

Glucosamine contents of milk hydrolysates from various mammals

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Abstract

It was reported in the authors' previous study that the glucosamine (GlcN) content of Asian elephant milk (516 mg GlcN/100 g milk) was markedly higher than that of cow, mare and human breast milk. Based on these findings, the GlcN levels of milk from other mammals, especially herbivores, were analyzed using the identical high performance ion exchange liquid chromatography as was employed in the previous study. The following mg GlcN/(100 g milk) values were obtained for milk hydrolysates from various mammals: Jersey cow 12; water buffalo 6; goat 10; hippopotamus 19; dog 24; bear 97; giant panda 72; seal 114; and dolphin 94. Additionally, mg GlcN/(g total amino acids), and mg GlcN/100 kcal were calculated. The milk from herbivores contained much lower levels of GlcN than elephant milk. Bear and marine mammals' milk exhibited moderate GlcN levels, probably because of their low moisture content. Giant panda milk displayed slightly increased GlcN levels and the highest value of mg GlcN/(g total amino acids), and mg GlcN/100 kcal. Free GlcN was not detected in any of the milk samples. The large interspecies differences in milk GlcN concentrations may be related to their ways of life and have important implications for human and animal health, especially in bone development and gut microbiota.

Keywords: glucosamine, hydrolysate, mammals, milk

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Introduction

Large amounts of glucosamine (GlcN) are present in cartilage, which is used for bone growth (Alberts *et al.*, 2015; Cooper *et al.*, 2013). GlcN is present in some oligosaccharide chains of glycoproteins, proteoglycans and glycolipids. Milk may contain little free GlcN, as is the case for free amino acids (AA), which account for <1% of the AA found in milk (Kiyosawa, 1998). On the other hand, the efficacy of pure GlcN supplementation in humans with bone disorders is still questionable; thus, bone development, renewal and GlcN metabolism may also differ among mammal species. GlcN was found to be the first “bifidus factor”, a selective growth substrate for intestinal bifidobacteria (Musilova *et al.*, 2014), and hence, it may affect gut microbiota.

We previously found that the hydrolysate of Asian elephant (*Elephas maximus*) milk contained 14-128 times that of GlcN human (*Homo sapiens*), mare (*Equus caballus*) and cow (*Bos Taurus*, Holstein) milk (Takatsu *et al.*, 2017). This difference may be associated with bone development, gut microbiota and animal health.

The present study was performed to examine whether milk from other mammals, especially herbivores except for elephants, exhibits high GlcN levels. Thus, 11 types of animal milk were examined using the same methods as in our previous study.

Materials and Methods

All experimental procedures were performed according to the ethical rules of the Japanese Association of Zoos and Aquariums, after consent to subject the milk samples to nutritional analyses was obtained from all of the relevant facilities.

Table 1 shows the donated milk samples. The samples were filtered through gauze-like fabric to eliminate grass or feed and then cooled. All milk samples were frozen as soon as possible and transported at -20°C. The reagents, standards and mixture solutions were supplied by Wako Pure Chemical (Osaka, Japan), Sigma-Aldrich (Saint Louis, MO, USA), Sigma-Fluka (Rockville, NY, USA), or Mitsubishi Chemical (Tokyo, Japan).

Table 1 Sources of milk samples

Name	Jersey cow	Water buffalo	Goat		Hippo	Dog	Bear	Giant panda		Seal ²	Dolphin 1	Dolphin 2
	<i>Bos taurus</i>	<i>Bubalus bubalis</i>	<i>Capra hircus</i>		<i>Hippopotamus amphibius</i>	<i>Canis lupus familiaris</i>	<i>Ursus arctos</i>	<i>Ailuropoda melanoleuca</i>		<i>Phoca largha</i>	<i>Pseudorca crassidens</i>	<i>Tursiops truncatus</i>
Number of samples	2	2	1	1	1	3	1	1	1	1	1	1
Donated by	Highland Ice Cream Factory	Kurkku Fields	Ichihara Elephant Kingdom mixed with commercial milk from Okinawa	Ichihara Elephant Kingdom	Kobe Oji Zoo	dog breeders	Noboribetsu Bear Park	Adventure World	Adventure World	Oturu Aquarium	Oarai Aqua World	Oarai Aqua World
Obtained at (days) ¹	240 and 299	30 and 202	pooled mix	97	125	6, 12, and 13	84	2 (colostrum)	8 (regular)	0, 3, and 5	21	7 (stillbirth)
Sample volume (ml)	both 100	both 50	100	100	10	8, 10, and 7	10	1	1	27, 41, and 14	10	mammals

1) Days after parturition

2) The three seal milk samples were mixed to create a single sample for use in the subsequent assays.

The nutritional contents of each type of milk were analyzed as follows: moisture by the gravimetric analysis, protein by the modified Dumas method, fat by the Röse-Gottlieb method, ash by the gravimetric method and carbohydrate determined by calculations. Energy levels were calculated using Atwater's formula: [(protein g +carbohydrates g)*4+fat g*9] kcal/g. These methods were based on the Association of Analytical Communities Methods for Analyzing Dairy Products (Latimer 2016).

For the AA and GlcN ion-exchange chromatography, the same methods as were used in the previous study were employed (Hitachi, 1990; Le Boucher *et al.*, 1997). Briefly, for the free GlcN chromatography, a sample was dissolved in sulfosalicylic acid and the resultant aggregates were filtered (JFRL, 2015). For the chromatography of the GlcN in the milk hydrolysates, a sample was hydrolyzed with 6 M hydrochloric acid (HCl) and then the hydrolysate was filtered and dried, before the precipitate was dissolved in HCl. The free and hydrolyzed samples were subjected to AA analysis using the L-8900 AA high-performance liquid chromatography (HPLC) system (Hitachi High-Tech Science Co., Tokyo, Japan) with post-column ninhydrin derivatization. The high-resolution 155-minute cycle described in the manufacturer's manual was employed (Hitachi, 1990; Le Boucher *et al.*, 1997). AA and GlcN concentrations of the samples were

calculated by comparing the area of each HPLC peak with those of the relevant standards. The GlcN peak was well separated from the peaks for other AA in the 155-minute-mode HPLC.

The nutritional contents of milk samples are usually compared based on their protein and energy levels (CODEX, 2007). Protein levels are related to the growth rate of baby cubs and energy levels are related to growth and feeding conditions; i.e., temperature, humidity and frequency of feeding. As the protein contents of the samples were slightly different from their total AA levels, GlcN mg/(g total AA) values were calculated for the milk samples because the total AA concentration data were obtained from the same chromatograms as the GlcN concentration data. The concentrations of Met, Cys and Trp were not measured because these AA account for <5% of the total AA concentration (Davis *et al.*, 1994), and quantifying them would have required the use of greater volumes of milk samples. Therefore, “the total AA level” does not include the levels of Met, Cys or Trp.

Results and Discussion

The nutritional contents of each type of milk; i.e., their moisture, protein, fat, ash and carbohydrate contents, and their calculated energy levels are shown in Table 2. Free GlcN was not detected in any of the samples (detectable level: 0.1 mg/100 g). The GlcN

levels (mg/100 g) of the animal milk hydrolysates are shown in Fig. 1. As outlined in the nutritional considerations for human infant formula criteria (CODEX, 2007), values relative to their total AA levels; i.e., their mg GlcN/ (g total AA), were calculated, and the GlcN contents of the examined samples relative to their total energy levels; i.e., their mg GlcN /100 kcal values, were also calculated for the milk samples (Fig. 2).

The milk from Jersey cows, water buffalos, goats and hippopotamuses had low GlcN concentrations, low GlcN levels relative to their total AA, and low GlcN levels relative to their total energy levels. The milk from dogs had a slightly higher GlcN level than that of herbivores and lower than that of bears and pandas for concentration in milk, AA and energy. Samples (1 ml) of giant panda milk were analyzed and the nutritional contents and energy data for these samples were determined by proportional calculations

based on the AA data for a milk sample collected from Dashuang (a giant panda) at 25 days after parturition (Table 2). The nutritional contents of Dashuang milk (g/100 g) were as follows: protein: 6.6, fat: 6.9, carbohydrates: 2.5, ash: 1.0, moisture: 83.0, total AA: 6.2 (excluding Met, Cys, and Trp), and energy (kcal/100 g): 99 (Zhang *et al.*, 2016). Although the giant panda milk had high moisture levels, as was seen in the herbivores, it contained the highest levels of GlcN in terms of its mg GlcN/(g total AA) and mg GlcN/100 kcal values. On the other hand, bear, seal and dolphin milk, which had low moisture levels (<75%), contained moderate levels of GlcN in terms of their mg GlcN/(100 g milk), mg GlcN/(g total AA), and mg GlcN/(100 kcal) values. These kinds of milk have higher milk solid levels and large amounts of oligosaccharides (Albrecht *et al.*, 2014; Boix-Amorós *et al.*, 2019; Li *et al.*, 2019).

Table 2 Total AA levels, energy levels and nutritional contents of the milk of various mammals

	Jersey cow	Water buff ³⁾	Goat	Hippo ⁴⁾	Dog	Bear	G panda ^{2 5)}	G panda ^{8 5)}	Seal	Dolphin1	Dolphin2
Number of samples	2	2	2	1	3	1	1	1	1	1	1
Total AA ¹⁾ g/100 g	4.9	3.7	3.7	6.4	7	11.3	4.6	5.3	10.3	16.8	16.3
Energy ²⁾ kcal/100 g	79	118	69	154	123	128	73	85	318	312	213
Protein g/100 g	3.6	5.4	4.1	6.0	7.6	12.4	4.9	5.7	11.4	16.2	17.6
Fat g/100 g	5.1	8.5	3.7	12.8	8.5	7.4	5.1	6.0	29.8	26.8	15.6
Carbohydrates g/100 g	4.7	4.9	4.8	3.8	4.0	3	1.9	2.2	1.1	1.4	0.6
Ash g/100 g	0.7	1.0	0.8	0.8	0.9	2	0.7	0.9	0.8	0.8	1.0
Moisture g/100 g	85.9	80.2	86.6	76.6	79.0	75.2	87.4	85.4	56.9	54.8	65.2
GlcN deviation among samples ⁶⁾	1.8	0.2	1.2		10.8						

1) The total AA levels did not include the levels of Trp, Cys or Met.

2) Energy levels were calculated using Atwater's formula (Latimer, 2016).

The nutritional content and energy data for the giant panda milk (collected 2 and 8 days after parturition, respectively) were obtained via proportional calculations based on the data for Dashuang's (a giant panda) milk given in the reference (Zhang *et al.*, 2016).

3) water buff: water buffalo

4) hippo: hippopotamus

5) G panda: giant panda

6) Deviation from the mean in cases involving two samples and the SD for cases involving three samples

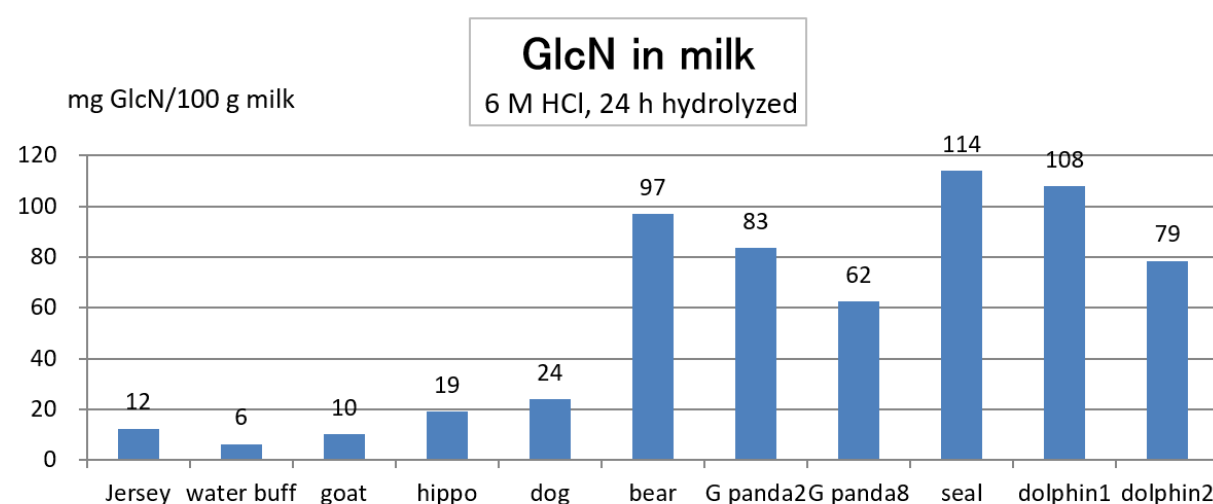


Figure 1 GlcN mg/100 g milk values of milk hydrolysates obtained via 24 h treatment with 6M HCl at 110°C
Jersey: Jersey cow, hippo: hippopotamus, G panda: giant panda, water buff: water buffalo

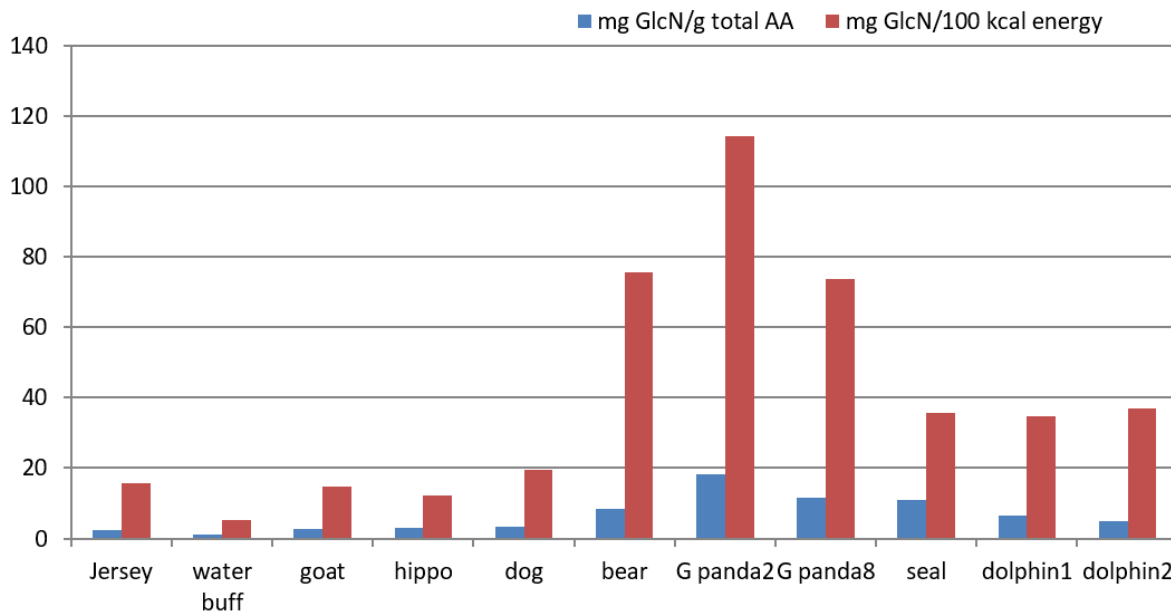


Figure 2 GlcN/(total AA) and GlcN/energy values of milk

Jersey: Jersey cow, hippo: hippopotamus, G panda: giant panda, water buff: water buffalo

The mg GlcN/(100 g milk), mg GlcN/(g total AA), and mg GlcN/(100 kcal) values in marine mammals milk were at moderate levels because they have low moisture, high total AA and great energy.

GlcN level deviations (mg/100 g milk) of 1.8, 0.2, and 1.2 from the mean were seen for Jersey cow milk, water buffalo milk and goat milk, respectively; and the standard deviation (SD) among the three dog milk samples was 10.8. These deviations were due to the timing of the sample collection (in days after parturition), the milking time and the differences among mammals. On the other hand, one milk sample was analyzed for each of the other mammals (Table 2). The mean GlcN levels are shown in Fig. 1.

The variations among species were somewhat marked; however, the GlcN level of elephant milk was >4.5 times higher than those of other species. Holstein cows and Jersey cows are of the same species but differ in their milk GlcN contents. These differences may have been caused by the long history of cow breeding by humans. Commercial milk from these two types of cows is sold in different categories. The levels of most milk components generally differ markedly among species (Jenness and Sloan, 1970); however, GlcN in elephant milk was 27-82 times greater than in herbivores in this study, although each AA in various milk has usually similar contents (Davis *et al.*, 1994). The large interspecies differences in the GlcN concentrations of milk observed in our previous and present studies may be related to the type of saccharides present, that is, oligosaccharides markedly exceed lactose in some kinds of milk (Messer and Urashima, 2002) and may have important implications for human and animal health. GlcN-containing oligosaccharides were reported to act as a growth substrate for intestinal bifidobacteria (Bode, 2012; Cavalli *et al.*, 2006; Musilova *et al.*, 2014).

Bone development starts with space filling by hyaluronan with 50% GlcN content, and the hyaluronan is gradually replaced by bone. Therefore,

GlcN is a key factor in bone development (Alberts *et al.*, 2015, Dammrich, 1991). The differences in bone to body weight ratios among species are not as great as the differences in GlcN content. However, the bone development rate/ body weight gain rate may be influenced by bone stress and there are other physiological differences in mammal species, for example, in the metabolic processes involved in bone development, gut microbiota and their digestive systems. The milk of African elephants, rhinoceroses, dugongs and manatees should be studied in the future, as these animals are most closely related to Asian elephants (McCullagh and Widdowson, 1970; Osthoff *et al.*, 2008).

The following mg GlcN/(100 g milk), mg GlcN/(g total AA), and mg GlcN/100 kcal values were obtained based on calculations performed in this study using data obtained in our previous study: Asian elephant milk: 516, 152, and 472, respectively; Holstein cow milk: 4, 1, and 6, respectively; mare milk: 12, 6, and 22, respectively; and human breast milk: 38, 47, and 59, respectively (Takatsu *et al.*, 2017).

Except for elephants, milk from herbivores contained lower levels of GlcN. Dog milk had a slightly higher GlcN level than herbivores. Bear and marine mammal milk contained moderate GlcN levels but the values were reduced relative to total AA and energy. In addition, giant panda milk had the highest GlcN level relative to its total AA and energy levels. Oligosaccharides and other saccharides containing GlcN showed strong associations with animal and human gut microbiota and health. Furthermore, differences in GlcN levels may influence bone development such as replacement of cartilage-derived hyaluronan (50%GlcN) with bone.

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