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EFFECTS OF FEEDING HEME IRON AND CATTLE BONE CALCIUM TO IRON AND CALCIUM DEFICIENT MICE.

Rumi Ogasawara, Kazuhisa Honda, Miki Suzuki, Masahiro Numata, Toyoo Nakamura and Tetsuji Nagao*

Central Research Institute, Itoham Food Inc., Ibaraki, 302-0104, Japan
*Hatano Research Institute, Food and Drug Safety Center, Kanagawa, 257-0025, Japan

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Background:
Valuable by-products are obtained from animals during slaughter and processing. In addition to the obvious monetary value that is derived from the processed by-products, the conversion of inedible parts of the animal into useful products performs a very important function from the standpoint of sanitation. Large volumes of blood and bone result from the slaughtering and the meat processing process. Both have nutritional value, however, bone usually winds up being used in seasonings, feeds and fertilizers; and blood is usually discarded at high cost.
In Japan, calcium and iron deficiencies have been a nutritional problem for a long time. It is known that supplementing calcium in the early years is effective in the prevention of osteoporosis. Iron deficiency causes anemia, which can be a serious problem, especially in women. However, it is difficult to amply supply these elements due to Japanese food customs and, as such, inorganic mineral calcium and iron supplements are often used. The drawback to this is that several inorganic minerals on the market which are sold in large quantities and at low prices have been shown to provide low levels of bioavailability[1)] and to even produce side effects[2)].
Cattle bone calcium comprised of hydroxyapatite has show bioavailability equal to the calcium found in milk, which is widely recognized as a good source of bioavailable calcium [3)]. Heme iron, which is a component of red blood cells, has been found to have five times the bioavailability of nonheme iron [1)] . The absorption of nonheme iron is thought to be inhibited by many ingredients found in human food [4)] , however, heme iron is absorbed intact as an iron porphyrin complex and the iron is released inside the intestinal mucosal cells without being inhibited by these ingredients [5)]. It follows that cattle bone calcium and heme iron derived from blood can be used as materials to supply these elements.

Objectives:
This investigation was carried out to investigate the effective utilization of cattle bones and cattle blood which are the main animal by-products in Japan.

Methods:
Fresh cattle blood was obtained from a slaughtering plant (Obihiro, Japan) and 0.5% sodium citrate was added as an anticoagulant. The blood was centrifuged to separate plasma and the blood cell fraction. The blood cell fraction, which almost completely consisted of red blood cells, was dissolved in water and digested by alkaline protease. After enzyme deactivation, the hydrolyzate was centrifuged to separate the heme rich precipitant. The precipitant was washed in water and centrifuged. The heme iron concentrate which resulted as a precipitant was resuspended in water and spray-dried. The heme iron concentrate powder contained approximately 1% iron, 10% of porphyrin and 70% of protein hydrolyzate.
Fresh cattle bones were obtained from a slaughtering plant (Kagoshima, Japan). The bones were suspended in water and boiled to remove the water soluble and lipid components. The boiled bones were washed with water to remove any remaining meat residues, then dried and ground into a powder. The powdered bone obtained by this process had a composition consisting of approximately 33% calcium and 16% phosphorus.
Male ICR mice (Charles River Inc., Kanagawa, Japan) were kept in individual cages with the temperature maintained at 24±1℃ and provided with feed and water *ad libitum*. The rats were fed a commercial stock diet (CE-2, Japan CLEA Co. Ltd., Tokyo, Japan) for a week to allow them to adapt to the new environment.
In Experiment 1, mice were grouped into 4 experimental groups: a group supplied with the standard diet (AIN93-G, ORIENTAL BIOSERVICE KANTO Inc., Ibaraki, Japan) for 5 weeks (S5); a group supplied with a calcium and iron deficient diet (calcium and iron removed AIN93-G) for 5 weeks (D5); a group supplied with a calcium and iron deficient diet for 5weeks and AIN93-G for 1 week (D5+S1); and a group supplied with a calcium and iron deficient diet for 5 week and AIN93-G for 3 weeks (D5+S3).
In Experiment 2, mice were grouped into 4 experimental groups: a group supplied with the AIN93-G for 6 weeks (S6); a group supplied with the AIN93-G for 8 weeks (S8); a group supplied with a calcium and iron deficient diet for 5weeks and with the experimental diet (bone calcium and heme iron added to the calcium and iron deficient diet) for 1 week (D5+E1); and a group supplied with the calcium and iron deficient diet for 5 weeks and with the experimental diet for 3 weeks (D5+E3).
After feeding, the mice were fasted for 24hr (09:00-09:00), and then anesthetized using a subcutaneous injection of sodium pentobarbital. Blood was collected to determine red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC), values which served as iron deficiency parameters. Plasma alkaline phosphatase activity, phosphorus and calcium concentration values were determined for use as the parameters for calcium deficiency. Plasma alkaline phosphatase activity, phosphorus and calcium concentrations were measured using an auto analyzer system (COVAS-FARA, Roche Diagnostic Systems, Montclair, NJ).
A histological examination of the small intestine and femur of each animal was performed. The small intestine and femur of each animal was removed, fixed in 10% neutral buffered formalin, and embedded in paraffin. Tissues were serially sectioned at 8 μm (microns) and stained with hematoxylin and eosin.
Statistical analysis was done using Student's t-test.

Results and discussion:

Body weight gain and feed consumption were significantly suppressed in mice on the calcium and iron deficient diets as compared to the standard diet.

As shown in Table 1, calcium deficiency parameters (alkaline phosphatase activity and phosphrus) were significantly increased in the D5 group. In addition, feeding on the experimental diet resulted in immediate recovery as compared to the standard diet. These results suggest that the cattle bone supplement was more effective than the calcium carbonate supplement in acting on the biological changes which resulted from the calcium deficiency.

On the other hand, as shown in Table 2, the iron deficiency parameters (HGB, HCT, MCV and MCH) were significantly decreased in group D5. However, these parameters were restored after feeding of the standard and experimental diet. The hematological changes caused by iron deficiency were restored to normal levels within a week. Significant differences correspondent to the type of iron supplement used were not noted in this study. The period of feeding on a normal diet after feeding on a deficient diet was too long to ascertain significant differences in effects between nonheme iron and heme iron.

Histological changes in small intestine and the femur specimens were noticed in the D5 group (data not shown). Intestinal tissue cells were markedly enlarged. Femurs also appeared to be thinner. The experimental diet showed more effectiveness in the recovery from these changes, however, side effects from nonheme iron related to the small intestine were not observed.. These results suggest that cattle bone calcium has much higher bioavailability than the calcium carbonate presently being used in large quantities in organic and inorganic foods as a calcium supplement. In addition, it is possible that the effects seen in the femur in the experimental diet were derived from not only the bone calcium, but also from the protein hydrolyzate contained in heme iron which is effective for the acceleration of bone formation and the prevention of bone resorption [6].

Conclusions:

These findings suggest the possibility of using cattle bone calcium and heme iron as a safe and highly bioavailable source for calcium and iron.

References

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Table 1 Effect of dietary iron and calcium type on calcium deficient parameters of iron and calcium deficient mice.

	Group	ALP (U/l)	P (mg/l)	Ca (mg/l)
Exp. 1	S5	114.4±29.1	7.3±0.9	7.8±0.5
	D5	437.5±76.0**	8.5±0.8*	7.4±0.5
	D5+S1	227.1±63.9**	8.1±1.2	7.2±0.5
	D5+S5	108.9±203	8.5±0.7*	8.4±0.3*
Exp. 2	S6	111.4±42.3	7.1±0.4	8.2±0.4
	S8	94.4±20.1	7.6±1.3	8.6±0.3
	D5+E1	127.6±39.9	7.9±1.1	8.5±0.3
	D5+E3	94.8±16.0	7.6±1.7	8.6±0.5

Means±SD. Statistical significance compared with S5 (Exp.1) or S6 (Exp.2) (*:$p<0.05$, **:$p<0.01$)

Table 2 Effect of dietary iron and calcium type on iron deficient parameters of iron and calcium deficient mice.

	Group	RBC ($\times 10^6$/mm^3)	HGB (g/dl)	HCT (%)	NCV (μm^3)	MCH (pg)	MCHC (%)
Exp. 1	S5	8.5±0.3	13.4±0.4	42.1±0.6	49.7±1.3	15.9±0.2	31.9±0.5
	D5	7.8±0.7	10.4±1.3*	32.8±3.9*	42.0±1.9*	13.4±0.6*	31.8±0.6
	D5+S1	9.7±0.4	14.2±0.5	44.4±1.4	45.8±1.3	14.6±0.3	31.9±0.5
	D5+S3	9.5±0.6	14.6±0.8	45.5±3.7	47.9±1.8	15.4±0.3	32.2±1.0
Exp. 2	S6	8.1±0.3	13.5±0.5	40.7±1.6	50.5±1.5	16.8±0.5	33.2±0.4
	S8	8.6±0.2	13.6±0.3	43.0±1.0	49.8±1.7	15.7±0.5	31.6±0.1
	D5+E1	8.5±0.2	13.8±0.9	41.4±2.1	48.7±1.9	16.2±0.7	33.3±0.6
	D5+E3	9.2±0.3	13.1±0.9	41.7±3.3	45.0±3.1	14.2±0.8	31.5±1.3

Means±SD. Statistical significance compared with S5 (Exp.1) or S6 (Exp.2) (*:$p<0.05$)