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Development of One-handed Milking Device to Collect Milk From Lactating Rats: Analysis of Feeding With Progressive Lactation

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Neonatal nutrition through mother's milk may be an important environmental factor in rats. Therefore, milk content should occasionally be measured to analyze its quality, but it is difficult to obtain sufficient amounts of rats' milk for analyses. In this study, we developed a new device for sampling milk from rats and used it to evaluate dietary effects during pregnancy and lactation on the growth performance of pups. In addition, certain substances（carp gallbladder extract and pumpkin heated water extract）were given to pregnant Wistar rats and their effects of stimulating mother's milk secretion were examined. Milk samples were obtained using the newly developed device on the 7th, 14th, and 21st postpartum days. The body weights of dams increased during pregnancy, but the milk volume and body weight（pups）were not significantly different. These findings suggest that the above-mentioned dietary supplementation during pregnancy and lactation may not improve milk quantity, but it is necessary to optimize this newly developed device further, even though the milk yield obtained in the present study was adequate.

Key words: lactation, rats, milker, functional food

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INTRODUCTION

Environmental pollution, especially carcinogenic substances and endocrine-disrupting chemicals, has become a major worldwide problem in recent years. The influences of such chemicals on babies through breast milk or cow milk are of great concern. Mother's milk is the only source of nutrition for suckling pups and is ideally suited to ensuring growth and development during the neonatal period. Investigations of the influence of genetic traits and parental eating habits on children have been performed among other factors. However, not only blood, but also milk, which can be supposed to be influential on children, is regarded as an important factor for evaluation［1］.

The ability to gather milk from the rat, a widely used experimental animal, may aid in elucidating the effects of chemicals on infants and their development. To test the composition of rat milk, it is typically gathered from the stomach of rat pups that have nursed once, since it is difficult to gather milk directly from the teats of dams［2, 3］. This milk has thus sometimes been mixed with saliva and gastric juice, and degraded for digestion, so measurement of its actual original composition is difficult. Moreover, milker devices are expensive and large［4］. Regular suction is another major shortcoming; it reduces the price of milk by lowering the quality and hampers proper milking. It is also time-consuming to apply gentle manual pressure and collect milk in capillary tubes［5, 6］. Against this background, our device

was produced to eliminate these problems.

In this study, we have newly developed a one-handed milking device for the sampling of milk in rats. In addition, carp gallbladder and pumpkin extracts, which have effects on milk production, have been used in folk medicine [7, 8, 9]. Those certain substances were given to pregnant Wistar rats and their effects of promoting mother's milk secretion were examined.

MATERIALS AND METHODS

Animals and diet

Male and female rats of the Wistar strain (8 weeks old) were obtained from CLEA Japan, Inc. (Tokyo, Japan). All rats were immediately placed in individual cages, fed a normal diet (CE-2; CLEA Japan, Inc., Tokyo, Japan), and allowed to acclimatize to their new circumstances. The rats were kept at a constant temperature (23 ± 2°C), humidity (55 ± 10%), and a 12-h light cycle (lights on at 07:00 and off at 19:00).

After 4 weeks of acclimatization, animals free of abnormal findings were selected for the experiment. Fifteen female rats over 12 weeks old with confirmed regular estrous cycles were fertilized by a male rat. Mating was confirmed the following morning by the presence of sperm in the vaginal smears and/or vaginal plugs in the litter pan. Pregnant dams were placed in individual nursing cages 2 weeks after the estimated date of vaginal plugs. Once declared pregnant, the rats were divided into three groups (n=5): 【1】 normal diet (control) group; 【2】 carp extract (1% extract essence from *Cyprinus carpio*; Icreo Co. Ltd., Tokyo, Japan)-treated group; and 【3】 pumpkin extract (1% extract essence from *Cucurbita*; Icreo Co. Ltd., Tokyo, Japan)-treated group. During the experiment, the animals were fed these diets. Food consumption and body weight were checked. The day of parturition was considered day 0 of lactation. Within 48 h after delivery, parturition litters were randomly reduced to 8 for each mother rat. Animal care and experimental procedures were approved by the Animal Research Committee of Shimane University and conducted according to the Regulations for Animal Experimentation at Shimane University.

One-handed milking device for milk collection

Milk samples were collected using a vacuum milking system, the one-handed device for milk collection (Fig. 1-A). The apparatus consisted of a milking pipe, a pressure adjustment pipe, an exhaust pipe, and a glass suction cup (made by Natsume Seisakusho Co. Ltd., Tokyo, Japan) connected to polythene tubing (Fig. 1-B). The shape of the milking pipe is like that of an elbow; its edges are applied to a teat of a rat, siphon adjustment is performed by opening and closing a pipe using the index finger, and the suction force is adjusted. The milker is connected to a pump. Siphon pressure adjustment is performed by opening and shutting a pipe (Fig. 1-C). The milking method involves using the index finger during suction in the milking state, having a sample pipe operated by one hand. We acquired a patent (Japan patent: JP 5187628) for this automated experimental milker for rats, through the Collaboration Center of Shimane University.

Milking procedure

Milk samples were obtained on the 7th, 14th, and 21st postpartum days. Pups were separated from their mother 4 h before sampling (Fig. 1-D). Body weights of the dams were measured before milking. The dams were anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg, i.p.). They were administered oxytocin (0.02 U/100 g BW; Sigma-Aldrich Co., LLC, Tokyo, Japan) just before sampling. After oxytocin injection, the mother's udder and periphery were wiped with gauze and moistened with alcohol. The teat was sucked for 15 minutes into the liner of the teat cup after the mammary gland had been gently massaged and milking had started (Fig. 1-E and F). Milk was stored frozen at -30°C until further analysis.

Statistical analysis

Data are expressed as mean ± S.E.M. Differences were tested using analysis of variance (ANOVA) and Scheffe's post hoc test. Analyses were performed using StatView (SAS Institute Inc., Cary, NC, USA). A p value of less than 0.05 was considered statistically significant.

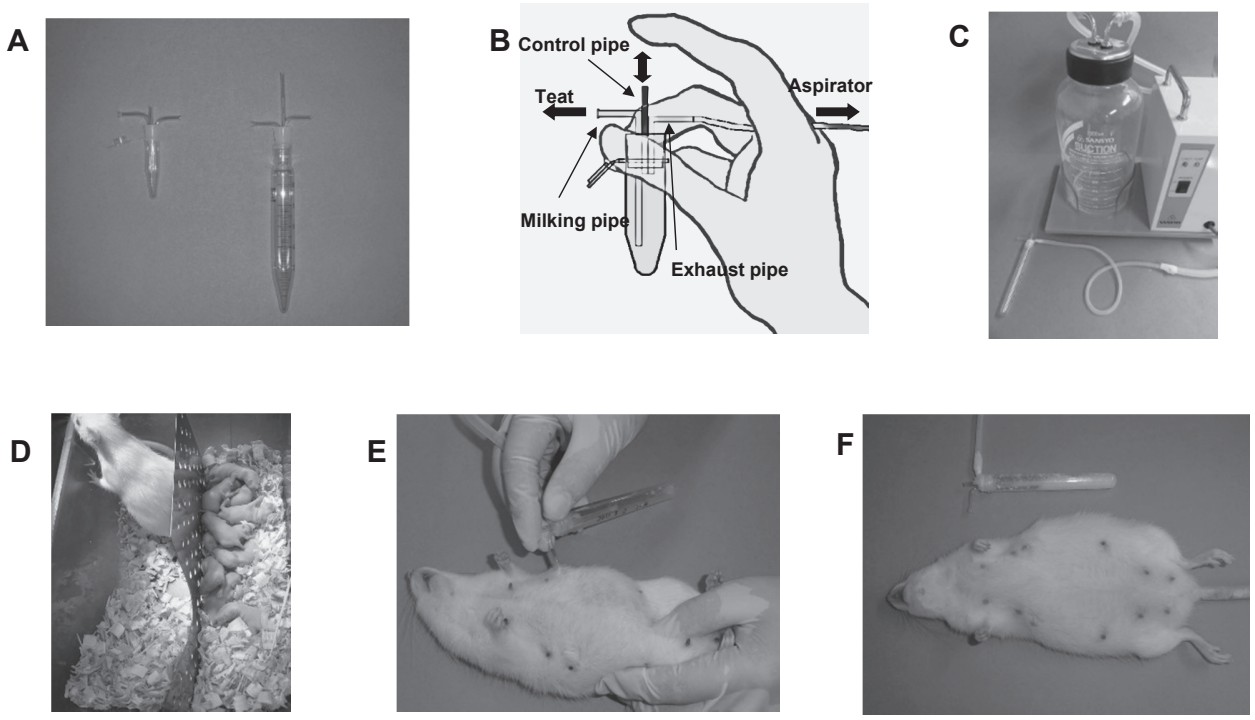

Fig. 1. How to use the one-handed milking device on lactating rats. (A) Device for the milking of mice (left) and rats (right). (B) The apparatus consists of a milking pipe, a pressure adjustment pipe, and an exhaust pipe. (C) The milker is connected to a pump. Siphon pressure adjustment is performed by opening and shutting a pipe. (D) Pups were separated from their mother 4~6 h before sampling. (E) Milk samples were collected using a vacuum milking system. (F) The milk extracted in 15 minutes (about 1000 μl).

RESULTS

Observation of general condition and body weight

Administration of the test materials revealed no clinical signs, adverse effects, or death during the observation period. Body weight changes among the groups are shown in Fig. 2. Until 4 weeks, there were no significant differences in the growth among the three groups.

Food consumption

Figure 3 shows that all groups showed an increase in food consumption, but the differences were not significant among the three groups. Food consumption increased to match the start of eating food by the pups.

Evaluation of the newly developed device and milk amount

As our goal was to develop our new device, we tested the strength of the negative pressure by tapping; it was possible to milk while the device was grasping the nipple and to collect milk. In addition, no signs of bleeding were observed in the milk. As shown in Fig. 4, there were no significant differences among the three groups. In the pumpkin extract group, the quantities of milk were 1.16 ± 0.09, 0.92 ± 0.15, and 1.55 ± 0.13 mL in the first, second, and third weeks, respectively. Milk quantity in the pumpkin extract group was greater in the third week, but there were no significant differences compared with 1.11 ± 0.24, 1.08 ± 0.14, and 0.97 ± 0.27 mL in the control group.

Body weight of the pups

Body weights of the pups are shown in Fig. 5. There were no significant differences in the growth among the three groups.

DISCUSSION

Our study confirmed that the newly developed one-handed milking device is adequate for the sampling of milk in rats. It was proved that this apparatus could be used easily due to its lightweight structure and handy operation in efficient lactation.

Direct and indirect methods of milk collection have previously been considered, for example, milking by hand into a collecting tube or the euthanasia of pups and the removal of milk from their stomach. Takahashi *et al*. [10] and Keen *et al*. [11] also mentioned that milking by massaging the teats is not promising and attractive because it requires excessive pressure, so the tubuli galactophori sometimes become blocked and signs of bleeding appear in the milk. Rodgers [12] developed a method of milking by negative pressure. Temple and Kon [13] produced pulsation in negative pressure using a vacuum pump in the suction through the operation of a piston. Those devices, however, were not popular and practicable owing to their noise and inconsistency during the operation. Watanabe [4] also developed a milker, but it was expensive. In contrast to these alternatives, the functioning of our newly developed device is based on suction produced by negative pressure either induced directly using a vacuum pump or pulsated by hand (Fig. 1). We are currently working to improve this device further in

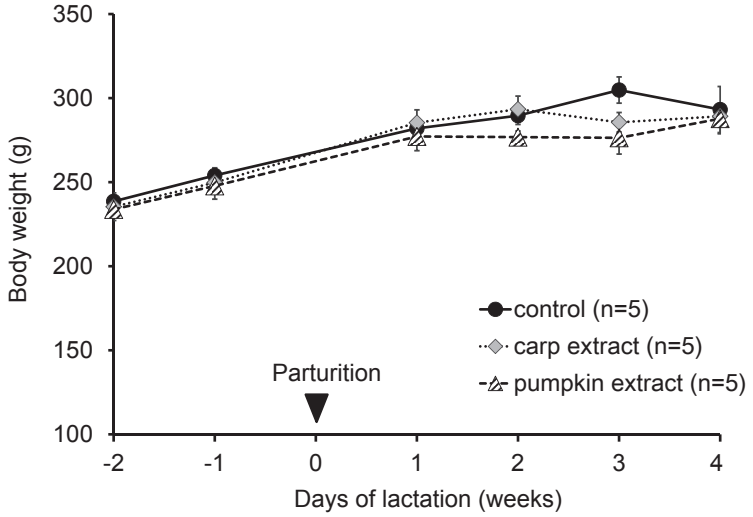

Fig. 2. Body weight during pregnancy and lactation of dams fed three different diets. Data are represented as mean ± SEM.

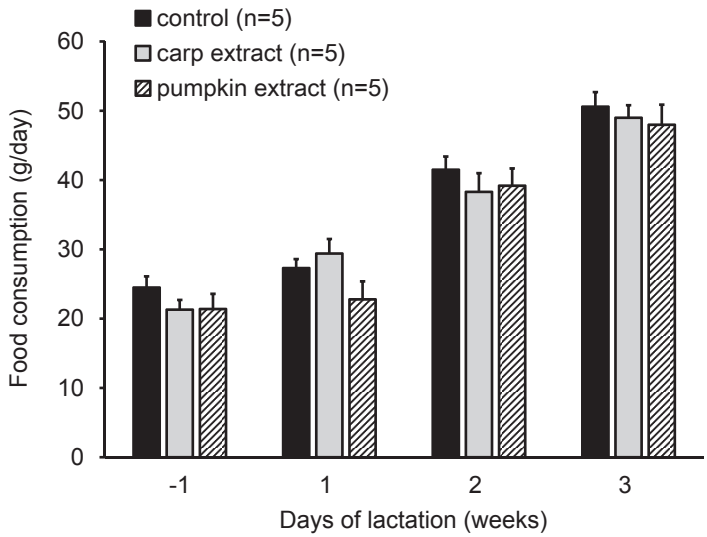

Fig. 3. Changes in food consumption of dams and pups fed three different diets. Data are represented as mean ± SEM.

spite of having obtained an adequate milking yield for chemical analysis.

The assessment of milk production in animals such as rats is difficult. In several reported studies, measurements of pup weight and weight gain were used to determine the milk yield indirectly. However, the direct determination of milk yield can be achieved by measuring pup weight during the suckling period and during a subsequent time course of separation of dams from their pups [14]. Some researchers have suggested that milk yield influences pup number and separation time [15, 16]. Separation periods of greater than 4 h should be avoided [6], but in order to obtain sufficiently large amounts of milk, most studies have employed separation periods of 6-9 h [5]. This study employed a standard period of maternal separation from the litter, in consideration of animal welfare.

Breastfeeding is essential for the survival of newborns. Women with deficient milk production have often used traditional plants to enhance milk production or yield. It was demonstrated that some

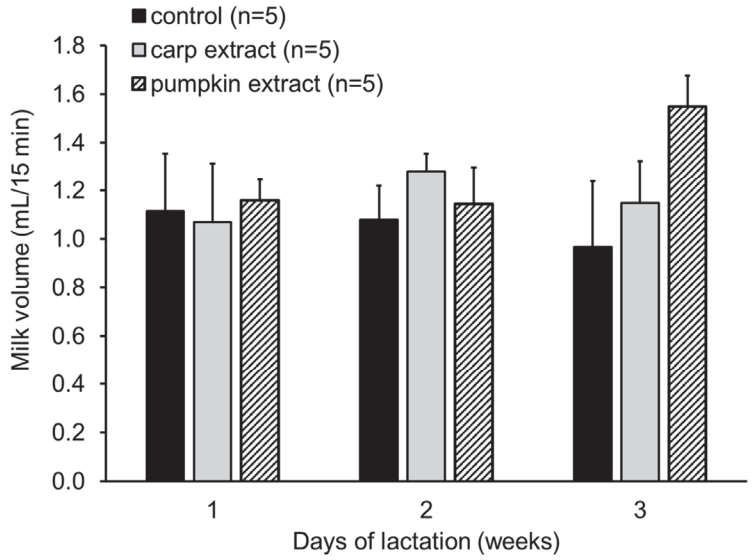

Fig. 4. Changes in milk volume of dams during the lactation period. Data are represented as mean ± SEM.

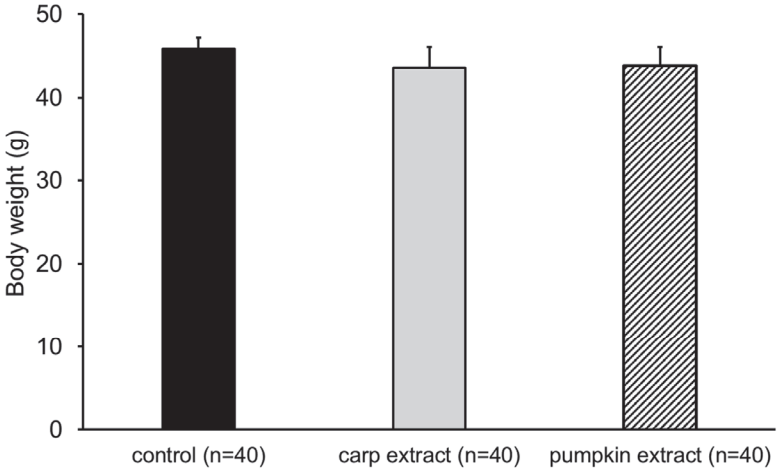

Fig. 5. Body weights of pups at 21 days of age. Data are represented as mean ± SEM. There were no significant differences among the 3 groups.

plants increase milk production via the induction of lactogenic hormones (prolactin), growth hormone, and casein accumulation in the mammary gland [17, 18]. Functional foods are those that, beyond basic nutritional functions, can produce beneficial health effects and should be safe for consumption without medical supervision. Carp gallbladder and pumpkin extracts, which have effects on milk production, have been used in folk medicine. However, their biological effects in the induction and stimulation of lactation remain unclear. We found no significant differences among the treatment groups, which may have been due to the small number of experimental rats. We think that additional experiments and reconsideration of the protocol should be considered.

In conclusion, the rat milking technique requires a great deal of patience and manual dexterity to achieve competence; however, with a very gentle touch and a good deal of patience, anyone can easily learn how to milk rats successfully. Our findings also suggest that the addition of dietary components with lactation-promoting activities during pregnancy and lactation may not improve milk quantity. However, it is necessary to optimize this newly developed device further, even though the obtained milk yield was adequate.

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