

## The Effect of Preferable Enrichments in the Laboratory Minipigs

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### ABSTRACT

Miniature pig (minipig) has been considered as an important laboratory animal in the developmental biotechnology researches with respect to xenotransplantation, stem cell, somatic cell nuclear transfer and embryo transfer. Given that the laboratory minipigs are normally housed at an indoor facility, they pass the time with lying or sleeping unless it is feeding time. Therefore, it is necessary to provide environmental enrichments to satisfy their innate needs and to lessen atypical behaviors caused by stress, on the purpose of welfare. We quantitatively investigated the type of preferable enrichment for the laboratory minipigs as well as its effect on their daily life. They presented a great interest to the pliable pail but a rapid loss of attraction to non-preferable enrichments. When the daily life of the single housed minipigs was quantified based on duration of playing or resting, they were more actively engaged in lively activities in the presence of enrichments. In addition, the provision of enrichments could effectively alleviate the conflicts during group housing when new pen mate was introduced, resulting in reduction of wound cases. We believe the considerations of animal welfare are essential to the conduct of better research because animals in the non-stressful environment will be more physiologically stable and provide more reliable results in the animal experiments.

(Key Words : Laboratory minipigs, Welfare, Enrichment, Stress, Housing)

### INTRODUCTION

Miniature pig (minipig) has been considered as an important laboratory animal in the preclinical study, biomedical protocols and new surgical technique development, due to their similarities with human in regards with the anatomical and physiological characteristics (Smith and Swindle, 2006). In addition, they have been recently employed in the developmental biotechnology as the organ donor of xenotransplantation into human, cell source of the stem cells and surrogate in the embryo transfer of somatic nuclear transfer embryos (Hur et al., 2012).

Given that pigs are the omnivores, they spend most of active time to search for food in the wild as a means of gratifying their hunger, curiosity and reduction of boredom; these behaviors are expressed by rooting, snouting, digging, foraging and chewing the food sources (Horback, 2014). However, the laboratory pigs are sedentary and usually pass the time with lying or sleeping unless it is feeding time. Therefore, it is necessary for them to provide environmental

enrichments to gratify their needs, on the purpose of welfare (Smith and Swindle, 2006). In addition, the wild pigs are innately social animals. Once the social dominance order is established, they show the reduction of aggressive interactions in their social groups, and are closely in contact with other members for social playing; if strange pigs are introduced in the stable group, a new social order is established upon new aggressive interaction (Horback, 2014). In contrast with the wild, the laboratory pigs are not able to occupy the enough chances for social relationship in case of single housing for the research setting. Furthermore, group housed pigs may prevalently encounter the situation for aggressive interactions to establish the dominance when the animals are regrouped with others.

Therefore, the welfare of laboratory minipig has been highly highlighted, based on the unique behavioral and husbandry considerations of pig species. Unless welfare is enough, atypical behavior with respect to less activity, play and explorative behavior as well as enhancement of aggressiveness

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to the other pigs can be more provoked (Horback, 2014; Telkänranta et al., 2014; Huntsberry et al., 2008). Although the appropriate environmental enrichments in the rodents and nonhuman primates were widely reported, the studies which enrichments can satisfy or encourage the unique behavior of the laboratory minipigs are now in progress (Hutchinson et al., 2005; Lutz and Novak, 2005). Several studies have shown the preference of rooting materials such as straw, compost, spruce chips, seed grass hay, peat and chopped straw in the farm pigs as enrichments, which are able to satisfy the rooting behavior (Jensen and Pedersen, 2007). Since the farm pigs are normally housed in the concrete floor, the provision of aforementioned rooting materials can be easily provided. However, the rooting materials are hard to be given to the laboratory minipigs living on the plastic grates floor because they can interfere with the manure or cleaning system in the facility and are not the specific pathogen free (SPF) substances; it is necessary to find the alternative enrichments in accordance with the preference of the laboratory minipigs. Therefore, the present study is aimed to quantitatively investigate the preferences and effects of the enrichment in the laboratory minipigs, based on duration of conflicting or playing or resting or sleeping, so as to support welfare and reduce stress as much as possible. We believe the considerations of animal welfare are essential to the conduct of better research because animals maintained in a non-stressful environment will be more physiologically stable and provide more reliable results in the experiments.

## MATERIAL AND METHOD

### 1. Ethics and animals

All procedures with the laboratory minipigs were approved by the Institutional Animal Care and Use Committee (IACUC). A total number of 32 healthy 6 to 12 months old SPF minipigs (Prestige Bioresearch, Singapore) were employed in the present study. The 8 groups (4 male and 4 female groups) with 3 minipigs or 8 groups (4 male and 4 female groups) of a single minipig were housed in case of group housing study or single housing study, respectively. In the group housing, similar size of minipigs was allocated to each pen. The each pen for the single housed minipig was closely placed for tactile, auditory and visual contact to each other.

### 2. General housing and husbandry

The minipig(s) was housed with a dimension of 210 cm length × 165 cm width × 125 cm height, consistent with the recommendation of space requirement for pig from Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) during acclimation and experiment period. The animal room environment was controlled to maintain the temperature at 24-30 °C, humidity at 50-80%, approximately 12 hours light/dark cycle with 150-300 lux and ventilation 10-20 times/hour. A standard certified nutrient and micro-ingredient composition of minipigs maintenance diet (Altromin 9029, Germany) was available to each minipig daily. Municipal water irradiated by ultraviolet light was provided to the minipigs *ad libitum*.

### 3. Preferable enrichments

The 4 types of widely recommended enrichments to the pigs, plastic bottle, metal chain, blanket and pail, were given to the single housed minipigs after an hour of feeding to investigate the preferable enrichment in the laboratory minipigs (Smith and Swindle, 2006; Smith et al., 2009). Because the novelty for an object is important for pigs to initiate the explorative behavior and the loss of novelty makes pigs habituated to the object, the 4 types of enrichments were rotated daily; the provided enrichments to a pen at the previous day was collected, was cleaned and was given to another pen (Van de Weerd et al., 2003; Gifford et al., 2007). Upon providing the enrichments to each pen, the caretaker recorded the playing time of minipig with the enrichments for an hour. The playing time was determined via recording by the stop watch in accordance with the previous report; the playing was defined as manipulating the provided object or housing structures where they were housed, with active behaviors such as walking, smelling, rooting, snouting, biting, pushing, kicking, licking and chewing (Horback, 2014, Telkänranta et al., 2014). These experiments were repeated in quadruplicates.

### 4. The effect of enrichments in minipigs' daily life

The daily life of the laboratory minipigs with or without enrichments was quantified to investigate the effect of enrichment, based on duration of playing or resting and sleeping. The 4 pens of the single housed minipigs occupied the all of 4 kinds of enrichments for a whole day, but other 4 pens did not. At the next day, the provisions or absences of enrichments were

changed to each other. The behavior of minipigs was recorded for both groups throughout the 24 hours using Closed Circuit Television (CCTV) footage and was evaluated by the caretaker. The resting time was defined as lying with no apparent activity, in accordance with the previous report (Jensen and Pedersen, 2007). This experiment was repeated in triplicates.

#### 5. The effects of enrichments in the group housing

The behavioral characteristics with or without enrichments were quantified in the group housed minipigs, based on duration of conflicting or playing or resting or sleeping. The aforementioned enrichments were continuously rotated to the each pen every hour during 12 hours to maintain the novelty of enrichments into the 4 group housed pens. In contrast, enrichments were not placed in other 4 group housed pens. At the next day, the provisions or absences of enrichments were changed to each other as well as a minipig per pen was randomly selected and was shifted to another pen to stimulate the aggressive interaction under the strange situation. In a same manner with the previous experiment, the behavior of minipigs was recorded by CCTV and was evaluated about their daily life. The conflicting time was defined as aggressive behaviors toward pen mates such as belly nosing, ear biting, tail biting and mounting (Horback, 2014). This experiment was repeated in quadruplicates. In addition, the number of conflict-relative wounds (abrasion, laceration, puncture, contusion, bleeding and abscess) was counted during the experiments, followed by proper treatments.

#### 6. Statistical analysis

Student's T test or One-way analysis of variance (ANOVA) with Duncan's post hoc test was used to analyze for significant differences by PASW software (SPSS, Chicago, IL, USA). A p value less than 0.05 was considered to be significant.

## RESULT

### 1. Preferable enrichments

When the 4 types of enrichments were provided, minipigs displayed the explorations with pushing against objects with their snouts, a behavior known as rooting, and/or chewing the objects (Fig. 1a). Of particular, they significantly presented the highest interests to pail than others (Fig. 1b). In case of the pail, minipigs could fit its own head in the pail, raising its curiosity which further stirred its interest for a long time. In contrast, minipigs showed chewing behavior to other objects first, thereafter, they quickly lost interests to the objects. The loss of novelty that meant pigs became habituated to objects was recorded within several minutes, except for the pail.

### 2. The effect of enrichments in minipigs' daily life

The durations of playing, resting and sleeping times in the single housed minipigs with or without enrichments were recorded and quantitatively analyzed (Fig. 2). Presence of enrichments significantly arose playing time with showing touching, snouting, chewing and rooting to the objects, and reduced boredom with no apparent activity. The sleeping time

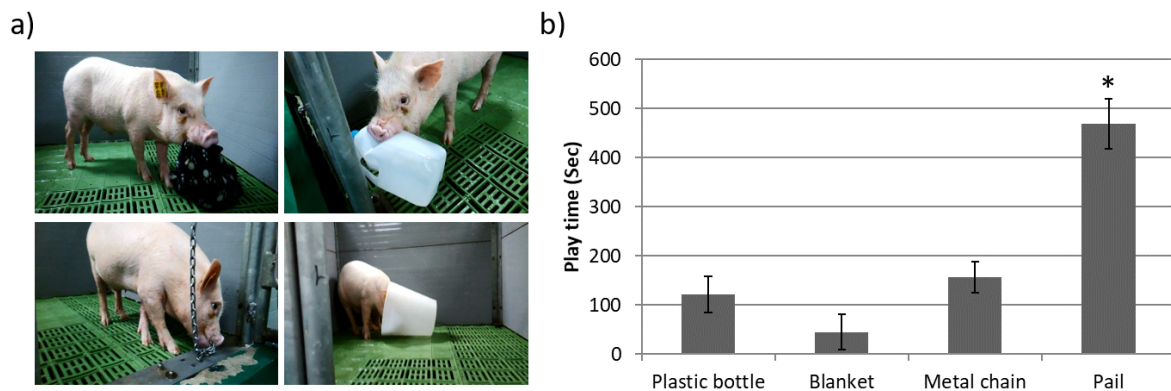


Figure 1. The investigation of preferable enrichments. (a) The 4 types of enrichments (plastic bottle, metal chain, blanket and pail) were separately given to the single housed minipigs for an hour. (b) The preference for each enrichment was quantitatively evaluated, based on duration of playing. Graphs were presented as the mean  $\pm$  SEM. Superscript indicated a significant ( $P < 0.05$ ) difference.

was not related with presence or absence of enrichments. This thus showed that minipigs preferred to spend more time for playing and are more actively engaged in lively activities in the presence of enrichments.

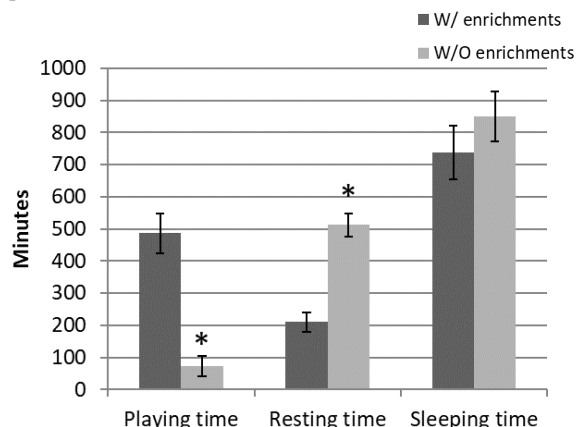


Figure 2. The effect of enrichments in minipigs' daily life. The daily life of minipigs with or without enrichments was quantified to investigate the effect of enrichment, based on duration of playing or resting or sleeping for a whole day. Graphs were presented as the mean  $\pm$  SEM. Superscripts indicated a significant ( $P < 0.05$ ) difference between two groups. W/ enrichment, minipigs with enrichments; W/O enrichment, minipigs without enrichments.

### 3. The effects of enrichments in the group housing

The durations of conflicting, playing, resting and sleeping time with or without enrichments in the group housed minipigs were recorded and quantitatively evaluated (Fig. 3a). The provision of enrichments could effectively alleviate the conflicts

during group housing when new pen mate was introduced, resulting in reduction of wound cases (Fig. 3b). Although the enrichments could not perfectly block the conflicts between strange minipigs, the group housed minipigs with enrichments significantly attempted to spend their daily life to explore and play with the enrichment rather than conflicts to establish the dominance in the pen, in comparison with the counterpart without enrichments; once minipigs with enrichments established the dominance, they spent more time to explore the newly and continuously provided enrichments. There was no a significance in the sleeping time between the groups with or without enrichments in the group housed minipigs, however, the amount of sleeping time tended to be smaller than the single housed minipigs (Fig. 2).

## DISCUSSION

In case of other large animal models, dogs and non-human primates (NHPs), there have been large experiences to understand their behavioral characteristics and wide investigations for proper welfare for them in the laboratory conditions (Ellegaard et al., 2010). Researches on pigs for their welfare have been mainly focused on the ethological approaches to evaluate behavioral needs of pigs, and have been widely conducted on the farm pig for the purpose of more economical production. However, the researches and experiences for welfares of the laboratory minipigs are more limited, even

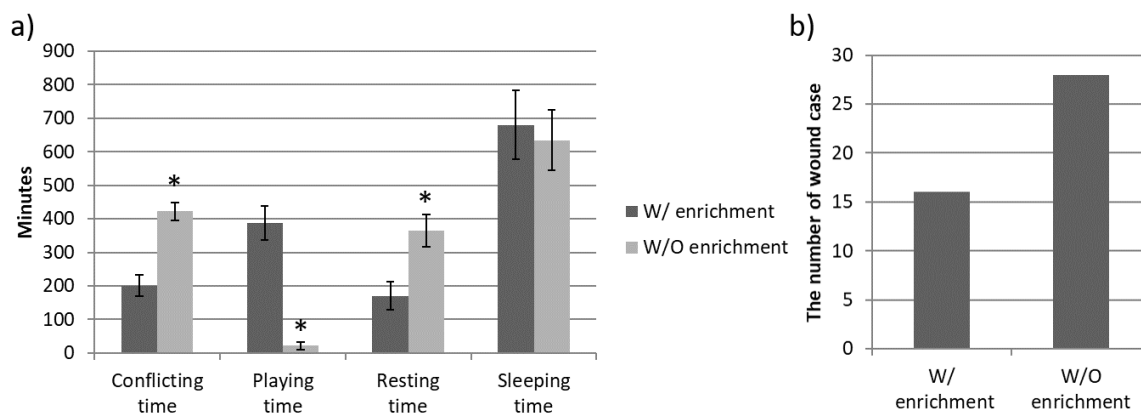


Figure 3. The effects of enrichments in the group housing. (a) The behavioral characteristics with or without enrichments were quantified in the group housed minipigs, based on duration of conflicting or playing or resting or sleeping for a whole day. Graphs were presented as the mean  $\pm$  SEM. Superscripts indicated a significant ( $P < 0.05$ ) difference between two groups. (b) The number of conflict-related wounds was counted during the experiments.

though they are highly important as the laboratory animal for the biomedical research and developmental biotechnology. Therefore, the present studies was carried out to quantitatively uncover the preference of enrichments of minipigs and its effects in the group housing, in order to satisfy their natural behavior, reduce the aggressiveness, minimize the housing problem in the facility and relieve the stress in the laboratory conditions.

The farm or wild pig can obtain the opportunity to manipulate objects by rooting, snouting, biting, pushing, kicking, licking and chewing to bedding/edible substrate (compost, straw, coconut fiber, mushroom peat, stacks of paper, cotton rope and cloth strips) or animal's environment itself (rock, sand, soil and grass), respectively (Blackshaw et al., 1997; Horback, 2014). When the pigs were limited from these behavior indoor, they showed aggressiveness such as destruction of housing structures (gate, water nipple, rubber mat and slatted floor) and conflicts toward pen mates (belly nosing, ear biting, and tail biting), which indicated the stress (Day et al., 1995; Horback, 2014, Munsterhjelm et al., 2010). Therefore, proper welfares have to be provided to satisfy their natural behaviors and to relieve the stress of the laboratory minipigs (Newberry, 1995). The concept of welfares implies that the laboratory animals are maintained in similar conditions with those experienced by their wild counterparts, resulting that the animals experience as little stress as possible under the given laboratory conditions (Ellegaard et al., 2010).

The type of housing is regarded as an important part of welfare in the laboratory animals. During the research with the laboratory minipigs, single housing has widely chosen, due to prevention of possible variables by pen mates and group housing such as conflict with pen mates, competition for feeds, inconvenience for monitoring to individuals and difficulty in handling during sampling. However, the single housed minipigs also experienced the chronic stress because they are social animal (Kanitz et al., 2004). Therefore, groups housing is recommended unless the minipigs are under specific experiment (Ellegaard et al., 2010). In case of groups housing, the behavioral characteristics of pigs are signified; the conflicts is unavoidable to establish of dominance in the society of pigs. In accordance with the present study, the provisions of enrichments could effectively diminish the aggressiveness (fig. 3); once establishing dominance within shorter conflict duration, the novel enrichments could enhance the playing time rather than the lasting conflicting time, in comparison with the

counterparts without enrichments. Meanwhile, it has been widely recommended that each pig should have the visual, olfactory and auditory contacts with each other to prevent social deprivation if the research requires the single housing (Smith and Swindle, 2006). The proper enrichments were additionally able to strengthen welfares in the single housed minipigs; the single housed minipigs without enrichments expressed higher boredom with no apparent activity, in contrast, the minipigs with enrichments showed higher active behaviors (fig. 2). Therefore, both single and group housing require the enrichments to enhance welfare of the laboratory minipigs.

Likewise, enrichments play a larger role in supporting welfare in the laboratory minipigs. The design of enrichments and method to use should be considered in accordance with the behavioral needs of minipigs. In case of design, it was well addressed that pigs had the interests for the chewable, ingestible, deformable and destructible enrichments (Van de Weerd et al., 2003). When several types of enrichments were provided to pigs, the playing time with cone or apple shaped object or ball was  $282 \pm 54$  or  $66 \pm 18$  or  $14 \pm 3$  seconds, respectively; the minipigs preferred to play with pliable cone than inflexible ball (Smith et al., 2009). In agreement with these studies, the laboratory minipigs in the present study showed similar results that they had the greatest interest to the pail similar with pliable cone but showed a rapid loss of attraction to non-preferable enrichments within several minutes (Fig 1b). These results implied the composition and shape of enrichment were necessary to be considered in accordance with their natural behavior. In terms of method to use, the novelty was important to maintain the explorative behavior because loss of novelty in the pigs took place rapidly with becoming habituated to enrichments as shown at figure 2b in the present study (Apple and Craig, 1992; Van de Weerd et al., 2003).

The advantage of enrichments aims to not only the reduction of aggressiveness and stress in the laboratory condition but also the health of minipigs. The playing with enrichments encouraged the physical development, cognitive abilities and coordination of skeletal muscles in the juvenile minipigs. In addition, it related to high levels of acetylcholine, glutamate and opioids, and low cortisol level in the body of various species (Berridge and Kringelbach, 2008; Burgdorf and Panksepp, 2007; Horback, 2014).

In conclusion, we quantitatively investigated the preferable enrichment and the positive effect of enrichments of the laboratory minipigs. Understanding and consideration of the

natural behavior of minipigs is important to ensure animal welfare in the laboratory condition. These results may contribute to better support of welfares in the laboratory condition to assure the reliable results in the animal experiments.

## DISCLOSURE STATEMENT

The authors declare that there are no conflicts of interest.

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