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https://doi.org/10.5109/23689

出版情報：九州大学大学院農学研究院紀要. 23 (3/4), pp.177-184, 1979-03. Kyushu University
バージョン：
権利関係：

J. Fac. Agr., Kyushu Univ., 23, 177-184 (1979)

Availabilities of Phosphorus Compounds as Dietary Phosphorus Sources for Red Sea Bream*

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(Received December 9, 1978)

Three experiments were conducted to compare the availabilities of eight phosphorus compounds as a phosphorus sources in the diet for red sea bream, *Chrysophrys major*. Phosphorus contents of the test diets were adjusted to 680 mg per 100 g of diet in each experiment. The fish were reared on the test diets at 25°C over a 77 day period (Exp. I), 62 day period (Exp. II), and an 88 day period (Exp. III). At the end of the feeding trial, ten fish from each group were selected for the chemical analyses of the blood serum, liver, and vertebrae. Sodium phosphates (mono-, di-, and tribasic), potassium phosphate monobasic, and calcium phosphate monobasic were more effective than calcium phosphates (di- and tribasic) to prevent the development of phosphorus deficiency symptoms. Calcium phytate was scarcely utilized as the dietary phosphorus source by red sea bream. Therefore, the water soluble phosphorus compounds, from which inorganic phosphorus is easily released, should be employed as the dietary phosphorus source for red sea bream.

INTRODUCTION

When sodium phosphate monobasic was used as the dietary phosphorus source, red sea bream fed the diet containing phosphorus at less than 680mg per 100 g level exhibited various phosphorus deficiency symptoms (Sakamoto and Yone, 1973; Sakamoto and Yone, 1978). On the other hand, in the diet of rainbow trout, *Salmo gairdneri*, (Ogino and Takeda, 1974; Takeda and Ogino, 1975) and carp, *Cyprinus carpio*, (Takeda and Ogino, 1975; Shitanda and Ukita, 1979), it has been reported that the physicochemical form of phosphorus compounds affects phosphorus absorption. Therefore, in this study, three experiments were conducted to compare the availabilities of eight phosphorus compounds in the diet for red sea bream.

MATERIALS AND METHODS

The dietary phosphorus sources used were: sodium phosphate monobasic, potassium phosphate monobasic, and calcium phosphate monobasic in experi-

* Contribution from Fish. Res. Lab., Kyushu Univ., No. 135. This work was orally presented to the Annual Meeting of Japan. Soc. Sci. Fish., at Tokyo in April, 1978.

ment I; sodium phosphate mono-, di-, and tribasic in experiment II; and calcium phosphate mono-, di-, and tribasic and calcium phytate (phytin) in experiment III. These phosphorus compounds were supplemented to test diets

Table 1. Composition of test diet.

Casein	52.0	L-Lys•HCl	0.6
Gelatin	11.0	L-Val	0.7
L-Phe	0.6	Gly	0:4
L-Arg•HCl	1.3	P. L. Oil[1]	9.0
L-cys	0.7	Dextrin	8.0
L-Try	0:2	Minerals[2]	8.0
L-His•HCl•H_2O	0.2	Vitamins[3]	3.0
DL-Ala	1.3	n-Cellulose	2:o
L-Asp•Na	1.0		

1) Alaska pollack liver oil purified by molecular distilation. 2) Shown in Table 2.
3) Halver's vitamin mixture (1957) +a-cellulose.

Table 2. Composition of mineral mixtures.

Experiment	I			
Phosphorus source	Without	1Na	1K	1Ca
NaH_2PO_4•$2H_2O$		30.805		—
KH_2PO_4			26.873	
$Ca(H_2PO_4)_2$•H_2O				24.886
Fe-citrate	1.485	1.485	1.485	1.485
α-Cellulose	99.103	68.298	72.230	74.217
P (mg/8g mineral mix.)		486	486	486
P (mg/100g diet)	194-	680	680	680

Experiment	II		
Phosphorus source	1Na	2Na	3Na
NaH_2PO_4•$2H_2O$	30.805	—	
Na_2HPO_4•$12H_2O$		70.718	
Na_3PO_4•$12H_2O$			75.058
Fe-citrate	1.485	1.485	1.485
α-Cellulose	68.298	28.386	24.046
P (mg/8g mineral mix.)	486	486	486
P (mg/100g diet)	680	680	680

Experiment	III				
Phosphorus source	Without	1Ca	2Ca	3Ca	Phytin
$Ca(H_2PO_4)_2$•H_2O		24.886		—	—
$CaHPO_4$•$2H_2O$			33.951	—	
$Ca_3(PO_4)_2$				30.625	
Ca-phytate					29.238
Fe-citrate	1.485	1.485	1.485	1.485	1.485
α-Cellulose	99.103	74.217	65.152	68.478	69.865
P (mg/8g mineral mix.)		486	486	486	486
P (mg/100g diet)	194-	680	680	680	680

at a 680 mg P per 100 g diet level. The composition of test diets and mineral mixtures are listed in Table 1 and Table 2, respectively. The fish were reared on the test diet at 25°C over a 77 day period (Exp. I), 66 day period (Exp. II), and an 88 day period (Exp. III). The methods of fish selection, grouping, care, preparation of diets, and feeding were the same as those reported previously (Yone *et al., 1974).* At the end of the feeding trial, ten fish from each group were selected. A blood sample was taken from each fish by a cardiac puncture for the chemical analyses of serum. Calcium and inorganic phosphorus content of the blood serum was quantified by orthocresolphthalein complexone (Conerty and Briggs, 1966) and molybdenum blue method (Tausky and Shorr, 1953), respectively. The blood serum levels of glucose, total protein, total bilirubin, total cholesterol, and urea-N (BUN), and the activities of glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), lactic dehydrogenase (LDH) , leucine aminopeptidase (LAP), and alkaline phosphatase (ALP) in the blood serum were determined with Rapid Blood Analyzer 3010 and Unikit (Chugai Pharmaceutical Co.). After the blood sampling, the fork length and the weight of entire body and liver were measured. From these values, the condition factor and the percentage of liver to body weight (hepatosomatic index) were calculated. The analytical samples of the liver and vertebrae were taken from each fish in the same amount, and were mixed thoroughly in each group. Moisture contents of the liver and vertebrae were determined by a 24 hour drying in an electric oven at 105°C. Lipid content of the liver was quantified by extraction with ethyl ether for 16 hours, and lipid in the vertebrae was extracted with methyl alcohol for 24 hours followed by a 16 hour extraction with ethyl ether. Glycogen content of the liver was determined by the method of Carroll *et al. (1956).* The phosphorus content of liver ***was*** analysed by molybdenum blue method (Tausky and Shorr, 1953) after wet ashing with nitric acid and hydrogen peroxide. The calcium and phosphorus content of vertebrae were quantified by orthocresolphthalein complexone (Conerty and Briggs. 1966) and molybdenum blue method (Tausky and Shorr, 1953), respectively, after dry ashing with an electric furnace at 450°C for 48 hours.

RESULTS AND DISCUSSION

Experiment I

No significant differences were recognized among the groups fed the diets with sodium-, potassium-, and calcium phosphate monobasic in the following determinations: the growth rate, feed efficiency, condition factor, and hepatosomatic index (Table 3) ; the blood serum levels of calcium, inorganic phosphorus, glucose, total protein, total bilirubin, total cholesterol, and BUN (Table 4); the activities of GOT, GPT, LDH, LAP, and ALP in the blood serum (Table 4) ; the moisture, lipid, glycogen, and phosphorus content of liver (Table 5) ; and the lipid, ash, calcium, and phosphorus content of vertebrae (Table 5). From these findings, it appears that sodium phosphate monobasic,

Table 3. Effect of dietary phosphorus compounds on the growth rate, feed efficiency, condition factor, and hepatosomatic index of red sea bream in experiment I.

Phosphorus source	Without	1Na	1K	1Ca
No. of fish				
at start	20	20	20	20
after 77 days	19	20	20	20
Average body weight (g) at after start 77 days	49.6±94. If13.8 4.5	109.3±13.649.8±5.1	107.4 49.6±±14.0 4.8	110.2±16.149.5±5.2
"t" test (5%)	S[1] 54		NS[2]	NS
Feed efficiency (%)	2.35±0.13	71	71	72
Condition factor[3]		2.44±0.05	2.42±0.07	2.46±0.10
"t" test (5%)	NS		NS	NS
Hepatosomatic index (%)[4]	1.68±0.35	1.72±0.39	1.63±0.33	1.54±0.32
"t" test (5%)	NS		NS	NS

[1] Significant. [2] Non-significant. [3] Body weight (g) x 100/{Folk length (cm)}^{3}.
[4] Liver weight (g) x 100/Body weight (g).

Table 4. Effect of dietary phosphorus compounds on the levels of chemical components and the activities of enzymes in blood serum of red sea bream in experiment I.

Phosphorus source	Without	1Na	1K	1Ca
Ca (mg/dl)	15.0	12.9	12.9	12.7
Inorganic P (mg/dl)	6.1	8.9	8.9	8.7
Ca/P ratio	2.5	1.4	1.4	1.5
Glucose (mg/dl)	79	65	65	67
Total protein (g/dl)	4.1	4.0	4.0	4.0
Total bilirubin (mg/dl)	1.2	0.7	0.9	0.7
Total cholesterol (mg/dl)	255	236'	231	210'
BUN (mg/dl)	12.5	10.5	11.7	13.5
GOT (K. U.)[1]	122	154	148	187
GPT (K.U.)[1]	65	62	67	95
GOT/GPT ratio	1.9	-2.5	2.2	2.0
LDH (W. U.)[2]	1635	1530	1527	1515
LAP (G.-R. U.)[3]	630	765	795	895
ALP (K.-A. U.)[4]	5.9	3.5	3.4	3.4

[1] Karmen Unit. [2] Wroblewski Unit. [3] Goldberg-Ruteinburg Unit.
[4] King-Armstrong Unit.

potassium phosphate monobasic, and calcium phosphate monobasic are equally available as a dietary phosphorus source for red sea bream.

Experiment II

In the group fed the diet supplemented with sodium phosphate dibasic, the feed efficiency, condition factor, and hepatosomatic index were lower than those of sodium phosphate monobasic and tribasic diet groups (Table 6), whereas the glucose content and activities of GOT, GPT, and LDH in the blood serum were higher (Table 7). However, the dibasic group was similar to the monobasic and tribasic groups in the following determinations: the blood se-

Table 5. Effect of dietary phosphorus compounds on the chemical components in liver and vertebrae of red sea bream in experiment I.

Phosphorus source	Without	1Na	1K	1Ca
Liver				
Moisture (%)	64. 5	67. 4	67. 3	68.2
Lipid (% db)[1)]	43. 1	30. 3	31.3	34.6
Glycogen (% db)	9.1	16.0	14. 7	12.6
P (mg/100g db)	589	651	639	640
Vertebrae				
Lipid (% db)	24.4	23.3	22.1	21.8
Ash (% db)	47. 5	50. 7	50. 4	50.1
Ca (mg/g ash)	347	354	353	352
P (mg/g ash)	172	177	176	175
Ca/P ratio	2. 0	2. 0	2.0	2.0

[1)] % on dry weight basis.

Table 6. Effect of dietary phosphorus compounds on the growth rate, feed efficiency, condition factor, and hepatosomatic index of red sea bream in experiment II.

Phosphorus source	1Na	2Na	3Na
No. of fish			
at start	20	20	20
after 62 days	20	19	20
Average body weight (g)			
at start	10. 3± 1.2	10. 3± 1.3	10. 3±1. 3
after 62 days	33. 4± 7.7	31. 3± 6.2	33. 9±6. 2
"t" test (5%)		NS	NS
Feed efficiency (%)	93	a5	86
Condition factor	2. 28±0. 11	2. 19±0. 10	2. 27±0. 10
"t" test (5%)	—	NS	NS
Hepatosomatic index (%)	2. 28±0. 52	1. 84±0. 51	2. 55±0. 44
"t" test (5%)	—	NS	NS

Table 7. Effect of dietary phosphorus compounds on the levels of chemical components and the activities of enzymes in blood serum of red sea bream in experiment II.

Phosphorus source	1Na	2Na	3Na
Ca (mg/dl)	11. 0	**11.2**	11.3
Inorganic P (mg/dl)	7.7	7.8	7.0
Ca/P ratio	1.4	1.4	1. 6
Glucose (mg/dl)	72	93. 6	75
Total protein (g/dl)	3. 5	0. 5	3.9
Total bilirubin (mg/dl)	230. 4		0. 4
Total cholesterol (mg/dl)		221'	261
BUN (mg /dl)	9. a	11.1	10.9
GOT (K.. U.)	55 14	la7 62	57 19
GPT (K.-U.)	3.9	3.0	3. 0
GOT /GPT ratio	1030	>2630	1590
LDH (W. U.)	721	737	711
LAP (G.-K. U.)	4.6	5.1	4.6
ALP (K.-A. U.)			

Table 8. Effect of dietary phosphorus compounds on the chemical components in liver and vertebrae of red sea bream in experiment II.

Phosphorus source	1Na	2Na	3Na
Liver			
Moisture (%)	65.3	64.5	64.2
Lipid (% db)	33.4	36.7	35.0
Glycogen (% db)	17.3	17.4	19.3
Vertebrae			
Lipid (% db)	22.6	22.1	22.8
Ash (% db)	51.2	51.3	51.3
Ca (mg/g ash)	354	355	354
P (mg/g ash)	188	188	187
Ca/P ratio	1.9	1.9	1.9

rum levels of calcium and inorganic phosphorus; the ALP activity in the blood serum; the moisture, lipid, and glycogen content of the liver; and the lipid and ash content of the vertebrae (Tables 7 and 8). Therefore, it can be considered that the fish fed the diet supplemented with sodium phosphate dibasic were not in the phosphorus deficiency state. The test diet supplemented with sodium phosphate tribasic could not be cut into cubes with a suitable size owing to its adhesiveness, so this diet was crushed in the sea water and given to fish. **A** part of this crushed diet was not consumed. Therefore, the feed efficiency of tribasic group was inferior to that of monobasic group, but other values were similar (Table 6). From these findings, it can be presumed that these three sodium phosphates (mono-, di-, and tribasic) are equally available as a dietary phosphorus source for red sea bream.

Experiment III

As shown in Tables 9, 10, and 11, the phytin group exhibited lower values than the calcium phosphate monobasic group in the following parameters: the growth rate and feed efficiency; the blood serum level of inorganic phosphorus ; the moisture, glycogen, and phosphorus content of liver; and the ash con-

Table 9. Effect of dietary phosphorus compounds on the growth rate, feed efficiency, condition factor, and hepatosomatic index of red sea bream in experiment III.

Phosphorus source	Without	1Ca	2Ca	3Ca	Phytin
No. of fish					
at start	15	15	15	15	15
after 88 days	14	15	15	15	14
Average body weight (g) at after start 88 days	152.4±19.8	82.7±6.1	172.1±21.8	82.5±6.3	170.1±23.4 82.6±5.8	169.3±35.0 82.5±5.6	157.4rt31.7	82.6i6.2
"t" test (5%)	S		NS	NS	NS
Feed efficiency (%)	57	70	70	72	62
Condition factor	2.38±0.06	2.45±0.08	2.49±0.07	2.56±0.14	2.44±0.15
"t" test (5%)	S	—	NS	S	NS
Hepatosomatic index (%)	1.64t0.69	1.62±0.22	1.68t0.32	1.72±0.46	1.70i0.22
"t" test (5%)	NS		NS	NS	NS

Table 10. Effect of dietary phosphorus compounds on the levels of chemical components and the activities of enzymes in blood serum of red sea bream in experiment III.

Phosphorus source	Without	1Ca	2Ca	3Ca	Phytin
Ca (mg/dl)	14.6	13.9	14.3	15.3	15.4
P (mg/dl)	5.8				6.4
Ca/P ratio	2.5	9.5	8.6	8.9	2.4
Glucose (mg/dl)	76'	74.0	77	79	74'
Total protein (g/dl)	4.2	0.9	4.3	4.a	4.6
Total bilirubin (mg/dl)	1.0		1.0	1.0	1.3
Total cholesterol (mg/dl)	240	189'	191	229	236
BUN (mg/dl)	14.6	9.9	10.3	11.9	a.7
GOT (K. U.)	80	79	76	80	76
GPT (K. U.)	42	37	36	40	37
GOT/GPT ratio	1.9	2.1	2.1	2.0	2.0
LDH (W. U.)	905	1140	1099	1120	1380
LAP (G.-R. U.)	538	679	661	646	712
ALP (K. -A. U.)	4.0	2.6	3.3	3.6	4.2

Table 11. Effect of dietary phosphorus compounds *on* the chemical components in liver and vertebrae of red sea bream in experiment III.

Phosphorus source	Without	1Ca	2Ca	3Ca	Phytin
Liver					
Moisture (%)	54.9	68.6	63.7	54.4	54.6
Lipid (% db)	62.8	29.9	41.6	62.2	60.1
Glycogen (% db)	5.1	14.4	10.4	6.1	a.5
P (mg/100g db)	346	700	608	429	375
Vertebrae					
Lipid (% db)	28.9	24.3	25.0	25.6	26.7
Ash (% db)	43.4	50.5	49.0	48.1	45.1
Ca (mg/g ash)	364	354	354	351	355
P (mg/g ash)	170	172	172	171	171
Ca/P ratio	2.1	2.1	2.1	2.1	2.1

tent of vertebrae. However, the Ca/P ratio and ALP activity in the blood serum and the lipid contents of liver and vertebrae were higher. These values for the phytin group were similar to those of the group fed the diet without phosphorus supplementation. It appears that phosphorus in calcium phytate is scarcely utilized by red sea bream. In the above determinations, the calcium phosphate di- and tribasic groups showed different values from the monobasic group, but the growth rates and feed efficiencies were similar. From these findings, it is reasonable to presume that phosphorus in calcium phosphates (di- and tribasic) is considerably utilized, but its utilization rate, especially in tribasic form, is inferior to that in calcium phosphate monobasic.

In rainbow trout (Ogino and Takeda, 1974; Takeda and Ogino, 1975) and carp (Takeda and Ogino, 1975; Shitanda and Ukita, 1979), it was proven that the absorption rate of phosphorus from soluble phosphorus compounds were higher than that from insoluble compounds and phytin. In this study, soluble phosphorus compounds (sodium phosphate mono-, di-, and tribasic, potassium phosphate monobasic, and calcium phosphate monobasic) were more effective

than insoluble compounds (calcium phosphate di- and tribasic) and phytin in preventing the development of phosphorus deficiency symptoms. Perhaps, the absorption rates of phosphorus from insoluble compounds are lower than those from soluble compounds in red sea bream also. The poor availability of phytin as the dietary phosphorus source may be due to the inability of inorganic phosphorus to be released. Therefore, the water soluble phosphorus compounds, from which inorganic phosphorus is easily released, should be employed as the dietary phosphorus source for red sea bream.

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