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Accelerated Article

Supplementation of Calves with Stabilized Orthosilicic Acid

Effect on the Si, Ca, Mg, and P Concentrations in Serum and the Collagen Concentration in Skin and Cartilage

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ABSTRACT

The bioavailability of silicon in stabilized orthosilicic acid was investigated in a double blind, placebo controlled supplementation study of calves maintained on a normal diet. The total dietary Si intake was increased by 4.9% in the form of stabilized orthosilicic acid. After 23 wk of Si supplementation, the serum Si concentration increased ($p = 0.0001$, $n = 29$) by 70% compared to control animals in spite of the low Si dose administered and the Si adequate diet. The individually administered Si dose was significantly associated with the serum Si concentration ($r = 0.44$, $p = 0.016$, $n = 29$). The collagen concentration in dermis was significantly higher ($p = 0.019$, $n = 4$) in the Si group and a positive correlation ($r = 0.72$, $p = 0.018$, $n = 9$) was found between the Si concentration in serum and the collagen concentration in cartilage. The calcium (Ca) and phosphorus (P) concentrations in serum were marginally higher for animals supplemented with Si compared to control animals. In serum, a significant linear relationship was found between the Si and the Ca concentration ($r = 0.31$, $p = 0.019$, $n = 59$), whereas the magnesium concentration correlated marginally with the Si concentration ($r = 0.25$, $p = 0.068$, $n = 59$). In summary, increasing the total dietary Si intake by 4.9% in the form of stabilized orthosilicic acid resulted in a 70% higher Si concentration in serum indicating a high bioavailability of Si in this supplement.

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The positive correlation between the serum Si concentration and the collagen concentration in cartilage and the serum Ca concentration, respectively, suggest the involvement of Si both in the formation of extracellular matrix components and in Ca metabolism.

Index Entries: Silicon supplementation; calcium; phosphorus; magnesium; orthosilicic acid; collagen; cartilage; dermis; skin.

INTRODUCTION

Silicon (Si) has been recognized as an essential element for diatoms *(1,2)*, Si accumulating plants such as *Equisetum arvense (3)*, and higher animals, although its biological function has only been partially elucidated *(4)*. Experiments in which rats and birds were maintained on a Si-deficient diet illustrated the requirement of Si in both bone mineralization *(5)* and the synthesis of connective tissue compounds, such as collagen and glycosaminoglycans *(6,7)*.

Information regarding Si metabolism and the bioavailability of Si in the diet is scarce. Recently it was shown that Si is solely present as a non-protein-bound low-mol-wt compound in serum *(8)*, which was in agreement with earlier findings of Adler et al. *(9)* and Carlisle *(4)*, who reported that Si is almost entirely present as free monosilicic acid ($Si[OH]_4$, orthosilicic acid) in serum. For urine, it was also suggested *(10)* that Si is present as undissociated $Si(OH)_4$.

Dietary silicon compounds, such as amorphous hydrated silica, are hydrolyzed into orthosilicic acid prior to the gastrointestinal absorption of Si *(11–15)*. Consequently, orthosilicic acid is suggested to have an important function in Si metabolism and is found both in fresh water (10–500 μ*M*, *[16]*) and in sea water (1–100 μ*M*, *[16]*). It is stable in dilute concentrations of about 10^{-4} *M*, but condenses into silica gels at higher concentrations and low pH *(17)*.

The bioavailability of Si in stabilized orthosilicic acid was investigated in the present study and was assessed by analyzing the serum Si concentration after 6 mo supplementation of calves maintained on a normal diet. The effect of Si supplementation on the collagen concentration in dermis (skin) and cartilage (trachea) was investigated since Si was reported to be involved in collagen synthesis *(4–7)*. The interrelationship of calcium, phosphor, magnesium, and silicon was evaluated in serum, since these elements are important for bone metabolism.

MATERIAL AND METHODS

Animals and Diet

Sixty commercial calves (1 wk old) were used in this study. Prior to the supplementation period and during the complete study they were

Table 1
Composition[a] of Commercial Skimmed Milkpowder Diet

Ingredient	Amount
Crude protein	22.7% (w/w)
Crude fat	21% (w/w)
Cellulose	0.1% (w/w)
Ash	7% (w/w)
Carbohydrates	29% (w/w)
Skimmed milkpowder	52% (w/w)
Moisture	4.5% (w/w)
Zincbacitracine (per kg)	80 mg
Vitamin mixture (per kg)	
vit A	25,000 IU
vit D3	4000 IU
vit E	50 IU
Copper (mg/kg)	10

[a]Communicated by the manufacturer (Schils B.V., The Netherlands). The diet was approved by the European Community (registration number EC 1725/79). Prior to consumption, the diet was dissolved in tap water (100 g/L). The analyzed concentration of Si, Ca, total phosphate, and Zn in the disolved milkformula diet is: 36.5 ppm Si, 642 ppm Ca, 0.148% (w/w) phosphate, 10 ppm Zn.

fed a commercial water-solubilized skimmed milkpowder diet (B. V. Schils, The Netherlands). The composition of this diet is given in Table 1. All the animals were raised in separate boxes and subjected to a 16-h light:8-h dark photoperiod.

Study Design

The calves were randomly allocated into two groups. Si was administered as stabilized orthosilicic acid (BioSil™,* BioMinerals N. V., B-9070 Destelbergen, Belgium) in one group (30 calves) and choline chloride (70% choline chloride in MilliQ water [UCB, Belgium]) was administered in the second group (30 calves) as a placebo since it is used as a stabilizing agent in BioSil. The composition of this liquid Si supplement is: 3% (w/v) soluble Si as stabilized orthosilicic acid, 71% (w/v) choline, and 21% (w/w) water. The chemical structure of the silicon compound in BioSil was verified. Fast Atom Bombardment Mass Spectrometry (FAB/MS) with glycerol as liquid matrix revealed a spectrum with a molecular cation at M/Z 104 (C^+) and an MC^+ adduct ion at M/Z243/245. This spectrum was identical to the spectrum of choline. ^{13}C-NMR analysis confirmed the presence of choline and the absence of any C-Si or C-

*Patent pending product.

Table 2
Daily Dose of Silicon (Si) and Choline Supplements Administered to Calves

Age, wk	Daily dose, mg	
	Choline [a] (= placebo)	Silicon[b]
1–7	350	17.5
8–13	700	35
14–17	1050	52.5
18–23	1400	70

[a]Choline was administered as choline chloride.
[b]Si was administered as stabilized orthosilicic acid (BioSil, BioMinerals N. V., B-9070 Destelbergen, Belgium). The choline dose in the placebo group was similar to the choline intake in the Si group since choline is used as a stabilizing agent in this Si supplement.

O bounds. Element analysis indicated 24 ± 2% (w/v) chloride and 9 ± 1% (w/v) nitrogen, which illustrated a ratio of chloride to nitrogen of 1:1.

The dose of Si and choline was adapted to the age of the calves since they gain more than 300% of their initial body weight in 24 wk feeding (Table 2). Each calf was administered Si or choline twice a day. Dilutions of the Si supplement were made with ultrapure water (Milli Q, Millipore, Belgium). The supplements were mixed for each calf separately in the diet just before consumption.

The body weight and shoulder height was determined at selected time intervals to evaluate the growth of the animals. Blood was taken prior to slaughtering (wk 23) in dry, trace element free polypropylene tubes (Falcon, Becton Dickinson, Belgium). The serum was removed and stored at –20°C. At 24 wk of age, the animals were slaughtered in random order. Dermis (skin) and cartilage (trachea) samples were taken from randomly selected animals and stored at –70°C in polypropylene containers for further analysis. One calf in the silicon supplemented group died at the age of 5 wk owing to a lung infection. This calf was considered as a dropout in the study.

Analytical Procedure

The Si concentration in serum was determined in one batch using atomic absorption spectrometry equipped with Zeeman background correction. A Perkin Elmer model 4100 ZL Zeeman atomic absorption spectrometer combined with a PE AS-70 autosampler and a EDL system 2 (Perkin Elmer, Germany) was used. Pyrolytic graphite coated tubes combined with pyrolytic graphite platforms were used *(18,19)*. Recovery experiments were carried out by spiking a total diet sample with 0.5, 1.0, and 2.0 ppm of a $(NH_4)_2SiF_6$ standard. The mean value for the recovery

was 97 ± 6% *(19)*. Feed samples were wet-ashed-digested in a teflon vial with nitric and perchloric acid *(20)*.

The total calcium *(21)*, phosphorus *(22)*, and magnesium *(23)* concentrations were determined spectrophotometrically (Hitachi model 747, Japan) with an automated method (Boehringer Mannheim, Germany) using respectively o-cresolphtaleïne complexone, phosphomolybdate, and xylidil blue (all from Boehringer Mannheim, Germany) as reagents.

The collagen concentration in tissue samples was determined by analyzing spectrophotometrically *(24)* the concentration of hydroxyproline after freeze-drying and acid hydrolysis with hydrochloric acid (12*M*, Merck, Belgium).

Samples were numbered with the ear-number mark of the calves and were analyzed blindly. All assays were performed in duplicate.

Statistical Analysis

Differences between means were evaluated with a one-tailed, unpaired, Student *t*-test. A p value smaller than 0.05 was considered to be significant. The association between two parameters was investigated with the Pearson correlation procedure. A Macintosh computer with a statistical package (Statview 512, BrainPower, Berkeley, CA) was used.

RESULTS

The milkpowder diet was analyzed to determine the basal Si intake (Table 1). At the age of 7 wk, the calves were administered 10 L of the solubilized diet. The calculated daily Si intake was 360 mg Si in the placebo group and 377.5 mg in the Si group (Tables 1 and 2). Consequently, the daily dose of Si administered as stabilized orthosilicic acid represented only 4.9% of the total intake. This percentage remained stable thereafter since both the amount of feed and the Si dose were increased with the age of the calves (Table 2). A 70% increase in the serum Si concentration was observed after 23 wk of supplementation in the Si group compared to the animals in the placebo group (Table 3). A significant linear relationship was found in the Si group between the individually administered Si dose and the Si concentration in serum (Fig. 1).

The effect of Si supplementation on the growth was measured by the increase in body weight and shoulder height (Table 4). After 23 wk of supplementation, a significant higher body weight was found in the Si group, whereas the shoulder height was similar for the animals in both dietetic groups.

A significant higher hydroxyproline concentration was found after 23 wk supplementation in dermis (Table 5), but not in cartilage. The Si concentration in serum correlated significantly with the hydroxyproline concentration in cartilage (Fig. 2).

Table 3
Effect of Supplementation with Stabilized Orthosilicic Acid on the Serum Silicon Concentration of Calves After 23 wk Supplementation

	Serum Si concentration, µg/L	
	Placebo group	Silicon group
	(n = 30)	(n = 29)
Mean ± SD	72.61 ± 23.61	125.21 ± 23.61[a]
Median	67	120
95% CI	63.45 – 81.76	113.98 – 136.43

[a]p = 0.0001 vs placebo.
The within variation for each dietetic group is shown by the 95% confidence range (95% CI).

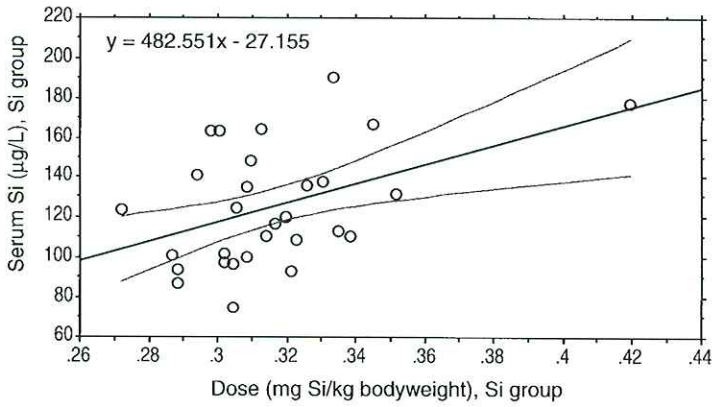

Fig. 1. Relationship between the individually administered Si dose per kg body weight and the serum Si concentration of calves in the silicon group (n = 29) after 23 wk supplementation. Both parameters were significantly correlated (r = 0.44, p = 0.016). Curved lines indicate 95% confidence bands.

A marginally higher Ca and P concentration was found in serum of animals supplemented with orthosilicic acid compared to control animals. However these differences were not statistically significant and the mean individual Ca:P ratio was similar for both dietetical groups (Table 6). A significant correlation was found in serum respectively for the Si versus the Ca concentration (r = 0.31, p = 0.019, n = 59; Fig. 3A) and the Ca:P ratio (r = 0.29, p = 0.025, n = 59), but not for the Si vs the P concentration (r = 0.17, p = 0.21, n = 59; Fig. 3C). The Mg concentration was marginally correlated with the Si concentration in serum (r = 0.25, p = 0.068, n = 59; Fig. 3B).

Table 4
Effect of Supplementation with Stabilized Orthosilicic Acid on the Growth of Calves

	Body wt, kg				Shoulder height, cm	
Age, wk	1	8	14	23	1	23
Placebo group (n = 30)	44.93 ± 3.61	75.93 ± 3.55	126.07 ± 6.52	214.47 ± 18.33	72.15 ± 1.85	99.15 ± 3.30
Silicon (n = 29)	44.62 ± 3.51	77.35 ± 5.12	129.17 ± 8.55	223.10 ± 17.12	71.0 ± 1.85	99.15 ± 3.35
p value	0.36	0.11	0.060	0.033	0.015	0.48

Values are given as mean ± SD.

Table 5
Effect of Supplementation with Stabilized Orthosilicic Acid on the Collagen Concentration in Dermis (Skin) and Cartilage (Trachea) of Randomly Selected Calves in the Silicon Group (5 Calves) and the Placebo Group (4 Calves) After 23 wk Supplementation

	Collagen[a] (mg hydroxyproline/dry matter)	
	Dermis	Cartilage
Placebo group (n = 4)	87.58 ± 6.90	70.59 ± 2.45
Silicon group (n = 5)	98.64 ± 6.05	72.10 ± 2.10
p value	0.019	0.163

[a]Collagen was determined by analyzing the concentration of hydroxyproline, a specific amino acid in collagen. Data given as mean ± SD.

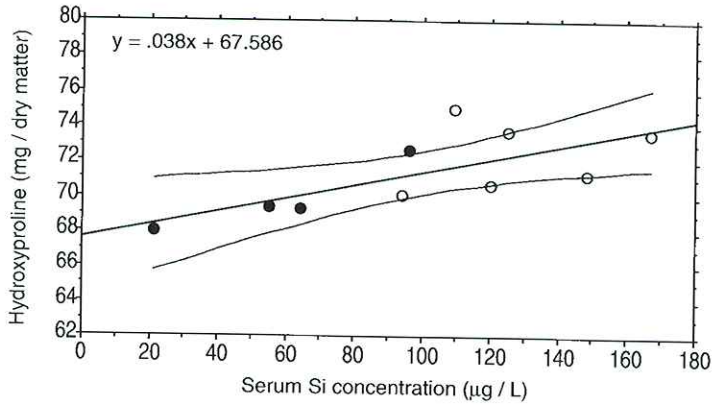

Fig. 2. Relationship between the individual serum Si concentration and the individual hydroxyproline concentration in cartilage (trachea) of calves in the placebo (closed circles) and the silicon group (open circles) after 23 wk supplementation. Both parameters were significantly correlated (r = 0.723, p = 0.018). Curved lines indicate 95% confidence bands.

DISCUSSION

The bioavailability of Si in stabilized orthosilicic acid is high considering the 70% increase of Si concentration in serum when the total dietary Si intake was raised with only 4.9% from the supplement. In addition, a significant dose-related effect between the Si intake from the Si supplement and the serum Si status was observed. Several silicon compounds such as sodium silicate *(4–7,25,26)*, silanol *(27)*, and aluminosilicates (i.e., zeolit A, *[28,29]*) were used in other supplementation

Table 6
Effect of Supplementation with Stabilized Orthosilicic Acid on the Ca, P, and Mg Concentration and the Ca:P Ratio in Serum of Calves in the Silicon Group (29 Calves) and the Placebo Group (30 Calves) After 23 wk Supplementation

Group	Total Ca, mg/L	P, mg/dL	Mg, mg/dL	Ca:P
Placebo (n = 30)	91.37 ± 19.02	8.91 ± 1.63	1.29 ± 0.38	1.02 ± 0.13
Silicon (n = 29)	98.03 ± 16.26	9.45 ± 1.50	1.35 ± 0.42	1.04 ± 0.11
p value	0.07	0.09	0.31	0.30

Values given as mean ± SD.

studies with rats *(25,26)*, birds *(28)*, and horses *(29)*. However, either purified diets were used yielding a low basal Si intake *(4–7,25)*, or high doses of supplements were added to an adequate Si diet *(26,28,29)*. The Si concentration in serum of hens supplemented with 306 mg Si as zeolit A, was increased 14-fold *(28)*, but the administered physiological dose for hens was more than 500-fold higher compared to the mean dose in the present study (0.3 mg Si/kg body weight, Fig. 1). Stabilized orthosilicic acid is most likely directly absorbed in the gastrointestinal tract, whereas silicates in the diet or administered as food supplements have to be hydrolyzed into orthosilicic acid prior to Si absorption *(11–15)*.

The comparable growth measured for both dietetical groups after 23 wk supplementation indicates the safety of stabilized orthosilicic acid as a food supplement. Furthermore, because no adverse drug reactions were observed during the complete study. Stabilized orthosilicic acid can be regarded as a safe supplement.

Bioavailability is not the same as absorption, since other processes such as the biotransformation of the absorbed Si into biologically active silicon compounds should also be considered. However, up to now there were no silicon-containing enzymes characterized. Therefore, the effect of supplementation with stabilized orthosilicic acid was investigated on a biological parameter (e.g., collagen) since Si was reported to be involved in collagen synthesis. The hydroxyproline concentration in dermis was significantly higher in the Si supplemented group compared to the control group in spite of the small number of analyzed samples. Carlisle reported that Si is required in the formation of extracellular matrix components such as collagen and glycosaminoglycan *(4–7)*. In fact, nutritional Si deficiency in chicks caused a significant decline of the collagen and the glycosaminoglycan concentrations in tibia *(6)*. Si supplementation stimulated also in vitro the rate of total hydroxyproline synthesis in epyphyseal cartilage of chick embryos *(30)*. Therefore, the present results suggest that supplementation with stabilized orthosilicic acid could stimulate the collagen formation in dermis even when calves are fed a nor-

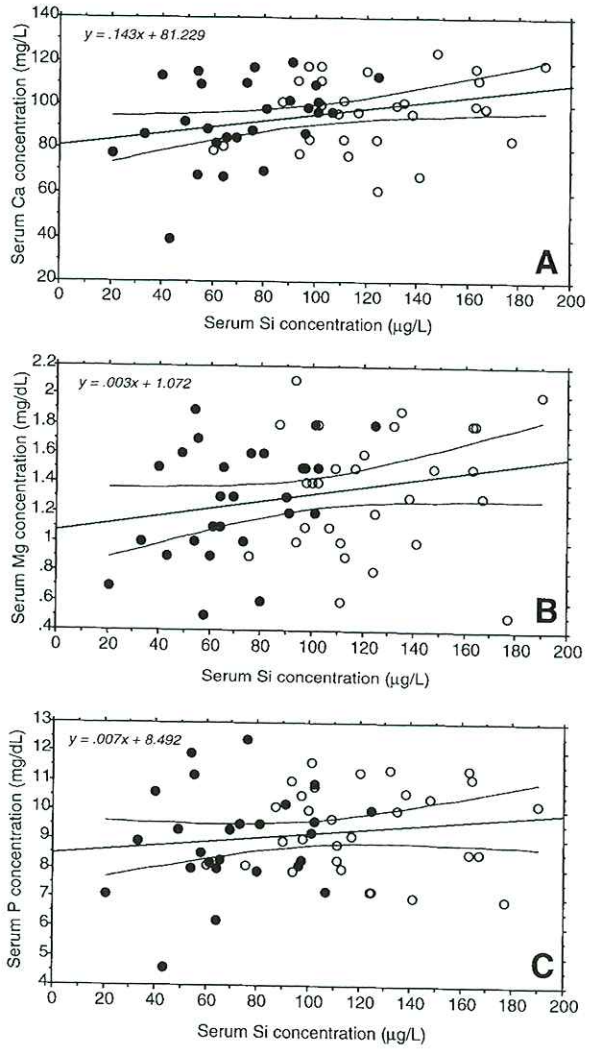

Fig. 3. Relationship between the individual Si and the Ca ([**A**], $r = 0.31$, $p = 0.019$), the Mg ([**B**], $r = 0.25$, $p = 0.068$), and the P ([**C**], $r = 0.17$, $p = 0.21$) concentration in serum of calves in the placebo ($n = 30$, closed circles) and the silicon groups ($n = 29$, open circles) after 23 wk supplementation. Curved lines indicate 95% confidence bands.

mal, Si-adequate diet. This milk powder diet is a standard diet containing a normal silicon concentration. The significant correlation between the hydroxyproline concentration in cartilage and the Si concentration in serum confirms the involvement of Si concerning collagen formation, which was suggested earlier for cartilage *(30)*.

A marginal higher Ca concentration was found in serum for calves, supplemented with stabilized orthosilicic acid compared to control animals. In addition, regression analysis revealed a significant relationship between the Si and the Ca concentration in serum. The effect of Si on the metabolism of Ca, P, and Mg was investigated in other studies *(4,5,25,26)*. Carlisle suggested that Si act as a trigger factor in bone mineralization since Si was found uniquely localized in active growth areas of young bone of rat and mice *(4,5)*. The concentration was shown to be high when the Ca:P ratio was below 1 but fell markedly as the calcification of the bone progressed. Dietary experiments with rats on a low Si diet illustrated that the Ca content of bone increased when Si was added to the diet *(4)*. These findings were confirmed in recent studies with rats showing a decreased concentration of Ca and Mg in femur when Si was lacking in the diet *(25)*, and an increased Ca concentration in serum when animals were administered high doses (0.1–0.4 mg Si/g body wt *[26]*) of sodium metasilicate. However, compared to these studies, the results in the present study were obtained with animals on a normal diet and after supplementation with a low Si dose (mean value: 0.3 mg Si/kg body wt), which was administered in the form of stabilized orthosilicic acid.

Up to now, a positive correlation was made between Si and Ca in urine, but not in serum *(10)*. The significant correlation in serum between these elements, which was found in the present study, suggests a possible role for Si in Ca metabolism. In this view, it should be noted that the dietary level of vitamin D_3 is important for the rate of Ca entry from the intestinal lumen into the enterocyte *(31)*, but was also reported to influence the Si concentration in serum *(28)*. Some studies report a positive effect of Si supplementation on bone density in horses *(29)*, rats *(27)*, and osteoporotic women *(32)*. Further study investigating important parameters such as alkaline phosphatase and calcitonin is certainly needed to evaluate the effect of Si supplementation on Ca metabolism, but such studies require the use of bioavailable silicon products such as stabilized orthosilicic acid.

In summary, increasing the total dietary Si intake by 4.9% in the form of stabilized orthosilicic acid resulted in a 1.7-fold higher Si concentration in serum, indicating a high bioavailability of Si in this supplement. The collagen concentration in dermis was significantly higher after Si supplementation, in spite of the low administered Si dose and the normal diet of the calves. These findings suggest a stimulation of the collagen formation in the dermis of animals on a normal diet, which is different from the experiments done by Carlisle, who used Si-depleted animals. The positive correlation between the serum Si concentration and the collagen concentration in cartilage and the serum Ca concentration, respectively, suggest the involvement of Si both in the formation of extracellular matrix components and in Ca metabolism.

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