

Crossover feeding test of a new milk mixture for giant panda (*Ailuropoda melanoleuca*) cubs

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Abstract

A milk substitute developed in 1988 (OM) has been used to feed giant panda cubs, but its contents do not match those of panda breast milk. OM contains 10% more protein than the breast milk, and so a novel milk substitute (NM) was developed based on breast milk data. NM has the following nutrition: protein, 38%; fat, 40%; carbohydrates, 12% (containing lactose, 7%); ash, 6%; and moisture, 3%; and it also contains lactoferrin, nucleotides, oligosaccharides and docosahexaenoic acid. In order to ensure that NM is safe before it is fed to premature newborns, a plan was made to subject twin cubs (Kaihin and Youhin, around one year old) to the first feeding test of NM. Here, a crossover feeding test of OM mixture (OMM) and NM-containing mixture (NMM) is described. Kaihin and Youhin were fed 800-1300 ml/d NMM and OMM, respectively, combined with 200 ml/d breast milk for 280-380 days after birth. Then, they were fed 1370-1570 ml/d OMM and NMM, respectively, without breast milk for the next 100 days. Both cubs exhibited constant growth of 90 g/day throughout the test, which is comparable with the growth of previous panda cubs (including twins). Moreover, no NMM-associated safety concerns were observed. Feces were excreted every 3-5 d during milk feeding, whereas they were excreted several times a day when bamboo was consumed. It could be concluded that NM can be fed safely to newborn panda cubs.

Keywords: feeding, giant panda, cub, milk

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Introduction

Milk substitutes are used to feed giant panda cubs in captivity. OM, a milk substitute for neonate panda cubs, was released in 1988 (Tanabe, 1995); however, it has a slightly higher protein content than panda breast milk (protein, 48%; fat, 41%; carbohydrates, 4%; ash, 4%; moisture, 3%; and lactose, nil) (Table 1). Other formulas have also been used to feed panda cubs, but their contents differ from those of panda breast milk (Edwards et al., 2006). While these substitutes have been successfully used for nursing, their effects on growth have not been reported in

detail. Zhang (2013) reported that the survival rates of giant panda newborns in captivity around the world at one year old were 71% (20/28) in 2012 and 86% (42/49) in 2013.

At Adventure World, OM has been administered to newborn panda cubs, a mixture of OM, dog milk powder, and human infant formula (OMM), during mid-term feeding for 10 years. Nine cubs grew well with the OMM; and Nakao et al. (2013) reported that the survival rate of panda cubs fed the OMM between 2001 and 2013 was 92% (11/12). Fig. 1 shows growth curves for another set of twins that were born in 2008, Meihin (a female) and Eihin (a male).

Table 1 Composition of the milk substitutes, milk mixtures, and breast milk (DM basis)

The composition of the four milk substitutes used as raw materials, and the mixtures fed to the cubs are shown.

OLD MILK, TD, and NL were mixed at a ratio of 4:5:4 and administered as the control mixture (OMM).

NEW MILK, TD, and NL were mixed at a ratio of 4:5:4 and administered as the test mixture (NMM).

The nutritional contents of the breast milk of Dashuang, YaYa, and Qi Zhen were reported previously (Zhang et al., 2016).

| Milk | Protein % | Fat % | Carbohydrates % | Ash % | Moisture % | Energy kcal/100 g | Lactose % |
|-----------------------------------|--------------|----------|--------------------|----------|---------------|----------------------|--------------|
| OLD MILK | 48 | 41 | 4 | 4 | 3 | 572 | 0 |
| NEW MILK | 38 | 40 | 12 | 6 | 3 | 561 | 7 |
| TD | 47 | 28 | 17 | 5 | 3 | 510 | 8 |
| NL | 13 | 20 | 62 | 2 | 3 | 479 | 0 |
| OLD MILK+TD+NL (4:5:4) | 37 | 30 | 27 | 4 | 3 | 519 | 3 |
| NEW MILK+TD+NL (4:5:4) | 34 | 29 | 29 | 5 | 3 | 516 | 5 |
| Dashuang (25 days after delivery) | 38.7 | 40.8 | 14.7 | 5.8 | 0.0 | 580 | 5.3 |
| YaYa (160 days) | 29.0 | 35.9 | 32.0 | 3.2 | 0.0 | 567 | 3.6 |
| QiZhen (154 days) | 25.9 | 50.1 | 21.5 | 3.2 | 0.0 | 640 | 1.6 |

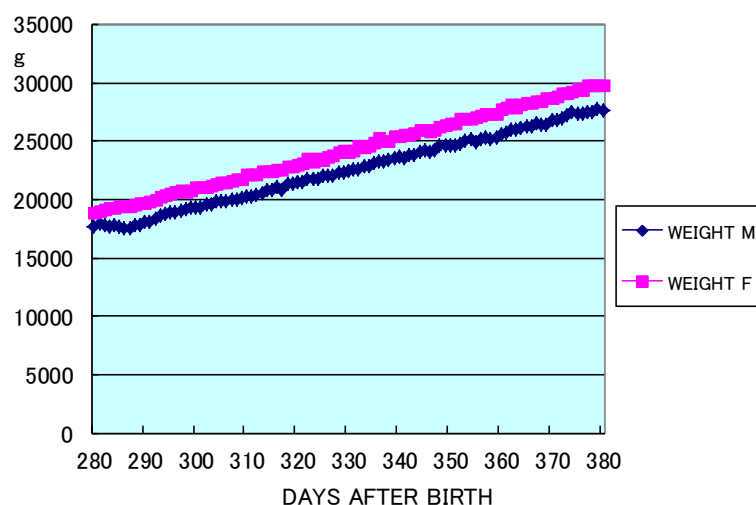


Figure 1 Growth curves of another set of twins reared at Adventure World

The growth curves of another set of twins, Meihin, a female, and Eihin, a male, who were born in 2008 and fed OMM during the same period (FH, d280-380), are shown.

Zhang et al. (2016) proposed NM as a milk substitute for nursing panda cubs that exhibited a closer nutritional profile to panda breast milk than previous milk substitutes. NM powder has the following nutritional profile: protein, 38%; fat, 40%; carbohydrates, 12%; ash, 6%; moisture, 3%; and lactose, 7%. It also contains docosahexaenoic acid/eicosapentaenoic acid, nucleotides, arachidonic acid, taurine, lactoferrin, and L-carnitine. There has been little opportunity to test the feasibility of feeding newborn panda cubs with NM because panda cubs are of global importance. There is similar difficulty in

performing experiments on neonates of other captive animals. A mixture of fish fillets and a milk substitute was fed to *Tursiops* neonates (Sweeney et al., 2010), but the comparison with breast milk was insufficient. In human infants, new ingredients should be fully studied in children and young adults before they are tested on neonates (Aggett et al., 2001; CEAI, 2004). For example, while probiotics are widely used in adults and young people, their use in infants aged from 0-6 months is still being studied (Bertelsen et al., 2016; FAO, 2001).

There are some nutritional differences between NM and OM (Table 1), but all of the ingredients of NM have been used to feed humans and animals for more than 30 years (the first safety step). Here, as a second safety step, a crossover feeding trial was carried out comparing NMM with OMM in weaning giant panda cubs. The third step will be a feeding test of NM involving neonates which will examine the safety of the new ingredients and the nutritional adequacy of the formula.

Materials and Methods

All of the procedures were performed in accordance with the Act on Welfare and Management of Animals, Act No.105, October 1, 1973, (Ministry of Environment, JAPAN), and with the ethical rules of the Japanese Association of Zoos and Aquariums.

Twin cubs (Kaihin, male, stud number: 782, Chinese name: HAI BING; and Youhin, female, stud number: 783, Chinese name: YANG BING; Xie, 2013) were subjected to a feeding test. Both pandas were born on August 11, 2010, at Adventure World. The feeding test was started when they were around one year old.

Milk mixtures (OMM or NMM) were administered at a concentration of 16.7%, 3-4 times a day. The OMM was composed of OM + Tube Diet (TD, Morinyu Sunworld Co. Ltd., Tokyo) + Non-Lacto (NL, Morinaga Milk Co., Tokyo) (ratio: 4:5:4). The NMM was composed of NM+TD+NL (ratio: 4:5:4) (Table 1). TD is a high-energy milk powder for dogs and cats with the following nutritional profile: protein, 47%; fat, 28%; carbohydrates, 16%; ash, 6%; moisture, 3%; and lactose, 8%. NL is an infant formula for lactose-intolerant human babies with the following nutritional profile: protein, 13%; fat, 20%; carbohydrates, 62%; ash, 2%; moisture, 3%; and lactose, nil.

In the first half of the test (FH), the pandas were fed their mother's breast milk 1-2 times a day until day 425 after birth (d425), but the volume of breast milk consumed could not be documented during the latter half of the test (LH). Table 1 shows the contents of breast milk samples which were previously obtained from three panda mothers (Zhang et al., 2016) because breast milk samples could not be collected in this study.

Both cubs were fed the OMM before the test and continued receiving the OMM or NMM in the interval between FH and LH (from d380-404). A

crossover feeding test of the milk substitutes was carried out from d279 to 380 (FH), and from d404 to 504 (LH). At the start of each period, the milk mixtures were gradually introduced over 10 days, i.e. from d279 to 288 and from d404 to 413. The milk mixtures were administered 3-4 times a day in FH and LH. Kaihin was fed the NMM from d279 to 380 and then fed the OMM from d404 to 504. Conversely, Youhin was fed the OMM from d279 to 380 and then fed the NMM from d404 to 504.

The pandas were given apples every day throughout the study, biscuits (Mazuri 5MA4, Purina Mills, USA) after d433, and carrots after d488. They were also given small amounts of bamboo shoots in FH. Furthermore, they took some of their mother's bamboo during LH (from d404 to 425) and were fed bamboo every day after d426. Probiotics (Bio-Three powder; a mixture of *St. faecalis*, *B. mesentericus*, and *C. butyricum*; 1-2 g/day; 1.2×10^7 colony-forming units; Toa Pharmaceutical Co., Ltd., Tokyo) were administered every day. On each day, the volume of milk consumed, amounts of other foods consumed, BW, frequency of excretion, quantity and condition (shape, moisture content, color, etc.) of the cubs' feces, luster of the cubs' hair, and the cubs' general health were assessed. The amount of water consumed was not measured.

Results and Discussion

Both pandas were fed 100-400 g/day breast milk during FH. The mean amounts (\pm standard deviation) of breast milk consumed by Kaihin and Youhin were 288 ± 65 g/d and 257 ± 96 g/d, respectively, which represent about a quarter of the administered OMM or NMM. The amount of breast milk consumed was only documented on 40 days during FH (Fig. 2); however, it seemed to be markedly lower in LH than in FH because breast milk consumption decreased during FH. No breast milk was administered from d426 as the mother and the twins were separated. The twins consumed similar amounts of the NMM or OMM (1065 ± 200 g/day) in FH and identical amounts (1368 or 1568 g/day) in LH (Fig. 3). In FH, the proportion of total energy provided by breast milk and the NMM was calculated to be 76% (volume*kcal/100g), $(1065 * 516) / (288 * 595 + 1065 * 516) = 0.76$, and more than 64% of energy was provided by the NMM during LH. The same values were obtained for the OMM (Tables 1-2, Figs. 2-3).

Table 2 Estimated nutritional contents of the foods consumed from d495 to d504

Data were estimated based on the composition of the milk substitutes; nutritional data of apples and carrots from nutritional tables (Council for Science and Tech, 2010); nutritional data of the Mazuri biscuits provided by the manufacturer; and data about the nutritional content of bamboo (Dierenfeld et al., 1982; Schaller, 1985). The amounts of each food consumed by each cub, and the contents and energy of the NMM are shown.

| Foods consumed by each cub | Weight g/day | Weight % | Protein % | Fat % | Carbohydrates % | Fiber % | Ash % | Energy % |
|-------------------------------------|-----------------|-------------|--------------|----------|--------------------|------------|----------|-------------|
| NMM 16.7%, d=1.0 | 1368 | 22 | 77 | 92 | 31 | 0 | 44 | 64.3 |
| Apples | 250 | 4 | 0 | 0 | 17 | 0 | 2 | 7.2 |
| Mazuri 5MA4 biscuits | 60 | 1 | 11 | 4 | 14 | 7 | 15 | 9.2 |
| Carrots | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0.2 |
| Bamboo (total) | 4590 | 73 | | | | | | |
| Bamboo (digestible) 20% 0.2*4590 | | | 12 | 4 | 38 | 93 | 39 | 19.1 |
| Total | 62.78 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

The other foods made smaller contributions to the pandas' nutritional intake in FH than during LH. The pandas ate 60 g/day (24-168 g) apples from d279 to 380 (Kaihin and Youhin ate 5708 g and 6480 g, respectively). The twins took small amounts of biscuits and carrots from their mother's supplies. In LH, the pandas consumed 120-250 g/day apples after d404, 10-60 g/day biscuits after d433, and 10 g/day carrots after d488. The pandas consumed 3-20 g of bamboo shoots on d304 and d309 in FH, consumed some bamboo with their mother from d404 to 425 in LH, and took 1600~12500 g/day of young bamboo after d426. The maximum total amount of food, including bamboo, consumed was estimated during d495-504 in LH (Table 2) from published composition data (Zhang et al., 2016; CST, 2015; Dierenfeld et al., 1982; Schaller et al., 1985).

Both twins were very healthy and demonstrated reasonable growth throughout the study; Kaihin and Youhin grew by 93 and 108 g/d in FH, and by 93 and 92 g/d in LH, respectively (Fig. 4).

Kaihin was more active, and thus, gained slightly less weight than Youhin. The cubs' growth rates were compared using the t-test. First, the 82 and 79 pieces of growth rate data obtained for Kaihin and Youhin in FH were compared, respectively, and the 44 and 43 pieces of growth rate data collected for the pandas in LH, respectively. Then, the 82 and 44 pieces of growth rate data collected for Kaihin in FH and LH were compared, respectively, and the 79 and 43 pieces of growth rate data obtained for Youhin in FH and LH, respectively. The four sets of growth data did not differ significantly ($t=0.01\sim0.44$, t -critical one tail=1.65~1.66 with $\alpha 0.05$). The twins' BW values at 12 months of age were comparable with those of healthy cubs, including twins, which had previously been raised on the OMM at Adventure World in the past 10 years. Both milk mixtures allowed the twins to grow appropriately, and no NMM-associated safety concerns arose during the test.

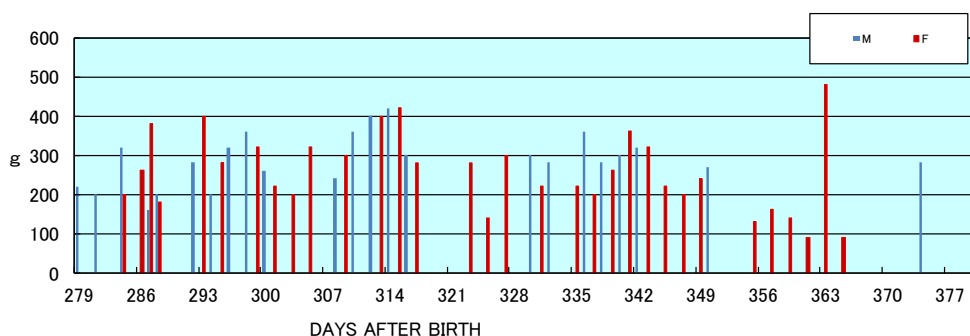


Figure 2 Volume of breast milk fed to the cubs (g/day)

Only data obtained during FH are shown, as data during the exhibition or free nursing periods could not be obtained. The pandas were fed breast milk one to three times a day in FH and LH until d425. M: the male cub, F: the female cub.

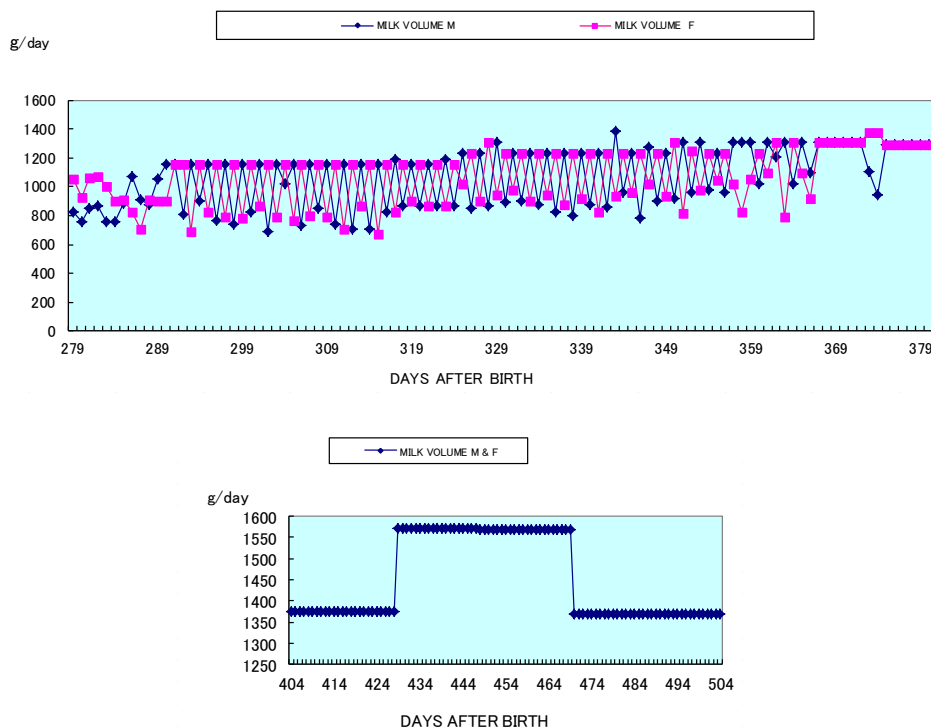


Figure 3 Milk mixture consumption

Both pandas were fed similar volumes of milk mixture in FH and identical amounts of milk mixture in LH. M: the male cub, F: the female cub.

The consumption of apples increased the amount of fecal matter produced by the cubs, and some pieces of apple were observed in the pandas' feces. Most of the young bamboo was excreted, but this did not affect the condition of the pandas' digestive tracts. The cubs' bamboo consumption was recorded, but as the degree of moisture evaporation from the bamboo changed daily, the accurate amount taken could not be determined, so the pandas' feces were monitored instead. The total amounts of collected feces are shown in Fig. 5. Kaihin and Youhin produced 1731 g and 3245 g of fecal matter during FH, respectively. In addition, they excreted feces every 5.1 days and 2.6 days, and the mean amount of fecal matter excreted per day was 87 g and 83 g, respectively. The amount of feces produced

by the pandas during FH was very small compared with the amount of milk solids they consumed, i.e. the cubs consumed 167 g/day milk solids, which would have produced 560 g/day of feces if none of the milk had been digested (assuming a fecal moisture content of 70%) (Schaller et al., 1985). Therefore, it was speculated that more than 95% of the milk was absorbed, as described previously (NAFR, 2009). The differences in fecal production in FH between the twins might have been due to variations in apple consumption. In LH, the feces excreted by the two pandas could not be collected separately after d432 because the amount of feces produced increased greatly after the twins started to consume bamboo regularly (Dierenfeld et al., 1982).

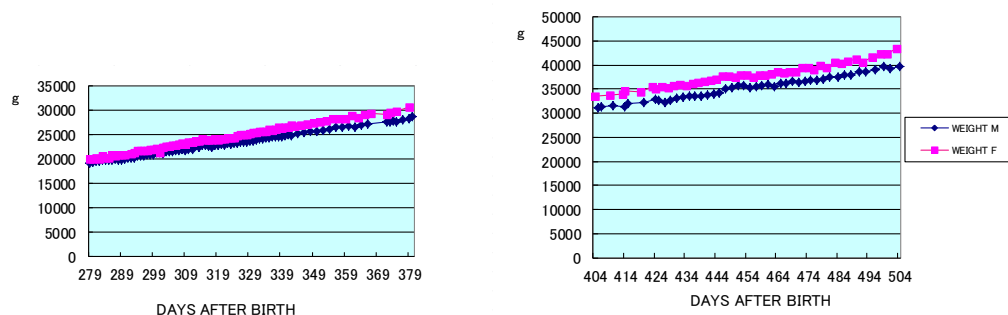


Figure 4 Growth curves for the twins

Separate growth curves for FH and LH are shown. The crossover feeding of the two mixtures did not result in any differences in BW gain. M: the male cub, F: the female cub.

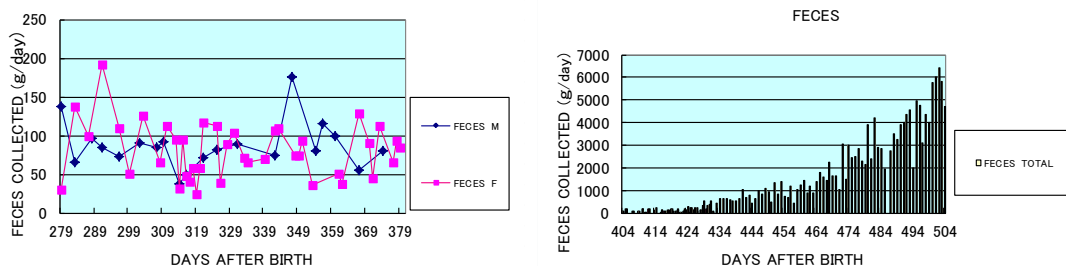


Figure 5 Weight of the collected feces

Most feces could be collected during FH, but the feces produced by each of the pandas were difficult to be gathered separately after d433 because the amount of feces produced by the pandas increased; therefore, only the total amount is shown in LH. M: the male cub, F: the female cub.

Kaihin suffered a fever from d321 to 322, and exhibited mouth breathing and coughing after consuming milk or sliced apples from d373 to 377. In addition, he consumed less milk on d374. Youhin excreted soft feces containing milk particles from d316 to 320. However, none of these issues were considered to be related to the milk mixtures. Both cubs grew well during LH, and no health-related issues or abnormal feces were observed in this period.

Growth is an essential endpoint in all safety assessment of new foodstuffs for human infants, and our findings suggest that the NMM meets this criterion. The normal growth demonstrated by the twins indicates that it is safe to conduct a nutritional study of formulas containing NM involving newborn panda cubs. Although it is impossible to carry out large clinical trials, small amounts of data should be accumulated, such as that reported in this study, in

order to build up a clearer picture of how wild panda cubs should be raised in captivity.

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บทคัดย่อ

การทดลองครอสโอเวอร์โดยการให้อาหารผสมนมชนิดใหม่แก่ลูกแพนด้ายักษ์ (*Ailuropoda melanoleuca*)

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อาหารทดแทนนม (OM) ที่ได้รับการพัฒนาขึ้นในปี 1988 ถูกนำมาใช้สำหรับเลี้ยงลูกแพนด้ายักษ์ แต่ส่วนประกอบของอาหารดังกล่าวยังไม่สามารถเทียบเท่ากับน้ำนมของแม่แพนด้าได้ OM มีปริมาณโปรตีนมากกว่านมแพนด้า 10% จึงมีการพัฒนาอาหารทดแทนนมตัวใหม่ (NM) ขึ้นมาโดยอ้างอิงจากข้อมูลของนมแพนด้า ซึ่ง NM มีส่วนประกอบของสารอาหาร ได้แก่ โปรตีน 38%, ไขมัน 40%, คาร์โบไฮเดรต 12% (ประกอบด้วยแลคโตส 7%), เกลือ 6% และ ความชุ่มชื้น 3% นอกจากนี้ ยังมีแลคโตเฟอริน นิวคลีโอไทด์ โอลิโกแซ็กคาไรด์ และกรดโดโคซาเฮกซาอีโนอิกด้วย เพื่อตรวจสอบความปลอดภัยก่อนให้อาหาร NM แก่แพนด้าแรกเกิด จึงได้ทำการทดลองป้อน NM ให้กับลูกแพนด้าฝาแฝด (โคชินและโยฮิน อายุประมาณ 1 ปี) ก่อน โดยทดลองป้อนอาหารแบบสลับกันระหว่างอาหาร OMM และอาหารที่มีส่วนประกอบของ NM (NMM) ทั้งโคชินและโยฮินได้รับ NMM และ OMM 800-1,300 มล./วัน ตามลำดับ โดยผสมกับนมแม่ 200 มล./วัน เป็นเวลา 280-380 วันหลังจากที่ลูกแพนด้าทั้งสองเกิด หลังจากนั้นลูกแพนด้าทั้งสองได้รับ OMM และ NMM 1,370-1,570 มล./วัน ตามลำดับ โดยไม่ผสมกับนมแม่ต่ออีกเป็นเวลา 100 วัน การทดลองพบว่า ลูกแพนด้าทั้งสองตัวมีอัตราการเติบโตอยู่ที่ 90 กรัม/วันตลอดระยะเวลาการวิจัย ซึ่งเทียบเท่ากับอัตราการเจริญเติบโตของลูกแพนด้าตัวอื่น ๆ (รวมถึงตัวที่เป็นฝาแฝดด้วย) และไม่พบว่ามีข้อกังวลด้านความปลอดภัยเกี่ยวกับ NMM นอกจากนั้น ยังพบว่าในระหว่างที่กินอาหารทดแทนนมลูกแพนด้ามีการขับถ่ายอุจจาระทุก ๆ 3-5 วัน ในขณะที่การกินต้นไผ่ทำให้ลูกแพนด้าขับถ่ายอุจจาระวันละหลายครั้ง ดังนั้น จึงสรุปได้ว่า NM มีความปลอดภัยสำหรับใช้ป้อนให้กับลูกแพนด้าแรกเกิด

คำสำคัญ: การให้อาหาร แพนด้ายักษ์ ลูกแพนด้า นม

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