. It gives the caretaker enough time to attend to each rabbit's needs. This interval also allows for proper documentation of reproductive data.

Table 2. The first breeding program planner for the year 2021

P (Parental Generation) ID	Breeding Date	Estimated Delivery Date
H01 x H04	2 to 4 February 2021 (3 days) 0800 to 1000 (2 hours)	4 March to 9 March 2021
H02 x H05	9 to 11 February 2021 (3 days) 0800 to 1000 (2 hours)	11 March to 16 March 2021
H03 x H06	16 to 18 February 2021 (3 days) 0800 to 1000 (2 hours)	16 March to 23 March 2021

During the observation period, the rabbits were monitored three times per day: early morning from 0800 to 0900, early afternoon from 1200 to 1300, and late evening from 1600 to 1700. The behaviours observed included feeding, drinking, and body care behaviours such as grooming, scratching, and resting. Reproductive and productive performance was also monitored, including the gestation period, litter size for each doe, and conception rate (CR), calculated according to a previous study (8). Other parameters observed included the kindling rate (%), pre-weaning mortality rate (30 days) (%), and the mortality rate of breeding rabbits (%). Reproductive and productive parameters in breeding rabbits are shown in Table 4. The hairless, blind, and deaf kits were placed in a container lined with tissue paper to observe their faeces and urine. The tissue was changed 3 times or more, daily. The litter's containers were separated and labelled based on their parents' ID. Adult rabbits were caged individually to prevent unwanted fights and were re-bred. Observation for any abnormality in behaviour, discharge and casualty was easier with individual cages. Weaning was carried out at 4 to 5 weeks of age for all does. During this period, any abnormalities in behaviour, discharge, or casualties were closely monitored, as individual cages allowed for better observation and intervention when necessary.

RESULTS

The mating process was observed closely. Upon introduction of the bucks to the does, the males displayed typical courtship behaviour, including sniffing and licking the females, with no signs of aggression or fighting. The males began spraying urine on the females, which is a common behaviour in rabbits to signal readiness for mating. In response, the females flattened their bodies against the floor, and the males started to grasp her and mount the partner. Throughout the entire process, no abnormal behaviour was noted, and all rabbits continued their usual feeding, drinking, and grooming activities without any disruptions.

All rabbits showed normal feeding and drinking behaviour and performed body care activities as usual. The summary of animal behaviour observed during the breeding period is shown in Table 3. There was no abnormal behaviour observed, particularly in female rabbits during pregnancy. Abdominal palpation was conducted on the 12th–14th days post-mating to confirm pregnancy as shown in Table 4. As a result of abdominal palpation, it was confirmed that all three does were pregnant. One doe, identified as H06, exhibited signs of nesting a day before giving birth. The doe plucks hair from her abdomen and builds a nest in the nest box. However, another two does (H05 and H04) showed no signs of nest building but showed signs of aggressive behaviour such as excessive scratching and thumping her hind legs when observed (H04). On the 27th and 28th days of pregnancy, a specially designed nest box, cut on one side, was placed in the doe cage to facilitate easier access. This modification allowed the doe to enter and exit the box with more ease. Kindling occurred during the early morning hours, and all 24 kits were born healthy, with no congenital defects. However, some kits were noticeably smaller than their siblings. After birth, the does remained with their kits for a few hours, after which all kits were removed from the doe's cage.

Table 3. The summary of animal behaviour observed during the breeding period

Behavioural parameter	Feeding	Drinking	Body care	Resting
In the early morning (0800- 0900am)	Rabbit eats more leaves than pellets.	Consumption of water from the nipple drinker.	Body grooming (female) Male sniffing, licking on female Male mounting on female	Resting on abdominal posture and lateral posture (both).
In the early afternoon (1200-1300pm)	Rabbit eats pellets.	Consumption of water from the nipple drinker.	Body grooming (both) Licking and nipping the fur (female) Seating and scratching the body (both)	Resting on abdominal posture and lateral posture (both).
In the late evening (1600- 1700pm)	Rabbit eats both leaves and pellets.	Consumption of water from the nipple drinker.	Body grooming (both) Licking and nipping the fur (female) Seating and scratching the body (both) Male mounting on female	Resting on abdominal posture and lateral posture (both).

Table 4. The reproductive and productive parameters in breeding rabbits

P (Parental Generation) ID	Abdominal palpation	Delivery Date	Gestation Length s (days)	Litter size at birth (head)	Conception Rate of P (%)	Kindling rate (%)	Pre-weaning Mortality (30 days)	Mortality Rate of P (%)
H01 x H04	18 February 2021	5 March 2021	±31	9	100	100	44.44 %	0
H02 x H05	25 February 2021	11 March 2021	±33	6	100	100	0	0
H03 x H06	4 March 2021	21 March 2021	±33	9	100	100	22.22%	0

Since this was the first pregnancy for all, they were unable to nurse all the kits effectively. The doe nursed twice daily with assistance from a caretaker, spending approximately 5 to 10 minutes per session, depending on the cooperation of the doe during nursing. The caretaker made sure that all kits got enough milk when the little bellies were round, even distended (Figure 1a and 1b). H06 gave birth to 9 kittens of various sizes, averaging from 1.3g to 1.9g. We found that the smallest kits had difficulty in feeding and needed extra care by the caretaker, even though H06's mother gave good cooperation and nursed well. The smallest kits were given extra milk from another two does. However, from our observation, we noticed that H04 had some difficulty feeding her kits and had some aggressive behaviour towards them. It seems H04 could not accept her kitten's presence and tried to hurt and caused some superficial injury to her kits during feeding. Injured kittens were treated with iodine and separated from other siblings for better monitoring and care. Kits need to be reared by a caretaker, but the death rate is high. H05 served as their foster mother because she had the smallest litter size compared to the other two. Additionally, some of the weaker or smaller kittens were fed milk replacers, as they struggled with direct feeding either from their biological mother or foster mother. All healthy kittens survived through the pre-weaning stage, as shown in Figure 1(c).

Figure 1. (a) LARU staff assisted the kitten in suckling the nipple, (b) hand-feeding the kittens, and (c) healthy kittens survived after pre-weaning

DISCUSSION

This was the first breeding program in 2021, and it was successful. All does used in this breeding program were bred with healthy kittens, and the total number of newborns was 24. Several factors influenced the success of this rabbit breeding, including reproduction, feeding, environment and management practices.

The 1:1 ratio was chosen for ease of monitoring the breeding performance of each animal since this is their first breeding program. The right time to mate is determined by checking the female's reproductive organs externally (4). The vulva becomes swollen and pink-purple or reddish-purple when she is receptive, and the vulva appears narrow and pale during anestrus. In our breeding program, we observed that the female's vulva turned red. The first mating attempt involved placing a female rabbit into the male's cage, which occurred on the first day, typically between 8:00 a.m. and 10:00 a.m. The mating process was observed. Upon introduction, the buck follows the doe, sniffing and licking her, and no fighting behaviour is observed. As mentioned by a previous study (7), the natural mating behaviour was observed in our program too. The female flattens her body to the ground as the males begin to spray her with his urine, and the males then grab her and mount the partner. After mating, ovulation typically occurred 10 to 13 hours later (4,9) before returning the doe to her enclosure. Although a single mating is often sufficient to stimulate ovulation, we choose to leave them together at least for 3 days with close supervision. The animals were observed to be comfortable with their partners, and no signs of aggressive or fighting behaviour were observed over the three days. This suggests that the rabbits were successfully acclimatised to one another, and their interactions were peaceful, allowing for a stable environment conducive to breeding. Another factor that limits the conception is the environmental factor. In extreme heat temperatures such as 30^{o}C to 32^{o}C, the rabbits tend to become temporarily sterile (6). The ambient temperature plays an important role in animal production, where high temperatures will affect the reproductive performance of rabbits and restrict their breeding season (10). Temperature also determines the gestation period. A previous study reported that the gestation length (days) was longer in summer at 31.71 days and 31.22 days during winter, where the experiment was done in a room with a temperature of 28.7^{o}C to 31.8^{o}C during summer and 13^{o}C to 20.4^{o}C during winter (11). On the contrary, Balabel (2004) found in his experiments that high temperature significantly decreased the gestation period to 29 days when he carried out his experiment in a natural room condition with 33^{o}C to 35^{o}C. However, the results of our findings found that the gestation period was 31 to 33 days with ambient temperature ranging from 24^{o}C to 34^{o}C during the mating period and 25^{o}C to 35^{o}C during kindling. The high range of room temperature in this study can be attributed to the fact that the rabbits were kept in a natural room environment, where the ambient temperature naturally influenced the temperature within the rabbit room. In this setting, fluctuations in the outdoor climate, such as changes in weather or season, would directly impact the internal temperature of the room, leading to variations in the conditions to which the rabbits were exposed. This could result in a wider range of temperatures during the study period, which may affect factors like breeding behaviour, gestation length, and litter size.

Temperature not only affects the gestation length, but it also affects the number of litter sizes. Previous studies (11,12) have shown that litter size numbers will decrease in higher temperatures; however, this was not reported to our breeding program. Although the temperature in Malaysia is relatively high compared to other regions, it does not appear to affect the litter size produced in our laboratory. We were able to maintain good husbandry practices, and even with the ambient temperature ranging from 24°C to 34°C, we were able to achieve a larger litter size compared to the two previous studies. The comparison data is summarised in Table 5.

Table 5. Comparison between gestation period, litter size and room temperature between three studies.

Data Source	LARU (2021)	Mady et al. (2018)	Balabel (2004)
Gestation period	31 to 33 days	31.71 to 31.2 days	29 days
Litter size	6 to 9	4.56	5
Room temperature (malting period)	24°C to 34°C	28.7°C to 31.8°C (summer) 13°C to 20.4°C (winter)	33°C to 35°C

Good management and care of the rabbit will determine the survival of the kittens, apart from being free from any disease. With competent veterinary care and diligent attention from the caretakers, these kittens were well cared for and nursed accordingly. However, hand-raising can be challenging, particularly during the weaning process, which is a critical time when young rabbits are vulnerable to illness.

At around 3 weeks old, we noticed that some of the kittens started showing abnormal performance of body weight and their health began to decline. Abnormal behaviour was observed, such as kits not suckling well or refusing when nursing, being dull and depressed, not active and starting to lose weight. One of them showed torticollis, indicating potential health problems. Moreover, competition among littermates for limited teats can exacerbate these issues, particularly in larger litters (13). An increase in litter size will decrease the milk available to each kit (14), and this limitation will affect their survival, leading to higher risks of malnutrition and mortality. Data in Table 3 showed that the pre-weaning mortality rate for H04 is 44.44% and 22.22% for H06. Both does gave birth to 9 kittens compared to H05, with 6 kittens who survived until weaning. The total number of kits that died during pre-weaning periods was 6; 4 kits for H04 and 2 kits for H06. The larger litter size in our data increased the abnormality and increased the pre-weaning mortality rate, as weaker kittens may struggle to compete for milk and nourishment. Similarly, a previous study (15) also reported high pre-weaning mortality in large litter sizes. As a result, the weaker competitors may experience starvation and are more likely to die. Therefore, good husbandry and management are essential to ensure that these young rabbits can live healthily until weaning. Several suggestions and recommendations can be implemented to reduce or potentially eliminate pre-weaning mortality rates in future studies, particularly given that larger litter sizes are associated with higher mortality rates. To ensure the health of the does before breeding, it is crucial to provide a balanced and nutritious diet. Well-nourished does are more likely to successfully nurse larger litters effectively, improving the survival chances of all kits.

Stress can significantly impact maternal behaviour, including nursing and bonding with the kits. Therefore, minimising stress factors such as overcrowding, excessive noise, or frequent disturbances can help ensure that does care for their litter, reducing the risk of abandonment or neglect. Additionally, providing larger or more comfortable nest boxes will ensure that all kits have enough space to stay warm, minimising the risk of chilling or overheating. Increasing monitoring and care for high-risk litters by regularly checking to ensure that all kits are receiving enough milk can also help. Offering extra care or supplementary feeding with a milk replacer may be beneficial for weaker kits to prevent malnutrition or dehydration, thereby lowering mortality rates. To reduce the risk of infections and diseases, the environment should be kept clean and hygienic, with regular cleaning of nesting areas and proper sanitation.

By implementing these suggestions in future studies, pre-weaning mortality rates can be reduced, leading to healthier and more productive rabbit populations in future breeding programs.

CONCLUSION

In conclusion, the initial rabbit breeding program in 2021 emphasised several key factors for achieving successful rabbit production. The program effectively implemented a 1:1 mating ratio and closely monitored natural mating behaviours. The gestation period was found to be between 31 and 33 days, with temperature fluctuations not significantly impacting litter size. Notably, the study observed that pre-weaning mortality rates were higher in does with larger litters, likely due to increased competition for milk and other related factors.

To address this in future studies, the program recommends optimising doe nutrition, minimising stress, providing adequate nesting space, increasing monitoring and care for high-risk litters, and maintaining a clean environment. These measures can help improve kitten survival rates and enhance overall rabbit production efficiency.

The 2021 rabbit breeding program was successful, with the three newly arrived does demonstrating strong reproductive performance during their first breeding cycle. While a positive correlation between large litter sizes and high pre-weaning mortality rates was observed, successful breeding outcomes were largely influenced by effective husbandry, care, and management practices.

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