

## PROCESSING AND PRODUCTS

### Mineral, Amino Acid, and Hormonal Composition of Chicken Eggshell Powder and the Evaluation of its Use in Human Nutrition

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**ABSTRACT** Chicken eggshell powder (ESP) might be an attractive source of Ca for human nutrition. To study its nutritional value, we analyzed minerals, amino acids, and hormones in commercially available Slovakian ESP. The mineral composition was compared with three Dutch ESP samples that differed in feed and housing, a Japanese ESP, refined CaCO<sub>3</sub>, and an oyster shell supplement.

Chicken eggshell powder contains high levels of Ca (mean  $\pm$  SD/g EPS: 401  $\pm$  7.2 mg) and Sr (372  $\pm$  161  $\mu$ g) when compared with recommended or estimated daily intakes for humans 51 to 70 yr of age. Levels of potentially toxic Pb, Al, Cd, and Hg were very low as were levels of V, B, Fe, Zn, P, Mg, N, F, Se, Cu, and Cr. Large differences in the levels of F, Se, Cu, Cr, and Sr in the Dutch

and Slovakian ESP indicated a strong influence of feed and environment. The small protein fraction of ESP contains high levels of Gly and Arg. Furthermore, small amounts of transforming growth factor- $\beta$ 1 (0.75 to 7.28 ng/g ESP), calcitonin (10 to 25 ng/g ESP), and progesterone (0.30 to 0.33 ng/g ESP) were detected. Estradiol-17 $\beta$  and calcitriol were below the detection limit of the methods used. Compared with ESP, refined CaCO<sub>3</sub> was found to contain increased levels of Cd, and the oyster shell supplement showed increased levels of Al and Cd.

Therefore, ESP seems to have a beneficial composition with about 39% of elemental Ca, relevant amounts of Sr, and low levels of Al, Pb, Cd and Hg. It may be used as a Ca source in human nutrition.

(Key words: eggshell, minerals, hormones, amino acids, human nutrition)

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## INTRODUCTION

Several Ca sources are available for food fortification (Faine, 1995). CaCO<sub>3</sub> is the most widely used Ca salt because 40% of the compound is well absorbable Ca. This Ca salt can be formulated from Ca(OH)<sub>2</sub> or chalk in the laboratory but can also be derived from fossilized or fresh shells (e.g., chicken eggshell and oyster shell) (Kärkkäinen et al., 1997). Natural Ca sources are of interest because they contain not only Ca but also other elements (e.g., Sr and F), which may have a positive effect on bone metabolism (Reginster et al., 1999). The safety of natural Ca supplements, however, has been questioned because they may contain relevant amounts of potential toxic elements such as Pb, Al, Cd, and Hg (Whiting, 1994).

Chicken eggshells, which first serve to protect and provide nutrients to the enclosed embryo (Solomon et al., 1994), have been used by humans for a long period as a food additive, but on a very modest scale. Lichtenstein (1948) mentioned a large store of Ca that was thrown away instead of being used for human nutrition. In 1953,

chicken eggshell powder (ESP) was shown to have antirachitic effects in rats (Bölönyi and Orsós, 1954). In vitro, ESP stimulated the growth of chicken embryo cartilage cells (Rovensky et al., 1994). In 1991, demembranized chicken ESP was studied (Makai and Chudacek, 1991) in an elderly population with osteoporosis. Use of the ESP resulted in decreased pain and increased bone mineral density (BMD). In 1995, comparable results were obtained from a Dutch pilot study with osteoporotic subjects (Schaafsma and Pakan, 1999). In piglets, the apparent absorbability of Ca from ESP was found to be at least as good as that from purified CaCO<sub>3</sub> (Schaafsma and Beelen, 1999).

Because it has been suggested that hormone-like activity is present in ESP (Rovensky et al., 1994; Stanciková et al., 1996), this might play a role in the effects on BMD and pain as mentioned before. Transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1) is thought to play a role in regulating BMD (Grainger et al., 1999). Calcitonin inhibits osteoclasts and reduces pain in subjects with a high bone turnover (Aurbach, 1988; Overgaard et al., 1992; Rong et al., 1996). Based

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**Abbreviation Key:** AAS = atomic absorption spectroscopy; BMD = bone mineral density; ESP = chicken eggshell powder; TGF- $\beta$ 1 = transforming growth factor- $\beta$ 1.

on the amino acid sequence, it has been suggested that chicken calcitonin might be one of the most potent calcitonins, and it is also effective in mammalian systems (Deftos, 1993). Of the steroid hormones, both progesterone and estrogen reduce bone resorption in postmenopausal women, and progesterone may also stimulate bone formation (Oursler et al., 1996). Calcitriol, which is the most active vitamin D<sub>3</sub> hormone, reduces serum parathyroid hormone and, as a consequence, reduces bone turnover and loss (Aurbach, 1988).

The main goal of this study was to provide insight into the amounts and variability in amounts of elements in ESP and the relevance of these amounts compared with recommended, estimated, or acceptable daily intakes. Additionally, three samples of Dutch ESP were analyzed to estimate the influence of housing and feed on the mineral composition of eggshell. Finally, the presence of a number of hormones in ESP was studied.

## MATERIALS AND METHODS

### Study Design

Twelve samples from different batches of Slovakian ESP<sup>2</sup> were analyzed for several elements. It was supposed that the eggs were derived from chickens with only small differences in feed and housing. Mean values and mean  $\pm$  2 SD intervals of the Slovakian ESP were compared with purified, food grade CaCO<sub>3</sub>,<sup>3</sup> oyster shell tablets,<sup>4</sup> a Japanese ESP,<sup>5</sup> and the recommended (Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 1997; National Research Council, 1989) or acceptable daily intakes (Dokkum van, 1985) for adults (51 to 70 yr of age). Only a small number of ESP samples were studied for the influence of feed and housing of chickens on eggshell mineral composition (n = 3), amino acid patterns of the protein fraction (n = 4, Dutch and Slovakian ESP), and the presence of hormones (n = 4 to 10, Slovakian ESP).

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<sup>20</sup>SPE 1 ml columns 100 mg; Varian, Houten, The Netherlands, NL-3994 DB.

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### Materials and Methods

Slovakian ESP was prepared from battery eggs by a patented process.<sup>2</sup> The final powder does not include the two inner membranes of the eggshell. Dutch ESP samples were prepared in the laboratory; two inner membranes were not included. Samples included those from brown, free-range, 'four cereal' eggs<sup>6</sup> (ES1) and 'Grass' eggs<sup>7</sup> (ES2) and those from brown, battery eggs<sup>8</sup> (ES3). 'Four cereal' chickens were fed a mix of four cereals (Viergranenmeel<sup>9</sup>) and were kept inside (with outside light) on a litter of sand and wood chips. 'Grass' egg chickens could walk outside (enjoy fresh air and sun), were able to eat grass and green vegetables, and received an additional feed consisting of cereals and leguminous plants (Legmeel Grashennen<sup>9</sup>). Dutch battery chickens received standard feed for laying hens (Legmeel A, B, and C<sup>9</sup>).

**Mineral Determination.** Total N, NH<sub>3</sub> (Kjeldahl<sup>10</sup>), Ca, Mg, Fe, Zn [flame AAS<sup>11</sup> (atomic absorption spectroscopy)], P (spectrophotometrically<sup>12</sup>), Cu, Pb, Al, Cd (graphite furnace AAS<sup>11</sup>), and Hg (flame ionization absorption spectroscopy<sup>11</sup>) were analyzed by the Research and Development Department of Friesland Coberco Dairy Foods. Chromium, V (graphite furnace AAS 430<sup>11</sup>), B (inductively couple plasma—mass spectroscopy), Elan 5000<sup>11</sup>], F [gas chromatographically (Fresen et al., 1968)], Se (fluorimetrically, RF-1501<sup>12</sup>), Sr (flame AAS<sup>11</sup>), and Si (inductively coupled plasma—atomic emission spectroscopy<sup>13</sup>) were analyzed by TNO Nutrition.<sup>14</sup>

**Amino Acids and Hormones.** Amino acid patterns were determined directly in ESP with a Hewlett Packard 1090 Aminoquant by Eurosequence B.V.<sup>15</sup> after vaporphase hydrolysis (standard hydrolysis condition: 5.7 N HCL for 2 h at 166 C) of protein samples (Schuster, 1988). With this method Trp is destroyed, the detection of Cys is not reliable because of instability, and Asn and Gln are deaminated into Asp and Glu. A precision error up to 10% is normal for amino acid analyses.

Transforming growth factor- $\beta_1$  was determined with Predicta® TGF $\beta_1$  (ELISA<sup>16</sup>) by VURCH.<sup>17</sup> For this analysis, a 12% ESP in PBS (pH 7.2) was prepared and stirred at 4 C for 24 h, followed by centrifugation (10,000 rpm, 4 C). The samples were activated as described for plasma samples (by acidifying with HCl for 1 h) prior to analysis of TGF- $\beta_1$ .

Calcitonin was determined in the water-soluble protein fraction of ESP, which was prepared according to the method of Hincke et al. (1992) by the Universital Hospital of Groningen.<sup>18</sup> A competitive enzyme immunoassay (EIAH 6003<sup>19</sup>), developed for the detection of salmon calcitonin, was used with a standard curve of chicken calcitonin.

Progesterone and estradiol-17 $\beta$  were determined by TNO Nutrition<sup>14</sup> after organic solvent extraction and cleaning up via Solid Phase Extraction.<sup>20</sup> Progesterone was analyzed with a commercially available RIA kit,<sup>21</sup> whereas estradiol-17 $\beta$  was analyzed with a RIA-kit from TNO Nutrition.<sup>14</sup>

**TABLE 1: Mineral composition of Slovakian and Dutch eggshell powder**

Component		Slovakian <sup>1</sup>	ES1 <sup>2</sup>	ES2 <sup>2</sup>	ES3 <sup>2</sup>
N	mg/g	3.96 (0.24)	4.01	3.90	4.02
NH <sub>3</sub>	mg/g	0.38 (0.11)	0.65	0.66	0.68
Ca	mg/g	401 (7.21)	385	393	387
Mg	mg/g	4.50 (0.50)	3.6	3.6	3.5
P (Total)	mg/g	0.99 (0.21)	1.50	0.20	1.90
Sr	μg/g	372 (161)	380	880	320
Zn	μg/g	5.13 (0.53)	5.0	5.0	.0
Fe	μg/g	22.4 (1.08)	23.0	23.0	22.5
Cu	μg/g	7.7 (1.37) <sup>3</sup>	10.0	13.0	7.5
B	μg/g	≤0.5 <sup>3</sup>	0.5	0.5	0.8
Cr	μg/g	0.12 (0.05) <sup>3</sup>	0.20	0.40	0.25
F	μg/g	3.75 (1.04) <sup>3</sup>	6.0	5.5	3.0
Se	ng/g	23.5 (5.0) <sup>3</sup>	45	50	53
V	μg/g	<0.5 <sup>3</sup>	<0.5	<0.5	<0.5
Pb	μg/g	<0.5	1.10	1.30	0.53
Al	μg/g	<5	2.0 <sup>4</sup>	2.0 <sup>4</sup>	2.0 <sup>4</sup>
Cd	ng/g	<50	18.0 <sup>4</sup>	24.0 <sup>4</sup>	3.0 <sup>4</sup>
Hg	μg/g	<0.2	<0.2	<0.2	<0.2

<sup>1</sup>Slovakian data (n = 12) are presented as means (SD). For B, V, Pb, Al, Cd, and Hg, the amounts were below the detection limit of the method.

<sup>2</sup>ES1 = Dutch 'four cereal' egg, and ES2 = Dutch 'Gras' egg, ES3 = Dutch battery egg.

<sup>3</sup>n = 6.

<sup>4</sup>Estimated amount.

Calcitriol was determined with a receptor assay from TNO Nutrition.<sup>14</sup> A calcitriol-containing fraction was recovered following a warm saponification of ESP, organic solvent extraction, and a washing procedure. Finally, the calcitriol was isolated from possible related components with solid phase HPLC. The method is described in detail in TNO's internal standard operating procedure.<sup>22</sup>

**Data Evaluation.** Twelve samples of Slovakian ESP were considered to have a composition minimally influenced by feeding of the chickens. Variability for each element was calculated as the mean  $\pm$  2 SD. The mineral compositions of the Dutch ESP (ES1, ES2, and ES3) were regarded to be different from the Slovakian ESP when they were out of this range.

Dutch samples were presumed to be different from each other in case the detected value of an element of one sample was outside a calculated interval (detected value  $\pm$  2 SD) of another sample. The SD used in the calculations was derived from the reference group or, when not available, was a standard estimate of variance of 10% of the detected value (Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 1997).

## RESULTS

**Mineral Composition.** The major elements in Slovakian ESP are Ca and Mg (Table 1). When compared with recommended or estimated daily intakes, however, Ca and Sr are the most important elements (Table 2; Figure

1). High variability was found in the amounts of P, Se, F, Cr, and Sr. The amounts of Pb, Al, Cd, Hg, B, and V were below the detection limit of the methods used. Of the Dutch ESP, increased levels of F, Cu, Pb, and Cd were found in both free-range ESP (ES1 and ES2). The level of Sr was relatively high in 'grass' ESP (ES2). Dutch ESP contained more Se than Slovakian ESP. No differences were found between the levels of the other elements.

**Amino Acids and Hormones.** No differences were found between the amino acid patterns of Slovakian and Dutch ESP. The ESP protein fraction is rich in Gly (mean: 79 mg/g protein) and Arg (mean: 108 mg/g protein), but absolute amounts are rather low because of the low protein content (mean: 1.8%) of ESP. Amino acid composition is available upon request.

Of the hormones analyzed, small amounts of TGF- $\beta$ 1 (0.75 to 7.28 ng/g ESP; n = 10), calcitonin (10 to 25 ng/g ESP; n = 4), and progesterone (0.30 to 0.33 ng/g ESP; n = 4) were detected. Estradiol-17 $\beta$  and calcitriol (n = 4) could not be detected. The absolute values found for calcitonin should be viewed with caution because of a low recovery (38%) of a spiked sample. Furthermore, some calcitonin may be lost during centrifugation because about 25% of the spiked amount was recovered in the pellet. Temperature during sample handling did not influence the amount of calcitonin.

**Comparison with Other Ca Supplements.** Slovakian ESP and Japanese ESP appear to have a comparable mineral composition with the exception of Mg (Table 2). The high content in the Japanese sample, however, might be due to enrichment of the powder. Compared with purified CaCO<sub>3</sub>, ESP contains an equal amount of Ca, more Sr, Se, and Mg, but less F. The oyster shell tablet studied, contained higher amounts of Mg (in part derived from enrichment), Sr, Fe, F, and B than did ESP. The Ca content in oyster shell was 29.5% compared with 39 to 40% in the other preparations. ESP had lower levels of V, Cr, Pb, Al, and Cd than did oyster shell and purified CaCO<sub>3</sub>.

## DISCUSSION

Slovakian ESP, prepared from demembranized chicken eggshells, has a high Ca content (Table 1), which is regulated within narrow limits. Although the amounts of Sr showed a wide range, they may contribute considerably to the normal daily intake (Table 2) and, as a consequence, may have a positive influence on bone metabolism in the long term. The levels of other elements are low, especially when compared with recommended or adequate dietary intakes (Table 2) and with the Ca content of ESP (Figure 1). Low levels of Pb, Al, Cd, and Hg may be an advantage of ESP over other natural Ca sources. For some of the elements (Sr, Cu, Cr, F, Se, Pb, Cd), a strong influence of feed and housing may be expected (Table 1). Based on the absolute, low amounts of protein and low levels of calcitonin, TGF- $\beta$ 1, and progesterone, no specific role seems likely. All together, ESP prepared from eggs of chickens on controlled feed and adequately housed, is of

<sup>22</sup>SOP nr DPHA/KRC/039.



**TABLE 2: Mineral composition of Slovakian eggshell powder and three commercially available Ca preparations**

Component		Slovakian eggshell <sup>1</sup>	RDA, DRI or ADI <sup>2</sup>	Calcipural <sup>3</sup>	Oyster shell <sup>4</sup>	Japanese eggshell powder <sup>5</sup>
Calcium	mg/g	386–415	1,200 mg	397	295	393
Mg	mg/g	3.5–5.5	320–420 mg	<0.03	78.8	28
P	mg/g	0.6–1.4	700 mg	0	0.15	0.1
Sr	mg/g	0.050–0.693	1–3 mg <sup>6</sup>	0.230	0.910	0.410
Zn	mg/g	0.004–0.006	12–15 mg	<0.001	0.005	0.004
Fe	mg/g	0.020–0.025	10–15 mg	0.036	0.42	0.027
Cu	mg/g	0.005–0.010	1.5–3.0 mg	0.008	0.007	0.009
B	μg/g	≤0.5	500–1000 μg	0.5	9	ND <sup>7</sup>
Cr	μg/g	0.03–0.20	50–200 μg	1.1	1.0	ND
F	mg/g	0.002–0.006	3.1–3.8 mg	0.025	0.070	ND
Se	μg/g	0.014–0.034	55–70 μg	0.008	0.145	ND
V	μg/g	<0.5	<10 μg	0.5	1	ND
Pb	μg/g	<0.5	430 μg	0.99	0.72	<0.5
Al	mg/g	<0.005	70 mg	0.007	4.4	<0.005
Cd	μg/g	<0.050	60 μg	0.657	0.062	<0.050
Hg	μg/g	<0.2	29 μg	<0.2	<0.2	<0.2

<sup>1</sup>Data are presented as means ± 2 SD (see Table 1).

<sup>2</sup>RDA = Recommended dietary allowances for ≥51 yr (National Research Council, 1989) for Zn, Fe, Cu, B, Cr, Se, and V; DRI = adequate dietary intake for ≥51 yr (Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 1997) for Ca, Mg, P, and F; and ADI = acceptable daily intake for a adult weighing 70 kg (Dokkum van, 1985) for Pb, Al, Cd, and Hg.

<sup>3</sup>Food-grade, purified Ca carbonate (Scora S.A., Caffiers, France).

<sup>4</sup>Oyster Ca (Vitasan; OTC Pharma BV, Gorinchem, The Netherlands). Ingredients: oyster Ca (280 mg Ca per tablet of 900 mg), vitamin D (10 IU per tablet), micro crystalline cellulose, magnesium stearate.

<sup>5</sup>Japanese eggshell powder (Furikake Tamago Calcium; Toyo Keiran, Tokyo, Japan).

<sup>6</sup>Estimated daily intake (Eastwood, 1997).

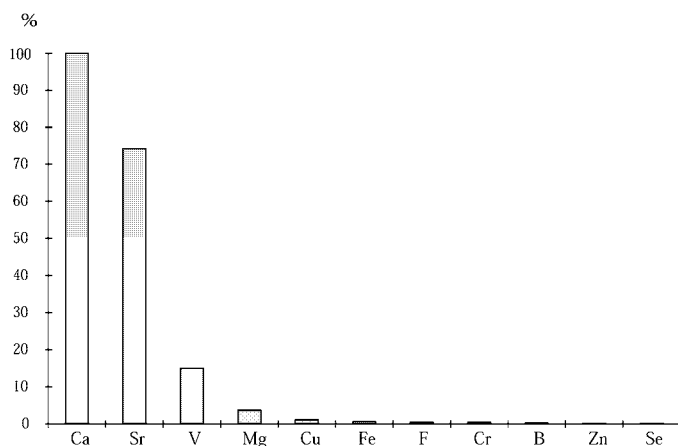
<sup>7</sup>ND = Not determined.

interest for human nutrition, especially as a source of Ca and Sr.

The amounts of Ca and Mg were found to be within rather narrow limits. This is a consequence of limited concentrations in the feed. Both elements affect egg production and shell strength (Atteh and Leenson, 1983). High as well as low levels of Ca have an adverse effect on egg production. Mg is important for egg weight and shell thickness, but high levels combined with low levels of Ca increase shell deformity (Atteh and Leenson, 1983; Nadi et al., 1984), which means that ESP can be considered

to have a rather stable amount of Ca. Approximately 39% is the maximum amount possible when Ca is present as CaCO<sub>3</sub> (calcite crystals) and when about 2% of the eggshell consists of organic matrix (Cortivo et al., 1982). With regard to Ca content, ESP is equal to purified CaCO<sub>3</sub>, which is widely used because of its high Ca content (Kärkkäinen et al., 1997). A piglet study (Schaafsma and Beelen, 1999) showed that Ca from ESP was as well absorbed as Ca from purified CaCO<sub>3</sub> or even better when both Ca sources were given in combination with soya protein. Therefore, ESP is a good source of highly bioavailable Ca.

Levels of Sr in the eggshell seem to be directly related to dietary levels (Weber et al., 1973). Although high intakes of Sr decrease shell thickness and egg production, reduce fertility, and increase embryonic mortality, a content up to 1,500 to 2,000 μg Sr/g of eggshell is not associated with these negative effects. In other words, the amounts, as determined in this study, can be improved significantly. In humans, Sr is suggested to have anabolic effects on bone (Reginster et al., 1999). The effective amounts are debated. Treatment with a pharmacological dosage of 170 mg/d increased BMD (Reginster et al., 1999). However, high intakes of Sr may lead to incorporation into hydroxyapatite crystals, which may result in a reduced bending strength in the long term (Christoffersen et al., 1997). In contrast, adhesion of Sr to hydroxyapatite crystals increases bending strength (Okayama et al., 1991). Therefore, in long-term strategies to prevent natural bone loss, chronic loading with low amounts of Sr may be preferred (Okayama et al., 1991). Normal daily intakes



**FIGURE 1:** Percentage contribution to recommended or estimated daily intakes when 1,200 mg Ca is consumed from eggshell powder [100% of USA dietary reference intake (1997)].

of Sr are estimated to be 1 to 3 mg (Eastwood, 1997), which means that ESP can importantly contribute to a higher intake of this bone-seeking element.

The elements F, Cu, and Se are known or are suggested to have positive effects on bone metabolism (Heaney, 1996; Reginster et al., 1999; Seifert and Watkins, 1997). Their amounts in ESP are probably strongly influenced by diet (Romanoff and Romanoff, 1949), which is also suggested by the differences we found between Dutch and Slovakian ESP. However, because the detected amounts are much lower than the advised amounts (Table 2), concentrations in ESP would have to increase by a factor of 100 to 1,000 for a relevant contribution. This seems quite impossible.

Low levels of Pb, Al, Cd, and Hg provide an advantage for ESP because natural Ca sources may be polluted with these elements (Whiting, 1994; Faine, 1995). In the purified CaCO<sub>3</sub> powder and oyster shell tablet (Table 2), the amounts of Pb, Al, and Cd were higher than in the Slovakian ESP. As indicated by the Dutch 'grass' ESP in particular, amounts of Pb and Cd probably reflect the amounts in the environment and feed, which means that levels can be controlled as is shown by the low amounts found in battery eggs. Although the 'high' amounts of Al in oyster shell and Cd in purified CaCO<sub>3</sub> were much lower than the acceptable daily intakes, they may suggest a well-considered problem. Recently, the negative effects of increased environmental Cd on bone metabolism have been reported (Staessen et al., 1999).

The role of amino acids from eggshell protein in bone metabolism is probably nil as the absolute amounts per gram of ESP are very low. This effect is probably also true for the hormones calcitonin, TGF- $\beta$ 1, and progesterone. Therefore, it is likely that neither amino acids nor the detected hormones from ESP play a role in the increased BMD and reduced pain as reported from small studies (Makai and Chudacek, 1991; Schaafsma and Pakan, 1999).

Chicken eggshell powder with its high percentage of Ca, relevant amounts of Sr, and controlled low levels of Pb, Cd, and Al should be a useful ingredient for human Ca enrichment strategies. Increases in BMD and reduction of pain as reported from two small studies in subjects with osteoporosis support this application (Makai and Chudacek, 1991; Schaafsma and Pakan, 1999). Levels of microelements, such as Sr, F, and Se in eggshell, can be improved and controlled via the feeding of the chickens. Therefore, a large store of wasted Ca (Lichtenstein, 1948) may be a tailor-made Ca source for postmenopausal women and the elderly population in particular.

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