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Effect of dietary chitosans on trace iron, copper and zinc in mice

Lintao Zeng [a], Caiqin Qin [a,b,*], Guanghui He [a], Wei Wang [a], Wei Li [a,b], Dongsheng Xu [a]

[a] Laboratory of Natural Polysaccharides, Xiaogan University, Xiaogan, Hubei 432000, China
[b] Hubei Key Laboratory of Biomass-Resource Chemistry and Environmental Biotechnology, Wuhan University, Wuhan 430072, China

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ABSTRACT

Dietary chitosans with different molecular weight M_w and the degree of deacetylation DDA (high molecular weight chitosan HCS with M_w 7.60×10^5 and DDA 85.5%, middle molecular weight chitosan MCS with M_w 3.27×10^4 and DDA 85.2%, chito-oligomer COS with M_w 0.99×10^3 and DDA 85.7% and water-soluble chitosan WSC with M_w 3.91×10^4 and DDA 52.6%) were used at the 1.05% level to feed mice for 90 days. Afterwards no pathological symptoms, clinical signs or deaths were observed. The body weight of mice in chitosan group and control group showed no significant difference. Although HCS, COS and WSC had no significant effect on the level of Fe, Zn and Cu in the tested mice's liver, spleen, heart and kidney, MCS significantly increased the level of Fe, Zn and Cu in liver. Therefore dietary ingestion of chitosan did not depress the level of Fe, Zn and Cu in mice.

1. Introduction

Chitosan, derived from crustacean or fungal chitin, is a polymer composed of D-glucosamine with some degree of *N*-acetyl-D-glucosamine. The D-glucosamine content in chitosan is indicated by the degree of deacetylation (DDA) (Hejazi & Amiji, 2003; Nah & Jang, 2002). The US FDA approved chitosan as a feed additive in 1983. Chitosan has been widely applied in functional food, food additive, pharmaceuticals, environmental protection and biotechnology (Shahidi, Arachchi, & Jeon, 1999; Vilai, Nijarin, Nilada, & Pachara, 2006). Chitosan with high molecular weight (M_w) is insoluble in water but soluble in acid, and it has been employed as a dietary fiber. Moreover, the bioactivities of water-soluble chitosan have been reported to have antitumor, cholesterol-lowering (Gallaher, Munion, Hesslink, Wise, & Gallaher, 2000; Ormrod, Holmes, & Miller, 1998), immuno-enhancing, antidiabetic (Hayashi & Ito, 2002), wound healing, antifungal and antimicrobial effects (Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004). Chitosan has also been reported to reduce lipid absorption in the intestine by binding with fatty acids and bile acids and then increasing their excretion. Chitosan inhibits the development of atherosclerosis in individuals with hypercholesterolemia by lowering the serum cholesterol levels. The molecular weight has important effect on the biological activity and absorption of chitosans in vivo (Chae, Jang, & Nah, 2005; Zeng, Qin, Wang, Chi, & Li, 2008).

A large dose intake of chitosan for 30 days was reported to contribute to a decrease in plasma vitamin E level, bone mineral content and growth retardation for mice (Deuchi, Kanauchi, Shizukuishi, & Kobayashi, 1995; Tanaka, Tanioka, Tanaka, Tanigawa, & Kitamura, 1997). One possibility for this is that macromolecular chitosan might form gels in the intestinal tract, which entrap lipids and other nutrients including fat-soluble vitamins and minerals (Koide, 1997; Sugano, Fujikawa, Hiratsuji, Nakashima, & Fukuda, 1980), but in vitro data by Muzzarelli et al. (2006) are against this hypothesis. The dietary chitosan might influence the metabolism of trace elements by accelerating the urinary excretion (Wada, Nishimura, Watanabe, Takita, & Innami, 1997). Considering the fact that chitosan is widely applied in foods, the four typical food-related chitosan samples with different M_w and water-solubility were used to investigate whether chitosans depress the level of trace elements in mice.

2. Experimental

2.1. Material and methods

Crude chitosan HCS were supplied by Golden-shell Biochemical Co., Ltd., China. The other chitosan samples (MCS, COS and WSC) were prepared in our laboratory. The characteristics of samples were listed in Table 1. Other reagents were of analytical grade. Kunming strain female mice (4 weeks old) weighing 20–26 g were purchased from Hubei Experimental Animal Center (China).

2.2. Ninety days feeding study in mice

Fifty healthy Kunming strain female mice were divided into four chitosan groups and one control (10/group, 5/cage). The chitosan groups were fed with diets containing 1.05% chitosan in addi-

* Corresponding author. Address: Laboratory of Natural Polysaccharides, Xiaogan University, Xiaogan, Hubei 432000, China. Tel.: +86 712 2345697; fax: +86 712 2345265.
E-mail address: qincaiqin@yahoo.com (C. Qin).

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Table 1
The characteristics of chitosan samples

Samples	DDA (%)	M_w	M_w/M_n
HCS	85.5	7.60×10^5	3.01
MCS	85.0	3.27×10^4	2.65
COS	85.9	0.99×10^3	1.07
WSC	52.6	3.91×10^4	2.78

tion to the basic mice diet, which contained 4.80% crude fiber, 4.25% crude fat, 19.1% crude protein, 12.13% amino acid, 1.08% Ca, 41.77 mg/kg P, 164.1 mg/kg Fe, and 54.12 mg/kg Zn. Diets and water were given ad libitum for a continuous period of 90 days. With monitored daily food intakes, all the animals were observed thoroughly and weighted weekly to check for any signs of toxicity.

After the mice were decapitated at the 90-day, the vital organs of each mouse were excised and observed grossly. Heart, liver, kidneys, spleen, thymus and lung were weighted and the percent ratios of organ to body weight were calculated.

2.3. Measurement of trace elements

The organ (0.1–0.2 g) was wet ashed with 5 ml of 65% nitric acid and 1 ml $HClO_4$, concentrated by evaporation and diluted with triple-distilled water. The mineral concentration was analyzed by atomic absorption spectrometry (TAS 986 Model, Beijing Purkinje General Instrument Co., Ltd., China), using standard conditions (Fe 0.2 nm, 4.0 mA and 1.6 L/min C_2H_2, Cu 0.4 nm, 3.0 mA and 1.6 L/min C_2H_2, Zn 0.4 nm, 3.0 mA and 1.6 L/min C_2H_2) and excitation lamps (Fe 248.3 nm, Zn 213.9 nm, Cu 324.8 nm). The element contents were expressed as micrograms of the element per gram of wet tissue weight (μg/g organ). Mean values and SD were determined by the SPSS program, and the significance of difference was estimated by the standard Student's *t*-test. A significant difference was accepted with $p < .05$.

3. Results

3.1. Ninety days feeding study in mice

Throughout the 90-day dietary feeding study, no deaths were found in all groups. During the experiment period, no significant abnormality in food intake, feces, hair and behavior were observed.

The mean body weights in each group versus time are presented in Fig. 1. The four chitosan samples did not cause any significant difference in body weight in comparison with the control.

As shown in Table 2, the four chitosan samples increased the thymus/body weight ratios and spleen/body weight ratios of mice, and water-soluble chitosan WSC significantly increased the thymus/body weight ratios after 90-day dietary feeding. Gross examination at necropsy did not reveal any treatment-related changes. In histopathology, gross examination did not reveal any abnormalities. Further, on microscopic examination, no treatment-related pathological lesions were evident in any tested organs.

3.2. Effect of feeding dietary chitosan on the trace element level in mice

Table 3 listed the Fe level in the liver, heart, spleen and kidney of the mice after feeding dietary chitosan samples for 90 days. The Fe level had no significant difference in the tested organs after feeding HCS, COS and WSC. However, the Fe level of liver and spleen increased after feeding MCS.

Table 4 listed the Zn level in the liver, heart, spleen and kidney of the mice in this experiment. COS, HCS and WSC had no significant effect on the Zn levels in these tested organs. However, MCS caused the elevation of Zn level in liver, spleen and heart.

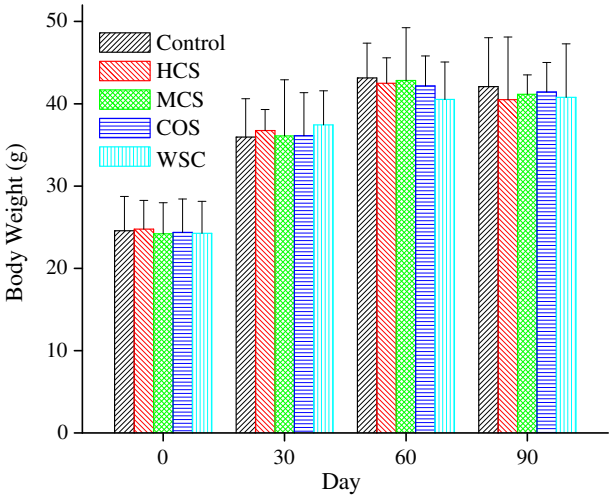

Fig. 1. Body weight changes of mice.

Table 5 listed the Cu level in the liver, heart, spleen and kidney of the mice in this experiment. There also was no significant difference of the Cu level after administration of HCS, COS and WSC. However, MCS increased the Cu level in liver and spleen.

4. Discussion

Chitosan residues have amine groups. Indeed, nitrogen atoms hold free electron doublets that can react with metal cations (Varma, Deshpande, & Kennedy, 2004). However, the amine groups are easily protonated in acidic solutions. The water-solubility of chitosan was dependent on the pH of solution. The four chitosan samples could be dissolved in the gastric acid, and the free $-NH_2$ translated into $-NH_3^+$, which had weak chelating ability with metal cations. The dissolved HCS macromolecules would precipitate with an increase in pH in the intestine from 4.8 to 8.2 (Xiang, 2003), which made the chitosan difficult to be further degraded and hard to be absorbed by the intestine.

The small intestine is the main site for the metal cation absorption. Yonekura, Tamura, and Suzuki (2004) reported that 1% dietary chitosan could increase zinc absorption in rats by formation of stable complexes with phytic acid. The free Zn^{2+}or water-soluble zinc complexes were better absorbed than the phytic acid–Zn complexes. Chitosan had no inhibitory effects on zinc apparent absorption in phytate-free diets. Our experimental results demonstrated that 1% plain chitosan HCS in diet had no significant effect on the levels of Fe, Zn and Cu in these tested organs. HCS was difficult to be absorbed by the intestine, and it could form gels in the intestinal tract entrapping nutrients. The amount of chitosan was much less than the protein and amino acid in diets, and these peptides

Table 2
Organ/body weight ratios of the mice (g/100 g body weight)

Organ	Control	HCS	MCS	COS	WSC
Heart	0.38 ± 0.05	0.41 ± 0.08	0.38 ± 0.06	0.40 ± 0.04	0.42 ± 0.08
Liver	4.28 ± 0.37	4.55 ± 0.34	4.38 ± 0.41	4.32 ± 0.45	4.49 ± 0.39
Spleen	0.30 ± 0.06	0.32 ± 0.05	0.33 ± 0.11	0.34 ± 0.05	0.37 ± 0.09
Thymus	0.15 ± 0.03	0.18 ± 0.05	0.19 ± 0.07	0.17 ± 0.07	0.22 ± 0.07[a]
Kidney	0.56 ± 0.06	0.63 ± 0.05	0.58 ± 0.07	0.56 ± 0.05	0.56 ± 0.06
Lung	0.47 ± 0.11	0.52 ± 0.13	0.53 ± 0.12	0.50 ± 0.14	0.55 ± 0.13

[a] $p < .05$.

Table 3
The Fe level in organs of the mice (μg/g organ, X ± S, $n = 10$)

	Liver	Spleen	Heart	Kidney
COS	149.0 ± 17.9	818.1 ± 177.9	214.4 ± 57.6	161.9 ± 23.9
MCS	167.7 ± 20.2[a]	958.4 ± 233.2[a]	226.1 ± 54.3	151.5 ± 20.1
WSC	148.7 ± 31.7	878.4 ± 131.7	222.7 ± 31.7	159.9 ± 17.7
HCS	161.5 ± 23.6	845.7 ± 243.6	216.1 ± 32.9	154.7 ± 23.6
Control	146.3 ± 19.3	843.6 ± 105.3	256.2 ± 50.1	157.3 ± 26.1

[a] $p < .05$.

Table 4
The Zn level in organs of the mice (μg/g organ, X ± S, $n = 10$)

	Liver	Spleen	Heart	Kidney
COS	29.90 ± 5.81	30.09 ± 6.44	39.18 ± 7.04	26.85 ± 6.18
MCS	54.81 ± 16.82[a]	44.97 ± 6.65[a]	65.71 ± 18.04[a]	25.59 ± 6.73
WSC	28.28 ± 9.25	31.03 ± 9.55	44.55 ± 11.72	24.19 ± 4.87
HCS	30.14 ± 4.72	30.72 ± 3.68	42.92 ± 14.75	24.16 ± 4.03
Control	31.64 ± 4.06	31.76 ± 5.23	38.29 ± 6.39	26.90 ± 7.43

[a] $p < .05$.

Table 5
The Cu level in organs of the mice (μg/g organ, X ± S, $n = 10$)

	Liver	Spleen	Heart	Kidney
COS	5.04 ± 0.97	5.89 ± 2.20	9.75 ± 2.48	5.15 ± 0.25
MCS	5.44 ± 0.10[a]	6.22 ± 2.18	10.04 ± 2.24	4.90 ± 0.28
WSC	5.16 ± 0.19	5.90 ± 2.40	9.52 ± 0.95	4.98 ± 0.17
HCS	5.27 ± 1.90	5.92 ± 2.23	9.73 ± 1.68	5.56 ± 0.60
Control	4.88 ± 0.27	5.57 ± 2.07	10.24 ± 1.51	5.40 ± 0.13

[a] $p < .05$.

and amino acids had better chelating effect with these metal cations in the intestine. MCS, LCS and WSC had no ability to form gels because of their low M_w.

The LCS and WSC were completely soluble in the intestine. The small molecules from LCS was absorbed rapidly by intestinal, and also was rapidly distributed to other tissues in the body. The larger chitosan molecules were degraded to some extent before absorption, and the absorbed chitosan molecules should be water-soluble small molecules. As the monomer of chitosan, glucosamine has been used in various forms for osteoarthritis in the Europe for decades and did not show any significant toxicological effects (McAlindon, LaValley, Gulin, & Felson, 2000).

The MCS molecules were degraded to smaller molecules in stomach and upper intestine, but the speed was slower than that of WSC because WSC had better water-solubility. The degraded chitosan molecules were not as easy to precipitate, and further degraded in lower intestine. The absorbed chitosan molecules from MCS were larger than that from LCS and WSC. The absorbed larger chitosan molecules were subject to rapider plasma clearance, and had higher liver accumulation level (Tai, Sheu, Lee, Yao, & Chaiang, 2000). The larger molecules needed to be further degraded and digested in liver, and then distributed to other tissues. The smaller molecules could better penetrate biomembranes to reach tissues, enter body fluids and be excreted in urine (Onishi & Machida, 1999).

Chitosan molecules possess the metal-complexing ability with Fe^{2+}, Zn^{2+} and Cu^{2+} in blood and liver around the neutral conditions. When the chitosan level in liver was higher, these levels of these metal cations should be higher. Therefore, MCS enhanced the levels of these metal cations in livers.

The tested elements Fe, Cu and Zn are essential to the normal functioning of an animal's metabolism (Wei & Chung, 1993). Key enzymes involved in the synthesis of erythrocytes contain Cu and it participates in the oxidizing chain in mitochondria. The deficiency of Cu may induce tiredness, hepatic and renal problems and anemia. Fe is an important constituent of succinate dehydrogenase. Aerobic metabolism is dependent on Fe, which is contained in hemoglobin and is essential for the synthesis of myoglobin and of several cellular enzymes, such as cytochromes, catalases and peroxidases. Weakness and anemia are the main effects of Fe shortage. Zn is contained in insulin and in several enzymes. It participates in various important processes for the metabolism of proteins, nucleic acids, carbohydrates and lipids. A level of Zn lower than normal may induces weakening of the immune defenses, with a consequent predisposition to allergies and skin infections (Cunningham-Rundles, 1996; Driessen, Hirv, & Rink, 1995).

5. Conclusion

A 90-day feeding study showed no pathological symptoms, clinical signs or deaths in mice during the period of feeding 1.05% dietary chitosan. Although the four chitosan samples had no significant effect on the body weight of the mice, they raised the thymus/body weight ratios and spleen/body weight ratios of mice in the tested groups, especially WSC significantly raised the thymus/body weight ratios. The WSC, COS and HCS samples had no significant influence on the level of Fe, Zn and Cu in liver, spleen, heart and kidney, but the MCS sample significantly increased the level of Zn, Fe and Cu in liver. It was concluded that the dietary ingestion of chitosan did not depress the level of Fe, Zn and Cu in mice.

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References

Chae, S. Y., Jang, M. K., & Nah, J. W. (2005). Influence of molecular weight on oral absorption of water soluble chitosans. *Journal of Controlled Release, 102*, 383–394.

Cunningham-Rundles, S. (1996). Zinc modulation of immune function: Specificity and mechanism of interaction. *Journal of Laboratory and Clinical Medicine, 128*, 9–11.

Deuchi, K., Kanauchi, O., Shizukuishi, M., & Kobayashi, E. (1995). Continuous and massive intake of chitosan affects mineral and fat-soluble vitamin status in rats fed on a high-fat diet. *Bioscience Biotechnology and Biochemistry, 59*, 1211–1216.

Driessen, C., Hirv, H., & Rink, L. (1995). Zinc regulates cytokine induction by superantigens and lipopolysaccharide. *Immunology, 84*, 272–277.

Gallaher, C. M., Munion, J., Hesslink, J. R., Wise, J., & Gallaher, D. D. (2000). Cholesterol reduction by glucomannan and chitosan is mediated by changes in cholesterol absorption and bile acid and fat excretion in rats. *Journal of Nutrition, 130*, 2753–2759.

Hayashi, K., & Ito, M. (2002). Antidiabetic action of low molecular weight chitosan in genetically obese diabetic KK-Ay mice. *Biological and Pharmaceutical Bulletin, 25*, 188–192.

Hejazi, R., & Amiji, M. (2003). Chitosan-based gastrointestinal delivery systems. *Journal of Controlled Release, 89*, 151–165.

Koide, S. S. (1997). Chitin-chitosan: Properties, benefits and risks. *Nutrition Research, 18*, 1091–1101 .

Kumar, M. N. V. R., Muzzarelli, R. A. A., Muzzarelli, C., Sashiwa, H., & Domb, A. J. (2004). Chitosan chemistry and pharmaceutical perspectives. *Chemical Reviews, 104*, 6017–6084.

McAlindon, T. E., LaValley, M. P., Gulin, J. P., & Felson, D. T. (2000). Glucosamine and chondroitin for treatment of osteoarthritis. *Journal of the American Medical Association, 283*, 1469–1475.

Muzzarelli, R. A. A., Orlandini, F., Pacetti, D., Boselli, E., Frega, N. G., Tosi, G., et al. (2006). Chitosan taurocholate capacity to bind lipids and to undergo enzymatic hydrolysis: An in vitro model. *Carbohydrate Polymers, 66*, 363–371.

Nah, J. W., & Jang, M. K. (2002). Spectroscopic characterization and preparation of low molecular, water-soluble chitosan with free-amine group by novel method. *Journal of Polymer Science, Part A Polymer Chemistry, 40*, 3796–3803.

Onishi, H., & Machida, Y. (1999). Biodegradation and distribution of water-soluble chitosan in mice. *Biomaterials, 20*, 175–182.

Ormrod, D. J., Holmes, C. C., & Miller, T. E. (1998). Dietary chitosan inhibits hypercholesterolaemia and atherogenesis in the apolipoprotein E-deficient mouse model of atherosclerosis. *Atherosclerosis, 138*, 329–334.

Shahidi, F., Arachchi, J. K. V., & Jeon, Y. J. (1999). Food applications of chitin and chitosans. *Trends in Food Science and Technology, 10*, 37–51.

Sugano, M., Fujikawa, T., Hiratsuji, Y., Nakashima, K., & Fukuda, N. (1980). A novel use of chitosan as a hypocholesterolemic agent in rats. *American Journal of Clinical Nutrition, 33*, 787–793.

Tanaka, Y., Tanioka, S., Tanaka, M., Tanigawa, T., & Kitamura, Y. (1997). Effect of chitin and chitosan particles on BALB/c mice by oral and parenteral administration. *Biomaterials, 18*, 591–595.

Tai, T. S., Sheu, W. H., Lee, W. J., Yao, H. T., & Chaiang, M. T. (2000). Effects of chitosan on plasma lipoprotein concentrations in type 2 diabetic subjects with hypercholesterolemia. *Diabetes Care, 23*, 1703–1704.

Varma, A. J., Deshpande, S. V., & Kennedy, J. F. (2004). Metal complexation by chitosan and its derivatives: A review. *Carbohydrate Polymers, 55*, 77–93.

Vilai, R., Nijarin, W., Nilada, K., & Pachara, C. (2006). Application of fungal chitosan for clarification of apple juice. *Process Biochemistry, 41*, 589–593.

Wada, M., Nishimura, Y., Watanabe, Y., Takita, T., & Innami, S. (1997). Accelerating effect of chitosan intake on urinary calcium excretion by rats. *Bioscience Biotechnology and Biochemistry, 61*, 1206–1208.

Wei, Y. Y., & Chung, C. (1993). Elemental analysis in liver, ascites, and blood of tumor-bearing mice. *Journal of Radioanalytical and Nuclear Chemistry, 171*, 383–400.

Xiang, J. Z. (2003). *Pharmacology*. Beijing: Science Press (in Chinese).

Yonekura, L., Tamura, H., & Suzuki, H. (2004). Chitosan and resistant starch restore zinc bioavailability, suppressed by dietary phytate, through different mechanisms in marginally zinc-deficient rats. *Nutrition Research, 24*, 121–132.

Zeng, L. T., Qin, C. Q., Wang, W., Chi, W. L., & Li, W. (2008). Absorption and distribution of chitosan in mice after oral administration. *Carbohydrate Polymers, 71*, 435–440.