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# Rapid milk intake of captive giant panda cubs during the early growth stages

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**Abstract.** Survival, especially the youth, is critical for the reproduction of a species. Giant panda (*Ailuropoda melanoleuca*) cubs are not well developed and are vulnerable at birth, and they have developed many survival strategy to assist with survival until adulthood, including rapid growth of their young. By analysing the changes in the daily milk intake and weight gain during the early stages of cub growth for 11 years (2004–2014) over 42 healthy giant panda cubs, we found that milk intake by the cubs increased rapidly during the first 10 days. After 10 days, the daily milk intake decreased gradually and stabilized beginning at 35 days. In addition, the cubs with lower birth weight exhibited higher daily milk intake, while those with higher birth weight consumed less milk per unit of body weight. This study explored the characteristics of daily milk intake during the early growth stage of giant panda cubs, offering insight into adaptations strategy of newborns in this species and providing valuable information for artificial rearing to improve the survival rate of captive panda cubs.

**Key word:** parental care, rapid development, body weight, survival strategy

## Introduction

The survival of newborns is a key step in the reproduction of animal species therefore, neonates should either be able to quickly adapt to the environment or be taken care of by their parents (Clutton-Brock 1991, König 1997, Nowak et al. 2000). For most herbivorous mammals, such as antelopes (Manski 1991), giraffes (Pratt & Anderson 1979) and bovids (Green 1986, Houwing et al. 1990), although the young are precocial, they stay with their mother for a long time, as their mother is their only food source and protects them from predators. On the other hand, the neonates of carnivores and omnivores, such as bears (Ramsay & Stirling 1988, Robbins et al. 2012), lions (Rudnai 1973), non-human primates (Savage et al. 1996), and even human beings (Lummaa & Clutton-Brock 2002), are generally altricial requiring more parental care before they can move around alone.

Giant panda (*Ailuropoda melanoleuca*) is one of the most well-known mammal species in the world, not only for their cuteness but also for their unusual biological features. They evolve many special physiological features to adapt on eating bamboo as their primarily food sources despite their digestive

system is still carnivore-like (Zhao et al. 2010, Xue et al. 2015), and they also have an extremely low level of daily energy consumption (Nie et al. 2015). The giant panda is also known for their smallest neonate-maternal weight ratio (1/900). Although the adult panda could grow to 150 cm in length and 160 kg in weight, the neonates of giant panda has a body length only around 15 cm and highest weight of no more than 250 g (Zhu et al. 2001), and they are also extremely underdeveloped – they have minimal motor ability, their eyes are not open, and they have no fur to keep themselves warm (Peng et al. 2001). They need their mother's care for at least three months before they can leave the den by themselves (Lu et al. 1994). Therefore, early parental care, especially feeding with milk, is critical to the survival of giant panda cubs, whether in captivity or in the wild (Ma et al. 2017). Giant panda cubs have been the subject of many studies, addressing topics such as their development and growth (Peng et al. 2001, Zhu et al. 2001, Che et al. 2015), milk nutrition (Liu et al. 2003, Nakamura et al. 2003, Zhang et al. 2016b), mother-infant interactions (Lu et al. 1994, Zhu et al. 2001, Snyder et al. 2003) and vocal communication (Stoeger et al. 2012, Baotic et al. 2014). However, only a few studies

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have addressed the amount of early milk intake and its effects on the growth rate of the cubs (Huang et al. 2004, 2011). Moreover, each of those works used only a single individual, from which it is impossible to extrapolate the features of this species.

The present study aims to investigate the pattern of milk intake by cubs in the captive population, which may provide basic information for the artificial rearing of those cubs who are abandoned by their mothers, thereby improving the quality of the breeding of captive giant pandas. By measuring the cubs' daily milk intake and weight, we were able to derive curves for those parameters based on the cubs' ages in days, in order to explore the characteristics of the cubs' growth during their early growth stages. Based on the previous studies on individual cubs, we hypothesize that cubs are fed relatively large amounts of milk after birth and that intake would regularly change with age and weight. In addition, because birth weight varies among giant panda cubs, we also expect that there would be differences in the amount of milk intake among the different birth weight groups.

## Material and Methods

### *Animals*

The data were collected from 42 giant panda cubs that were raised by their mothers at the Chengdu Research Base of Giant Panda Breeding, from their day of birth to the age of 120 days, during 2004 to 2014.

All cubs were healthy during the data collection period, and their growth rates fit the previous data for the growth of giant panda cubs. All research complied with the legal requirements of the Chinese government and the China Wildlife Conservation Association's principles for the ethical treatment of endangered species. All experimental procedures in this study were assessed and approved by the Research Department, Veterinary Department, and Animal Husbandry Department of the Chengdu Research Base of Giant Panda Breeding and were performed in accordance with its guidelines and rules.

### *Data collection*

In order to ensuring the survival of newborn cubs, the guidelines and rules of Chengdu Research Base of Giant Panda Breeding forbid anyone to move them away from the delivery house nor do any invasive experiment on those newborn cubs, which makes their body weight to be the only index of their growth and development in this experiment. To ensure the accuracy of milk intake measurements at each feeding, cubs under the care of the mother were taken away

carefully for defecation and weighing before being returned (excretion before milk intake, followed by weighing). They were then returned to the mother for feeding. After they stopped taking milk, they were weighed a second time (accuracy 0.1 g). However, sometimes the mother refused to feed the cubs, and these cubs were then fed artificially with milk that had been collected from mothers in previous birthing instances at corresponding periods. When milk intake is sufficient, the cub stops sucking and swallowing, giving small but clear cries and remaining active, and the cub's abdomen is clearly distended after milk intake.

All individuals during the data collection period received artificial nursing without a certain pattern as a results of ensuring the survival rate, thus all data were pooled together to further analyses. Daily milk intake represents the weight gain from suckling and artificial feeding. Total milk intake in a day (from the time of birth to the same point the next day) was designated as the daily milk intake of the cub at the current age in days. If milk intake was not recorded on a given day because of extenuating circumstances, the data for that day were excluded from further analysis.

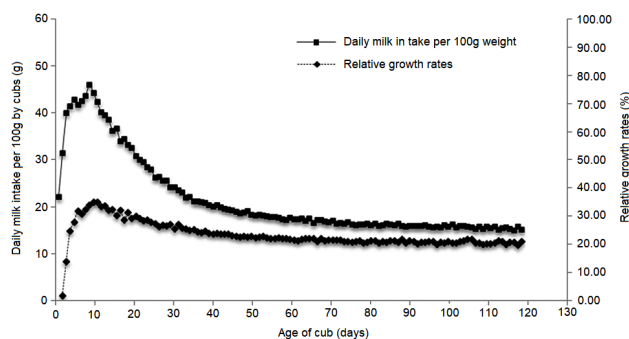
### *Data processing*

As proposed by (Zhang & Wang 2003), the mean  $W_0$ , which is defined as the body weight of the cubs at the birth day, over 170 newborns during 20 years, was  $144.9 \pm 40.59$  g (mean  $\pm$  SD). Based on these values, we divided the cubs into four groups:  $W_0 < 100$  g (actual  $W_0$  range: 51-98 g,  $n = 6$ , accounting for 14.3 %),  $150 \text{ g} > W_0 \geq 100$  g (actual  $W_0$  range: 107-149 g,  $n = 17$ , accounting for 40.5 %),  $200 \text{ g} > W_0 \geq 150$  g (actual  $W_0$  range: 150-183 g,  $n = 15$ , accounting for 35.7 %), and  $W_0 \geq 200$  g (actual  $W_0$  range: 202-219 g,  $n = 4$ , accounting for 9.5 %).

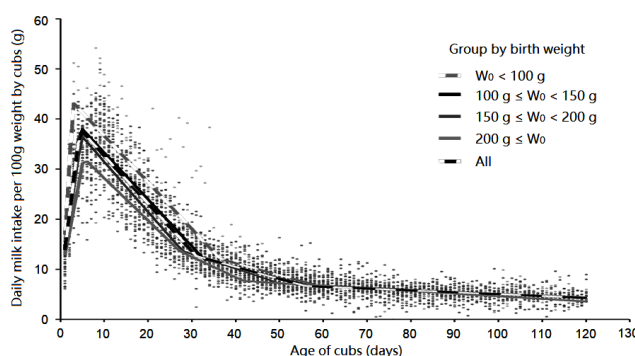
Due to the substantial differences in birth weight among the study subjects (51-219 g), daily milk intake was converted to daily milk intake per 100 g of body weight (Zhang & Wang 2003) and (Huang et al. 2004, 2011) with the following formula:

$$Fn = \frac{100 \times Gn}{Wn}$$

where  $n$  is the age in days (the day on which the cub was born is expressed as 1<sup>st</sup>, followed by 2<sup>nd</sup>, 3<sup>rd</sup>.....  $n^{\text{th}}$ );  $Fn$  (g) represents the daily milk intake per 100 g of body weight on the  $n^{\text{th}}$  day after birth;  $Gn$  (g) is the milk intake on the  $n^{\text{th}}$  day; and  $Wn$  (g) is the cub's weight on the  $n^{\text{th}}$  day, measured at a time of day close to its birth time. The weight gain is expressed as the relative growth rate.



**Fig. 1.** The daily milk intake and the relative growth rate for by age (days). The curves with squares show the daily milk intake per 100 g weight for each age in days. The curves with rhombuses show the relative growth rate for each age in days.



**Fig. 2.** The daily milk intake during the first 120 days for different weight classes. The curves represent the different weight classes. The points in the same shade as a given curve represent the daily milk intake of each individual in that weight class.

While processing the data, we noticed that the  $F_n$  value varied significantly along with the age in days. To compare the differences in  $F_n$  between different ages in days, the age distribution (in days) of giant panda cubs was divided into several stages according to the preliminary analysis of the curve relating  $F_n$  to age in days: 1 day, 2 days, 3-14 days, 15-31 days, 32-54 days, and 55-120 days, of which the first three classes were considered the early stages and the other three were regarded as the later stages. Both breakpoints and age dividers were determined by segmented regression analysis. Then, the milk intake per 100 g of body weight for a given stage was calculated by averaging the  $F_n$  values within that stage. If the data

for an individual were missing on any day within a stage, all the data for that individual in that stage were discarded.

### Statistical analysis

For changes in daily milk intake of different birth weight classes in different age (days) classes, a mixed linear model was adopted for analysis in SPSS, version 19 (SPSS Inc., Chicago, Illinois, U.S.A.). The Bonferroni modified differential test method was adopted for paired comparisons of individual daily milk intake between different age (days) classes and different birth weights. To check the relationships between daily milk intake per 100 g ( $F_n$ ) and age (days) among different birth weight classes, segmented regression analysis to obtain the age (days) node, the intercept and the slope of the linear regression equation was performed by using R 3.24. The Pearson correlation coefficient was calculated to measure the correlation between daily milk intake per 100 g of body weight and daily relative rate of weight gain and age (days) of cubs over 120 days. A significance threshold of  $p < 0.05$  was used, with  $p < 0.01$  considered extremely significant.

## Results

The numbers of individuals at each stage are presented in Table 1. It can be seen that discarded data were mostly from the first stage and the final stage, which suggested that most of the data for the middle days are available for analysis and that the results should be useful, although some individuals' data were excluded.

### Milk intake increased rapidly during the early stages

The daily milk intake of giant panda cubs and correlated growth data are presented in Fig. 1. These data showed that these two parameters changed nonlinearly; thus, segmented regression was adopted for linear fitting. The first change node (age in days) of milk intake per 100 g of body weight occurred between 3 and 5 days (2.731-4.631); before that, daily milk intake increased rapidly, with a slope of 16.21,

**Table 1.** Birth weight classes ( $W_0$ ) and numbers of cubs at different ages. The table presented the number of cubs of different classes measured at different ages.

Cub age (days)	1	2	3-14	15-31	32-54	55-120
$W_0 < 100$ g	5	6	6	5	5	4
$150 \text{ g} > W_0 \geq 100$ g	12	16	17	17	17	8
$200 \text{ g} > W_0 \geq 150$ g	12	15	15	15	15	4
$W_0 \geq 200$ g	4	4	4	4	4	2

**Table 2.** Results of segmented regression analysis on cubs' daily milk intake per 100 g of body weight during the first 120 days. This table presents the parameters of the change in the curves for daily milk intake of cubs of different weight classes.

	$W_0 < 100\text{ g}$	$150 > W_0 \geq 100\text{ g}$	$200 > W_0 \geq 150\text{ g}$	$W_0 \geq 200\text{ g}$	All
Breakpoints	2.731	4.326	4.631	4.484	4.396
	36.668	32.725	27.685	34.266	30.749
	62.729	56.913	50.887	79.634	54.122
Intercept 1	-0.862	5.437	5.904	6.024	6.758
Slope 1	16.21	7.67	6.677	5.736	7.113
Intercept 2	45.95	42.67	41.47	35.11	42.23
Slope 2	-0.930	-0.937	-1.002	-0.751	-0.955
Intercept 3	19.70	19.07	21.62	12.90	20.73
Slope 3	-0.214	-0.215	-0.2855	-0.103	-0.256
Intercept 4	8.137	9.153	9.164	4.462	9.051
Slope 4	-0.030	-0.041	-0.041	0.003	-0.040
Adjusted R <sup>2</sup>	0.884	0.912	0.895	0.873	0.880

**Table 3.** Mixed linear model analysis of giant panda cubs' daily milk intake per 100 g of body weight during the first 120 days. The table presents the differences between different weight classes, \* $p < 0.01$ .

	df	F	$p$
Intercept	1, 34.899	2572.981	$< 0.001^*$
Birth weight classes	3, 96.893	4.265	0.007*
Age class	5, 101.501	525.716	$< 0.001^*$

decreasing sharply afterward until the second node, between 28 and 37 days (27.685-36.668), when the decrease began to flatten. At the third node, between 51 and 80 days (50.887-79.634), daily milk intake per 100 g of body weight was flat, with a slope in the range of -0.041-0.003 (Table 2). According to the mixed linear model analysis, there was a significant difference in overall daily milk intake among the different age classes (Table 3), of which intake was the highest for the age class 3-14 days and lowest for 55-120 days (Table 4) (Bonferroni-corrected  $p < 0.01$ ).

During days 1-9, milk intake per 100 g of body weight was positively correlated with age in days ( $r = 0.832$ ,  $p < 0.01$ ,  $n = 9$ ), while during days 9-120, a negative

correlation was observed ( $r = -0.804$ ,  $p < 0.01$ ,  $n = 113$ ). The peak value occurred at 9 days ( $37.51 \pm 5.73$  g). From 6 to 19 days, the relative weight increase peaked for the entire period ( $8.92 \pm 1.19\%$ ), and the peak values were reached at 10 and 11 days (10.72 %) (Fig. 1).

Similarly, the relative weight for cubs increased ( $r = 0.916$ ,  $p = 0.000$ ,  $n = 10$ ) from 1 to 10 days along with the daily milk intake per 100 g of body weight, and the rate of relative weight increased even more rapidly than daily growth (extremely significant positive correlation with age in days,  $r = 0.857$ ,  $p = 0.01$ ,  $n = 10$ ). After 10 days, the relative increase in weight decreased along with the decrease in daily milk intake per 100 g of body weight, presenting a significant positive correlation with a gradual decrease ( $r = 0.981$ ,  $p < 0.01$ ,  $n = 110$ ), whereupon the relative weight gain rate increased slowly (significantly negative correlation with age in days,  $r = -0.848$ ,  $p < 0.01$ ,  $n = 110$ ) (Fig. 1).

Those results suggested that the giant panda cubs, regardless of their birth weight, consume a large amount of milk during the early stages, which contributed to their fast growth rate in the early stages.

**Table 4.** Giant panda cubs' milk intake (g) per 100 g of body weight during the first 120 days. The data are shown as the mean  $\pm$  SD. Mean values with different superscript capital letters in each row indicate significant differences ( $p < 0.05$ ) between age classes within each weight class. Mean values with different superscript small letters within parentheses in each column indicate significant differences between weight classes within each age class ( $p < 0.05$ ).

Cub age (days)	1	2	3-14	15-31	32-54	55-120
$W_0 < 100\text{ g}$	$15.44 \pm 3.02^{\text{D(a)}}$	$31.38 \pm 4.60^{\text{B(a)}}$	$38.73 \pm 6.96^{\text{A(a)}}$	$24.30 \pm 8.03^{\text{C(a)}}$	$10.92 \pm 4.57^{\text{D(a)}}$	$5.71 \pm 1.53^{\text{E(ab)}}$
$150\text{ g} > W_0 \geq 100\text{ g}$	$11.26 \pm 3.64^{\text{C(a)}}$	$21.91 \pm 3.86^{\text{B(b)}}$	$33.90 \pm 5.75^{\text{A(b)}}$	$20.84 \pm 6.02^{\text{B(b)}}$	$9.82 \pm 2.61^{\text{C(b)}}$	$5.73 \pm 1.79^{\text{D(ab)}}$
$200\text{ g} > W_0 \geq 150\text{ g}$	$11.07 \pm 2.88^{\text{C(a)}}$	$19.36 \pm 5.03^{\text{B(b)}}$	$32.02 \pm 5.92^{\text{A(c)}}$	$18.59 \pm 5.36^{\text{B(c)}}$	$9.34 \pm 2.29^{\text{C(bc)}}$	$5.86 \pm 1.59^{\text{D(a)}}$
$W_0 \geq 200\text{ g}$	$10.99 \pm 1.90^{\text{CD(a)}}$	$17.50 \pm 5.01^{\text{BC(b)}}$	$27.62 \pm 4.61^{\text{A(d)}}$	$17.85 \pm 6.26^{\text{B(c)}}$	$8.66 \pm 2.57^{\text{D(c)}}$	$5.50 \pm 1.72^{\text{E(b)}}$
Mean	$11.79 \pm 3.37$	$21.93 \pm 6.07$	$33.32 \pm 6.53$	$20.19 \pm 6.42$	$9.64 \pm 2.87$	$5.73 \pm 1.69$



### *Cubs of different birth weight show different daily milk intake*

According to the mixed linear model analysis, there were also significant differences in daily milk intake among the different birth weight classes (Table 3). Specifically, the daily milk intake of the  $W_0 < 100$  g group was significantly higher than those of the  $200 \text{ g} > W_0 \geq 150$  g and  $W_0 \geq 200$  g groups (Bonferroni-corrected  $p < 0.05$ ), while the other groups showed no significant differences (Bonferroni-corrected  $p > 0.05$ ). According to paired comparisons of all birth weight classes, in the  $W_0 < 100$  g group, all classes showed significant differences except classes one day and 32-54 days. In the  $150 \text{ g} > W_0 \geq 100$  g group, classes one day and 32-54 days and two days and 15-31 days were not significantly different, while the other classes showed significant differences. In the  $200 \text{ g} > W_0 \geq 150$  g group, classes one day and 32-54 days and two days and 15-31 days were the only non-significant comparisons among classes. In the  $W_0 \geq 200$  g group, classes one day and two days, one day and 32-54 days, and two days and 15-31 days exhibited no significant differences, while all other comparisons were significantly different (Fig. 2 and Table 4).

Based on the paired comparisons of milk intake in different age classes among different weight classes, daily milk intake in the one day class did not differ significantly among the different weight classes. In the two days class, the  $W_0 < 100$  g group showed significantly higher daily intake than the other groups. In the 3-14 days class, different weight classes exhibited significant differences in milk intake. In the 15-31 days class, except for the groups  $200 \text{ g} > W_0 \geq 150$  g and  $W_0 \geq 200$  g (which were similar), the groups were significantly different from each other. In the 32-54 days class, groups  $150 \text{ g} > W_0 \geq 100$  g and  $200 \text{ g} > W_0 \geq 150$  g and  $200 \text{ g} > W_0 \geq 150$  g and  $W_0 \geq 200$  g were not significantly different from one another, while the other groups were significantly different. In the 55-120 days class, only groups  $200 \text{ g} > W_0 \geq 150$  g and  $W_0 \geq 200$  g were significantly different, while the other groups were very similar (Fig. 2 and Table 4).

These data show that the milk intake of giant panda cubs was related to their birth weight, with the cubs with low birth weight consuming more milk and those with high birth weight consuming less milk.

## **Discussion**

### *Milk intake at early stages largely matches the requirement for cubs' growth*

The results of the present study showed that the giant panda cubs rapidly increased their milk intake during

the first 10 days in order to quickly increase their body weight, which corresponds to the idea that the early stages of cub development are critical for their survival (Lindström 1999).

For bears and giant panda, the body development of a newborn is nearly at the foetal stage, which means that they do not have basic survival skills such as keeping warm and defecation, and they are also vulnerable to diseases due to their undeveloped immune systems (Ramsay & Stirling 1988, Oftedal et al. 1993, Peng et al. 2001). In these cases, maternal assistance is highly necessary (Snyder et al. 2003). This kind of nurturing may result in mothers staying with the cubs 24 h per day to keep them safe for a period of approximately one to two weeks, without feeding (Oftedal et al. 1993, Atkinson & Ramsay 1995), which may cause the mothers to lose 40 % of their body weight (Oftedal 1993). To maintain their ability to feed their cubs and reduce their bodies' nutrient consumption, because they do not have abundant internal nutrient stores, the mother should cease fasting and search for food as soon as possible. Therefore, the cubs should grow quickly during the early stages and master the most basic skills in a short period of time, which could reduce dependence on the mothers so that both mother and cub can be prepared for the approaching winter in order to improve the probability of survival (Wang et al. 1981, Lu et al. 1994). The idea that cubs need to grow quickly during early stages may also be supported by the nutritional changes in the mother's milk (Liu et al. 2005, Zhang et al. 2015, 2016a); it has been shown that panda milk contains significantly more protein at the early stages (3~6 days) than at the later stages (7~23 days), while other components such as lactose and vitamins did not vary much. As protein is important for the growth of bones, muscle and visceral organs (Reichling & German 2000), high protein content in the milk at early stages may significantly improve the growth of newborns.

### *Different milk intake for cubs with different birth weight increases the survival rate*

Despite the similar milk intake curve, the cubs of different birth weight classes consumed various relative amounts of milk, which may be a strategy to produce higher survival rates for both giant panda mothers and cubs.

The lower birth weight of some newborns indicates that they are less developed than those of average birth weight, meaning that they are weaker, and have a higher mortality (McCormick 1985). Therefore, cubs with lower birth weight need more nutrition to grow

as fast as possible to catch up with the cubs of average birth weight in order to reach levels of development at which their survival would be fairly certain. This requires that they consume larger amounts of milk than cubs with higher birth weights. Their rapid growth also could reduce their dependence on the mother, leaving more time for the mother to forage, which could improve the survival of both mother and cubs (Zhu et al. 2001).

Because none of the newborn panda cubs are fully developed, they should consume as much milk as possible in order to obtain enough nutrition to grow. Thus, it is surprising to find that cubs with high birth weights ( $W_0 > 200$  g) consume less milk per unit of body weight than other classes. One possible explanation for this phenomenon may be due to the mother panda. It is known that the giant panda mothers need to nurse the cubs all day and fast for approximately two weeks (Zhi et al. 2000), during which they consume most of the energy that was stored before delivery and lose large amounts of weight. However, giant pandas' diets show extremely low energy digestibility (Finley et al. 2011), requiring pandas to eat large quantities of food and maintain a low daily energy expenditure to ensure the balance of their basic metabolism (Nie et al. 2015). If the amount of milk intake per 100 g of body weight were the same for cubs with high birth weight as for average cubs, the mother would need to provide more milk to feed them. This situation would result in consumption of additional energy and

would worsen the physical condition of the mother panda when she begins to forage, which may reduce the quality of subsequent breeding periods and finally decrease the survival of the cubs. As the cubs with high birth weight are developed better than other cubs, their mothers may not provide extra milk to feed them, thereby ensuring that the mothers could remain healthy enough to nurse. Thus, the cubs with high birth weight show lower relative milk intake than cubs of average birth weight.

Sufficient intake of milk, especially colostrum, is extremely important for cub survival, which has been confirmed after many years of raising giant pandas in captivity (Zhang & Wei 2006). Our present study investigates the conditions under which giant panda cubs are fed and reveals other important information to establish feeding standards for the observed period. Such information could provide a scientific reference for artificial interventions during captive giant pandas breeding.

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