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RESEARCH ARTICLE

Silkworm (*Bombyx mori*) larva converts less bioavailable plant-based zinc in mulberry (*Morus alba*) leaf to more bioavailable animal-based zinc

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Abstract

This is the first report on the *in vitro* bioavailability of zinc (Zn) in silkworm (*Bombyx mori*) larva powder (SLP) developed for human consumption. We used the chick bioassay, which has been extensively employed to evaluate the relative Zn bioavailability of animal feeds and foodstuffs, using $ZnSO_4 \cdot H_2O$ as the 100% reference standard. Multiple regression analysis of total tibia Zn against supplemental Zn intake yielded a slope-ratio relative bioavailability value of 175% for Zn in SLP, which was significantly higher ($P < 0.05$) than that of mulberry (*Morus alba*) leaf (91%). SLP is an excellent efficacious source of very highly bioavailable Zn.

Keywords

bioavailability – *Bombyx mori* – *Morus alba* – mulberry – silkworm – zinc

1 Introduction

With increasing protein demand globally, many sustainable alternate sources of protein are being developed, including insects. The consumption of insects, or entomophagy, contributes positively to the environment and to health and livelihood of humans (van Huis *et al.*, 2013). Thus, many large food companies and small start-up companies across the globe are investigating the feasibility of industrializing insects for food and feed, and some are already on the market.

Historically, silkworm (*Bombyx mori*) has been consumed in Asian countries, including Japan. A report dated back in 1919 by the Japanese Ministry of Agriculture and Commerce shows how silkworms at various parts of the life stage (pupa and larva) were consumed for nutritional and medicinal purposes (Miyake, 1919). Silkworm pupae are a byproduct of sericulture and were most popular for human consumption since they have high content of proteins (Tomotake *et al.*, 2010). On the contrary, silkworm larvae have not been very popular for human consumption since larvae are not a byproduct *per se*. They are at the stage just before producing valuable silk cocoon so farmers are reluctant to consume them. Silkworm larvae are also known to have high content of proteins, lipids, vitamins, and minerals (Finke, 2002). However, their nutritional value and nutrient bioavailability have not been fully explored.

Because silkworms only eat the leaves of mulberry plant (*Morus alba*), they are known to accumulate nutrients and substances from mulberry. For example, mulberry leaf is rich in 1-Deoxynojirimicin (DNJ), a known potent α-glucosidase inhibitor (Asano *et al.*, 1994), silkworm larvae are indeed rich in DNJ (Yin *et al.*, 2010).

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Mulberry leaf is rich in zinc (Zn), iron, and calcium (Srivastava *et al.*, 2006), and so are silkworm larvae (Finke, 2002).

Zinc is an important nutrient in human and animal nutrition. Zinc is essential in many aspects of cellular metabolism and physiological functions and supports healthy growth and development (Roohani *et al.*, 2013). Dietary sources of Zn include red meat, poultry, seafood, grains, and various inorganic Zn salt supplements such as Zn-sulfate and Zn-gluconate.

Zinc bioavailability is affected by multiple factors. The source and the chemical form seem to largely affect digestibility and absorbability. In general, animal-based Zn is more bioavailable than plant-based Zn. The reason is that the plant-based Zn is mostly bound to an anti-nutrition factor, phytic acid, and the presence of which makes it less digestible. On the other hand, animal-based Zn is often bound to proteins (enzymes, transporters, and storage proteins) which is more digestible in the mammalian alimentary tract (Mwangi *et al.*, 2018; Welch, 1993). Furthermore, the *in vitro* solubility and availability of Fe and Zn from various insect species (not including the silkworm) have been compared with those from sirloin beef using a Caco-2 cell model (Latunde-Dada *et al.*, 2016). It was found that Fe and Zn solubility was significantly higher from the insect samples than from beef, implicating higher bioavailability of Zn in insects.

No information is available on the forms of Zn in silkworm larva and their bioavailability. Thus, we hypothesized that Zn in silkworm larva is more bioavailable than Zn in mulberry leaf, which is the only source of Zn consumed by the silkworm.

There is a limited number of methods to access Zn bioavailability, including *in vitro* animal and isotopic human studies (Dias *et al.*, 2017). We chose the chick bioassay which has been extensively used to evaluate relative Zn bioavailability of animal feed and foodstuffs against $ZnSO_4 \cdot H_2O$ as the 100% reference standard (Aoyagi *et al.*, 1995; Smiricky-Tjardes *et al.*, 2024; Wedekind and Baker, 1990).

2 Material and methods

Preparation of silkworm larvae powder (SLP)

The 5th instar mulberry silkworms were provided by a local silk farmer. They were harvested on Day 4 or at approximately 3 g body weight and frozen. Frozen silkworms were pulverized and blended with hot water at 95 °C for at least for 10 min but not longer than 20 min. Silkworm emulsion was quickly frozen and freeze-dried until the moisture level fell below 5% within 30 h. Freeze-dried silkworm was pulverized (mesh 0.3 mm) to produce SLP.

TABLE 1 Composition of Zn-deficient basal diet

Ingredient	%
Soy concentrate (63.9% CP)	31.00
Dextrose	28.96
Cornstarch	28.96
Soybean oil	5.00
Zn-free mineral mix[b]	5.37
DL-Methionine	0.20
L-Threonine	0.10
Purified vitamin mix[c]	0.20
Choline chloride (99%)	0.20
DL-tocopheryl acetate	0.002
Ethoxyquin	0.0125

Zinc concentration was 14 mg/kg from analysis. Zn-free mineral mix as percentage of the diet: $Ca_3(PO_4)_2$, 2.80; K_2HPO_4, 0.90; NaCl, 0.88; $MgSO_4 \cdot H_2O$, 0.35; $CaCO_3$, 0.30; $MnSO_4 \cdot H_2O$, 0.065; $FeSO_4 \cdot 7H_2O$, 0.0415; KI, 0.004; $CuSO_4 \cdot H_2O$, 0.002; $Na_2MoO_4 \cdot 2H_2O$, 0.0009; H_3BO_3, 0.0009; $CoSO_4 \cdot 7H_2O$, 0.0001; Na_2SeO_3, 0.0002. Purified vitamin mix provided per kg diet: thiamin · KCl, 20 mg; niacin, 50 mg; riboflavin, 10 mg; Ca-pantothenate, 30 mg; vitamin B12; 0.04 mg; pyridoxine · HCl, 6 mg; biotin, 0.6 mg; folic acid, 4 mg; menadione, 2 mg; ascorbic acid, 250 mg; cholecalciferol, 600 IU; retinyl acetate, 5200 IU.

Preparation of experimental diet

A Zn-deficient semi-purified diet based on dextrose, cornstarch, and soy protein concentrate was formulated to contain approximately 14 mg Zn per kg (Table 1). There were 9 dietary treatments in the experiment. Diet 1 was the Zn deficient base diet. Diets 2-3 contained 4 and 8 mg/kg of Zn, respectively, from feed grade $ZnSO_4 \cdot H_2O$ (36% Zn as determined by analysis). Diets 4-5 contained 4 and 8 mg/kg of Zn, respectively, of added Zn from SLP (57 mg/kg Zn from analysis). Diets 6-7 contained 5 and 10 mg/kg of Zn, respectively, of added Zn from soybean meal (SBM) which contained 71 mg/kg Zn from analysis. Diets 8-9 contained 4 and 8 mg/kg, respectively, of added Zn from mulberry leaf powder (MLP) purchased from Toyotama Healthfood (Tokyo, Japan) which contained 29.7 mg/kg Zn from analysis. All Zn additions were made in place of cornstarch in the base diet.

Chick bioassay

Commercial Ross 308 broiler male chicks were used. All chicks were fed a corn SBM starter diet (120 mg of Zn/kg from analysis) from 0 to 3 days of age. All chicks were then fed the Zn-deficient base soy protein concentrate diet (Table 1) until 7 days of age. After an overnight withdrawal of feed, chicks were weighed, wing-banded, and assigned randomly to 9 experimental groups at Day 8 post-hatching. Seven replicate groups of five chicks per pen were fed each of the 9 experimental diets from 8 to 21 days post-hatching.

At day 21 post-hatching, all chicks were weighed and then were euthanized with carbon dioxide gas and the right tibia bone was removed, autoclaved, cleaned of adhering tissue, and they were pooled by replicate, and dried at 105 °C for 24 hours. Dry tibiae were weighed and ashed in a muffle furnace. The ashed tibiae were then sent to the University Missouri Experiment Station Laboratory for the analysis of Zn concentration using flame atomic absorption spectrophotometry.

Weight gain and feed intake were calculated for each pen of chicks. Total bone ash in mg/tibia and bone ash as a percent concentration were calculated. In addition, supplemental Zn intake, tibia ash Zn concentration, and total bone Zn were calculated.

Statistical analysis

All data were initially analyzed by analysis of variance and differences among treatment means were determined using the least significant difference test at $P < 0.05$. Then, most importantly, total tibia Zn was regressed on supplemental Zn intake to determine bioavailability of the of the Zn in SLP, SBM, and MLP relative to the Zn in feed grade $ZnSO_4 \cdot H_2O$ using the multiple regression slope-ratio method (Finney, 1978).

3 Results

Weight gain increased with increasing dietary Zn and the increase for SLP was greater than for the other Zn sources (Table 2). Feed intake generally increased with increasing added Zn. Bone weight and bone ash also generally increased with increasing added Zn. As expected, supplemental Zn intake increased with increasing added Zn. Tibia ash Zn concentration and total tibia Zn increased greatly and linearly with increasing added Zn. For total tibia Zn, the key and most important parameter, the values for chicks fed SLP were higher than the other Zn sources.

For the multiple regression analysis of total tibia Zn on supplemental Zn intake, an excellent R^2 value of 0.91 was obtained. Dividing the regression coefficients for SLP, SBM and MLP by the regression coefficient for $ZnSO_4 \cdot H_2O$ yielded a slope-ratio bioavailability value of 175, 33 and 91% for Zn in SLP, SBM and MLP, respectively (Table 2 and Figure 1). The relative bioavailability value for Zn in SLP was significantly higher than $ZnSO_4 \cdot H_2O$, SBM, and MLP ($P < 0.05$).

4 Discussion

This is the first report on the *in vitro* bioavailability of the Zn in SLP, which was developed for human consumption by Morus (Tokyo, Japan). Zinc content in SLP was 57 mg/kg, which is similar if not higher to the values previously reported across other eatable insects (Finke, 2002).

The main conclusion of this study was that the bioavailability of the Zn in SLP was higher than the Zn in the inorganic $ZnSO_4 \cdot H_2O$, which is often used as the Zn source in multi-mineral supplements. Moreover, the Zn in SLP (animal-based Zn) was about twice more bioavailable than the Zn in MLP (plant-based Zn) as we had hypothesized. It can be said that bioconversion of mulberry Zn by the silkworm enhanced Zn bioavailability. This would be the similar reason as in the iron (Fe) bioavailability differences between animal-based heme Fe versus plant-based nonheme Fe (Hurrell and Egli, 2010). Insect Fe, however, is not considered as a heme Fe as the Fe is found in ferritin and holoferritin in insects (Mwangi *et al.*, 2018). Further investigation is warranted for other trace mineral bioavailability enhancement by the silkworm.

Protein (more specifically: amino acids such as histidine and methionine) is known to enhance Zn absorption (Lönnerdal, 2000). Silkworm larvae powder has a high protein content (approximately 60% from analysis). Therefore, the high protein content in SLP might have promoted the uptake of Zn compared to the lower protein content (approximately 20% DM) in MLP.

In conclusion, SLP is an excellent efficacious source of very highly bioavailable Zn.

TABLE 2 Zinc bioavailability in silkworm larvae powder, soybean meal, and mulberry leaf powder

Suppl. Zn	Zn source	Weight gain	Intake		Tibia			RBV (%)
(mg/kg)		(g)	Feed (g)	Suppl. Zn (mg)	Dry weight (mg)	Zn conc. (μg/g bone ash)	Total Zn (μg/tibia)*	
0		169.9^{f}	336.2^{d}	0^{f}	728^{e}	74.94^{e}	23.12^{g}	
4	$ZnSO_4 \cdot H_2O$	296.7^{d}	425.8^{c}	1.70^{e}	1042^{c}	96.19^{d}	41.64^{e}	100
8	$ZnSO_4 \cdot H_2O$	460.0^{b}	537.2^{b}	4.29^{bc}	1302^{b}	104.14^{c}	66.12^{bc}	
4	Silkworm larvae powder	427.7^{b}	539.1^{b}	2.15^{d}	1302^{b}	106.57^{c}	68.84^{b}	175
8	Silkworm larvae powder	550.0^{a}	644.7^{a}	5.15^{a}	1521^{a}	133.72^{b}	102.26^{a}	
5	Soybean meal	243.3^{e}	355.1^{d}	1.77^{e}	858^{d}	79.69^{de}	31.12^{f}	33
10	Soybean meal	321.2^{d}	411.6^{c}	4.11^{c}	1021^{c}	86.51^{d}	41.29^{e}	
4	Mulberry leaf powder	373.9^{c}	555.6^{b}	2.22^{d}	1095^{c}	104.41^{c}	54.88^{d}	91
8	Mulberry leaf powder	315.6^{d}	565.5^{b}	4.52^{b}	852^{d}	152.71^{a}	59.71^{cd}	
Pooled SEM		14.9	16.7	0.11	38.0	2.49	2.28	

Data represent mean values per chick of seven replicate groups of five chicks fed experimental diets from 8 to 21 days post-hatching; average initial weight was 107.0 g. Means within a column without common superscript letter differ ($P < 0.001$). Relative bioavailability (RBV) determined by multiple linear regression and slope-ratio methodology.

* Linear ($P < 0.01$) response to $ZnSO_4 \cdot H_2O$ and multiple linear regression of total tibia Zn as a function of supplemental Zn in $ZnSO_4 \cdot H_2O$ (X_1), silkworm larvae powder (X_2), soybean meal (X_3) and mulberry leaf powder (X_4) was $Y = 28.96 + 8.46 \pm 0.78X_1 + 14.85 \pm 0.65X_2 + 2.80 \pm 0.81X_3 + 7.76 \pm 0.73X_4$, $R^2 = 0.91$.

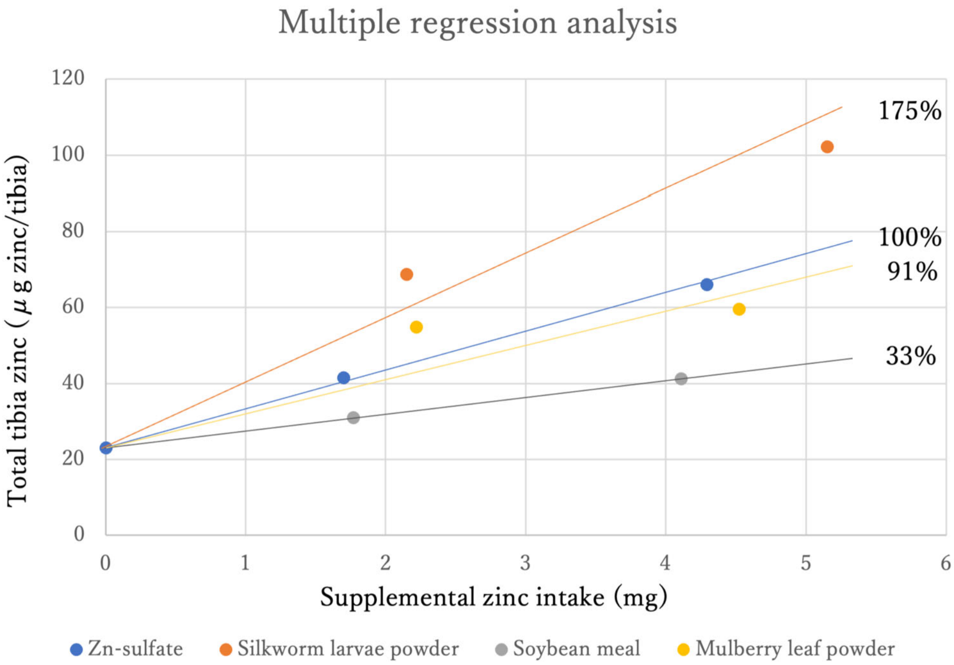

FIGURE 1 Illustration of relative Zn bioavailability from the multiple linear regression of total tibia Zn as a function of supplemental Zn from $ZnSO_4 \cdot H_2O$ (X_1), silkworm larvae powder (X_2), soybean meal (X_3) and mulberry leaf powder (X_4).

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