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Effects of Dietary Calcium Levels, Ca/P Ratios, and Calcium Components on the Calcium Absorption Rate in Carp

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Abstract

Effects of different dietary calcium levels, Ca/P ratios, and calcium components on the calcium absorption rates in the digestive tract of carp were examined. The calcium absorption rates were obtained by using test diets contained chromic oxide. The calcium absorption rates of test diets of different calcium levels (0.09–1.24 %) with a constant phosphorus level (0.68%) increased from 21.8 to 82.8% with an increase in dietary calcium level. At both 0.38 and 0.68% dietary calcium levels, the highest absorption rate was obtained when the Ca/P ratio was adjusted to 1.0. When a dietary calcium component was changed from Ca-lactate to $CaCO_3$ or $Ca_3(PO_4)_2$ at 0.38% calcium and 0.68% phosphorus levels, the absorption rate was significantly reduced from 58.0 to 27.2 or 37.2%, respectively.

Introduction

External sources of calcium for fishes are ambient water and food. Freshwater fishes, as compared with marine fishes, are considered to have a higher ability for calcium absorption because the calcium level of their serum is maintained much at a higher level than that of freshwater whereas that of marine fishes' serum is lower than that of seawater. They seem able to absorb calcium at need from either ambient water or food dependent on its amount available.

It was reported that rearing carp on a low calcium diet did not affect their growth and bone calcium contents, when the rearing water was rich in calcium.[1] In goldfish[2] and brook trout[3], on the other hand, the dependence on food calcium increased when the water had a low calcium level. However, quantitative analyses on actual absorption of food calcium in the digestive tract of fishes have not been studied. Previous studies on the dietary calcium absorption were indirect and were performed on the basis of analyses of the whole body; the absorption rate was 8–15% in the goldfish[4] and the most effective dietary Ca/P ratio was unity in the brook trout.[3]

The purpose of this study was to establish the ability of food calcium absorption in the digestive tract in carp when they are maintained in low calcium water. We examined the effects of different levels of dietary calcium, Ca/P ratios and different sources of dietary calcium compounds on calcium absorption rates.

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Materials and Methods

Materials and rearing conditions

One-year old carp (*Cyprinus carpio*) were obtained from a commerical dealer and reared on a commerical diet for more than a month until use. Ten fish (80–150 g in body weight) were kept in a polypropylene aquarium (54 1) with filtered and recirculated dechlorinated tapwater at 25±1°C. The water had calcium and phosphorus concentrations of 5 mg/l and 0.007 mg/l respectively, and was renewed daily to keep these concentrations.

Test diets

The test diets (Table 1) contained about 1% chromic oxide as an inert reference substance. The mineral mixture was modified from Halver.[5] The composition used for the standard test diet contained 0.38% calcium as Ca-lactate and 0.68% phosphorus, or a Ca/P ratio of 0.6 (Table 2). In other test diets, the calcium and phosphorus contents were changed by substitution of minerals (Ca-lactate, KH_2PO_4, $NaH_2PO_4 \cdot 2H_2O$) and α-cellulose. Test diets with a mineral mixture in which no calcium or phosphorus were added still contained 0.09% calcium or 0.38% phosphorus derived from other ingredients. In the experiment to observe the effects of different calcium compounds on the calcium absorption rate, Ca-lactate

Table 1. *Composition of the test diets*

Casein (vitamin-free)	40 (parts in weight)
Dextrin	20
Potate starch	20
α-Cellulose	9
Soybean oil[1]	3
Cod liver oil	2
Mineral mixture	4
Vitamin mixture[2]	2
Chromic oxide	1

1) Vitamin E (40 mg/100 g diet) was added.
2) after Halver[5]

Table 2. *Mineral mixture of the standard test diet*[1]

NaCl	4.35 (g)
$MgSO_4 \cdot 7H_2O$	13.70
$NaH_2PO_4 \cdot 2H_2O$	8.72
KH_2PO_4	23.98
Ca-lactate	46.28
Fe-citrate	2.97
$AlCl_3 \cdot 6H_2O$	0.018
$ZnSO_4 \cdot 7H_2O$	0.357
$MnSO_4 \cdot 4\text{–}6H_2O$	0.080
CuCl	0.011
KI	0.017
$CoCl_2 \cdot 6H_2O$	0.105

1) modified from Halver[5]

was replaced with $CaCO_3$ or $Ca_3(PO_4)_2$. These test diets were pressed into pellets, dried and stored at −10°C. Experimental fish were fed one of the test diets for seven days at a rate of 2.4% of their body weight three times (10:00, 13:00, 16:00) a day.

Calcium absorption rate

On the seventh day of feeding, feces was collected by pressing the fish abdomen at 14:00 or 16:00 (1 or 3 hours after the last feeding). It was possible that the feces contained calcium derived from imbibed water, so that possibility was checked by adding phenol red to the aquarium water at a concentration of 0.001% as a marker. This was done just before the feeding 24 or 25 hours prior to collection of feces. Based on a preliminary observation, the diet was excreted as feces within 24 hours.

The calcium absorption rate was calculated by the formula:

Ca absorption rate=(Ca intake–Ca in feces)× 100/Ca intake (%)
where Ca intake=Ca from diet+Ca from water
where Ca from diet=Ca/Cr ratio of diet × Cr in feces
Ca from water=Ca/phenol red ratio of water × phenol red in feces

The rate thus obtained indicates the net calcium absorption rate, as a result of absorption and excretion of calcium in the digestive tract.

Chemical analyses of diets and feces were conducted on wet digested samples. Calcium in diets, feces and rearing water was determined by atomic absorption spectrometry, phosphorus in diets and feces with the method of Fiske and Subbarow[6] and phosphorus in water by the molybdenum blue method.[7] Chrome in diets and feces was analyzed by the method of Furukawa and Tsukahara[8] and the phenol red in water and feces was determined by modifying the method of Oide and Utida.[9]

Results

It is evident that, both at 1 and 3 hours after the last feeding, the greater part of calcium intake in fish fed on the standard test diet was derived from the diet and that from water was only a few per cent (Table 3). With all the other test diets, the percentages of calcium from diets were always more than 97%.

The calcium absorption rates of the standard test diet determined from feces collected at 1 and 3 hours after the last feeding were 52.8% at 1 hour and 58.0%

Table 3. *Percentages of calcium intake from the standard test diet and the ambient water at 1 and 3 hours after the last feeding*

After feeding (h)	1	3
Number of fish	8	9
Ca from diet (%)[1]	96.8±0.8	97.9±0.7
Ca from water (%)[1]	3.2±0.8	2.1±0.7

1) Mean±S.E.M.

at 3 hours (Table 4) and were not significantly different ($P<0.05$, Student's t test). Therefore, in the remaining experiments, the feces was collected 3 hours after the last feeding.

It was clear that the calcium absorption rates of test diets of different calcium contents with a constant phosphorus level increased with an increase in the dietary calcium content and, hence, of the Ca/P ratio (Table 5).

Table 4. *Calcium absorption rate of the standard test diet[1] at 1 and 3 hours after the last feeding*

Sampling time	14:00	16:00
After feeding (h)	1	3
Number of fish	8	9
Ca absorption rate (%)[2]	52.8±3.9	58.0±6.1

1) Standard test diet contained 0.38% Ca and 0.68% P (Ca/P=0.6).
2) Mean±S.E.M.

Table 5. *Calcium absorption rates of test diets of various calcium contents*

Ca in diet (%)	0.09	0.38	0.68	1.24
P in diet (%)	0.68	0.68	0.68	0.68
Ca/P ratio	0.1	0.6	1.0	1.8
Number of fish	5	9	9	3
Ca absorption rate (%)[1]	21.8±3.3	58.0±6.1	70.2±2.6	82.8±1.8

1) Mean±S.E.M. at 3 hours after the last feeding

When the Ca/P ratio was adjusted to 0.3 or 1.0 by increasing (1.13%) or decreasing (0.38%) the standard level of phosphorus without changing the level of calcium (0.38%), the calcium absorption rates were significantly different ($P<0.05$) between the 1.0 and 0.3 Ca/P ratios (Table 6). When calcium was increased from 0.38 to 0.68% in combination with the previous three phosphorus levels, the calcium absorption rates were significantly different ($P<0.01$) between the 1.0 and 0.6 Ca/P ratios (Table 6). At both calcium levels, the highest absorption rate was obtained when the Ca/P ratio was adjusted to 1.0.

The calcium absorption rate in the standard test diet containing Ca-lactate was 58.0% and the rate was significantly reduced when Ca-lactate was replaced with different calcium compounds ($CaCO_3$ or $Ca_3(PO_4)_2$) without changing the amounts of calcium and phosphorus (Table 7).

Table 6. *Calcium absorption rates of test diets of various Ca/P ratios*

Ca in diet (%)	0.38	0.38	0.38	0.68	0.68	0.68
P in diet (%)	0.38	0.68	1.13	0.38	0.68	1.13
Ca/P ratio	1.0	0.6	0.3	1.8	1.0	0.6
Number of fish	8	9	9	7	9	5
Ca absorption rate (%)[1]	71.5±3.8[2]	58.0±6.1	46.9±7.4	65.9±5.3	70.2±2.6[3]	52.7±5.4

1) Mean±S.E.M.
2) Significantly different from the rate in 0.38% Ca and 1.13% P ($P<0.05$)
3) Significantly different from the rate in 0.68% Ca and 1.13% P ($P<0.01$)

Table 7. *Calcium absorption rates of test diets containing different calcium compounds as calcium components*[1]

Ca compound	$CaCO_3$	$Ca_3(PO_4)_2$	Ca-lactate
Number of fish	7	8	9
Ca absorption rate (%)[2]	27.2±3.1[3]	37.2±4.6[4]	58.0±6.1

1) Each test diet contained 0.38% Ca and 0.68% P (Ca/P=0.6).
2) Mean±S.E.M.
3) Significantly different from Ca-lactate ($P<0.05$)
4) Significantly different from Ca-lactate ($P<0.01$)

Discussion

The result that most of the calcium intake was derived from the diet supports that freshwater fish drink little water.[9] The calcium absorption rates calculated herein represent approximate rates of dietary calcium absorption by carp.

The calcium absorption rates in the intestines of rats (120–180 g in body weight) are reported in a range of 60–70%.[10] The rates obtained in the present study overlap this range and suggest that the digestive tract of carp is comparable to that of rat in the ability to absorb dietary calcium. Goldfish and brook trout absorb more calcium from food in low calcium water (5 mg/l) than in high calcium water (22 and 50 mg/l, respectively).[2,3] If carp are similar to them, the absorption rates in low calcium water (5 mg/l) obtained in this study would indicate higher rates than that in high calcium water. Tomiyama[4] reported a calcium absorption rate of 8–15% in goldfish, but that cannot be compared directly with the present results. He calculated the rate on the basis of the levels of accumulated calcium in the fish body except viscera when fish were reared in high calcium water (76.8 mg/l).

The results in Table 5 show that at a fixed phosphorus level the quantity of absorbed calcium increased directly at higher dietary calcium levels in the present range. Phillips[3] found that dietary Ca/P ratio of unity was most effective for utilization of the minerals in brook trout. On the other hand, Podoliak and Holden[11] observed that the accumulation of dietary calcium in the body of rainbow trout was higher in a diet containing no phosphorus than in a diet whose Ca/P ratio was 1. The present results agree with Phillips at the 0.38 and 0.68% calcium levels, and suggest the importance of the level of phosphorus for the absorption of dietary calcium.

One of the reasons for low absorption rates of $CaCO_3$ and $Ca_3(PO_4)_2$ may be low solubilities of these compounds. The solubility of Ca-lactate is 10.5 whereas those of $CaCO_3$ and $Ca_3(PO_4)_2$ are 0.0014 and 0.0025, respectively. Because the pH of the fluid in the digestive tract of carp, a stomachless fish, is about 7,[12] the solubilities of these compounds are not enhanced. It is possible that calcium in fish meal, which mainly derived from bone minerals, may not be absorbed efficiently from that diet by carp.

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