The Effects of Various Protein Sources to Iron Deficient Condition

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SUMMARY

The effect of various animal protein sources to iron deficient condition in the weanling rat was investigated. Wistar-strain male rats (n=46) were divided into 7 groups, and fed on normal casein diet, the iron deficient diet with casein, egg, chicken, pork, beef powder and fish meal as protein sources. Hemoglobin level, liver weight, iron and zinc concentrations in serum were decreased on iron deficient condition, but the hemoglobin levels in the iron deficient diet with egg, chicken, pork and beef powder were higher than in the iron deficient diet with casein and fish meal. Heart weight of the egg diet was lowest among the iron deficient groups. Hepatic iron level was also decreased on iron deficient condition. reversely, hepatic copper (especially in the mitochondrial and nuclear fractions) was increased on iron deficient condition, but that in the rat fed on egg diet tended to be lower in other groups. Hepatic xanthine oxidase, monoamine oxidase and urate oxidase activities in the iron deficient groups were higher than the normal group and the differences among several proteins were not clear.

INTRODUCTION

Iron is present in all cells of the body and plays a key role in many biochemical reactions, and also present in several enzymes that are responsible for the activation of oxigen (oxidase and oxygenase) for the transport of oxigen (hemoglobin and myoglobin). The iron content in the large variety of metalloemzyme amounts to 6 to 8mg. About 3mg of iron is present in plasma and binds to transferrin (specific iron transporting protein).

A varying amount of iron is stored as ferritin or hemosiderin in several tissues and organs (especially liver, spleen and bone marrow).

Dietary iron deficient anemia, namely nutritional anemia is know as the major health problem in many developing countries, and iron deficient anemia is also common nutritional problem among infants, children and women of reproductive age in the developed countries.

The present studies were performed to investigate the effects of various protein sources to iron deficient condition with hemoglobin value and iron, copper and zinc concentrations in serum, organs and hepatic cell, and hepatic metalloenzyme activities using albino rats.

MATERIALS AND METHODS

Weanling Wistar-strain male rats (about 50g, 20-day old) were housed in stain-less steel cages in a temperature, humidity and light controlled room (22±1°C, 60—70%, 12 hours light/dark cycle).

The rats were randomly divided into 7 groups (n=46) and supplied normal casein as control, iron deficient diet with casein, egg, chicken, pork, beef and fish meal as protein sources (freeze-dried),

respectively. Extra blood in the chicken, pork and beef was excluded in order to adjust iron levels in all iron deficient diets.

The composition of experimental diets is presented in Table 1. The rats were fed their respective ration for 30 days.

Table 1. The composition of diets (%)

	NC	DC	DE	DK	DP	DB	DF
Protein							
Casein	22	22	19.5	20.8	20.7	20.7	19.5
Egg Powder[a]	–	–	5.0	–	–	–	–
Chicken Powder	–	–	–	2.5	–	–	–
Pork Powder	–	–	–	–	2.6	–	–
Beef Powder	–	–	–	–	–	2.6	–
Fish Meal[a]	–	–	–	–	–	–	3.6
Carbohydrate							
Starch, Corn	32	32	29.5	30.7	30.7	30.7	30.9
Sucrose	30	30	30	30	30	30	30
Oil, Corn	5	5	5	5	5	5	5
Mineral Mix.[b]	4	4*	4*	4*	4*	4*	4*
Vitamin Mix.[c]	2	2	2	2	2	2	2
Cellulose	5	5	5	5	5	5	5
Concentration							
Iron (μg/g)	53	14	14	13	15	14	15
Copper(μg/g)	22	22	21	21	24	19	20
Zinc (μg/g)	15	14	15	14	18	15	17

[a] Provided by Nihon Nosan Kogyo Co., Ltd.; [b] According to A. E. Harper: J. Nutr., 68 (1959); [c] Takeda Panvitan Powder; * $Fe(C_6H_5O_7)\ 6H_2O$ was excluded.
NC: Normal Casein, DC: Iron-Deficient Casein, DE: Iron-Deficient Casein+Egg Powder, DK: Iron-Deficient Casein+Chicken Powder, DP: Iron-Deficient Casein+Pork Powder, DB: Iron-Deficient Casein+Beef Powder, DF: Iron-Deficient Casein+Fish Meal.

At the end of the observation, rats were killed by exsanguination, blood was collected in a tube and centrifuged to seperate the serum. After the liver was perfuged by physiological saline solution for enzyme assays, organs (liver, kidney, spleen and heart) were immediately removed. The concentrations of iron, copper and zinc in organs were determined using the atomic absorption/flame emission spectrophotometer, those in serum were measured by the method of Klauder *et al.*[1] and hemoglobin level was estimated by the method of Cyanmethemoglobin[2]. Hepatic metalloenzymes, namely xanthine oxidase (X.O.) activity and urate oxidase (U.O.) activity were determined by the method of Bergmeyer *et al.*[3] and monoamine oxidase (M.A.O.) activity was determined by the

method of kraml[4].

The mode of Hepatic cell fractionation was indicated in Fig. 1.

Students t-test was used to determine significant differences between group means. The probability level for significance was 5% or less.

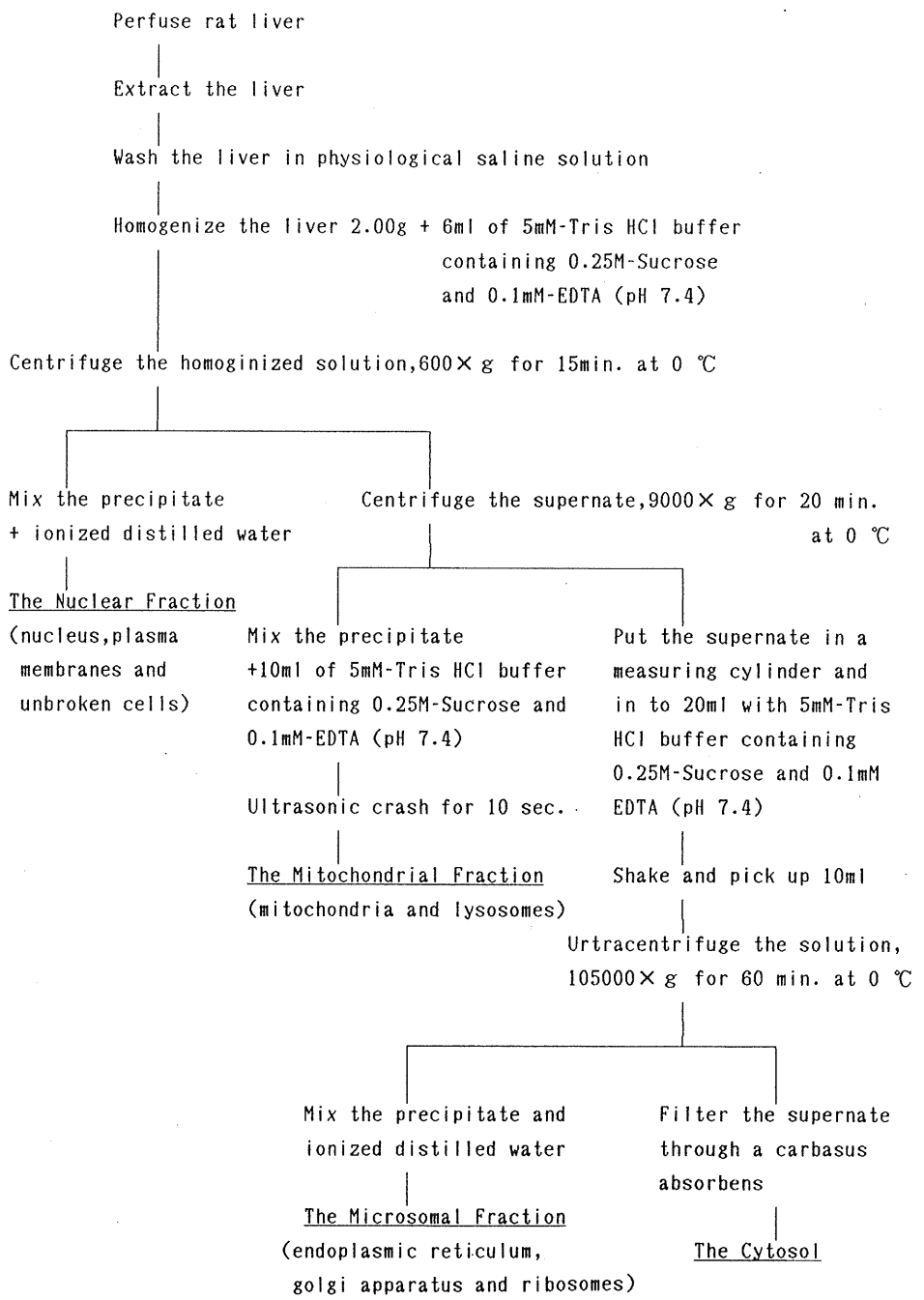

Fig. 1. The Mode of Cell Fractionation.

RESULTS AND DISCUSSION

Hemoglobin Level (Fig.-2)

Hemoglobin levels were significantly lower in the iron deficient groups than in the normal group, and indicated the different values by use of various protein sources. The DE, DK, DP and DB groups showed higher levels than those of the DC and DF groups.

Cook *et al.*[5] reported that iron absorption from standard meal is reduced when powdered egg is substituted for ground beef, and that iron uptake from a semisynthetic meal is not stimulated by powdered egg as it is by muscle meats. But Miller *et al.*[6] suggested that the anemic rats readily utilized the iron of spray dired egg yolk for hemoglobin regeneration. Our result is in agreement with Miller's result.

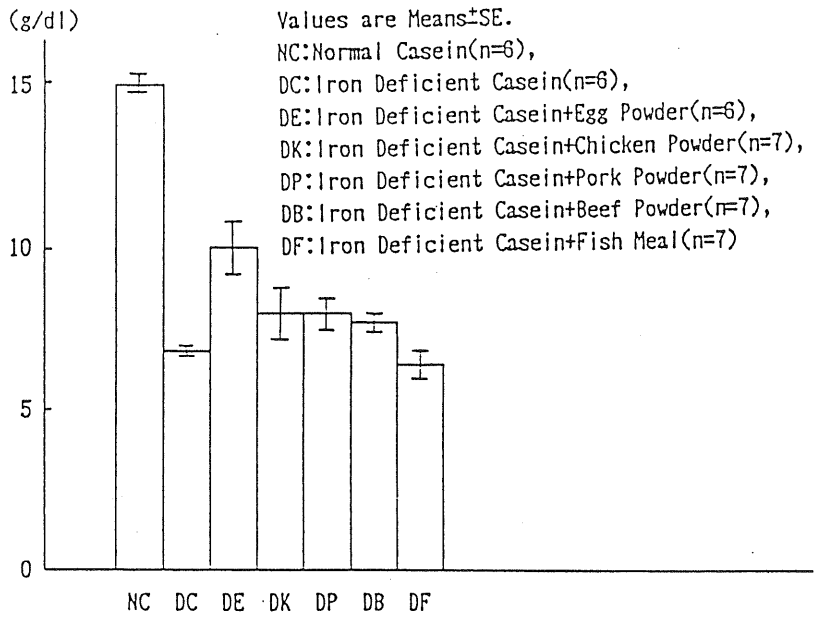

Fig. 2. The hemoglobin levels.

Concentrations of Iron, Copper and Zinc in Serum (Table-2)

Iron deficient condition indicated significant decreases of iron and zinc concentrations in serum.

Copper concentration showed the gentle decrease, comparison to iron and zinc concentrations. But the copper concentrations of the DC and DE groups were similar to that of the normal group, and this result has obtained in our previous study[7].

Organ Weight (Table-3)

The liver weights of the iron deficient groups were lower than the weight of the normal group, but that of the DP group was the same value as the normal group.

The heart and spleen weights were higher in the iron deficient groups than in the normal group, and

Table 2. The concentrations of iron, copper and zinc in serum (μg/dl)

	Iron	Copper	Zinc
NC (n=6)	$274.4 \pm 10.4^{a, b, c, d, e, f}$	$100.5 \pm 6.1^{g, h, i, k, l, m}$	$195.0 \pm 7.2^{p, q, r, s, t, u}$
DC (n=6)	167.7 ± 24.3^{a}	$93.4 \pm 6.4^{k, l, m}$	168.8 ± 10.7^{p}
DE (n=6)	157.0 ± 15.2^{b}	$96.8 \pm 9.9^{n, o}$	170.1 ± 6.3^{q}
DK (n=7)	140.2 ± 10.6^{c}	$72.8 \pm 3.8^{g, k}$	152.4 ± 6.9^{r}
DP (n=7)	145.2 ± 5.4^{d}	$65.5 \pm 5.9^{h, l, n}$	$163.8 \pm 5.6^{s, v}$
DB (n=7)	150.1 ± 10.8^{e}	$71.8 \pm 3.4^{l, m, o}$	$148.3 \pm 5.6^{t, v}$
DF (n=7)	157.3 ± 7.1^{f}	$74.2 \pm 9.7^{j, n}$	148.0 ± 11.8^{u}

Values are Means ± DE.

NC: Normal Casein, DC: Iron-Deficient Casein, DE: Iron-Deficient Casein+Egg Powder, DK: Iron-Deficient Casein+Chicken Powder, DP: Iron-Deficient Casein+Pork Powder, DB: Iron-Deficient Casein+Beef Powder, DF: Iron-Deficient Casein+Fish Meal. Matching superscript letters denote significant differences, $P < 0.05$.

Table 3. The organ weights (Wet weight / 100g of body weight)

	Liver	Kidney	Heart	Spleen
NC (n=6)	$4.7 \pm 0.2^{a, b, c, d, e}$	0.42 ± 0.01^{a}	$0.31 \pm 0.01^{a, b, c, d}$	$0.29 \pm 0.01^{a, b, c, d, e}$
DC (n=6)	$4.0 \pm 0.1^{a, f}$	$0.46 \pm 0.01^{a, b, c, d}$	$0.48 \pm 0.02^{a, e}$	$0.40 \pm 0.02^{a, f}$
DE (n=6)	$4.0 \pm 0.2^{b, g}$	0.40 ± 0.01^{b}	$0.36 \pm 0.01^{a, e, f, g}$	0.32 ± 0.02^{f}
DK (n=7)	$4.0 \pm 0.1^{c, h}$	0.43 ± 0.01^{e}	0.41 ± 0.01^{a}	0.37 ± 0.03^{b}
DP (n=7)	$4.4 \pm 0.1^{f, g, h, i, j}$	0.40 ± 0.01^{c}	$0.40 \pm 0.01^{b, e}$	0.35 ± 0.02^{c}
DB (n=7)	$3.8 \pm 0.1^{d, i}$	0.43 ± 0.01^{f}	$0.43 \pm 0.01^{c, f}$	0.36 ± 0.02^{d}
DF (n=7)	$3.7 \pm 0.1^{e, j}$	$0.39 \pm 0.01^{d, e, f}$	$0.42 \pm 0.02^{d, g}$	0.37 ± 0.02^{e}

Values are Means ± SE.

NC: Normal Casein, DC: Iron Deficient Casein, DE: Iron Deficient Casein+Egg Powder, DK: Iron Deficient Casein+Chicken Powder, DP: Iron Deficient Casein+Pork Powder, DB: Iron Deficient Casein+Pork Powder, DF; Iron Deficient Casein+Fish Meal. Matching superscript letters on individual organ denote significant differences, $P < 0.05$.

the DC group showed the highest value among the iron deficient groups. This phenomenon has obtained in previous study[7],and we estimated that the iron deficient condition was generated the compensatory hypertrophy of the heart. Heart weight in the DE group was lower than in other iron deficient groups, because the hemoglobin level of the DE group tended to be higher.

Concentrations of Iron, Copper and Zinc in Organs (Table-4)

Iron deficient condition was also accompanied with the decreases of iron concentrations in all organs, especially the remarkable decrease was observed in the liver.

Numerous researchers have investigated that the metabolism of iron and copper is interelated, and as a copper containing ferroxidase, ceruloplasmin catalyzes the conversion of iron from ferrous and ferric before iron is transported from the liver for hemoglobin synthesis[8,9,10]. And it has been also known that iron deficiency produces a increase of hepatic copper level in rats, although the mechanism responsible for accumulation of hepatic copper during iron deficiency is presently unknown. similarly, iron deficient groups in this study indicated a increase of hepatic copper, as compared with that of the nomal group, however hepatic copper level in the DE group was lower than in other iron deficient groups, this result may show that the rats fed on egg diet are hard to get into iron deficient anemia, which is consistent with previous our report[7].

In all organs, zinc concentration was not so changed by iron deficient condition, but hepatic zinc level in the DP group was lower than in other groups.

Iron, copper and Zinc concentrations in Hepatic Cell (Table-5)

Iron, copper and zinc concentrations in all fractions of hepatic cell showed much the same tendency as those in the whole liver.

Namely, in regard to iron concentration in hepatic cell, iron deficient groups showed the decrease. Espesialy, the concentrations of the DE, DP and DB groups were lower lower than those of the DC and DF groups in the mitochondrial fraction. Most of iron in mitochondrial fraction of the rats fed on egg, pork or beef diet which indicated the high Hb levels, may be mobilized to plasma for Hb synthesis.

Reversely, copper concentration in the all fractions was increased by iron deficient condition. Especialy, copper accumulated in the nuclear and mitochondrial fractions. This result is consistant with the previous report[11], but Sherman *et al.*[12] reported that the greatest increase in hepatic copper in iron deficient pups was found in the cytosol.

The zinc concentration in hepatic cell was little changed in all fractions.

Hepatic Metalloenzyme Activities (Table-6)

Topham *et al.*[13,14,15] posturated that xanthine oxidase is a iron containing flavoprotein and catalyzes the oxidation of iron that binds to transferrin and this reaction is similar to the reaction catalyzed by ceruloplasmin in the blood stream to mobilize iron from tissues.

Kelly *et al.*[16] reported that iron deficiency caused an increase in hepatic xanthine oxidase activity. In this study, xanthine oxidase activities of all groups were little changed in the mitochondrial fraction, but iron deficient groups showed the high xanthine oxidase activities in the

Table 4. The concentrations of iron, copper and zinc in organs (μg/g of dry weight)

		Liver	Kidney	Spleen	Heart
[Fe]	NC (n=6)	488.4 ± 41.8$^{a, b, c, d, e}$	315.8 ± 13.2$^{f, g, h, i}$	1026.2 ± 15.9$^{k, l, m, n, o}$	402.4 ± 45.4$^{p, q, r, s}$
	DC (n=6)	165.9 ± 25.7^{a}	224.5 ± 13.6^{f}	668.8 ± 80.6^{k}	237.7 ± 15.0^{p}
	DE (n=6)	222.1 ± 20.0^{b}	233.0 ± 23.0^{g}	612.3 ± 46.8^{l}	307.5 ± 26.8^{t}
	DK (n=7)	223.0 ± 14.2^{c}	293.3 ± 26.6^{j}	542.4 ± 17.9^{m}	287.4 ± 30.5^{q}
	DP (n=7)	207.9 ± 24.1^{d}	246.2 ± 20.6^{h}	585.5 ± 27.1^{n}	228.2 ± 11.7^{r}
	DB (n=7)	216.9 ± 34.2^{e}	242.3 ± 10.5^{i}	496.0 ± 20.5$^{l, n}$	276.4 ± 13.6^{s}
	DF (n=7)	158.6 ± 15.4$^{b, c}$	181.7 ± 10.4$^{f, h, i, j}$	556.6 ± 46.2^{o}	230.2 ± 7.4$^{s, t}$
[Cu]	NC (n=6)	21.6 ± 3.2$^{a, b, c, d, e, f}$	38.0 ± 4.2^{g}	15.2 ± 3.1	24.0 ± 0.7$^{h, i, j, k}$
	DC (n=6)	65.9 ± 14.6^{a}	31.8 ± 2.2	14.3 ± 1.4	28.9 ± 1.4^{h}
	DE (n=6)	60.0 ± 11.7^{b}	31.2 ± 1.7	14.0 ± 1.2	30.2 ± 1.3^{i}
	DK (n=7)	109.3 ± 19.3^{c}	29.4 ± 2.8	12.6 ± 1.0	34.3 ± 1.7^{j}
	DP (n=7)	78.2 ± 11.8^{d}	26.7 ± 1.1^{g}	14.0 ± 1.6	29.2 ± 1.4^{j}
	DB (n=7)	79.4 ± 10.1^{e}	29.7 ± 1.2	11.1 ± 1.6	31.2 ± 2.7
	DF (n=7)	78.5 ± 7.7^{f}	30.0 ± 1.4	15.2 ± 1.4	30.6 ± 0.8^{k}
[Zn]	NC (n=6)	129.8 ± 3.0$^{a, b}$	112.9 ± 2.5	114.3 ± 2.7^{k}	102.4 ± 1.7$^{o, p}$
	DC (n=6)	131.5 ± 2.8$^{c, d}$	122.3 ± 3.5^{h}	118.8 ± 4.4^{l}	98.3 ± 3.1
	DE (n=6)	146.0 ± 11.0$^{e, f}$	114.6 ± 2.1	114.2 ± 0.7^{m}	106.5 ± 8.7
	DK (n=7)	124.1 ± 4.1^{g}	123.5 ± 4.9^{i}	136.0 ± 5.4$^{k, l, m, n}$	92.0 ± 3.1^{o}
	DP (n=7)	109.5 ± 2.5$^{a, c, e, g}$	115.1 ± 2.4	134.9 ± 11.9	94.6 ± 2.1^{p}
	DB (n=7)	121.8 ± 1.6$^{a, c, e}$	120.2 ± 3.3^{j}	140.5 ± 11.6	95.4 ± 2.5
	DF (n=7)	114.9 ± 4.7$^{b, d, f}$	110.5 ± 0.6$^{h, i, j}$	116.9 ± 2.5^{n}	97.8 ± 1.9

Values are Means ± SE.

NC: Normal Casein, DC: Iron-Deficient Caselin, DE: Iron-Deficient Casein+Egg Powder, DK: Iron-deficient Casein+Chicken Powder, DP: Iron-Deficinet Casein+Pork Powder, DB: Iron-Deficient Casein+Beef Powder, DF: Iron-Deficient Casein+Fish Meal. Matching superscript letters denote significant differences, $P < 0.05$.

Table 5. The iron, copper and zinc concentrations in hepatic cell (μg/g)

		N.[1]	Mt.[2]	Ms.[3]	Ct.[4]
[Fe]	NC (n=6)	32.54 ± 3.80$^{a, b, c, d}$	10.50 ± 0.77$^{e, f, g, h}$	24.82 ± 3.14$^{l, m, n, o, p}$	35.13 ± 2.84$^{q, r, s, t, u}$
	DC (n=6)	12.08 ± 0.96^{a}	6.44 ± 0.59$^{i, j, k}$	7.23 ± 0.80$^{l, n}$	18.04 ± 3.72^{q}
	DE (n=6)	14.00 ± 0.40^{b}	3.93 ± 0.30$^{e, i}$	4.59 ± 0.16$^{l, m, o}$	11.88 ± 0.49^{r}
	DK (n=7)	12.86 ± 0.46^{c}	5.53 ± 0.77^{f}	6.91 ± 0.84^{m}	16.04 ± 3.00^{s}
	DP (n=7)	11.44 ± 0.44$^{c, d}$	3.63 ± 0.30$^{g, j}$	5.26 ± 0.38$^{n, o}$	11.00 ± 2.09^{t}
	DB (n=7)	13.04 ± 0.32^{d}	4.48 ± 0.32$^{h, k}$	6.60 ± 0.48^{o}	10.12 ± 0.44^{r}
	DF (n=7)	8.64 ± 0.40$^{a, b, c, d}$	6.87 ± 0.81$^{e, g, h}$	5.64 ± 0.75^{p}	11.28 ± 1.99^{u}
[Cu]	NC (n=6)	2.78 ± 0.38$^{a, b, c, d}$	0.35 ± 0.01$^{f, g, h, i, j}$	0.17 ± 0.01$^{k, l, m, n, o}$	1.72 ± 0.08$^{p, q, r, s, t}$
	DC (n=6)	7.72 ± 0.74^{a}	2.22 ± 0.28^{f}	0.52 ± 0.03$^{k, o}$	2.95 ± 0.18^{p}
	DE (n=6)	9.50 ± 2.00^{b}	2.06 ± 0.23^{g}	0.60 ± 0.01^{l}	3.54 ± 0.38^{q}
	DK (n=7)	21.04 ± 4.18$^{a, b, c, e}$	2.88 ± 0.54^{h}	1.01 ± 0.16$^{k, l, n}$	3.76 ± 0.47^{r}
	DP (n=7)	9.42 ± 1.46^{c}	2.50 ± 0.19^{i}	0.79 ± 0.21^{m}	3.44 ± 0.61^{s}
	DB (n=7)	13.90 ± 2.48^{d}	2.78 ± 0.39^{j}	0.60 ± 0.09^{n}	3.55 ± 0.47^{t}
	DF (n=7)	6.26 ± 0.98$^{d, e}$	7.39 ± 0.22$^{f, g, h, i, j}$	0.79 ± 0.10^{o}	4.17 ± 0.26^{p}
[Zn]	NC (n=6)	8.02 ± 0.86	2.37 ± 0.14$^{b, c}$	2.09 ± 0.06$^{h, i}$	15.13 ± 0.52^{p}
	DC (n=6)	7.96 ± 0.18	2.60 ± 0.14	2.29 ± 0.10$^{j, k}$	15.86 ± 0.86^{q}
	DE (n=6)	8.68 ± 0.92	2.85 ± 0.10$^{b, d}$	2.64 ± 0.08$^{h, l, m}$	16.68 ± 0.47
	DK (n=7)	8.66 ± 0.58	2.60 ± 0.11^{e}	2.50 ± 0.11^{i}	14.98 ± 0.55$^{r, s}$
	DP (n=7)	7.32 ± 0.32^{a}	2.30 ± 0.08$^{e, f, g}$	2.30 ± 0.11$^{l, n}$	15.26 ± 0.68$^{r, t}$
	DB (n=7)	8.62 ± 0.48^{a}	2.80 ± 0.11$^{c, f}$	2.46 ± 0.27^{o}	16.90 ± 0.78
	DF (n=7)	8.78 ± 1.50	2.92 ± 0.21^{g}	1.45 ± 0.12$^{i, k, m, n, o}$	19.06 ± 1.08$^{p, q, s, t}$

Values are Means ± SE.

[1]The Nuclear Fraction, [2]The Mitochondrial Franction, [3]The Microsomal Franction, [4]The Cytosol.

NC: Normal Casein, DC: Iron-Deficient Casein, DE: Iron-Deficient Casein+Egg Powder, DK: Iron-Deficient Casein+Chicken Powder, DP: Iron-Deficient Casein+Pork Powder, DB: Iron-Deficient Casein+Beef Powder, DF: Iron-Deficient Casein+Fish Meal. Matching superscript letters denote significant differences, $P < 0.05$.

Table 6. The hepatic metalloenzyme activities

	The Mitochondrial Fraction			The Cytosol
	X.O.[1]	M.A.O.[2]	U.O.[3]	X.O.
NC (n=6)	5.23 ± 0.23	0.31 ± 0.02$^{a, b, c,}_{d}$	2.55 ± 0.24$^{h, i, j,}_{k}$	0.51 ± 0.12$^{o, p, q,}_{r, s, t}$
DC (n=6)	5.19 ± 0.37	0.51 ± 0.03$^{a, e, f}$	4.63 ± 0.27$^{h, l, m}$	1.49 ± 0.47^{o}
DE (n=6)	5.06 ± 0.27	0.52 ± 0.03$^{b, g, h}$	4.43 ± 0.24^{l}	1.77 ± 0.38^{p}
DK (n=7)	5.37 ± 0.35	0.39 ± 0.02$^{e, g, i,}_{j}$	3.31 ± 0.20$^{l, n}$	1.56 ± 0.23^{q}
DP (n=7)	4.97 ± 0.38	0.35 ± 0.02$^{f, h, k,}_{l}$	3.41 ± 0.07$^{j, m}$	1.51 ± 0.20^{r}
DB (n=7)	4.95 ± 0.19	0.55 ± 0.02$^{c, i, k}$	4.30 ± 0.31$^{j, n}$	2.39 ± 0.44^{s}
DF (n=7)	4.88 ± 0.28	0.55 ± 0.05$^{d, j, l}$	4.74 ± 0.77^{k}	2.22 ± 0.49^{t}

Values are Means ± SE.
[1] Xantine Oxidase (μg/g Protein/60 min.) [2] Monoamine Oxidase (μg/g Protein/10 min.)
[3] Urate Oxidase (μg/g Protein/2 min)
NC: Normal Casein, DC: Iron-Deficient Casein, DE: Iron-Deficient Casein+Egg Powder, DK: Iron-Deficient Casein+Chicken Powder, DP: Iron-Deficient Casein+Pork Powder, DB: Iron-Deficient Casein+Beef Powder, DF: Iron-Deficient Casein+Fish Meal. Matching superscript letters denote significant differences, $P < 0.05$.

cytosol, as compared with the activity of normal group.

Monoamine oxidase activities in the iron deficient groups were higher than the normal groups in this study. However, previous our study didn't show the same result[17]. In explanation of this result, it may be considered that the monoamine oxidase activity was influenced by iron deficiency of the rats, moreover the activity ratio patern may be influenced by the inhibitory metabolic factors which compensate the effect of the iron deficiency, which was reported by Onda[18].

Urate oxidase activities of the iron deficient groups were also higher than the activity of the normal group.

In general, urate oxidase has been shown to respond the level of dietary protein. But our result indicated that the iron deficiency acted on the increas of urate oxidase activity.

In conclusion, we might suggest that various animal proteins have good effects on iron deficient rat, and there were differences among the effects of various protein sources to iron deficient condition.

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