

Reducing Effect of Ingesting Tannic Acid on the Absorption of Iron, but Not of Zinc, Copper and Manganese by Rats

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Interest in the beneficial effects of polyphenols, including tannic acid (TA), is increasing, although, these compounds also have adverse effects; for example, on the absorption of iron (Fe), and possibly other trace minerals. We examined the effect of a graded dose of TA on the absorption of Fe and compared with that of zinc (Zn), copper (Cu) and manganese (Mn) in rats. We also investigated the effect of TA on cecal fermentation which plays a role in absorption. In Experiment 1, to set the optimum dose of Fe, male Sprague-Dawley rats (weighing 70–90 g) after acclimatization were fed with different levels of dietary Fe (5, 10, 20, 30 and 35 mg/kg). We observed that the hematocrit (Ht), serum Fe concentration and transferrin saturation (%) were each reduced in those rats fed less than 20 mg/kg Fe in a dose-dependant manner. In Experiment 2, the rats were fed with test diets containing the minimum required level of Fe, 30 mg/kg diet, with (5, 10, 15 and 20 g/kg diet) or without TA for a period of three weeks. Feeding a diet containing more than 10 g TA/kg diet, but not 5 g TA/kg diet, reduced the hemoglobin concentration (Hb), Ht and serum Fe concentration due to decreased Fe absorption. In contrast, the Zn, Cu and Mn absorption was not affected by TA feeding. It is also demonstrated that liver Fe, but not the Zn, Cu and Mn contents, were lower in the TA groups than in the TA-free control group. Feeding TA slightly decreased the pH value of the cecal contents with an increase in the major short-chain fatty acid pool. About 15% of the ingested TA were recovered in the feces of each TA-fed group. Our results demonstrate that more than 10 g TA/kg diet induced anemia by reducing the Fe absorption, although there was no effect on the absorption of other important trace minerals. Our findings suggest that the usual intake of polyphenols is relatively safe, but that a high intake by supplementation or by dietary habit of tannin affects only the Fe level.

Key words: tannic acid; mineral element; intestinal absorption

Polyphenolic compounds are known for their many beneficial effects on health, mainly from their antioxidative properties. Tea and red wine are believed to be important sources of these compounds. In contrast, tannins, a group of polyphenolic compounds that are richly contained in tea and red wine, are reported to be toxic to animals if consumed in large amounts;¹ They reportedly reduced growth,² bound with protein,³ increased fecal excretion and nitrogen⁴ and inhibited digestive enzymes.⁵ The most serious problem with tannin is its effect on reducing iron (Fe) absorption when included in the diet at a high level, multiple galloyl groups being responsible for this inhibition of Fe absorption from foods.⁶ Evidence suggests that tannins may be partly responsible for the low bioavailability of Fe in many vegetable foods.⁷ Tea⁸ and coffee,⁹ when taken with composite meals, dramatically reduce Fe absorption. Red wine¹⁰ and some polyphenol-rich vegetable¹¹ have also been reported to inhibit Fe absorption. However, there have been no reports on Fe absorption and metabolism [hemoglobin (Hb), hematocrit (Ht) *etc.*] with regard to ingesting tannic acid (TA). Also, there is very little information on the effect of phenolic compounds on the trace element status other than Fe. Greger and Lyle¹² have found a reduction in zinc (Zn) absorption with minimal change in copper (Cu) absorption from tea.

Cecal fermentation is an important factor for mineral absorption. Sakai *et al.*¹³ have recently shown that cecal fermentation was responsible for the recovery of Fe-deficient anemia induced by gastrectomy in rats. We have also demonstrated that the large intestine has a role in Zn absorption.¹⁴ Dietary polyphenols have been reported to influence intestinal microflora and their fermentative capacity toward other food components,¹⁵ which in turn has the possibility to affect mineral absorption. This is another possible mechanism for the influence on mineral absorption of polyphenolics.

The aim of the present study was to investigate (i) the effect of various levels of Fe (Expt. 1) and (ii) the effects of different amounts of TA on the absorption of trace

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Abbreviations: TA, tannic acid; Tf, transferrin saturation; TIBC, total iron binding capacity; UIBC, unsaturated iron binding capacity

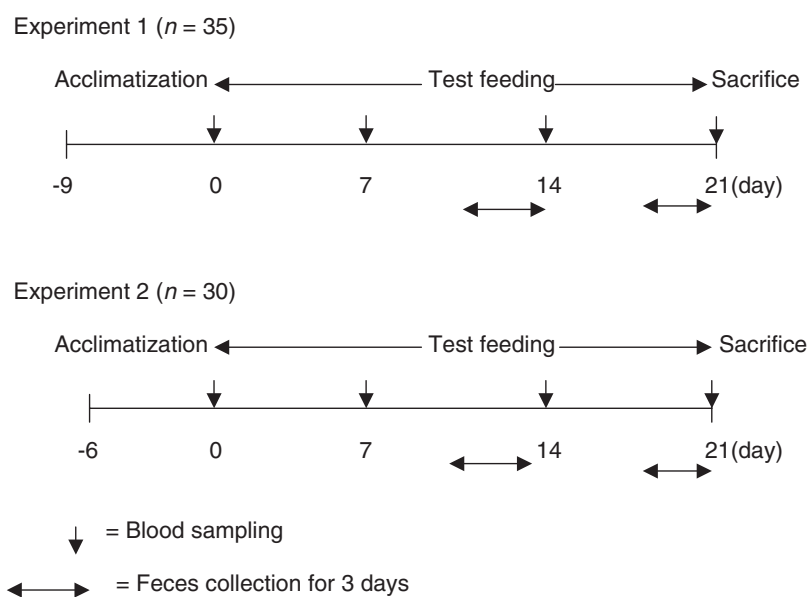


Fig. 1. Experimental Protocol of Expt. 1 and 2.

minerals, including Fe, Zn, Cu and Mn, in rats (Expt. 2). Expt. 1 was done to set Fe level to the minimum requirement for the second experiment and to evaluate the effect of TA on the body Fe status and Fe absorption. The impact of TA on cecal fermentation was also evaluated in this study.

Materials and Methods

Animals and diets.

Expt. 1. Male Sprague-Dawley rats (Clea Japan, Tokyo, Japan) of 4 weeks old (70–90 g) were acclimatized for nine days with free access to a stock diet¹⁶ (Table 1) and de-ionized water. The rats were then assigned to five groups of seven rats each based on their body weight and Hb and Ht values by a randomized block design. Each group of animals was fed on a test diet containing one of five levels of Fe (5, 10, 20, 30 or 35 mg/kg diet) for 3 weeks, the level of 35 mg Fe/kg diet being taken as the standard (control) based on the AIN-93G formulation (Table 1).

Expt. 2. The rats were assigned to five groups of six rats each according to the parameters in Expt.1 after a seven-day acclimatization period. The rats were fed on the basal diet (control diet with 30 mg Fe/kg diet) or on a diet containing one of four levels of TA (T-0125 Sigma-Aldrich Cheme, Steinheim, Germany; 5, 10, 15 or 20 g TA/kg diet) for 3 weeks (Table 1). TA was added to the basal diet containing 30 mg Fe/kg at the expense of the whole diet.

In Expt. 1, feces were collected during days 12–14 and days 19–21 of the test period to evaluate the Fe and Zn absorption, and in Expt. 2, whole feces were collected during days 11–13 and days 19–21 to assess the Fe, Zn, Cu and Mn absorption. In both experiments, the body weight and food intake was measured daily.

Table 1. Composition of the Stock and Test Diets (g/kg)

	Test diets*
Casein ^a	200
Dextrin ^b	466
Corn oil	50
Mineral mixture (Fe-free) ^c	35
Vitamin mixture ^c	10
Choline bitartrate	2.5
L-Cystine	3.0
Ferric citrate <i>n</i> -hydrate	0.177
Crystallized cellulose ^d	50
Sucrose	to make 1 kg

* The compositions of the stock and test diets were the same except for the Fe concentration. In Expt. 1 and 2, 35 mg Fe/kg was used to prepare the stock diet. Ferric citrate *n*-hydrate at 0.026, 0.056, 0.116, 0.177 & 0.207 g/kg (5, 10, 20, 30 and 35 mg Fe/kg) was added in Expt. 1, while in Expt. 2, 0.177 g/kg ferric citrate *n*-hydrate (30 mg Fe/kg diet) was added to the test diets with or without tannic acid (TA; T-0125; Sigma-Aldrich Cheme, Steinheim, Germany; 5, 10, 15 and 20 g/kg).

^a Alacid (New Zealand Dairy Board, Wellington, New Zealand)

^b Pine-Dex # 4 (Matsutani Chemical Industry, Hyogo, Japan)

^c Prepared according to the AIN-93G formulation (Reeves *et al.*, 1993).¹⁶

^d Crystallized cellulose (50 g/kg diet, Avicel PH102; Asahi Chemical Industry Co., Tokyo, Japan) was added to the stock and test diets in both experiments. Crystallized cellulose and TA were added to the diets at the expense of the whole diet.

Blood samples were obtained from the tail vein to determine the Hb concentration and Ht just before and at 1-week intervals after starting the test diets. The rats were housed in individual stainless-steel cages at a constant temperature (22–24°C), humidity (40–60%) and lighting (20:00 to 08:00 h) during the experiment.

On the final day of the experiment, the rats were killed by exsanguination under anaesthesia (50 mg sodium pentobarbital/kg body weight; Abbott, Chicago, IL, USA) and blood was collected from the abdominal aorta. The serum was separated by centrifuging at

3,000 × g for 10 min, and stored at −40°C until assayed for the mineral concentration. The cecum was removed with its contents. The liver was also collected after removing blood from the portal vein by perfusing with saline. Both organs were weighed, immediately frozen in liquid nitrogen and stored at −40°C until needed for subsequent analyses. The weight of the cecal contents was evaluated by subtracting the weight of the cecal wall from the total weight of the cecum.

This study was approved by the Hokkaido University Animal Use Committee, and the rats were maintained according to the guidelines of Hokkaido University for the care of laboratory animals.

Analytical methods. The Hb concentration was determined by using a commercial assay kit (Hemoglobin B-test, Wako Pure Chemical Industries, Osaka, Japan). The Ht value was measured by centrifuging (15,000 × g) blood with a Hematocrit MC-201 centrifuge (Hitachi, Tokyo, Japan).

The serum samples collected at the end of the study were used to determine the serum Fe concentration and unsaturated iron-binding capacity (UIBC) by commercial assay kits (Fe c-test and UIBC-test; Wako Pure Chemical Industries, Osaka, Japan). The total iron-binding capacity (TIBC) and transferrin saturation (Tf) level was calculated from the measured values.

The feces were freeze-dried, weighed and milled to a fine powder. Each diet (5 g) and feces (1.5 g) were dry-ashed in a muffle furnace (Eyela TMF-3200; Tokyo Rikakikai Co., Tokyo, Japan) at 550°C for 18 h after a linear increase in temperature up to 550°C for 6 h. After adding 5.49 mol/l HCl and heating until dry, the mineral concentrations of each ashed sample were measured with an atomic absorption spectrophotometer (AA-6400F; Shimadzu Seisakusyo, Kyoto, Japan) after appropriately diluting the sample with 0.82 mol/l HCl. The freeze-dried liver sample (1.8 g) was measured by the same procedure as that for the feces to determine the mineral concentrations.

We estimated the mineral contents in each test diet for both experiments. In Expt. 1, the Fe concentrations in the test diets were 4.70, 9.12, 19.2, 28.3 and 33.4 mg/kg diet for the 5, 10, 20, 30 and 35 mg Fe/kg diet groups, respectively. The determined mean values for the Fe, Zn, Cu and Mn concentrations in Expt. 2 were 28.2, 28.1, 5.56 and 9.43 mg/kg diet.

The cecal weight with its contents was measured, and the cecal wall was washed with saline and also weighed. The collected cecal contents were diluted with four volumes of de-ionized water and homogenized with a teflon homogenizer. The pH value was measured with a semiconducting electrode (ISFET pH sensor 0010-15C, Horiba, Kyoto, Japan) as the cecal pH. The total-mineral concentration in the homogenate was determined after dry-ashing by using the same procedure as that for the feces. The soluble-mineral concentration in the cecal supernatant was obtained after centrifuging the homo-

genate (30,000 × g at 4°C for 20 min) and then deproteinizing with 9 mol/l perchloric acid. The mineral concentrations in the dry-ashed cecal homogenate and deproteinized supernatant fraction were measured by atomic absorption spectrometry. The contents of organic acids (acetic, propionic and butyric) in the cecal homogenate were measured by ion-exclusion chromatography, using an HPLC system equipped with a solvent delivery system (SLC-10 AVP; Shimadzu), a double ion-exchange column (Shim-pack SCR-102h, 8 × 300 mm; Shimadzu) and an electroconductivity detector (CDD-6A; Shimadzu), after washing the homogenate with chloroform.¹⁷⁾

The TA concentration in the feces was determined by the AOAC¹⁸⁾ method. TA was first extracted from a 0.5 g fecal sample at 60°C for 45 min. with 5 ml methanol. The TA concentration of the extract was then spectrophotometrically measured by using the Folin-Denish reagent in a saturated Na₂CO₃ solution at 700 nm.

Calculation and statistical analyses. Each value was calculated as follows:

Fe (Zn, Cu and Mn) absorption (%) = 100 × [Fe (Zn, Cu and Mn) intake − fecal Fe (Zn, Cu and Mn) excretion]/Fe (Zn, Cu and Mn) intake.

The total TA concentration in the fecal extracts (mg) was determined from the absorbance of each sample in the TA groups, which was subtracted from the average absorbance of the extract in the TA-free group.

The effects of the Fe and TA levels in the diets were analysed by one-way ANOVA, and the Hb concentration and Ht by two-way ANOVA. Duncan's multiple range test¹⁹⁾ was used to determine whether a mean value was significantly different ($P < 0.05$). These statistical analyses were done by the General Linear Models procedure in the Statistical Analysis Systems program (SAS version 6.07, SAS Institute, Cary, NC, USA).

Results

Expt. 1

Effect of Fe level on growth

The body weight gain was lower in the rats fed with less than 10 mg Fe/kg diet than in the rats of other groups. The food intake of the rats fed with 5 mg Fe/kg was lower than that of the rats fed with 35 mg Fe/kg diet (Table 2).

Hematological parameters

The Hb concentration and Ht in the rats fed with 5 mg Fe/kg diet quickly became much lower than those of the rats fed with 30 and 35 mg Fe/kg diet in week 1 (Fig. 2A and B, respectively). In week 2, the Hb concentration and Ht were lower with 10 mg Fe/kg diet than with 30 and 35 mg Fe/kg diet. Finally, in week 3, the Hb concentration in the rats fed with 5 and 10 mg Fe/kg diets was further reduced, and the Ht in the rats fed with

Table 2. Body Weight Gain and Food Intake of Rats Fed on the Diets Containing Different Levels of Iron (Fe) for 3 Weeks

	5 mg Fe/kg	10 mg Fe/kg	20 mg Fe/kg	30 mg Fe/kg	35 mg Fe/kg	P value
Body weight gain (g/d)	7.50 ± 0.287 ^b	7.62 ± 0.346 ^b	8.58 ± 0.161 ^a	8.62 ± 0.119 ^a	8.80 ± 0.293 ^a	0.002
Food intake (g/d)	21.0 ± 0.390 ^c	22.2 ± 0.555 ^b	23.4 ± 0.430 ^a	23.9 ± 0.260 ^a	23.6 ± 0.645 ^a	< 0.001

Each value is the mean ± SE ($n = 7$). Means in a row not sharing a letter differ at $P < 0.05$.

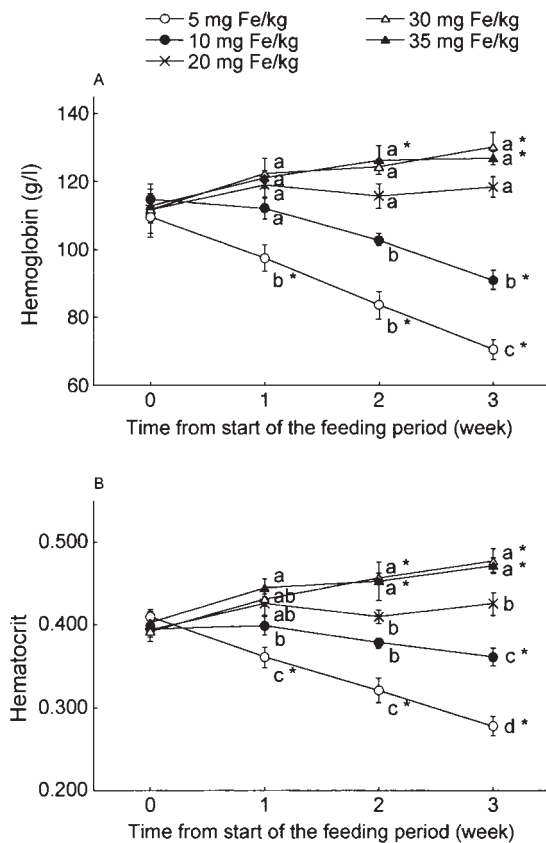


Fig. 2. Hemoglobin Concentration (A) and Hematocrit (B) in Rats Fed on Diets Containing Different Levels of Fe at 0, 1, 2 and 3 Weeks in Expt. 1.

Each value is the mean ± SE ($n = 7$). P values estimated by two-way ANOVA were (A) < 0.001 for the diet (D), 0.041 for the time (T) and < 0.001 for $D \times T$; (B) < 0.001 for D, 0.355 for T and < 0.001 for $D \times T$. Means not sharing a letter differ between groups within the same day ($P < 0.05$). * Different from the value at week 0 in each group ($P < 0.05$).

less than 20 mg Fe/kg diet was lower than the level in the rats fed with 30 and 35 mg Fe/kg diet. During all weeks, the Hb concentration and Ht in the 5 mg Fe/kg diet group were lower than those in the 10 mg Fe/kg diet group. The Hb concentration and Ht in the 30 and 35 mg Fe/kg diet groups, but not in the 20 mg Fe/kg group, increased from the start of the feeding. There was no difference between the 30 and 35 mg Fe/kg diet groups.

The serum Fe concentration and Tf level were lower, and UIBC and TIBC were higher in the rats fed with less than 20 mg Fe/kg than in the rats fed with 30 and 35 mg Fe/kg diet (Fig. 3).

Net absorption of Fe and Zn

The net absorption of Fe by the rats fed on the diets

containing the lower levels of Fe was lower than that by the rats fed with 30 and 35 mg Fe/kg diet in the 1st and 2nd balance periods, being dependent on the Fe-deficient level in the diets (Fig. 4). The absorption rates in the different Fe-fed groups were similar (the rates for the 30 and 35 mg Fe/kg diet groups were 51.3 and 48.7%, respectively). We also evaluated the net Zn absorption and there was no significant trend, although the absorption during the 2nd period was clearly lower than that in the 1st period. The respective mean values for net Zn absorption were 1.12, 1.13, 1.06, 1.23 and 1.02 mg/3d, $P = 0.535$ in the 1st balance period, and 0.681, 0.502, 0.687, 0.796 and 0.667 mg/3d, $P = 0.214$ in the 2nd balance period for the 5, 10, 20, 30 and 35 mg Fe/kg diet groups.

Expt. 2

Effects of dietary TA on the growth parameters and fecal output

The body weight gain and food intake was not influenced by TA feeding (Table 3). Feeding with TA increased the fecal dry weight in the 1st balance period. There were significant differences between the control, 5 g TA/kg diet and 10, 15 and 20 g TA/kg diet-fed rats. A similar, though not significant, tendency was observed in the 2nd balance period.

Hematological parameters

There were no significant changes in the Hb concentration and Ht among the groups in week 1. In week 2, however, the Ht of the 15 and 20 g TA/kg diet-fed groups was lower than that of the control group (Fig. 5B). We were unable to measure Hb concentrations in week 2 due to loss of the samples. In week 3, Hb concentration was less reduced in the 10, 15 and 20 g TA/kg diet -fed groups than that in the control group (Fig. 5A). These reductions were TA dose-dependent. Week 3 Ht were synchronised with the week 3 Hb concentrations. In week 3, the Hb concentration and Ht of the control group and the Hb concentration of the 5 g TA/kg diet group, but not of the other groups, were higher than the week 0 (Fig. 5A and B).

The serum Fe concentration of the 10, 15 and 20 g TA/kg diet groups was lower than that of the control and 5 g TA/kg diet groups at the end of the test period (Table 4), while the serum Zn level remained unchanged (data not shown).

Absorption of Fe, Zn, Cu and Mn in the balance study

The rate of Fe absorption was strikingly reduced in the 10, 15 and 20 g TA/kg diet groups compared to that in the control group during the 1st balance period, while

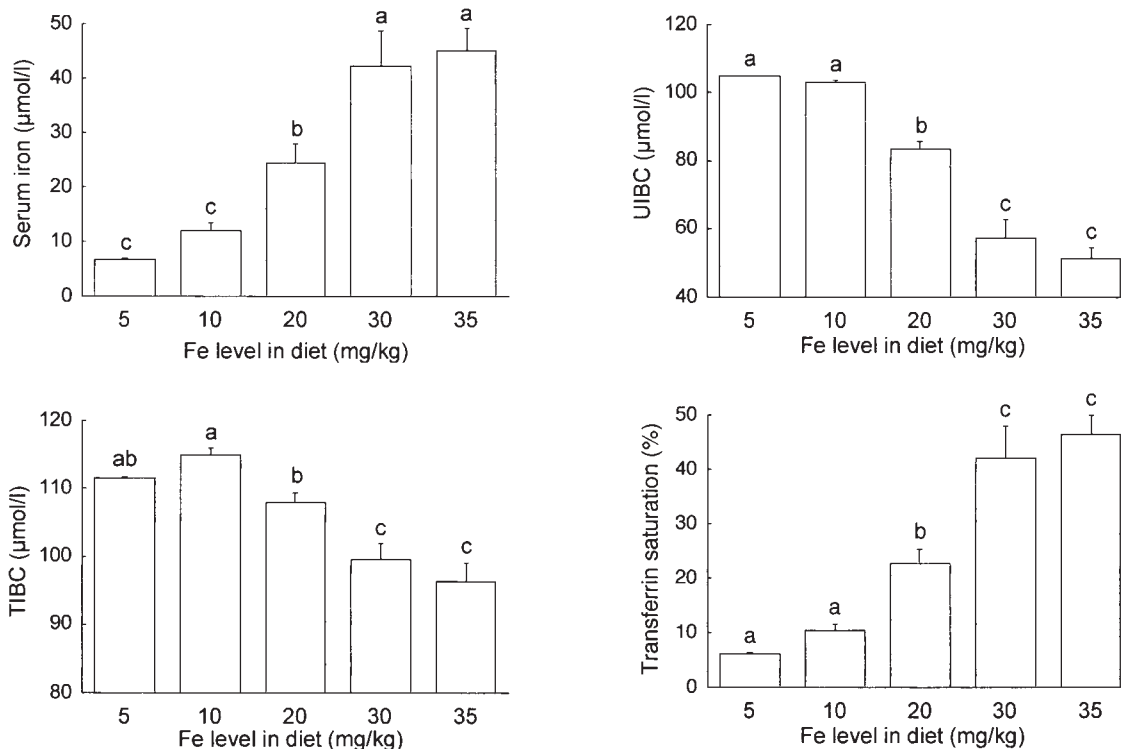


Fig. 3. Serum Fe Concentration, Unsaturated Fe-binding Capacity (UIBC), Total Fe-binding Capacity (TIBC) and Transferrin Saturation (Tf) in Rats Fed on Diets Containing Different Levels of Fe in Expt. 1.

Each value is the mean \pm SE ($n = 7$). P values for the Serum Fe Concentration, UIBC, TIBC and Tf (%) estimated by one-way ANOVA were < 0.001 . Means not sharing a letter differ between groups ($P < 0.05$).

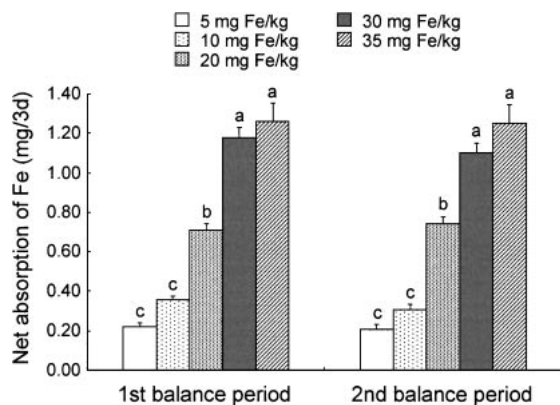


Fig. 4. Net Fe Absorption in Rats Fed on Diets Containing Different Levels of Fe in the 1st and 2nd Balance Periods in Expt. 1.

Each value is the mean \pm SE ($n = 7$). P values estimated by one-way ANOVA for the Fe Absorption were 0.324 and 0.784 in the 1st and 2nd balance periods, respectively. Means not sharing a letter differ between groups ($P < 0.05$).

the Fe absorption in the 5 g TA/kg diet group was also reduced from the control level (Fig. 6). The respective reduction of Fe absorption was 56.9% and 53.3% in the 15 and 20 g TA/kg diet groups from the level of the control group during the 1st balance period. In the 2nd balance period, the reduction in Fe absorption was more dose-dependent than that in the 1st period. The Fe absorption rates of the 15 and 20 g TA/kg diet groups were markedly lower than that of the control group in the 2nd period. The Fe absorption in the 2nd balance period was reduced by 52.5% and 45.9% in 15 and 20 g TA/kg diet groups, respectively, from the control level.

In contrast to the Fe absorption, no level of TA feeding had an effect on the Zn, Cu and Mn absorption during either balance period; Zn absorption was 24.9, 29.5, 27.8, 28.0 and 27.7%, $P = 0.807$ in the 1st period and 24.4, 23.1, 23.9, 20.0 and 21.2%, $P = 0.860$ in the 2nd period in the control, 5, 10, 15 and 20 g TA/kg diet groups, respectively; Cu absorption in the 1st period was

Table 3. Body Weight Gain, Food Intake and Fecal Dry Weight for the 1st and 2nd Balance Periods of Rats Fed on the Diets with or without Tannic Acid (TA) for 3 Weeks

	Control	5 g TA/kg	10 g TA/kg	15 g TA/kg	20 g TA/kg	P value
Body weight gain (g/d)	8.11 \pm 0.443	7.79 \pm 0.351	7.93 \pm 0.369	7.54 \pm 0.218	7.36 \pm 0.343	NS
Food intake (g/d)	21.1 \pm 0.732	21.0 \pm 0.660	20.3 \pm 1.05	20.4 \pm 0.501	20.3 \pm 0.795	NS
1st period fecal wt. (g/3d)	5.26 \pm 0.210 ^c	5.80 \pm 0.357 ^{bc}	6.74 \pm 0.606 ^{ab}	7.11 \pm 0.278 ^a	7.15 \pm 0.353 ^a	< 0.011
2nd period fecal wt. (g/3d)	5.52 \pm 0.562	5.65 \pm 0.227	6.00 \pm 0.534	6.39 \pm 0.259	6.62 \pm 0.409	NS

Each value is the mean \pm SE ($n = 6$). Means in a row not sharing a letter differ at $P < 0.05$, NS, not significant, $P \geq 0.05$.

27.0, 27.9, 26.6, 29.6 and 28.5%, $P = 0.927$ and 32.0 , 26.3 , 25.7 , 19.8 and 26.0% , $P = 0.393$ in the 2nd period; Mn absorption in the 1st period was 15.1 , 15.5 , 13.3 , 13.6 and 11.4% , $P = 0.785$ and 16.3 , 15.2 , 17.8 , 9.30

and 11.3% , $P = 0.545$ in the 2nd periods, respectively.

Trace minerals in the liver and cecum

The liver weight in the TA-fed groups was lower than that in the control group (data not shown). The Fe content in the liver of the rats fed with diets containing TA was much lower than that of the control group (Table 4), whereas the liver Zn, Cu and Mn contents were not affected by TA feeding at any level. The overall mean values for the Zn, Cu and Mn liver contents were 269 , 68.4 and $21.5 \mu\text{g/liver}$, the respective P values being 0.099 , 0.708 and 0.871 ($n = 30$). The concentration of soluble Fe was decreased and that of Cu and Mn was increased by feeding the TA diets. There was no significant difference found in the soluble Zn concentration (Table 5).

Effects of TA on the cecal fermentation and fecal output

The acetic and propionic acid pools were higher in the TA-fed rats than in the control group (Table 6). However, the butyric acid pool tended to be increased

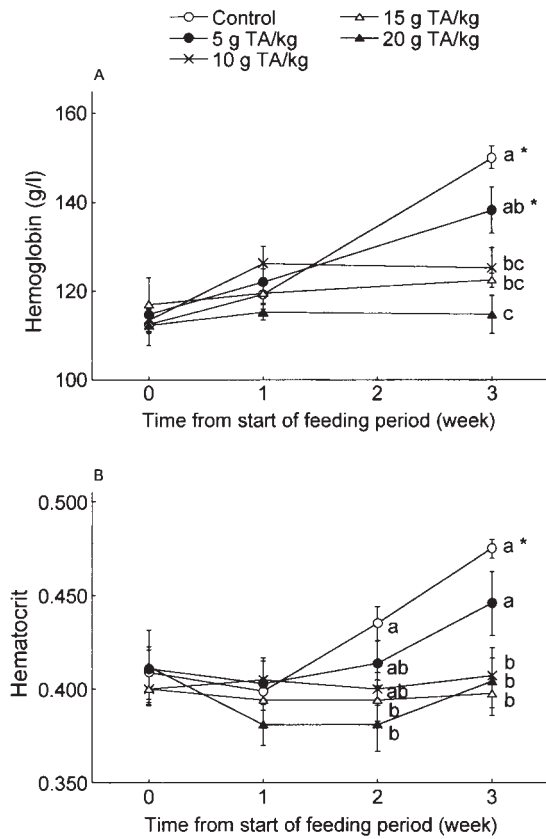


Fig. 5. Hemoglobin Concentration (A) and Hematocrit (B) in Rats Fed with or without Tannic Acid (TA) in Expt. 2.

Each value is the mean \pm SE ($n = 6$). P values estimated by two-way ANOVA were (A) 0.005 for the diet (D), < 0.001 for the time (T) and 0.043 for $D \times T$; (B) < 0.001 for D, 0.013 for T and 0.089 for $D \times T$. Means not sharing a letter differ between groups within the same day ($P < 0.05$). * Different from the value at week 0 in each group ($P < 0.05$).

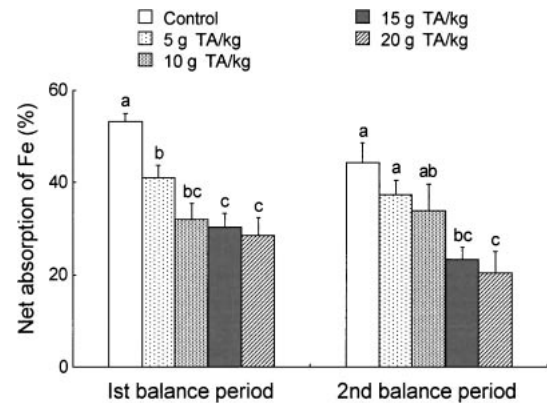


Fig. 6. Net Absorption of Fe in Rats Fed on Diets with or without Tannic Acid (TA) in the 1st and 2nd Balance Periods in Expt. 2.

Each value is the mean \pm SE ($n = 6$). P values estimated by one-way ANOVA for Fe Absorption were 0.004 and < 0.001 in the 1st and 2nd balance periods, respectively. Means not sharing a letter differ between groups ($P < 0.05$).

Table 4. Serum Iron (Fe) Concentration and Liver Fe Content of Rats Fed on the Diets with or without Tannic Acid (TA) for 3 Weeks

	Control	5 g TA/kg	10 g TA/kg	15 g TA/kg	20 g TA/kg	P value
Serum Fe conc. ($\mu\text{mol/l}$)	51.2 ± 2.57^a	44.6 ± 6.84^a	24.6 ± 6.62^b	22.7 ± 2.50^b	12.2 ± 2.48^b	< 0.001
Liver Fe cont. ($\mu\text{g/liver}$)	605 ± 55.9^a	274 ± 25.6^b	267 ± 16.1^b	206 ± 12.8^b	236 ± 8.53^b	< 0.001

Each value is the mean \pm SE ($n = 6$). Means in a row not sharing a letter differ at $P < 0.05$.

Table 5. Soluble Concentrations of Fe, Zn, Cu and Mn in the Cecal Contents of Rats Fed on the Diets with or without Tannic Acid (TA) for 3 Weeks

	Control	5 g TA/kg	10 g TA/kg	15 g TA/kg	20 g TA/kg	P value
Soluble concentration ($\mu\text{mol/g}$ of wet cecal contents)						
Fe	0.082 ± 0.010	0.052 ± 0.008	0.056 ± 0.014	0.051 ± 0.004	0.054 ± 0.006	NS
Zn	0.106 ± 0.016	0.081 ± 0.017	0.084 ± 0.009	0.080 ± 0.005	0.094 ± 0.009	NS
Cu	0.007 ± 0.010^c	0.020 ± 0.005^b	0.031 ± 0.004	0.030 ± 0.004^{ab}	0.035 ± 0.005^a	< 0.001
Mn	0.037 ± 0.007^b	0.043 ± 0.006^b	0.081 ± 0.009^a	0.072 ± 0.011^a	0.066 ± 0.003^a	0.022

Each value is the mean \pm SE ($n = 6$). Means in a row not sharing a letter differ at $P < 0.05$, NS, not significant, $P \geq 0.05$.

Table 6. Pools of Various Organic Acids in the Cecal Contents and Weight and pH Value of the Cecal Contents of Rats Fed on the Diets with or without Tannic Acid (TA) for 3 Weeks

	Control	5 g TA/kg	10 g TA/kg	15 g TA/kg	20 g TA/kg	<i>P</i> value
($\mu\text{mol}/\text{cecal content}$)						
Acetic acid	83.9 \pm 5.77 ^b	82.3 \pm 16.3 ^b	103 \pm 7.38 ^{ab}	118 \pm 12.2 ^{ab}	128 \pm 16.7 ^a	0.007
Propionic acid	20.1 \pm 1.57 ^c	16.7 \pm 4.59 ^c	35.7 \pm 3.91 ^b	48.8 \pm 2.91 ^a	42.5 \pm 3.70 ^{ab}	< 0.001
Butyric acid	17.0 \pm 2.15	15.0 \pm 2.7	17.9 \pm 4.63	29.0 \pm 9.14	29.7 \pm 10.5	NS
Total SCFA	121 \pm 7.63 ^b	114 \pm 21.3 ^b	157 \pm 10.5 ^{ab}	196 \pm 18.4 ^a	200 \pm 19.8 ^a	< 0.001
Cecal content (g)	2.77 \pm 0.259 ^d	3.86 \pm 0.701 ^{cd}	4.67 \pm 0.259 ^{bc}	6.35 \pm 0.599 ^{ab}	7.53 \pm 0.755 ^a	< 0.001
pH	7.00 \pm 0.108 ^a	6.76 \pm 0.092 ^{ab}	6.47 \pm 0.097 ^c	6.43 \pm 0.060 ^c	6.51 \pm 0.053 ^{bc}	0.007

Each value is the mean \pm SE ($n = 6$). Means in a row not sharing a letter differ at $P < 0.05$, NS, not significant, $P \geq 0.05$.

by TA feeding. The weight of the cecal contents was substantially increased and the pH value slightly decreased with increasing TA in the diets. The cecal wall weight was not affected by TA feeding. The mean values of the cecal wall weight were 0.177, 0.103, 0.144, 0.201 and 0.205 g in the control, 5, 10, 15 and 20 g TA/kg diet groups, respectively. The P value was 0.246.

There was no difference in the percentage of fecal TA output among the experimental groups. The average recovery rate of TA in the feces of the 5, 10, 15 and 20 g TA/kg diet groups was 13.3, 14.4, 18.4 and 15.6%, respectively, in the last 3 days of the experiment. The P value of ANOVA was 0.295.

Discussion

Evidence has been presented that TA has both enhancing²⁰⁾ and suppressing²⁾ effects on food intake. Vohra *et al.*²¹⁾ have reported that a TA level as low as 0.5% in the diet depressed the growth of chicks. However, in our study, TA feeding up to the 2% level did not have any significant effect on the body weight gain or food intake (Table 3). Other reports have failed to show any effect of a high level of TA on growth.^{5,22)} The absence of growth retardation in the present study shows that TA did not markedly reduce the absorption of those nutrients essential to growth such as nitrogen, energy sources and some major minerals and vitamins.

It has been reported that anemia was not caused by 5% TA feeding.²⁾ However, there is evidence to suggest that 2% black tea reduced the Hb concentration and Ht in rats.²³⁾ We have shown here that 10 g TA/kg diet, but not 5 g TA/kg diet induced anemia and that no further significant effect was apparent by feeding 10–20 g TA/kg diet (Fig. 4). The reduced levels of the blood parameters by feeding TA were almost the same as those in the 20 mg Fe/kg diet group in Expt. 1. These results show that the ingestion of TA at a level higher than 10 g TA/kg diet (1%) induced severe Fe-deficiency anemia. Previous studies have not yet evaluated the minimum dietary level of polyphenolics to induce anemia.^{2,23)} Reports indicate that the average intake of polyphenolics has been estimated at 1 g/d for humans.^{24,25)} The meal size for humans is about 25 times that for rats (500 g vs. 20 g). From the results of our study on rats, 2.5 g/d of polyphenolics may not induce anemia in humans. This

level is much higher than the usual intakes of polyphenolics. However, applying the factor for extrapolating to humans from animal studies, usually 1/10–1/5, which is used for the evaluation of NOAEL (no-observed-adverse-effect-level) of nutrients from animal studies, the level comes close to the usual intake by humans. This evaluation suggests that some individuals should take care with their Fe intake when they have TA-containing foods in their diet.

The results of the present study also demonstrate that, TA decreased the Fe absorption in a dose-dependent manner, this being confirmed by the blood parameters. The inhibition of Fe absorption may have been due to the presence of multiple galloyl residues bearing²⁶⁾ a trihydroxybenzene structure, which bind Fe with high affinity.²⁷⁾ Experiments in human have shown that polyphenols strongly inhibit Fe absorption.¹⁰⁾ These results confirm that the anemia discussed here was induced by the reduced Fe absorption resulting from TA-added to the test diets.

To date, very few studies have documented the effect of TA on Zn, Cu and Mn absorption. In the present study, we provide novel information that dietary TA did not decrease Zn, Cu and Mn absorption, in contrast to the clear reduction in Fe absorption. The liver content of these minerals support these findings; *i.e.* the Fe but not Zn, Cu or Mn content was decreased by feeding a TA-containing diet. Greger & Lyle¹²⁾ have reported that black tea and catechin decreased the Zn absorption in rats. Moreover, Fraile & Flynn²⁸⁾ have also found that feeding TA reduced the Mn absorption in rats. The reported decrease may be related to differences in TA dose. The reason for the different effects of TA on Fe and other trace minerals is still unknown. We speculate that the chelating property of TA to Fe is different from that to other trace minerals. Another possibility is that differences in the solubility among mineral sources might have caused different effects of TA on the absorption of Fe and other minerals. However, we used ferric citrate, which is the standard Fe source for rats and dissolves slowly in water, whereas the Zn, Cu and Mn sources (carbonates) and TA were rapidly soluble in the presence of gastric acid. This latter possibly is unlikely to account for the difference of TA effect on the absorption of these trace elements.

This study also investigated the effect of TA on the

cecal fermentation, because several previous works have shown that the large intestine was involved in the absorption of the trace minerals, Fe, Zn and Cu.^{13,14,29} Sakai *et al.*¹³ have shown that the enhancement of cecal fermentation restored gastrectomy-induced anemia in rats. The results of our study are inconsistent with those of Sakai is that we do not find a clear relationship between mineral absorption and changes in the cecal parameters. The cecal-soluble concentrations of Cu and Mn were increased, whereas those of Fe and Zn were decreased by TA feeding. Thus it is possible that the increased cecal absorption of Cu and Mn compensated for the reduced absorption of these minerals in the small intestine of the TA-fed rats.

We have shown that TA feeding increased the cecal acetate, propionate and SCFA pools with reducing pH value, indicating changes in the cecal fermentation process (Table 6). Polyphenolic compound-induced changes in fermentation have been reported. *In vitro* fermentation with quercetin³⁰ resulted in the production of a high level of acetic acid with a reduction in other SCFA levels. The findings from our study are somewhat inconsistent with this report, suggesting that the effect of TA on cecal microflora is different from that of quercetin.

We found that the fecal output of TA was only 15%. This compound may be indigestible and non-absorbable in the small intestine. Previous results on polyphenol absorption remain insufficient and are often controversial.³¹ *In vivo* studies using rats and humans have indicated that the bioavailability of polyphenols can vary widely, probably depending on both the experimental system and the chemical structure of the polyphenolic compounds.^{32,33} Bravo *et al.*⁴ have shown in rats, that only a small amount of TA (3.1%) was recovered in the feces, which is lower than the figure in our results. Approximately 85% of ingested TA disappeared from the intestine, suggesting that the most of the ingested TA was hydrolyzed in the large intestine and absorbed as gallic acid or was further degraded. Nakamura *et al.*³⁴ have recently reported that more than 60% of TA remained in an intact form after oral ingestion, but that some was hydrolyzed to gallic acid by bacterial tannase in the intestine and further metabolized to 4-*O* methylgallic acid, pyrogallol and resorcinol. The hydrolysis of TA may liberate chelating Fe in the cecum, and 'free' Fe is absorbable because more than two gallic acid residues on a TA molecule are necessary to allow chelation. Enhancement of the cecal fermentation process by indigestible sugars may aid the recovery of this decrease in Fe absorption. This may be true for humans. Our basic results for the effect of TA on Fe absorption and cecal fermentation may provide useful information for human studies to confirm these rat studies.

In conclusion, dietary TA reduced Fe absorption, but not that of other major trace elements in the diet and induced severe Fe-deficient anemia without any retar-

dation of growth. We also found that the lowest dose of phenolics, which was still a high level in ordinary food, did not induce anemia. TA possesses both positive and negative health effects, so, we need to know the correct dose of TA before recommending its intake by particular population groups. Human studies are warranted to confirm the results of these rat studies.

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