

## Analysis of the breast milk of giant pandas (*Ailuropoda melanoleuca*) and the preparation of substitutes

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**ABSTRACT.** The first milk substitute for giant panda cubs was developed in 1988 based on limited data about giant panda breast milk and that of certain types of bear. Mixtures of other formulas have also been fed to cubs at some facilities. However, they are not of sufficient nutritional quality for promoting growth in panda cubs. Here, we report analysis of giant panda breast milk and propose new milk substitutes for cubs, which were developed based on the results of our analysis. The Chengdu Research Base of Giant Panda Breeding obtained breast milk samples from three giant pandas. Up to 30 ml of breast milk were collected from each mother by hand. Then, the milk samples were frozen and sent to Nihon University. The levels of protein, fat, carbohydrates, ash, moisture, vitamins, minerals, total amino acids, fatty acids, lactose and other carbohydrates in the milk were analyzed. The breast milk samples exhibited the following nutritional values: protein: 6.6–8.5%, fat: 6.9–16.4%, carbohydrates: 2.5–9.1%, ash: 0.9–1.0% and moisture: 67–83%. We designed two kinds of milk substitutes based on the data obtained and the nutritional requirements of dogs, cats and rodents. The nutritional composition of the milk substitutes for the first and second stages was as follows: protein: 38 and 26%, fat: 40 and 40%, carbohydrates: 13 and 25%, ash: 6 and 6% and moisture: 3 and 3%, respectively. In addition, the substitutes contained vitamins, minerals, taurine, docosahexaenoic acid, lactoferrin, nucleotides and other nutrients.

**KEY WORDS:** breast, giant panda, milk, substitute

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It is difficult to gather sufficient amounts of giant panda breast milk for analysis, and hence, there are little data about its nutritional content. During a post-mortem examination of a giant panda, Lyster [20] obtained a small amount of milky fluid from the animal's mammary gland and studied it. In addition, Hudson *et al.* [12] analyzed the protein, fat, ash and moisture content of giant panda breast milk (total volume: 2 ml) obtained at 8–9 months after delivery in Madrid Zoo. Similarly, Nakamura *et al.* [22] analyzed the carbohydrate, protein, fat, fatty acid, ash and mineral content of a 3-ml sample of panda breast milk acquired at Adventure World (Shirahama, Japan). Finally, Liu *et al.* [19] collected 11 milk samples from 3 mothers (about 3 ml each) and analyzed their lactose, protein and vitamin levels; however, they did not report the fat or ash content of the samples. All of the above-mentioned data were derived from small samples (volume: 1–3 ml). Therefore, the reported values exhibited marked

variations, so it would be inappropriate to use them as the basis for producing breast milk substitutes for giant panda cubs.

There are a lot of data about the variations in the nutritional characteristics of cow's milk, which has been extensively examined [4, 17, 32]. The nutritional composition of cow's milk has been found to vary according to the number of months after delivery that the milk was collected, the duration of the suckling period, the time of day that the milk was collected, the inter-suckling interval, seasonal changes, the suckling technique used and inter-individual differences. Commercial cow's milk is a mixture of milk from numerous cows, and hence, the abovementioned variations are offset, and only some seasonal variations and differences among species remain.

Giant panda mothers with single cubs sometimes stop nursing their cubs in captive environments, and very few mothers with twin cubs nurse both of them. So, milk substitutes are essential for promoting the proliferation of captive giant pandas. Tanabe *et al.* [28] developed the first substitute formula for giant panda cubs in 1988. Their substitute contained a little bit more protein than giant panda breast milk. Edwards *et al.* [10] subsequently recommended a mixture of human infant formula powder and dog milk powder, but it had a lower protein content than giant panda breast milk.

In 2004, the Chengdu Research Base of Giant Panda

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Table 1. Analytical methods

General	moisture	Gravimetric analysis, ISO5537:2004
	protein	Semi-micro-Kjeldahl method, AOAC Official Method 991.20
	fat	Röse-Gottlieb gravimetric method, AOAC Official Method 945.48
	ash	Gravimetric analysis, AOAC Official Method 945.46
	carbohydrates	Calculations, AOAC Official Method 990.20
	energy	Calculations, modified Atwater method, with 4 & 9
	lactose, trisaccharides	HPLC, RI method, using lactose and maltotriose standards
	other saccharides	HPLC, RI method, peak area comparisons with maltotriose
Total amino acids	Amino acid hydrolysis and analysis, AOAC Official Method 994.12	
Taurine	HPLC, AOAC Official Method 991.20	
Fatty acid comp.	Gas chromatography, AOCS Official Method Ce 1b-89	
Minerals	Ca	ICP-AES, AOAC Official Method 984.27
	P	ICP-AES, AOAC Official Method 984.27
	K	ICP-AES, AOAC Official Method 984.27
	Na	ICP-AES, AOAC Official Method 984.27
	Cl	Potentiometric titration, AOAC Official Method 984.26
	Mg	ICP-AES, AOAC Official Method 984.27
	Fe	ICP-AES, AOAC Official Method 984.27
	Cu	ICP-AES, AOAC Official Method 984.27
	Mn	ICP-AES, AOAC Official Method 984.27
	Zn	ICP-AES, AOAC Official Method 984.27
	Vitamins	VA
VE		HPLC, Vitamin (Japan) 73 (11): 1999, 649–657
VB1		HPLC, Vitamin (Japan) 73 (11): 1999, 649–657
VB2		HPLC, Vitamin (Japan) 73 (11): 1999, 649–657
VC		HPLC, Vitamin (Japan) 73 (11): 1999, 649–657

The analytical methods used in this study are listed together with appropriate references.

Ca: calcium, P: phosphorus, K: potassium, Na: sodium, Cl: chlorine, Mg: magnesium, Fe: iron, Cu: copper, Mn: manganese, Zn: zinc, VA: retinol, VE: alpha-tocopherol, VB<sub>1</sub>: vitamin B<sub>1</sub>, VB<sub>2</sub>: vitamin B<sub>2</sub>, VC: ascorbic acid.

Breeding collected breast milk samples by hand, froze and stored them, and then used them to feed orphan/twin cubs. In addition, some frozen milk samples were sent to Nihon University for analysis in 2006 and 2007. This is the first analysis of giant panda breast milk involving samples of more than 20 ml to provide data that could be used to design a breast milk substitute for giant panda cubs.

## MATERIALS AND METHODS

*Panda milk collection:* By training the panda mothers, it became possible to collect milk directly from the panda mothers' nipples with sterile cups. Suckling was successfully performed for periods lasting tens of min, and temporal variations in the contents of the breast milk collected by suckling were equalized. The milk from each mother was collected into 10-ml plastic tubes, before being frozen in a refrigerator and kept at  $-20^{\circ}\text{C}$  until the analysis. There are a lot of restrictions on the use of giant panda breast milk for research and analysis, because it is mainly collected to aid the proliferation of the species, nurse orphans and provide breast milk to cubs whose mother's breast milk supplies are depleted on delivery.

The samples were obtained from a panda mother named Dashuang (Stud Number: 453) on day 25 after delivery, a panda mother named YaYa (Stud Number: 362) on day 160

after delivery and a panda mother named Qi Zhen (Stud Number: 490) on day 154 after delivery. The pandas were described in the 2013 International Studbook for the Giant Panda [33]. In total, 30, 24 and 31.5 ml of milk were collected from Dashuang, YaYa and Qi Zhen, respectively (Table 2).

*Compositional analysis of the milk samples:* The three frozen samples collected in Chengdu, China, were sent by air to Nihon University and analyzed at the Analytical Research Center of Morinaga Milk (Zama, Japan). The standard analyses for cow's milk outlined by the International Dairy Federation, the methods for analyzing milk recommended by the Association of Analytical Communities and the procedures for evaluating vitamin levels described by Kodaka [16] were employed.

The methods used are listed in Table 1. The moisture content; protein content; fat content; ash content; carbohydrate and energy content; lactose, trisaccharide and other saccharide levels; vitamin levels; total amino acid levels; taurine levels; fatty acid profiles; mineral levels; and chloride levels of the milk samples were analyzed with gravimetric analysis, the semi-micro-Kjeldahl method, the Röse-Gottlieb gravimetric method, gravimetric analysis, calculations, refractive index high performance liquid chromatography (RI-HPLC), HPLC, amino acid hydrolysis and analysis, HPLC, gas chromatography, inductively coupled plasma atomic

Table 2. Nutritional contents of the three breast milk samples

Mother panda			Date of milking	Days after delivery	Volume ml	Moisture	Protein	Fat	Carbohydrates	Ash	Lactose	Trisaccharides	Other saccharides	Total saccharides
Name	Stud #	Age (years)												
Dashuang	453	7	Oct 22, 2004	25	30	83.02	6.57	6.92	2.50	0.99	0.90	0.90	1.20	3.01
YaYa	362	16	Feb 9, 2006	160	24	71.62	8.22	10.19	9.07	0.90	1.02	0.94	4.84	6.79
Qi Zhen	490	8	Jan 8, 2007	154	31.5	67.23	8.47	16.4	7.03	0.87	0.53	0.44	not calculated	not calculated

The moisture, protein, fat, carbohydrate, ash, lactose, trisaccharide and other saccharide levels of the three breast milk samples were analyzed. Data are shown as percentages.

emission spectroscopy and potentiometric titration, respectively. Almost all of these methods are standard procedures and are routinely used for analyzing fresh cow's milk and dairy products [6]. Lactose and trisaccharides were analyzed after filtering with molecular weight (MW) 10,000 cut ultrafiltration (UF) filter, using RI-HPLC; i.e., a Shodex KS801 column eluted with water and RI detection. The peaks were identified using lactose and maltotriose standards, and the area of each peak was quantified. The peak areas of other saccharides were estimated by comparing them with the area of the maltotriose peak using RI detection.

*Development of milk substitutes:* We designed two milk substitutes for administering to giant panda cubs during the periods from soon after birth to 90 days (the first stage) and from 90 days to 500 days (the second stage). The protein, fat and ash contents of the formula for the first stage were decided based on the data obtained for Dashuang's breast milk, and the contents of the formula for the second stage were based on the data obtained for YaYa's and Qi Zhen's milk. We mainly focused on the protein and fat contents of the breast milk substitutes, because these are the most important characteristics in terms of nutrition and energy density. However, we also examined the formulas' lactose contents, as some animals are lactose intolerant. We adjusted the formulas' casein to whey protein ratios to 50:50 based on those seen in cow's, dog's and cat's milk [2, 3]. The formulas' vitamin and mineral concentrations were decided based on our findings, the nutritional requirements of dogs and cats [1, 7], and the nutritional requirements of humans outlined in the CODEX STAN [15]. In addition, we also referred to a previous study about the vitamin requirements of rodents [27], because the levels of many vitamins in the collected samples could not be analyzed.

## RESULTS

*Nutritional contents:* The milk samples collected from Dashuang, YaYa and Qi Zhen exhibited moisture contents of 83.02%, 71.62% and 67.23%, respectively. In addition, they displayed protein concentrations of 6.57%, 8.22% and 8.47%; fat contents of 6.92%, 10.19% and 16.40%; and carbohydrate levels of 2.50%, 9.07% and 7.03%, respectively. Furthermore, they had lactose concentrations of 0.90%, 0.90% and 0.53% and trisaccharide levels of 0.90%, 0.94% and 0.44%, respectively. The samples from Dashuang and YaYa had total saccharide concentrations of 3.01% and

6.79%, respectively (Table 2). We showed the original RI-HPLC chromatogram with Dashuang's and YaYa's breast milk (Fig. 1). Peaks with each retention time were the following. The 10–12 min peaks: oligosaccharides with MW less than 10,000; 13.5 min peak: trisaccharides calibrated with maltotriose; 15.4 min peak: lactose calibrated with lactose monohydrate; 17.2 min peak: monosaccharides which had the same retention time with glucose and fructose; and 22.1 min peak: substances with small molecules. The energy contents of the three samples (kcal/100 g of dry matter (DM)) were calculated to be 580 kcal/100 g of DM, 567 kcal/100 g of DM and 640 kcal/100 g of DM, respectively (Table 6). Furthermore, the vitamin concentrations of the three samples were as follows: vitamin A: 403 IU/100 ml, 597 IU/100 ml and 383 IU/100 ml, respectively; vitamin E: 1.46 IU/100 ml, 1.32 IU/100 ml and 1.41 IU/100 ml, respectively; vitamin B1: 0.04 mg/100 ml, 0.05 mg/100 ml and 0.06 mg/100 ml, respectively; vitamin B2: 1.35 mg/100 ml, 0.70 mg/100 ml and 0.70 mg/100 ml, respectively; and vitamin C: 9.17 mg/100 ml, 3.84 mg/100 ml and 8.87 mg/100 ml, respectively (Table 3).

Mineral data for the breast milk samples are shown in Table 3. The mineral concentrations of the samples collected from Dashuang, YaYa and Qi Zhen were as follows (all data are shown as mg/100 ml values): calcium: 160, 236 and 227, respectively; phosphorus: 147, 185 and 175, respectively; potassium: 201, 124 and 96, respectively; sodium: 66.2, 34.0 and 24.2, respectively; chlorine: 205, 109 and 62, respectively; magnesium: 17.1, 11.2 and 8.8, respectively; iron: 1.23, 0.82 and 0.85, respectively; copper: 0.10, 0.07 and 0.07, respectively; manganese: 0.01, 0.01 and 0.01, respectively; and zinc: 0.68, 1.13 and 1.36, respectively.

The total amino acid concentrations of the three samples were 6.6, 7.6 and 7.5 g/100 ml, respectively (Table 4), which were comparable with their protein levels (Table 2). In addition, the samples exhibited taurine levels of 50, 35 and 22 mg/100 ml, respectively. The frequencies of various types of fatty acids are shown in Table 5.

*Composition and preparation of the developed milk substitutes (powder):* The nutritional characteristics of the novel milk substitute powders are shown in Table 6. The composition of the novel formula for the first stage was as follows: 38% protein, 40% fat, 13% carbohydrates, 6% ash and 3% moisture (energy: 564 kcal/100 g of powder).

The novel formula for the second stage contained 26% protein, 40% fat, 25% carbohydrates, 6% ash and 3% mois-

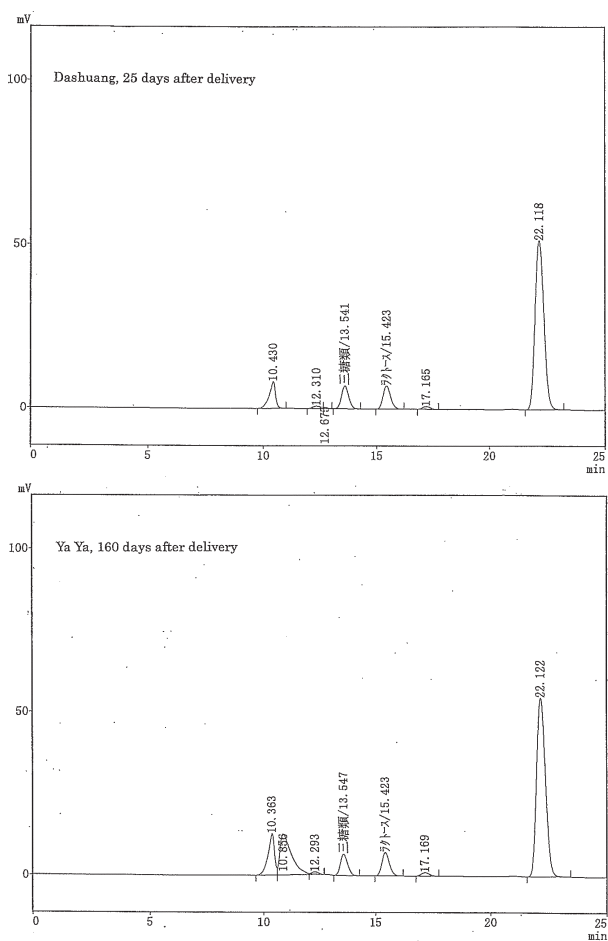


Fig. 1. RI-HPLC chromatogram of saccharides. This is the original HPLC chart with Dashuang's and Ya Ya's breast milk. Peaks with each retention time are 10–12 min: oligo saccharides <math>\text{MW}<10,000</math>; 13.5 min: trisaccharides calibrated with maltotriose; 15.4 min: lactose calibrated with lactose monohydrate; 17.2 min: monosaccharides which have the same retention time with glucose and fructose; and 22.1 min: substances with small molecules.

ture (energy: 564 kcal/100 g of powder). Nutritional data on the DM of the three breast milk samples, the nutritional requirements of dogs and cats, and the vitamin requirements of rodents are shown in Table 6 for reference.

Main ingredients of the substitutes are milk products (casein, whey protein, milk fat and skim milk powder), vegetable oils, dextrin, vitamin mixture and mineral mixture.

## DISCUSSION

**Analytical methods:** We employed the standard analytical methods that are used to examine cow's milk and dairy products [6, 29], but did not perform feed analysis. These two methods are not markedly different except with regard to the data they provide about the levels of dietary and crude fiber in a food. As milk contains very little fiber, the analytical methods used to assess cow's milk and dairy products

are reliable for analyzing giant panda milk with the aim of designing a substitute. We did not provide standard deviation values for the data, because we could not repeat the assays several times. Protein, fat, ash and moisture level measurements usually exhibit analytical variations of  $\leq 12\%$  [8]. As the samples' carbohydrate levels were calculated from these data, our carbohydrate data are considered to exhibit analytical variations of  $>12\%$ .

**Moisture and milk solids:** The moisture levels of the giant panda breast milk samples examined in the present study (67–83%) were similar to the values obtained by Nakamura (78.4%) [22]. The other previous studies of giant panda breast milk provided little information about its moisture content [12, 19, 20]. As for breast milk from other animals, bear's milk has a lower moisture content than giant panda milk [23, 24], while milk from herbivores sometimes exhibits higher moisture levels [14] (Table 7). This is reasonable, because panda mothers eat a lot of bamboo, which increases the moisture content of their milk. Protein and other contents on total energy have not great differences between giant panda and bears, and main differences seem to be moisture and fat contents [11, 13, 14] (Table 7). These may be because of the efficiency for energy acquisition and growth during the hibernation.

The amount of milk solids differed among the samples collected from the three pandas, which makes sense as the concentration of milk solids in cow's milk increases as time passes after delivery [17, 31, 32]. Nursing with breast milk continues nearly a year and half. Pandas grow from 100 g at birth to 20 kg during 8 months, but on the other hand, some kind of dogs and cats grow from 100 g at birth to 1–2 kg during a month. Cubs have 1/1,000 body weight of the mothers, and they cannot walk for months. It should be allowed with the bamboo trees surrounding and protecting them. The milk composition soon after delivery and after one year should have a great difference like kangaroo, but unfortunately, we could not make clear tendency of the variation, as we got only milk samples on 25 days and around 160 days for assay. We could not find any unique nutritional factors in the breast milk, too, which may be secreted by mother pandas, and it is a subject of further investigation.

**Protein:** Ueno Zoological Garden [28] developed a panda milk formula in 1988 based on a combination of data about bear's breast milk and giant panda's breast milk [12], because little data about giant panda's breast milk were available. Since then, several studies have obtained data about giant panda's breast milk, but they did not cover enough nutritional components to enable appropriate breast milk substitutes to be developed.

In a textbook about caring for giant pandas, Edwards [10] recommended a formula for feeding giant panda cubs. It was composed of human infant formula powder and dog milk powder in a 50:50 ratio. The formula's contents were as follows: 23% crude protein, 35% fat, 35% carbohydrates, 4% ash and 3% moisture (energy: 547 kcal/100 g of DM) (Table 6). The fat, ash and energy content of the formula were similar to those described in the present study. However, the protein content (23%) of the formula was only two

Table 3. Vitamins and minerals detected in the breast milk samples

Panda	VA	VE	VB1	VB2	VC	Ca	P	K	Na	Cl	Mg	Fe	Cu	Mn	Zn
	IU/100 ml		mg/100 ml			mg/100 ml									
Dashuang	403	1.46	0.04	1.35	9.17	160	147	201	66.2	205	17.1	1.23	0.10	0.01	0.68
YaYa	597	1.32	0.05	0.70	3.84	236	185	124	34	109	11.2	0.82	0.07	0.01	1.13
Qi Zhen	383	1.41	0.06	0.76	8.87	227	175	96	24.2	62	8.8	0.85	0.07	0.01	1.36

Units: IU/100 ml of milk for vitamins A and E and mg/100 ml of milk for the other components.

Table 4. The total amino acid and taurine levels of the breast milk samples (mg/100 ml)

	Arg	His	Ileu	Leu	Lys	Met	Phe	Thr	Trp	Val	Cys	Tyr	Ala	Asp	Glu	Gly	Pro	Ser	Total amino acids	Taurine
Dashuang	326	146	277	606	378	144	277	264	74	424	163	251	302	532	1,320	80	594	417	6,575	50
YaYa	376	170	336	687	456	174	327	285	76	469	191	310	319	602	1,570	77	675	459	7,559	35
Qi Zhen	369	167	345	680	464	123	320	282	86	913	110	302	290	712	1,440	76	342	451	7,472	22

Table 5. Fatty acid compositions of the breast milk samples (%)

%	C4	C6	C8	C10	C12	C14	C14:1	C15	C16	C16:1	C17	C18	C18:1	C18:2	C18:3	C20	C20:1	Others
Dashuang	0.43	0.15	0.08	0.12	0.24	4.59	0.22	0.16	27.78	3.94	0.21	6.15	27.05	14.54	2.42	0.08	0.69	11.13
YaYa	0.52	0.39	0.11	0.11	0.22	5.85	0.36	0.13	31.85	5.80	0.16	6.29	21.24	9.16	11.50	0.11	0.31	5.89
Qi Zhen	0.43	0.53	0.20	0.15	0.44	7.93	0.45	0.10	36.72	7.04	0.10	3.79	17.30	10.72	7.68	0.08	0.33	6.02
Cow	3.7	2.4	1.4	3.0	3.3	10.9	0.9	1.6	30.0	1.5	1.1	12.0	23.0	2.7	0.4	0.2	0.2	1.7
Human	0.0	0.0	0.1	1.1	4.8	5.2	0.1	0.0	21.2	2.3	0.0	5.4	40.9	14.1	1.4	0.2	0.5	2.7

thirds of that of Dashuang's breast milk (38.7%) and previously examined samples [12, 19, 22]. The abovementioned substitute is similar to the second stage formula, and it might be appropriate to feed cubs with a combination of breast milk and formula during captive feeding. The protein content of breast milk substitutes has the greatest impact on their suitability for nursing cubs, because it directly affects the cubs' body weight gain and health. Thus, when zoos use the abovementioned formula to feed giant panda cubs soon after birth, they should pay careful attention to the cubs' health.

In this study, we could not have a chance to analyze the ratio of casein and whey protein with other animals. It is deeply related to the digestibility with cubs, so we should have more information of the proteins to design milk substitutes. However, we have used casein and whey protein hydrolyzates in the substitutes, and they somehow relieve the problem of digestibility.

**Total amino acids:** Previous studies obtained data on the free amino acid and total amino acid levels of breast milk [18]. Total amino acids include free amino acids plus protein-bound amino acids. Free amino acids usually only comprise about 1% of the total amino acids in milk [4]. When we consider the nutritional needs of giant panda cubs, the concentrations of total amino acids, especially essential amino acids, in breast milk are important. At present, it is not known which amino acids are essential for giant pandas. However, we can speculate about this based on the essential amino acid profiles of human, dog and cat breast milk. In addition, it was reported that the total amino acid profiles of breast milk do not vary markedly among different animals [9].

We compared the concentrations of amino acids found

in the three breast milk samples collected in this study with the concentrations of provisional essential amino acids in breast milk from other animals (Table 6). The amino acid levels of the breast milk samples from the three pandas were about 3 times greater than the minimum essential amino acid requirements of dogs and cats. Similarly, breast milk from dogs and cats contains about three times more amino acids than the respective minimum requirements [2, 3].

**Fat and fatty acid composition:** In the milk collected from Dashuang, YaYa and Qi Zhen, fat accounted for 40.8%, 35.9% and 50.1% of the DM, respectively (Table 6). The fat content of Dashuang's milk was the same as that of bear's milk, and its fat/protein ratio of 1.05 was lower than those reported by Hudson (1.24) [12] and Nakamura (1.46) [22].

The milk samples collected in this study exhibited similar fatty acid profiles to those reported in previous studies [20, 22]. Lyster did not detect C4, C6 or C20 fatty acids in their samples, and Nakamura only detected C14 fatty acids at trace levels. However, no marked differences were detected between the overall fatty acid profiles of these samples and our samples. In addition, the fatty acid composition of the collected giant panda's milk was similar to that of cow's milk, human breast milk and other animals' milk [5, 25, 30]. Compared with cow's milk, the main differences exhibited by giant panda's milk seem to be that it lacks the short chain fatty acids produced in the cow rumen and has the different level of linoleic acid. Ruminants have microorganisms in their stomach, and they break some part of double bonds on linoleic acids in feed [21]. We make milk substitutes with vegetable oils and adjust essential fatty acid contents, so we suppose it is cleared on developing milk replacers, although

Table 6. Nutritional characteristics of the novel milk substitutes (powder or dry matter)

Table 6-1. General nutritional characteristics

Nutrient	Protein	Fat	Carbohydrates	Ash	Moisture (%)	Energy (kcal)	Lactose (%)
Novel milk for 1st stage	38	40	13	6	3	564	5.0
Novel milk for 2nd stage	26	40	25	6	3	564	2.5
Dashuang	38.7	40.8	14.7	5.8	0.0	580	5.3
YaYa	29.0	35.9	32.0	3.2	0.0	567	3.6
Qi Zhen	25.9	50.1	21.5	3.2	0.0	640	1.6
AAFCO dog growth	>22.0	>8.0					
AAFCO cat growth	>30.0	>9.0					
PANDA MILK since 1988	47.5	40.5	4.4	4.2	3.4	572	0.0
Edwards 2006	23	35	35	4	3	547	

Table 6-2. Essential amino acids and fatty acids

Nutrient	mg/100 g																		
	Arg	His	Ileu	Leu	Lys	Met +Cys	Met	Phe +Tyr	Phe	Thr	Trp	Val	Cys	Tyr	Taurine	Linoleic acid	4 $\alpha$ -Linolenic acid	Arachidonic acid	DHA
Novel milk for 1st stage	1,300	860	1,800	3,200	2,600	1,200	1,000	2,600	1,500	1,600	500	2,100	310	1,000	200	5,000	600	20	17
Novel milk for 2nd stage	890	590	1,230	2,200	1,780	820	680	1,780	1,030	410	340	1,440	210	680	200	5,000	600	20	17
Dashuang	1,920	860	1,630	3,570	2,230	1,810	850	3,110	1,630	1,550	440	2,500	960	1,480	290	5,630	940		
YaYa	1,320	600	1,180	2,420	1,610	1,290	610	2,240	1,150	1,000	270	1,650	670	1,090	120	3,120	3,920		
Qi Zhen	1,130	510	1,050	2,080	1,420	710	380	1,900	980	860	260	2,790	340	920	70	5,370	3,650		
AAFCO dog growth	620	220	450	720	770	530		890		580	200	480				1,000			
AAFCO cat growth	1,250	310	520	1,200	830	1,100	620	880	420	730	250	620			100	500		20	
PANDA MILK since 1988	1,752	1,332	2,720	4,635	4,044	1,961	1,460	4,788	2,360	2,395	865	3,168	501	2428	100	11,500	1,190	20	

Table 6-3. Minerals

Nutrient	mg/100 g											
	Ca	P	K	Na	Cl	Mg	Fe	Cu ( $\mu$ g/100 g)	Mn	Zn	I	Se
Novel milk for 1st stage	1,000	800	1,000	500	1,000	80	7	700	0.9	12	0.2	0.010
Novel milk for 2nd stage	1,000	800	1,000	500	1,000	80	7	700	0.9	12	0.2	0.010
Dashuang	940	870	1,180	390	1,210	100	7.2	589	0.06	4.0		
YaYa	830	650	440	120	380	40	2.9	247	0.04	4.0		
Qi Zhen	690	530	290	70	190	30	2.6	214	0.03	4.2		
AAFCO dog growth	1,000	800	600	300	450	40	8	730	0.50	12	0.15	0.011
AAFCO cat growth	1,000	800	600	200	300	80	8	500	0.75	7.5	0.035	0.010
PANDA MILK since 1988	900	570	360	140	330	50	5.5	300	0.10	2.5	0.04	

Table 6-4. Vitamins

Nutrient	mg/100 g																
	IU VA	IU VD	IU VE	$\mu$ g VK	$\mu$ g VB <sub>1</sub>	$\mu$ g VB <sub>2</sub>	Pantothenic acid	Niacin	VB <sub>6</sub>	Folic acid	Biotin	$\mu$ g VB <sub>12</sub>	Choline	Ascorbic acid	Lacto-ferrin	Nucleotides	Milk oligo sacch.
Novel milk for 1st stage	2,000	300	20	20	1.5	2.5	4.0	15.0	1.5	0.40	0.01	5.0	130	30	80	7	50
Novel milk for 2nd stage	2,000	300	20	20	1.5	2.5	4.0	15.0	1.5	0.40	0.01	5.0	130	30	80	7	50
Dashuang	2,375		8.6	0.20	8.00									54			
YaYa	2,102		4.7	0.20	2.50									14			
Qi Zhen	1,170		4.3	0.20	2.30									27			
AAFCO dog growth	500	50	5	0.10	0.22	1.0	1.14	0.1	0.018			2.2	120				
AAFCO cat growth	900	75	3	10	0.50	0.40	0.5	6.0	0.4	0.080	0.007	2.0	240				
PANDA MILK since 1988	5,000	1,000	14	20	1.5	2.0	6.0	15.0	1.5	0.50	0.01	5.0	150	200			
Rodent AIN 1993	400	100	7.5	90	0.5	0.6	1.5	3.0	0.6	0.2	0.02	2.5	100				

Novel milk for 1st/2nd stage: the milk substitutes (powder) developed in this study, Dashuang, YaYa, and Qi Zhen: the breast milk samples from each panda (dry matter), AAFCO dog growth: AAFCO dog food nutrient profiles (dry matter) [7], AAFCO cat growth: AAFCO cat food nutrient profiles (dry matter) [7], Panda milk since 1988: the 1st milk substitute developed by Tanabe [28], Edwards 2006: a formula recommended in a textbook [10], Rodent AIN 1993: the vitamin requirements for rodents outlined by the AIN (moisture content=6.6%) [27].

we do not yet know the minimum essential level of linoleic acid with giant panda.

**Lactose and other carbohydrates:** The lactose contents of the breast milk from the three pandas ranged from 0.5–1.0%, which were lower than the lactose levels seen in the breast milk of dogs (3%) and cats (4%) [2, 3]. Trisaccharides were detected in the samples from all three pandas, and the total

saccharide levels of Yaya's breast milk sample, which was obtained around 160 days after delivery, were 2.3 times as high as those of Dashuang's sample, which was collected 25 days after delivery (Fig. 1 and Table 2). The ratios of total saccharides to lactose in the samples from Dashuang and YaYa were 3.01/0.90=3.3 and 6.79/1.02=6.7, respectively. These ratios are not greater than the ratio of 8.3 calculated

Table 7. The compositions of breast milk from humans and other animals (g/100 ml)

Animal	Energy	Protein	Fat	Carbohydrates	Ash	Solids	Moisture	Lactose
	kcal/100 ml	g/100 ml						
Human	65	1.1	3.5	7.2	0.2	12.0	88.0	6.0
Cow	67	3.3	3.8	4.8	0.7	12.6	87.4	4.8
Dog	136	9.6	9.5	3.1	1.0	23.2	76.8	3.1
Cat	90	7.0	4.8	4.8	1.0	17.6	82.4	4.8
Bear	247	8.9	22.6	2.1	1.4	35.0	65.0	
Small hippo	74	3.8	4.4	4.8	0.7	13.7	86.3	4.8
Goat	65	2.9	3.8	4.7	0.8	12.2	87.8	4.7
Whale	429	11.0	42.3	1.0	1.4	55.7	44.3	

Solids=protein + fat + carbohydrates + ash. Energy (kcal/100 g)=(protein + carbohydrates) g/100 g\*4 kcal/g + (fat) g/100 g \*9 kcal/g. Lactose is included in carbohydrates.

by Nakamura from peak area data obtained using the phenol sulfuric acid method [22]. Subtracting total saccharides from total carbohydrates resulted in values of -0.51 and +2.28 for the samples from Dashuang and YaYa, respectively. These differences might have been due to the method used to determine the samples' carbohydrate levels, especially the effects of removing moisture from the concentrated milk solid samples, the filtering out of polysaccharides before the total saccharide analysis and the organic acid contents of the samples. We have had some difficulty to use hazardous reagents, and we hesitated to use phenol sulfuric acid method. But, the subtracting method has its weakness, especially on concentrated materials. In the next opportunity, we should confirm the correlation between the two methods taking organic acids and others into consideration. On the other hand, the differences between the two samples, which were collected on day 25 and day 160 after delivery, might have been due to changes related to preparing the cubs for weaning and their subsequent diet of bamboo shoots and leaves. Unfortunately, Qi Zhen's original HPLC data sheet was lost, and the data could not be estimated. The breast milk of other animals also contains many kinds of oligosaccharides [22], which probably play a role in promoting health and growth.

**Vitamins:** There is little available information about the vitamin content of giant panda's breast milk. Liu [19] examined its vitamin A, D and E concentrations. However, the reported levels were much lower than those seen in the present study. The nutritional requirements of various animals during growth have been outlined previously, e.g., those of dogs and cats have been described by the NRC [1], those for humans were mentioned in the CODEX STAN [15], and those for rodents were reported by the American Institute of Nutrition (AIN) [27]. Table 6 shows the analyzed data and minimum requirements in mg/100 g powder or DM. The milk collected from Dashuang on day 25 displayed higher vitamin levels than the samples obtained from YaYa and Qi Zhen around day 160. Similar patterns can be seen in many animals. The colostrum produced soon after delivery exhibits a thick consistency and contains many components [26]. The vitamin B<sub>2</sub> and E levels of the milk samples from the three pandas were higher than those recorded in other animals, while other vitamins were present at similar concentrations (mg/100 g) to the requirements outlined for dogs,

cats and humans. Further studies are needed to explain why the samples collected in the present study exhibited high levels of vitamins B<sub>2</sub> and E. It is possible that the feed given to panda mothers increases the levels of vitamins in panda breast milk.

**Minerals:** Liu *et al.* [19] and Zhang *et al.* [34] reported mineral data for giant panda breast milk. They both detected lower concentrations of calcium and phosphorus than were found in the present study. The mineral concentrations (in DM) of Dashuang's milk did not differ markedly from the mineral requirements of dogs and cats [1, 7], or rodents [27] (Table 6).

**Milk substitutes proposed:** Tanabe's and Edwards' formulas each represent important steps in the development of giant panda milk substitutes, and in this study, we proposed new substitutes, which we consider will aid the proliferation of giant pandas. This gradual approach to the development of giant panda breast milk substitutes is necessary, because it is not possible to obtain sufficient reliable data about wild animal's milk in a single study. After preparing the milk substitutes, we carried out feeding tests on giant panda cubs at Adventure World from 2011 to 2012. We are going to submit a further report about the results of these feeding tests.

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