*Iran Agricultural Research 22: 138-152 (2003)*

# NOTE

# EFFECT OF SUPPLEMENTAL CITRIC ACID AND PHYTASE ON GROWTH PERFORMANCE AND BODY COMPOSITION OF COMMON CARP *(CYPRINUS CARPIO)*

**F. SHIRMOHAMMAD, J. POURREZA AND N. MAHBOOBI[1]**

Department of Animal Science, College of Agriculture, Isfahan University of Technology, Isfahan, I.R. Iran.

(Received: July 12, 2003)

## ABSTRACT

An experiment was conducted to investigate the effect of supplemental citric acid and microbial phytase on the growth performance and body composition of common carp (*Cyprinus carpio)*. In a completely randomized design with 3×3 factorial arrangement, the effects of three levels (0, 1.5 and 3%) of citric acid and three levels (0, 500 and 1000 FYT $kg^{-1}$ diet) of microbial phytase were tested. Two hundred and sixteen fish, averaging 207 g, were divided into 27 pens, 8 fish per pen. Nine experimental diets were given to three replicates of 8 fish for a period of 8 weeks (plus two weeks for adaptation). Supplemental citric acid had no significant effect on plasma cholesterol, triglyceride, phosphorus, liver fat, carcass dry matter and protein, feed conversion ratio, protein efficiency ratio, carcass index, specific growth rate and apparent digestibility of phosphorus. Addition of 3% citric acid significantly ($P<0.05$) increased body ash (3.13%) and phosphorus (0.41%). Body fat was reduced significantly ($P<0.05$) due to supplemental citric acid, although, there were no further significant effects associated with the amount of citric acid given. Feed conversion ratio (2.25, FCR), protein efficiency ratio (1.29), specific growth rate (0.77), percent age of body weight growth (54.3%) and phosphorus digestibility (53.7%) were improved significantly ($P<0.05$) due to supplemental phytase. Phosphorus digestibility was not significantly affected by the amount of phytase fed. The highest body ash and P, protein

---

1. Former Ph.D. Student, Professor and Associate Professor, respectively.

efficiency ratio and specific growth rate were obtained numerically with 1000 FYT $kg^{-1}$ and 3% citric acid in the diet (3.4, 0.44, 1.3 and 0.8%, respectively). The lowest percent body fat and FCR, 6.1% and 2.21, respectively, were also observed with this diet. These results indicated that addition of 3% citric acid to the diet of common carp improved carcass composition and provided better digestive tract conditions for microbial phytase without any positive effect on growth.

**Key words**: Body composition, Citric acid, Common carp, Phytase, Phytate.

**تحقیقات کشاورزی ایران**

۲۲: ۱۳۸-۱۵۲(۱۳۸۲)

# اثر مکمل سیتریک اسید و فیتاز بر رشد و ترکیب بدن کپور ماهی

**فاطمه شیرمحمد، جواد پوررضا و نصراله محبوبی صوفیانی**

به ترتیب، دانشجوی دکتری، استاد و دانشیار گروه علوم دامی دانشکده کشاورزی و منابع طبیعی دانشگاه صنعتی اصفهان، اصفهان، جمهوری اسلامی ایران.

## چکیده

آزمایشی به منظور بررسی اثر مکمل سیتریک اسید و فیتازِ میکروبی بر رشد و ترکیب بدن کپور ماهی انجام شد. در این آزمایش اثر سه سطح سیتریک اسید (صفر، ۱/۵ و ۳ درصد) و سه سطح فیتاز میکروبی (صفر، ۵۰۰ و ۱۰۰۰ واحد در کیلوگرم جیره) در طرح پایه کاملاً تصادفی در قالب آزمایش فاکتوریل بررسی شد. تعداد ۲۱۶ ماهی با میانگین وزن ۲۰۷ گرم در ۲۷ قفس و در هر قفس ۸ ماهی قرار داده شد. هر کدام از ۹ جیره آزمایشی به سه تکرار ۸ ماهی برای مدت ۱۰ هفته (دو هفته دوره سازگاری در نظر گرفته شد) داده شد. مکمل سیتریک اسید تاثیری بر کلسترول پلاسما،

تری گلیسرید، فسفر، چربی کبد، ماده خشک و پروتئین لاشه، ضریب تبدیل غذایی، نسبت بازده پروتئین، وزن لاشه، سرعت رشد روزانه و قابلیت هضم ظاهری فسفر نداشت. افزودن ۳ درصد سیتریک اسید به طور معنی داری ($P<0.05$) خاکستر بدن (۳/۱۱ درصد ) و فسفر بدن (۰/۴۱ درصد) را افزایش داد. چربی بدن به طور معنی داری با به کار گیری مکمل سیتریک اسید کاهش یافت ($P<0.05$) اما تفاوت بین سطوح سیتریک اسید بر این پارامترها معنی دار نبود. ضریب تبدیل غذایی (۲/۲۵) ضریب بازده پروتئین (۱/۲۹) سرعت رشد روزانه (۰/۷۷)، درصد اضافه وزن بدن (۵۴/۳ درصد) و قابلیت هضم فسفر (۵۳/۷ درصد) به طور معنی داری با مکمل فیتاز، بهبود یافت. قابلیت هضم فسفر به طور معنی داری، تحت تاثیر مکمل فیتاز قرار نگرفت. بالاترین میزان خاکستر و فسفر بدن، نسبت بازده پروتئین و سرعت رشد روزانه با سطوح ۱۰۰۰ واحد فیتاز و ۳ درصد سیتریک اسید در جیره به دست آمد (به ترتیب، ۳/۴، ۰/۴۴، ۱/۳ و ۰/۸ درصد). پایین ترین درصد چربی بدن و ضریب تبدیل غذایی با همین سطوح فیتاز و سیتریک اسید به دست آمد (به ترتیب، ۶/۱ و ۲/۲۱ درصد). این نتایج نشان داد که اضافه کردن ۳ درصد سیتریک اسید به جیره کپور ماهی شرایط بهتری را برای فعالیت فیتاز میکروبی در دستگاه گوارش فراهم کرد اما اثر مثبتی بر رشد نداشت.

## INTRODUCTION

Common carp (*Cyprinus carpio)* is one of the most popular warm water fish cultured in many parts of Iran. The flesh quality of common carp is usually dependent on several factors including age, growth rate and diet composition (10). Deficiencies of methionine and phosphorus also increase fat deposition (19). Body fat content influences the palatability of fish (10). Supplemental phosphorus (P) increases the body protein content and reduces fat, indicating a role for P in fat metabolism and protein deposition (8).

Fish meal, plant by-products and mineral supplements are three major P sources in diets (13). The P in fish meal is poorly utilized by carp due to absence of an acidic stomach (5). Being an agastric animal, carp does not utilize P which is mainly bound to phytic acid in plant protein sources (21)

and the availability of phytate-P is about 40% for carp (8). However, it has been reported that the addition of citric acid (CA) to carp diets increases the digestibility of minerals by reducing digestive tract pH (17). Addition of phytase to diets can release inorganic P from phytate, hence improving P availability and thereby decreasing fecal P (22). Microbial phytase is most active at pH 2.5 and 5.5 (22). Supplementation of diets with organic acid can result in improved feed efficiency and daily weight gain (14). Hence, the effectiveness of microbial phytase may be increased if used together with organic acid in the diet of farm animals.

The objective of the present study was to evaluate the efficiency of CA on phytase activity regarding phytate P utilization, fish performance, body composition and some blood parameters.

## MATERIALS AND METHODS

The experiment was conducted at the Karaskan Fisheries Research Center in Isfahan Province, Iran. Two hundred and sixteen common carps (*Cyprinus carpio*) weighing 207±24 were divided into 27 groups, 8 fish per group. Triplicate groups were allotted to each pen of 1 $m^3$, a slow water supply to meet the fish $O_2$ requirements and water evaporation. Each experimental diet was randomly assigned to triplicate groups which were fed by hand twice a day at a level of 20 (g $kg^{-1}$) × $W^{0.8}$ (18). During the experimental period, water temperature and dissolved oxygen level were 19-20°C and 6.5-9.2 mg $l^{-1}$, respectively. The experimental period lasted for 8 weeks, plus two weeks for adaptation.

Nine isocaloric and isonitrogenous diets were formulated to meet the nutritional requirements (12) using three levels of CA (0, 1.5 and 3%) and three levels of phytase (Ronozyme™ P) (0, 500 and 1000 FYT $kg^{-1}$ diet). Ingredients of the test diets are shown in Table 1. The test diets were prepared as cold-extruded moist pellets (5 mm diameter). Two weeks before the end of the experiment, chromic oxide ($Cr_2O_3$) was added to all test diets as an inert marker at a concentration of 0.2% for determination of phosphorus digestibility.

The fish were not fed on the day after the experiment. Five fish were randomly selected from each pen and anesthetized by MS-222, immediately

upon capture. Blood samples (2 ml) were drawn into syringes from the caudal blood vessels of each fish and pooled. Fecal samples were collected from the end part of the small intestine of the dissected fish and pooled. Liver samples were also collected from five fish per pen and pooled. Five gutted fish from each replicate were completely minced, mixed and pooled. All samples were stored frozen at -17 °C .

Proximate chemical composition of whole body was determined by the methods of AOAC (1). Plasma cholesterol and triglyceride concentrations were determined using commercial kits (Ziest Chem Diagnostics) by the methods of Roeschlau *et al.* (15) and Fossati and Prencipe (7), respectively. Plasma P was measured using the method of Tietz (25). The dietary and fecal chromic oxide contents were determined spectrophotometrically at 440 nm (6).

Table 1. Formulation and composition of basal diets (%).

| Ingredients | C0P0 | C1.5P0 | C3P0 |
|---|---|---|---|
| Fish meal (Kilka) | 15.00 | 15.00 | 15.00 |
| Soybean meal (mechanically extracted) | 53.91 | 54.48 | 55.04 |
| Wheat flour | 27.15 | 24.65 | 22.10 |
| Sunflower oil | 1.89 | 2.35 | 2.81 |
| Mineral Premix† | 0.30 | 0.30 | 0.30 |
| Vitamin Premix§ | 0.30 | 0.30 | 0.30 |
| DL-Methionine (98%) | 0.41 | 0.41 | 0.41 |
| Molasses | 1.00 | 1.00 | 1.00 |
| Vitamin E (acetate) | 0.02 | 0.02 | 0.02 |
| Vitamin C (ascorbic acid) | 0.02 | 0.02 | 0.02 |
| Citric acid¶ | | 1.50 | 3.00 |

All diets were isocaloric, isonitrogenous and isophosphorus (DE, 12.12 MJ kg $^{-1}$, CP, 35%, and total phosphorus, 0.73%).

† Amount supplied (mg kg$^{-1}$ of diet): Mn, 1200; Fe, 60; Zn, 120; Cu, 12; I, 1.2; Se, 0.24.

§ Vitamin mixture supplied the following (mg or IU kg$^{-1}$ of diet): Vit. A, 10800 IU; $D_3$, 2400 IU; E, 21.6 IU; $K_3$, 2.4 IU; Thiamin, 2.16; Riboflavin, 7.9; Niacin, 12; Pyridoxin, 3.6; Folic acid, 1.2; $B_{12}$, 15; Biotin, 0.12; choline chloride, 600; and adequate antioxidant.

¶ 0627: Mallinckrodt Chemical works, St. Louis, MO.

1 Two levels of phytase (P, Hoffmann. La Roche Inc.) (500 and 1000 FYT $kg^{-1}$) were added to each diet (one phytase unit is the activity of phytase that generates 1 micromole of inorganic phosphorus per minute from an excess of sodium phytate at pH 5.5 and 37°C). The experimental diets were: 1) 0% citric acid+0 phytase units (C0P0), 2) 0% citric acid + 500 phytase units (C0P500), 3) 0% citric acid + 1000 phytase units (C0P1000 ), 4) 1.5% citric acid+0 phytase units (C1.5P0), 5) 1.5% citric acid+500 phytase units (C1.5P500), 6) 1.5% citric acid+1000 phytase units (C1.5P1000), 7) 3% citric acid+0 phytase units (C3P0), 8) 3% citric acid+ 500 phytase units (C3P500), 9) 3% citric acid+1000 phytase units (C3P1000 ).

The feeding trial was analyzed according to a completely randomized design in a 3 ×3 factorial arrangement, with three levels of CA and phytase supplements. Two-way ANOVA was used to test the effects of CA and phytase supplements (16). Duncan's (4) multiple range test was used to compare means when a significant F was found. Linear regression was determined between some significant parameters to investigate any interrelationships. Final body weight was included as a covariate in the statistical model. Treatment effects were considered significant at $P<0.05$.

## RESULTS

### Growth Parameters

Final weight and percentage of body weight gain (BWG) were increased significantly ($P< 0.05$) due to phytase supplement but not for CA. Interaction between CA and phytase on BWG percentage was not significant (Table 2). Addition of phytase at level of 1000 FYT $kg^{-1}$ to the basal diet resulted in significant ($P<0.05$) increases in specific growth rate (SGR) (Table 2). The difference between the diet P500 and P1000 was also significant ($P<0.05$), while there was no significant difference between the basal and P500 diets (Table 2). Interaction between phytase and CA on SGR was not significant.

Supplemental phytase but not CA (Table 3) improved FCR and PER ($P<0.05$). The diet P1000 showed a better FCR and PER than control (P0) and P500 diets. The regression equations were:

FCR=2.91- 0.0023X (phytase), $R^2=0.48$; $P<0.05$

PER=0.98+0.001X (phytase), $R^2=0.48$; $P<0.05$

Table 2. The effects of supplemental phytase and citric acid on growth of common carp.

| | Final Weight (g) | | | | Weight Gain (%)† | | | | SGR (%)§ | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Phytase (FYT $kg^{-1}$) | | 0 | 500 | 1000 | | 0 | 500 | 1000 | | 0 | 500 | 1000 |
| | Overall | 300.1b | 298.3b | 326.6a | Overall | 44.1b | 44.6b | 54.3a | Overall | 0.65b | 0.67b | 0.77a |
| Citric Acid (%) | | | | | | | | | | | | |
| 0 | 313.1 | 298.4 | 298.7 | 320.6 | 46.7 | 43.0 | 43.7 | 53.3 | 0.68 | 0.64 | 0.65 | 0.76 |
| 1.5 | 308.9 | 303.8 | 300.3 | 319.1 | 47.8 | 45.8 | 44.4 | 53.2 | 0.69 | 0.67 | 0.66 | 0.76 |
| 3 | 302.9 | 298.0 | 309.7 | 326.1 | 48.5 | 43.3 | 45.9 | 56.3 | 0.72 | 0.64 | 0.71 | 0.80 |
| Model | P>F | | | | P>F | | | | P>F | | | |
| Phytase (P) | 0.0001 | | | | 0.0007 | | | | 0.0008 | | | |
| Citric Acid (CA) | 0.18 | | | | 0.752 | | | | 0.470 | | | |
| P×CA | 0.063 | | | | 0.918 | | | | 0.767 | | | |
| SEM | 2.145 | | | | .997 | | | | 0.011 | | | |

abcd Means followed by similar letter (s) are no. significantly different (DMRT, P>0.05).

† Weight gain (%) =100× [(final BW-initial BW) / initial BW].

§ Specific growth rate = 100× [$L_n$ final BW- $L_n$ intial BW]/duration.

Table 3. The effects of supplemental phytase and citric acid on performance criteria of common carp.

| | FCRT (g:g) | | | | PER[§] (g:g) | | | | APD (%)[¶] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Phytase (FYT $kg^{-1}$) | | 0 | 500 | 1000 | | 0 | 500 | 1000 | | 0 | 500 | 1000 |
| | Overall | 2.8a | 2.8a | 2.2b | Overall | 1.03b | 1.02b | 1.3a | Overall | 40.8b | 49.4a | 53.7a |
| Citric Acid (%) | | | | | | | | | | | | |
| 0 | 2.6 | 2.8 | 2.8 | 2.2 | 1.1 | 1.03 | 1.01 | 1.3 | 48.5 | 42.6 | 48.9 | 54.1 |
| 1.5 | 2.6 | 2.6 | 2.8 | 2.3 | 1.1 | 1.09 | 1.02 | 1.24 | 48.9 | 38.3 | 53.7 | 54.6 |
| 3 | 2.7 | 3.0 | 2.9 | 2.2 | 1.1 | .96 | 1.04 | 1.32 | 46.4 | 41.4 | 45.5 | 52.3 |
| Model | P>F | | | | P>F | | | | P>F | | | |
| Phytase (P) | 0.002 | | | | 0.001 | | | | 0.002 | | | |
| Citric acid (CA) | 0.808 | | | | 0.988 | | | | 0.692 | | | |
| P×CA | 0.824 | | | | 0.764 | | | | 0.641 | | | |
| SEM | 0.065 | | | | 0.004 | | | | 1.25 | | | |

abcd Means followed by similar letter (s) are not significantly different (DMRT, P>0.05).

† Feed conversion ratio= DM feed Intake/wet weight gain.

§ Protein efficiency ratio=biomass gain/protein intake.

¶ Apparent phosphorus digestibility=100-[100 × ($Cr_2O_3$ (diet) / $Cr_2O_3$ (excreta)] × [P (excreta) /P (diet)]

Interactions between phytase and CA on FCR and PER were not significant (Table 3). Carcass index was not affected by citric acid and phytase supplements and remained fairly constant at all levels (data not reported). Apparent phosphorus digestibility (APD) was not affected by CA supplement, but APD of the diets supplemented by two levels of phytase (500 and 1000) improved significantly over the control diet (Table 3). The regression equation was:

APD=42.77+0.115X (phytase), $R^2$=0.49; P<0.05

Interaction between phytase and CA on APD was not significant.

## Body Composition and Hematological Data

Dietary supplementation with CA and phytase at the levels used in the present study, did not affect the body protein content, dry matter and liver fat content significantly, so data have not been reported. The effect of dietary CA on body ash content was significant. Fish fed the diet containing 1.5 and 3% CA had higher (P<0.05 ) body ash contents than those fed the control diet (Table 4). No significant interaction was observed between phytase and CA regarding the ash content.

The phosphorus content of the body was increased significantly (P<0.05) as a result of CA supplementation (Table 4). Fish fed the diet containing 3% CA showed the highest phosphorus level compared to fish given the control diet (0.41 vs 0.27%). The regression equation was:

Carcass phosphorus=0.252+0.215X (CA), $R^2$=0.58; P<0.01.

Supplemental phytase had no significant effect on body P and the interaction between phytase and CA on body P was not significant. Consumption of the diet C3P1000 resulted in numerically higher P content (0.44%) as compared to C0P1000 (0.24%) (Table 4).

Citric acid reduced the body fat content significantly (P<0.05) (Table 4). Dietary supplementation with phytase as high as 1000 FYT $kg^{-1}$ did not seem to affect body fat content. Interaction between phytase and CA on body fat was not significant (Table 4). The regression equation of body phosphorus on body fat was:

Carcass fat=8.32-4.707X (carcass phosphorus) $R^2$=0.52; P<0.02.

Supplemented CA and phytase had no effect on plasma cholesterol, triglyceride and P, so data have not been reported.

Table 4. The effect of supplemental phytase and citric acid on body composition of common carp.

| | | Ash (%) | | | | Phosphorus (%) | | | | Fat (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Phytase (FYT $kg^{-1}$) | | 0 | 500 | 1000 | | 0 | 500 | 1000 | | 0 | 500 | 1000 |
| | Overall | 2.83 | 3.09 | 2.96 | Overall | 0.34 | 0.36 | 0.35 | Overall | 6.59 | 6.67 | 6.75 |
| Citric Acid (%) | | | | | | | | | | | | |
| 0 | 2.61b | 2.57 | 2.95 | 2.31 | 0.27b | 0.27 | 0.31 | 0.24 | 7.11a | 6.94 | 6.85 | 7.53 |
| 1.5 | 3.14a | 2.81 | 3.46 | 3.15 | 0.36a | 0.30 | 0.39 | 0.38 | 6.74ab | 6.56 | 7.07 | 6.58 |
| 3 | 3.13a | 3.12 | 2.85 | 3.42 | 0.41a | 0.44 | 0.37 | 0.44 | 6.16b | 6.25 | 6.09 | 6.12 |
| Model | P>F | | | | P>F | | | | P>F | | | |
| Phytase (P) | 0.517 | | | | 0.776 | | | | 0.893 | | | |
| Citric Acid (CA) | 0.038 | | | | 0.001 | | | | 0.034 | | | |
| P×CA | 0.190 | | | | 0.197 | | | | 0.658 | | | |
| SEM | 0.088 | | | | 0.013 | | | | 0.137 | | | |

abcd Means followed by similar letter (s) are not significantly different (DMRT, P>0.05).

Values (ash, P and fat) are on wet-weight basis.

## DISCUSSION

Phytase increased weight gain and CA enhanced this effect numerically. Schafer *et al.* (18) demonstrated a similar growth increase in carp fed with phytase supplemented diets. A part of the effect of phytase could be due to its role in releasing more P from phytate and enhancing energy metabolism for better growth and FCR (8). In fact, improvement in growth and FCR with higher levels of phytase (1000 FYT $kg^{-1}$) might be explained by release of minerals from phytate complexes, increase in the utilization of myo-inositol after its release from phytate, or a possible increase in the digestibility of starch, or a combination of these mechanisms (22).

Supplemental phytase improved FCR, which is in agreement with the results of Zyla *et al.* (27). PER was also increased as a result of phytase supplementation which confirms the finding of Vielma *et al.* (26). Additionally, combination of phytase and CA also improved PER numerically. It seems that CA enhanced phytase activity, thereby, allowing more phytate to be hydrolyzed. Consequently, the inhibitory effects of phytate on proteolytic enzymes may be reduced or alternatively, more $Ca^{2+}$ may be available as cofactor for these enzymes and ultimately better protein digestion might be achieved (20).

Although it has been reported that CA is capable of increasing apparent digestibility of many of the minerals in fishmeal (23), in the present study no effects on ADP was recorded. Radcliffe *et al.* (14) also reported no significant effect of added CA on ADP in pigs. On the other hand, the positive effect of phytase on increasing ADP obtained in the present study confirms the findings of Maenz *et al.* (11) in broilers. They demonstrated that addition of 1000 units $kg^{-1}$ of microbial phytase to the broiler feeds could on average increase the ADP from 12 to 30%. Our findings showed that addition of 1000 FYT $kg^{-1}$ to the diets improved the ADP from 40.8 to 53.7% in carp.

The positive effect of CA supplementation on reduction of body fat could possibly be related to its effects on phosphorus retention. Existing reports show that a low phosphorus diet causes an accumulation of fat in tissues of fish (18). In fact, phosphorus deficiency results in the inhibition of β-oxidation and fat accumulation (24). Although phytase supplementation was not associated with a decrease in fat content, a non-significant

reduction in body fat content was noted as a result of combination of CA and phytase. This could be attributed to the change of pH in the digesta and hence a better phytase performance, improving P availability and retention.

Supplemental CA and phytase had no effect on plasma cholesterol, triglyceride and phosphorus levels, which is not in agreement with the findings of Sugiura *et al.* (23).

Adding phytase to the diet had no effects on body P and ash content when no CA was included in the diet. However, the addition of 3% CA to the diet supplemented with 1000 FYT $kg^{-1}$ of phytase showed the highest body P and ash content numerically. Similar results were also reported for rainbow trout (2). This shows that CA promoted more favorable conditions for digestion by reducing stomach pH, hence suggesting a better phytase performance and consequently a better digestibility of phytate P and release of other divalent elements including Ca, Mg, etc. Acidification of diets due to added CA was 6.46, 5.42 and 4.88, for the levels of 0, 1.5 and 3% CA, respectively. Ineffectiveness of added phytase on plasma and body phosphorus and ash content might be due to use of plasma P in mitochondrial respiration rather than retention in body or due to increase of urinary P excretion (2), but in the present study we didn't measure the concentration of P in the effluent. Similar findings were reported by Vielma *et al.* (26). The results of the present study are in agreement with the reports of Cain and Garling (3), who showed higher ash content in fish fed with a phytase supplemented soybean diet. Lower body fat contents due to consumption of the CA supplemented diet suggest a higher P availability and a higher fat oxidation, hence providing more energy for a better growth rate. These findings confirm the reports by Horl *et al.* (9).

## CONCLUSIONS

Supplemental phytase improved FCR, PER, specific growth rate and P digestibility, while, CA resulted in increased body ash and phosphorus levels and reduced body fat. A combination of phytase and CA was more effective numerically than each one alone.

## ACKNOWLEDGEMENT

The financial support of the Isfahan University of Technology is acknowledged. The authors thank the Akbarie Company, Tehran, Iran, for providing phytase supplements.

## LITERATURE CITED

1. Association of Official Analytical Chemists. 1990. Official Methods of Analysis, 15th ed., Association of Official Analytical Chemists, Washington, DC.
2. Bureau, D.P. and C.Y. Cho. 1999. Phosphorus utilization by rainbow trout (*Oncorhynchus mykiss*): Estimation of dissolved phosphorus waste output. Aquaculture 179: 127-140.
3. Cain, K.D. and D.L. Garling. 1995. Pretreatment of soybean meal with phytase for salmonid diets to reduce phosphorus concentrations in hatchery effluents. Prog. Fish. Cult. 57: 114-119.
4. Duncan, D.B. 1955. Multiple range and multiple F tests. Biometrica, 11:1-42.
5. Fauconneau, B., H. Alami-Durente, M. Laroche, J. Marcel and D. Vallot. 1995. Growth and meat quality relations in carp. Aquaculture 129: 265-297.
6. Fenton, T.W. and M. Fenton. 1979. Determination of chromic oxide in feed and feces. Can. J. Anim. Sci., 58: 631-635.
7. Fossati, P. and L. Prencipe. 1982. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin. Chem. 28: 2077-2080.
8. Hepher, B. 1990. Nutrition of pond fishes. Cambridge University Press, Cambridge, 388 p.
9. Horl, W.H., W. Kreusser, H. Rambausek, A. Heidland and E. Ritz. 1983. Glycogen metabolism in phosphorus depleted rats. Miner. Electrolyte Metab. 9: 113-118.
10. Kaushik, S.J. 1995. Nutrient requirements, supply and utilization in the content of carp culture. Aquaculture 129: 225-241.
11. Maenz, D.D., M. Carmen, C.M. Engele-Schaan, R.W. Newkirk and H.L. Classen. 1999. The effect of minerals and mineral chelators on the formation

of phytase-susceptible forms of phytic acid in solution and a slurry of canola meal. Anim. Feed. Sci. Tech. 81: 177-192.

12. National Research Council. 1989. Nutrient requirements of warm water fishes (Ed. R.P.Wilson). National Academy Press, Washington, DC.
13. Nordrum, S., T. Asgard, K.D. Shearer and P. Arnessen. 1997. Availability of phosphorus in fish bone meal and inorganic salts to Atlantic salmon (*Salmo salar*) as determined by retention. Aquaculture 157: 51-61.
14. Radcliffe, J.S., Z. Zhang and E.T. Kornegary. 1998. The effects of microbial phytase, citric acid, and their interaction in a corn-soybean diet for weanling pigs. J. Anim. Sci. 76: 1880-1886.
15. Roeschlau, P., E. Bernt and W. Gruber. 1974. Enzymatic determination of total cholesterol in serum. J. Clin. Chem. Clin. Biochem. 12: 226-227.
16. SAS Institute, 1993. SAS User's Guide: Statistics. SAS Institute, Inc., Cary, NC.
17. Satoh, S. 1991. Common Carp (*Cyprinus caprio*). In: Wilson, R.P. (Ed.). Handbook of Nutrient Requirements of Finfish. CRC Press, UK, 55-68 p.
18. Schafer, A., W.M. Koppe, K.H. Meyer-Burgdroff and K.D. Gunther. 1995. Effects of microbial phytase on the utilization of native phosphorus by carp in a diet based on soybean meal. Wat. Sci. Tech. 31: 149-155.
19. Schwarz, F.J., M. Kirchgessner and U. Deuringer. 1998. Studies on the methionine requirement of carp (*Cyprinus carpio* L.). Aquaculture 161: 121-29.
20. Selle, P.H., V. Ravindran, R.A. Caldwell and W.L. Bryden. 2000. Phytate and phytase: Consequences for protein utilization. Nutr. Res. Rev. 13:255-278.
21. Silva, S.S.D. and T.A. Anderson. 1995. Fish Nutrition in Aquaculture. CRC Press, UK, 319 p.
22. Simons, P.C.M., H.A.J. Versteegh, A.W. Jongbloed, P.A. Kemme, P. Slump, K.D. Bos, M.G.E. Wolters, R.F. Beudeker and G.J. Verschoor. 1990. Improvement of phosphorus availability by microbial phytase in broilers and pigs. Br. J. Nutr. 64: 525-540.
23. Sugiura, S.H., F.M. Dong and R.W. Hardy. 1998. Effects of dietary supplements on the availability of minerals in fish meal-preliminary observations. Aquaculture 160: 283-303.

24. Takeuchi, M. and J. Nakazoe. 1981. Effect of dietary phosphorus on lipid content and its composition in carp. Bull. Jap. Soc. Sci. Fish. 47:347-352.
25. Tietz, N.W. 1986. Textbook of Clinical Chemistry. W.B. Saunders, Co., Philadelphia, PA.
26. Vielma, J., T. Makinen, P. Ekholm and J. Koskela. 2000. Influence of dietary soy and phytase levels on performance and body composition of large rainbow trout (*Oncorhynchus mykiss*) and algal availability of phosphorus load. Aquaculture 183: 349-362.
27. Zyla, K., D.R. Ledoux, A. Garcia and T.L. Veum. 1995. An *in vitro* procedure for studying enzymic dephosphorylation of phytate in maize-soybean feeds for turkey poults. Br. J. Nutr. 74:3-17.