

SUPPLEMENTARY FILES

1

2 **Preparation for AA (Met, Cys and Trp) analyses**

3 The samples used to analyze the levels of Met and Cys were prepared in glass tubes
4 containing 100 mg or 200 mg milk supplemented with 3 ml performic acid solution
5 (formic acid: hydrogen peroxide solution=9:1), which were incubated at 5°C in a
6 refrigerator for 16 hr. Next, each tube was dried out, 3 ml of 6M HCl were added,
7 and then the samples were hydrolyzed for 18 hr at 110°C. After the hydrolyzation
8 procedure, we prepared filtered samples in the same manner as for the other AA.

9 The samples used to assess the concentration of Trp were prepared using 1 g milk
10 supplemented with 7.8 g BaOH·8H₂O, 4.5 ml water and 0.5 ml of 60%
11 thioldiethylene glycol, and were hydrolyzed in an evacuated glass tube for 12 hr at
12 110°C. Then, they were neutralized to slight alkalinity with 1M NaOH and topped
13 up to 100 ml with water. After the neutralization procedure, we prepared a filtered
14 sample in the same manner as for the other AA.

15 **The UKP was not found in a free form in milk**

16 We collected 1 g of milk (which contained about 30 mg protein) in a flask and then
17 mixed it with 15 ml water, before adding 1 g of 5-sulfosalicylic acid dihydrate and
18 filling the flask up to 20 ml with water. Then, the mixture was sonicated for 5 min,
19 before being filtered through a 0.2- μ m fine filter (Millex-LG, Merk Millipore). The
20 filtered solution had a pH of 2.2. Then, we analyzed the AA in the samples using the
21 155-min mode of the AA analysis system. We only detected a small amount of free

22 AA (22-46 mg/100 g) and <0.1 mg/100 g free UKP during HPLC performed in the
23 155-min mode (Table S1).

24 **The UKP did not represent a peptide and was not found in a free form in milk**

25 We initially wondered whether the UKP was a peptide, but hydrolyzing a
26 smaller volume of elephant's breast milk did not change its UKP content, so we
27 assumed that UKP was not a peptide. There was only a small amount of free UKP in
28 the unhydrolyzed milk (Table S1).

29 **Trichloroacetic acid (TCA) treatment**

30 We collected 0.1 g of elephant's breast milk in a tube, added 0.1 ml of 10%
31 (w/v) TCA, mixed it in a vortex mixer, and then centrifuged it at 4°C and 21500 g
32 for 15 min. The resultant supernatant (sup) and precipitate (ppt) were hydrolyzed
33 with 6M HCl for 24 hr. Then, we analyzed the AA in the hydrolysates using the
34 155-min mode of the AA analysis system. The UKP (Ala equivalents) was detected
35 at concentrations of 200 mg/100 g and 35 mg/100 g in the sup hydrolysate and ppt
36 (mainly protein) hydrolysate, respectively, which showed that the sup contained 5.7
37 times more UKP than the ppt (Table S1). The UKP in elephant's milk might be
38 mainly derived from mono-, oligo- or polysaccharides or glycolipids, rather than
39 from glycoproteins.

40 **Alkaline hydrolysis did not produce any UKP or GlcN**

41 In the Trp assay, we hydrolyzed Zuze's milk with 4M BaOH for 12 hr at 110°C.
42 No UKP peak was detected during the HPLC assay for Trp (Table S1). When the
43 GlcN standard was hydrolyzed with BaOH and subjected to HPLC, GlcN was
44 degraded, and hence, was not detected.

45

Table S1

Name, days after delivery	mg/100 g	TCA hydrolysis (ppt)	TCA hydrolysis (sup)		Free AA		Alkaline hydrolysis
		Zuze, d19	Zuze, d19	Pooly, d338	Zuze, d19	Pooly, d307	Pooly, d307
Asp/Asn		207	33	11.2	2.5	11.4	234
Thr		192	21	0.8	0.6	0.9	9
Ser		136	20	0.3	0.6	0.5	20
Glu/Gln		488	70	22.1	12.5	22.5	522
Gly		45	13	2.8	0.4	3.8	109
Ala		102	10	1.7	0.5	2.2	142
GlcN		78	446	0.0	0.0	0.0	0
Val		168	18	0.5	0.3	0.5	137
Cys		25	0	nd	nd	nd	nd
Met		43	5	nd	nd	nd	37
Ile		116	16	0.2	0.3	0.3	34
Leu		241	29	0.3	0.4	0.2	254
Tyr		142	12	0.3	0.4	0.4	152
Phe		132	13	nd	0.4	0.2	140
Trp		0	0	0.1	nd	nd	49
Lys		197	25	0.2	1.5	0.9	185
His		62	11	0.4	0.3	0.4	39
Arg		127	14	0.4	0.5	0.5	11
Pro		120	15	1.5	0.4	1.7	260
Total amino acids		2,542	325	43	22	46	2,334

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47 **Table S1 legend** AA and GlcN concentrations of elephant's milk samples subjected

48 to various processes

49 The table shows the following data: 1) The AA concentrations of the hydrolysates of
50 the sup and ppt obtained after the TCA treatment of elephant's milk

51 The milk was first treated with TCA, and then the sup and ppt were hydrolyzed
52 with HCl.

53 2) The free AA levels of the sup of unhydrolyzed milk samples

54 Milk samples were treated with sulfosalicylic acid, and their sups were assayed.

55 3) The AA concentrations of milk that had been subjected to alkaline hydrolysis
56 with 4M BaOH in the Trp assay

57 All data are shown as mg/100 g.