

Response of Rainbow Trout (*Oncorhynchus mykiss*) Growing from 50 to 200 g to Supplements of Dibasic Sodium Phosphate in a Semipurified Diet^{1,2,3}

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ABSTRACT Effects of increasing dietary concentrations of phosphorus on growth, feed intake, feed conversion, composition of gain and concentration of inorganic phosphate in plasma were studied in rainbow trout. Twelve groups of 20 trout initially weighing 53 ± 0.6 g/fish were fed semipurified diets containing 19.6 MJ digestible energy per kilogram of dry matter. Twelve levels of phosphorus ranging from 1.03 to 10.96 g/kg dry matter were achieved by replacing inorganic binder with dibasic sodium phosphate in 11 graded levels. Nonlinear responses of trout to increasing dietary phosphorus concentration determined over 53 d were described using exponential functions. Feed intake, growth rate and feed conversion ratio as well as plasma inorganic phosphate concentration increased with increasing dietary phosphorus concentration. The concentrations of phosphorus, calcium, magnesium and potassium in weight gain increased, whereas concentrations of lipids and energy in weight gain decreased with increasing dietary phosphorus concentration. Concentrations of protein and sodium in weight gain were unaffected. Different concentrations of dietary phosphorus were required for achieving 95% of the plateau value determined for desired traits. In growth rate and phosphorus deposition, the required phosphorus concentrations were 3.7 and 5.6 g/kg dry matter, respectively. However, dietary phosphorus was utilized most efficiently (88%) at a dietary concentration of 2.5 g/kg dry matter. At the dietary phosphorus concentration that resulted in maximum phosphorus deposition (5.6 g/kg dry matter), phosphorus utilization was about 60%. Supplemental phosphorus from dibasic sodium phosphate was completely available to trout which must be considered in formulating recommendations. Based on this work, 0.25 g available phosphorus/MJ digestible energy is recommended for trout diets. *J. Nutr.* 126: 324–331, 1996.

INDEXING KEY WORDS:

- requirement • rainbow trout • phosphorus
- blood plasma • body composition

Effects of different levels of dietary phosphorus (P) in trout were studied by Ogino and Takeda (1978) us-

ing fish weighing a maximum of 3.6 g. The authors concluded that the level of available P required to maintain a normal growth was 7 to 8 g/kg of the fish diet. So far, the P requirement of heavier trout has not been studied, which might explain why the NRC (1993) recommends 6 g P/kg trout feed according to studies performed with Atlantic salmon.

In trout fed diets sufficient in P, concentration of inorganic phosphate (P_i) in blood serum or blood plasma varies widely between 2.3 and 6.5 mmol/L according to the studies of Björnsson and Haux (1985), Cowey et al. (1977) and Knox et al. (1981 and 1982). Several factors could cause this wide range, e.g., feeding intensity, duration of the fastening periods or stress situations before blood sampling. Dietary P concentration, however, obviously did not affect concentration of P_i in plasma of fish fed above the phosphorus requirement (Cowey et al. 1977). Response in plasma P_i concentration to low dietary P supply in trout has not yet been described. From studies in rats (Harrison et al. 1986), swine (Fox et al. 1978, Sommerville et al. 1985) and ruminants (Rodehutscord et al. 1994), it is apparent that the concentration of P in plasma or serum drastically decreases when diets low in P are fed.

It was the objective of the present work to study the effects of increasing dietary concentrations of P in heavier trout with body mass up to 200 g. Rates of feed intake and growth as well as composition of gain and plasma P_i concentrations were determined.

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MATERIALS AND METHODS

Semipurified diets were fed with wheat gluten (2.5 g P/kg dry matter) and crystalline amino acids as the only sources of amino acids to arrive at a low P concentration in the basal diet (Table 1). A very similar diet containing extruded corn instead of gelatinized wheat starch was shown to enable growth and protein retention in trout comparable to diets based on fish meal as the main source of amino acids (Rodehutschord et al. 1995c). In eleven graded levels, dibasic sodium phosphate (Na_2HPO_4) was added to the basal diet, replacing corresponding amounts of the inorganic binder. Hence, analyzed concentration of P ranged from 1.03 to 10.96 g P/kg dry matter. Analyzed concentrations of crude protein ($\text{N} \times 6.25$) and lipids were 300 and 207 g/kg dry matter, respectively. Concentration of digestible energy (DE) was 19.6 MJ/kg dry matter, as calculated from concentrations of DE in individual components determined previously in our department. Fish oil, sunflower oil and warmed beef tallow were stirred with the binder before being added to the other components in a drum mixer. Mixtures were moistened in a cutter to a sticky paste which was pelleted by forcing it through 3-mm screens of a mincer. The moist pellets were stored at -18°C until feeding.

Two hundred sixty rainbow trout (*Oncorhynchus mykiss*) initially weighing 53 ± 0.6 g/fish (mean \pm SE) were taken from the population reared in our department. Up to the beginning of the experiment, trout had been fed a commercial diet (Aminoforte, Rheinkrone, Wesel, Germany). Groups of 20 trout each were placed in twelve 250-L round plastic tanks which were part of a circulatory system and which were continuously supplied with water in parallel. Approximately 70% of the outflowing water recirculated after clarification and aeration. Water temperature ranged between 16.5 and 17.5°C. Concentration of P in communal water used here was always lower than 5 mg/L, and Ca concentration ranged between 40 and 50 mg/L. Body mass of all fish in each group was recorded at the beginning of the experiment after the fish were anesthetized in a solution of 1,1,1-trichloro-2-methyl-2-propanol-hemihydrate and at the end of the experiment after the fish had been killed in 4-ethyl-aminobenzoate. Thirty-six hours before killing, feed was withheld. In the five largest fish of each experimental group, blood was withdrawn from the heart muscle immediately after killing using a heparinized syringe. Blood was centrifugated for 20 min with $10,000 \times g$ and plasma was stored at -18°C for analyses.

One additional group of trout was killed for base-line measurements at the beginning of the experiment. After freezing at -18°C , fish of the experimental groups as well as of the base-line group were cut into small pieces by use of a ribbon saw, forced repeatedly through a mincer, homogenized in a cutter and freeze-

TABLE 1
Composition of diets fed to rainbow trout

Component	Amount
	g/kg dry matter
Wheat starch, gelatinized	300
Wheat gluten	268
Amino acid premix ¹	97
Fish oil	100
Sunflower oil	53
Beef tallow	52
Binder ^{2,3}	80
Minerals ⁴	30
Vitamins ⁵	10
Choline chloride (50%)	10

¹ Amino acid mixture supplied the following (g/kg diet): L-lysine · HCl, 29.4; DL-Methionine, 6.2; L-threonine, 9.6; L-tryptophan, 2.3; L-arginine, 13.1; L-valine, 9.6; L-leucine, 11.8; L-isoleucine, 7.5; L-phenylalanine, 3.5; L-histidine · HCl · H₂O, 4.0.

² Sipernat® 50S, Degussa AG, Hanau, Germany.

³ Monosodium-phosphate replaced variable parts of the binder in each of 11 mixtures.

⁴ The mineral premix contained the following (g/kg): Ca, 166; K, 78; Na, 19; Mg, 13; Fe, 1.1; Mn, 1.2; Zn, 1; Co, 1.08; I, 0.27; Cu, 0.24; Se, 0.04; Al, 0.09; Mo, 0.01; F, 0.01.

⁵ For composition see Rodehutschord et al. (1995c).

dried. The composition of gain of experimental groups was calculated from differences between experimental groups and the base-line group as described previously (Rodehutschord et al. 1995c).

Each group was fed one of the experimental diets for 53 d. Trout were fed to satiation twice daily during the week and once daily on weekends. Thawed feeds were offered by hand until pellets were first seen to sink to the bottom of the tank. Thus, feed losses could be avoided almost completely. General care, handling and maintenance of trout followed the procedures approved by the Animal Welfare Commissioner of the University of Bonn in accordance with the German Animal Welfare Law.

In diets and body homogenates, dry matter (105°C), ash (550°C), total N (macro-Kjeldahl), lipids (petroleum ether extract after HCl treatment) and heat of combustion (adiabatic bomb calorimetry) were determined according to the official methods (Naumann and Bassler 1976). Analyses of elements were done in filtered ash solutions. Phosphorus was determined photometrically as orthophosphate using the vanado-molybdate method (Naumann and Bassler 1976). Calcium, magnesium, sodium and potassium were determined by atomic absorption spectrophotometry. Plasma was deproteinized with trichloroacetic acid and concentration of P_i was determined using the vanado-molybdate method described in the test kit No. 124974 (phosphorus/phospholipids) of Boehringer, Mannheim, Germany.

Exponential functions were calculated to fit the experimental data because the response to a limiting di-

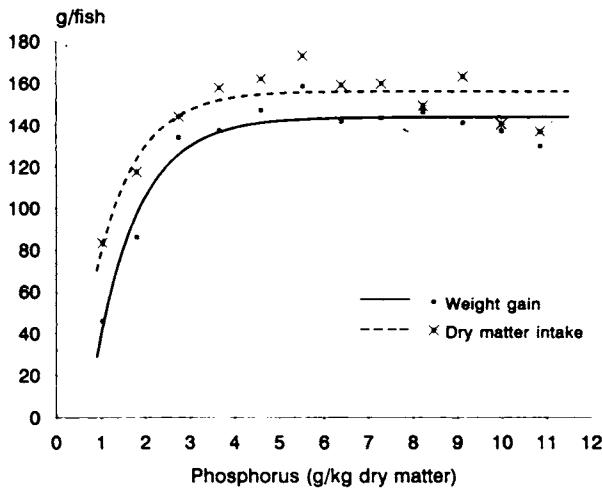


FIGURE 1 Effect of dietary phosphorus on body weight gain and dry matter intake of trout during the 53 d of the experiment (initial body mass 53 g/fish). Each point represents a group of 20 fish. The parameters of equations are summarized in Table 2.

etary component is nonlinear. For traits which increased with increasing dietary concentration of P, the equation employed for describing the respective response was

$$y = a(1 - e^{-b(x-c)}).$$

Lipid and energy concentration in gain, on the contrary, reacted negatively to increasing dietary P concentration. Therefore, the equation employed was

$$y = a + de^{-bx}$$

where x = dietary concentration of P (g/kg dry matter), a = plateau value of the respective curve, b = parameter characterizing the steepness of the curve, c = dietary P concentration at $y = 0$, and d = maximum response to supplemental dibasic sodium phosphate.

Model parameters were estimated by the least-squares principle (Rawlings 1988, Seber and Wild 1989). The resulting nonlinear equations were solved iteratively by the Levenberg-Marquard method (Press et al. 1987). Calculations were performed using the program BFIT (H. P. Helfrich, Seminar of Mathematics, Faculty of Agriculture, University of Bonn), which implements this method.

RESULTS

In each of the groups fed diets containing 1.03, 2.75 and 10.96 g P/kg dry matter, one fish died after 37, 21 and 36 d, respectively. In the group fed 1.81 g P/kg dry matter, one fish died after 34 d and two fish died after 47 d.

Figures 1, 2, 3 and 4 show the response of trout to increasing levels of dietary phosphorus for some of

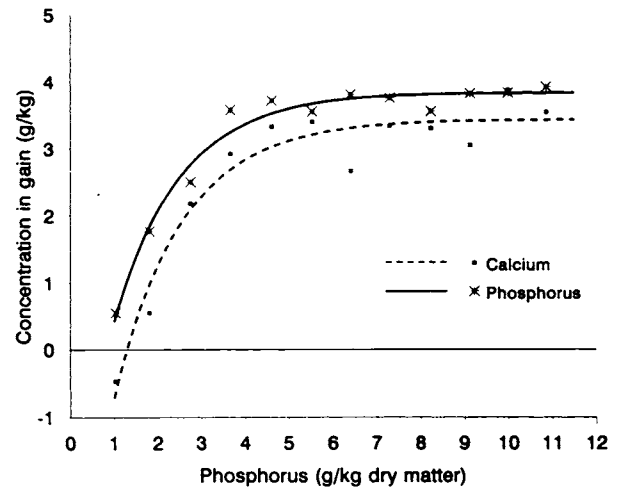


FIGURE 2 Effect of dietary phosphorus on concentration of phosphorus and calcium in gain of trout after 53 d of the experiment (initial body mass 53 g/fish). Each point represents a group of 20 fish. The parameters of the equation are listed in Table 2.

the traits investigated. All results are summarized in Table 2, where estimated parameters are listed for all traits in which a response was detectable. Concentrations of protein and sodium in weight gain were not influenced by dietary phosphorus; the concentrations were (mean \pm SE) 160 ± 1.6 and 0.82 ± 0.01 g/kg, respectively. Body weight gain as well as dry matter intake (Fig. 1) and feed conversion ratio increased with increasing dietary phosphorus concentration and clearly reached a plateau within the investigated range of dietary phosphorus. The phosphorus concentration in weight gain increased from ~ 0.5 g/kg in fish fed the basal diet to 3.8 g/kg in fish fed sufficient phos-

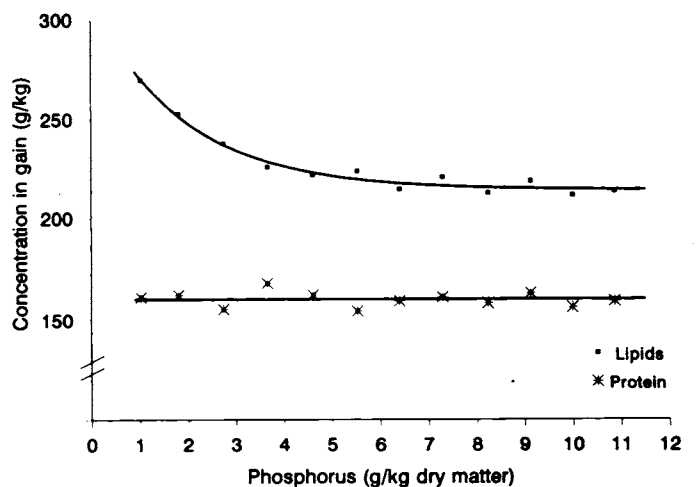


FIGURE 3 Effect of dietary phosphorus on concentration of protein ($N \times 6.25$) and lipids in gain of trout after 53 d of the experiment (initial body mass 53 g/fish). Each point represents a group of 20 fish. The parameters of equations are summarized in Table 2.

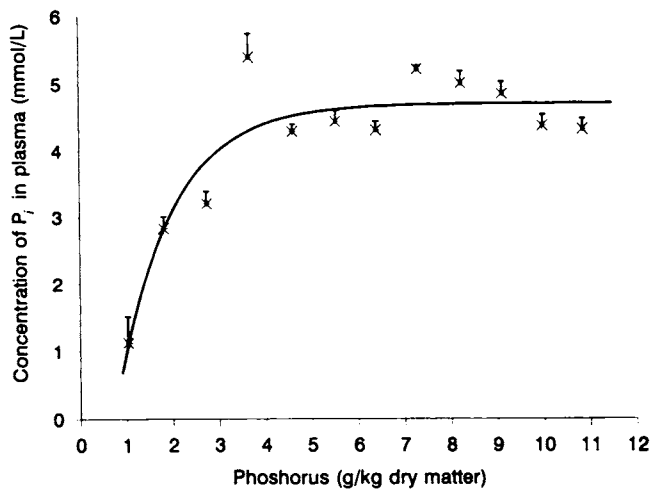


FIGURE 4 Effect of dietary phosphorus on concentration of inorganic phosphate (P_i) in blood plasma of trout sampled 36 h after the last meal of the experiment (five fish per treatment, mean and SE). The parameters of the equation are given in Table 2.

phorus (Fig. 2), and total phosphorus deposition increased from 25 mg/fish to ~550 mg/fish. Concentrations of calcium, magnesium and potassium also increased, whereas concentrations of lipids (Fig. 3) and energy decreased concurrently. Concentration of P_i in blood plasma of trout rose from 1.1 mmol/L when the basal diet was fed to a plateau of 4.7 mmol/L (Fig. 4).

DISCUSSION

Nonlinear equations are regarded as most appropriate for evaluating results of dose-response experi-

ments (Cowey 1992, Fuller and Garthwaite 1993, Kirchgeßner et al. 1992, Mercer et al. 1989). Efficiency of supplemented P decreased with increasing dietary P concentration, resulting in plateaus which could be described by exponential functions. Theoretically, leaching of P from the diets into the tank water might have influenced responses of trout. However, feed was introduced very carefully by hand into the tanks and was ingested immediately by trout which means that water could not enter the pellets. Therefore, reduction in either absorption rate or intermediary utilization of absorbed P or both must be the reason for plateaus in performance, resulting in increased excretion of either unabsorbed or endogenous P or both.

Unlike the broken-line model (Robbins et al. 1979), the exponential function does not give a well-defined value for "requirement" but leaves space for interpretation. For each of the traits evaluated with exponential functions, plateau values were calculated which describe a theoretical maximum or minimum that can be approached by continuously increasing the dietary phosphorus concentration (Parameter *a* in Table 2). As efficiency of supplementing P decreases with increasing dietary concentration of P, the respective phosphorus concentration required to approach a certain percentage of this theoretical maximum or minimum was calculated from these equations. Alternate calculations were arbitrarily done for 90, 95 and 98% of the plateau value (summarized in Table 3) but can also be done for any chosen level using the parameters listed in Table 2. Obviously, the P concentration required for reaching a certain percentage of the plateau depends on the trait chosen. As a consequence of increased concentration of P in the diet, concentrations of P, Ca and Mg in weight gain reach a plateau later

TABLE 2

Parameters estimated by fitting the experimental data to an exponential curve¹

	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>r</i> ²	Root MSE
Dry matter intake (g/fish)	156	1.08	0.35	—	0.82	11.4
Body weight gain (g/fish)	144	1.0	0.68	—	0.93	9.11
Feed conversion ratio (g gain/g dry matter)	0.93	1.07	0.20	—	0.89	0.04
Concentrations in body weight gain:						
Energy (MJ/kg)	12.4	0.40	—	3.20	0.82	0.35
Lipids (g/kg)	214	0.52	—	95.5	0.98	3.11
Phosphorus (g/kg)	3.84	0.66	0.83	—	0.98	0.19
Calcium (g/kg)	3.44	0.65	1.29	—	0.94	0.36
Magnesium (g/kg)	0.28	0.71	0.55	—	0.93	0.02
Potassium (g/kg)	2.55	1.53	0.43	—	0.96	0.07
Phosphorus deposition (mg/fish)	549	0.65	1.05	—	0.96	41.1
Concentration of inorganic phosphate in plasma (mmol/L)	4.70	0.84	0.71	—	0.84	0.52

¹ Abbreviations used: *a*, *b*, *c*, and *d*, estimated parameters of the following functions: $y = a(1 - e^{-b(x-c)})$ and $y = a + d e^{-bx}$, where *x* = dietary concentration of P (g/kg dry matter), *a* = plateau value of the respective curve, *b* = parameter characterizing the steepness of the curve, *c* = dietary P concentration at *y* = 0 and *d* = maximum response to supplemental dibasic sodium phosphate; MSE, mean square error.

TABLE 3

Dietary phosphorus concentration required to reach x% of plateau value of performance traits in rainbow trout

	x% of plateau		
	90	95	98
	g/kg dry matter		
Dry matter intake	2.5	3.1	4.0
Body weight gain	3.0	3.7	4.6
Feed conversion ratio (gain/feed)	2.4	3.0	3.9
Energy concentration of gain	2.4	4.2	6.5
Lipid concentration of gain	2.9	4.2	6.0
Phosphorus concentration of gain	4.3	5.3	6.7
Calcium concentration of gain	4.9	5.9	7.4
Magnesium concentration of gain	3.8	4.8	6.1
Potassium concentration of gain	1.9	2.4	3.0
Phosphorus deposition	4.6	5.6	7.0
Plasma inorganic phosphate concentration	3.5	4.3	5.4

than do feed intake or weight gain of trout. This indicates a capacity of the skeleton to buffer changes in P supply. For high gain in body weight, P deposition need not be at its maximum. For reaching 95% of the plateau in weight gain, only 85% of the plateau of P concentration in gain was required with fish showing no signs of impaired health. From this work, the necessity for maximum mineralization of the skeleton cannot be concluded. This demonstrates that the "requirement for P" must not be one single value but depends on what is considered to be a physiologically or economically important trait.

The plateau concentration of P in weight gain found in this work is at the lower limit of the range found in the literature (3.8 to 4.5 g/kg as reviewed by Wiesmann et al. 1988). This might be explained by the high concentration of lipids in weight gain caused by the high concentration of dietary lipids (Rodehutscord et al. 1995c, Storebakken and Austreng 1987). Pfeiffer (1978) determined concentrations of minerals in trout weighing 300 to 430 g. As compared with his results, the plateau concentration found for Ca in weight gain in this work was slightly higher, and plateau concentrations found for Mg and K in weight gain as well as concentration of Na in weight gain were comparable.

As a result of increasing concentration of dietary P, not only the concentration of P but also concentrations of Ca, Mg, and K in weight gain increased, although the concentrations of these elements in the diets were equal. In trout weighing 7–40 g, Frenzel and Pfeiffer (1982) also found an increased Ca retention when dietary P concentration was increased. It must be assumed that increased retention of Ca, Mg and K resulted from increased retention of P, which is confirmed by the parallel regression lines estimated for P and Ca concentration in gain (Fig. 2). The ratio of plateau concentrations for Ca and P in gain of 0.9:1 is

< half of the ratio found in mammals but is normal for trout (Pfeiffer 1978). The negative concentration of Ca in gain of trout fed the basal diet is due to the fact that trout lost 21 mg Ca/fish during the experiment although they gained 46 g in body mass.

The plateau in plasma P_i concentration found in this work is higher than concentrations found by Björnsson and Haux (1985) but lower than those found by Cowey et al. (1977), both studies performed with sufficient concentrations of dietary P. Cowey et al. (1977) varied P concentration in the diet from 11 to 40 g/kg dry matter and found no significant differences in plasma P_i concentrations. From their results it can be concluded that dietary P concentration does not affect concentration of plasma P_i as long as dietary P is sufficient, which is confirmed by the results presented here. Björnsson and Haux (1985) withheld trout feed for either 10 or 15 d before blood sampling and found no differences in P_i concentration of plasma (2.55 vs. 2.47 mmol/L). However, it must be assumed that the wide range in plasma P_i concentration found in the literature is at least partly due to differences in time between the last meal of the fish and blood sampling. If plasma concentrations are to be used as indicators, this point has to be considered and seems to be worth further investigation.

In the situation of deficient P supply, obviously trout do not maintain constant plasma P_i concentrations. As shown in Fig. 4, P_i concentration in plasma of trout fed the basal diet was only ~25% of the plateau concentration. Comparable responses in plasma concentrations of trout to deficient dietary supply were also found for Mg (Cowey et al. 1977). In lactating goats and cows, changes in dietary P concentration from sufficient to extremely deficient also caused a drastic decrease in P_i concentration within 10 d after switching P intake (Rodehutscord et al. 1994). However, the level found during P depletion in these animals was about 0.2 mmol/L, which was remarkably lower than the lowest value found in trout in the present work. Correspondingly, the level determined in ruminants fed sufficient dietary P (1.5–2.5 mmol/L) is ~ half the plateau value found in this work. When ruminants highly depleted of P were changed to adequate P intake, plasma P_i increased to values of ~4–5 mmol/L within 10 d but then returned to normal concentrations without further variation in P supply (Rodehutscord et al. 1994). In contrast, trout in this study maintained comparably high plasma P_i values as long as dietary P was adequate.

As an effect of increasing dietary P, both feed intake and gain in body mass were considerably increased. Whether increased intake was an effect of higher growth rate or if it was the primary response of trout resulting in higher growth rate remains unexplained. Additionally, efficiency of utilization of DE was about one-third lower in trout fed the basal diet (Fig. 5), and concentration of lipids in weight gain was greater than

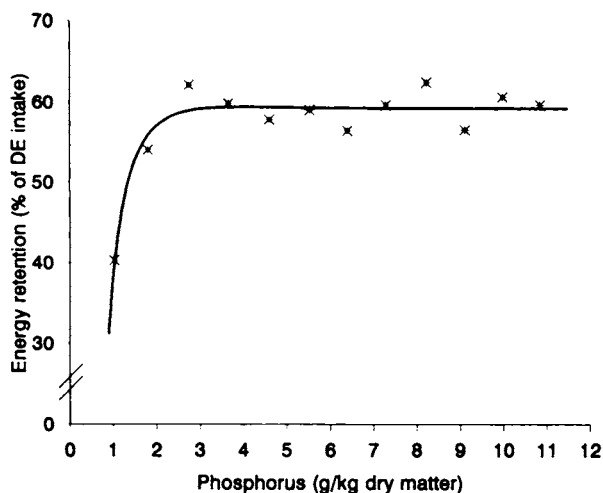


FIGURE 5 Efficiency of utilization of digestible energy (DE) in trout fed diets with increasing levels of phosphorus calculated as follows: (energy retention/DE intake) × 100. Each point represents a group of 20 fish.

in trout fed diets containing adequate P (Fig. 3). This indicates disturbances in intermediary energy metabolism which should be discussed in relation to reduced P_i concentration of blood plasma because several enzymatic processes are induced by P_i . Nothing is known about effects of dietary P on intracellular concentrations of P, but it can be assumed that they follow changes of P_i concentrations found in plasma. Takeuchi and Nakazoe (1981) fed carp (*Cyprinus carpio*) diets either sufficient or deficient in P for 6 wk followed by 3 wk of food deprivation before killing the fish. During the non-feeding period, lipid concentration in fish decreased between one- and two-thirds that seen in fish previously fed sufficient P, whereas in fish fed deficient P, lipid concentration decreased only slightly. The authors suggest that β -oxidation of fatty acids was inhibited by deficiency of dietary P which is in agreement with the above hypothesis. In ruminants, feed intake has been repeatedly shown to be reduced when P intake was low. From the studies of Milton and Ternouth (1985) in sheep, it can be concluded that negative effects of P deficiency in ruminants have to be attributed to alterations in plasma P_i concentration rather than to disturbances in the environment of rumen microbes.

Phosphorus intake of trout could be best described with the following linear regression: P intake (mg/fish) = $149 \times (\text{g P/kg dry matter} + 0.091)$. Dividing the function for deposition of P by this function for P intake results in a function describing the efficiency of utilization of P as an effect of dietary P concentration (Fig. 6). As dietary P originated mainly from dibasic sodium phosphate, a maximum in utilization of P of ~88% was reached. This maximum is obtained at a dietary P concentration which is ~ half of that concentration required to reach 95% of the plateau in P deposition. Therefore, high growth rates are connected

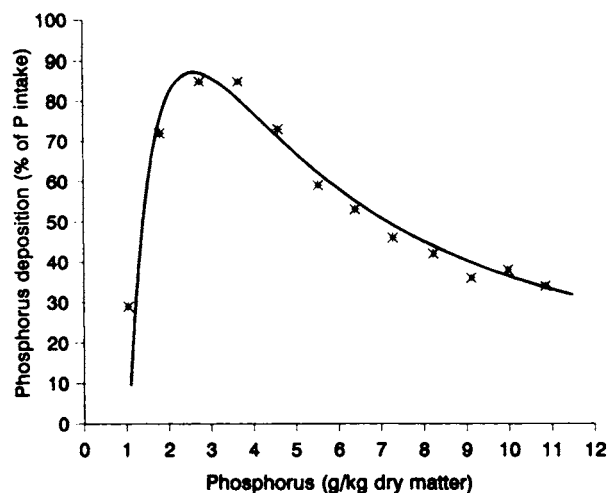


FIGURE 6 Efficiency of utilization of dietary phosphorus as affected by the dietary level of phosphorus (function calculated as the function of phosphorus deposition divided by the function of phosphorus intake). Each point represents a group of 20 fish.

with suboptimal utilization of dietary P. At a dietary concentration of 5.3 g/kg dry matter, which is required for reaching 95% of the plateau in P deposition, dietary P was utilized at only 64%. Comparable results could be obtained in experiments investigating the effects of supplemental dietary methionine, arginine and threonine (Rodehutsord et al. 1995a and 1995b).

Phosphorus excretion of trout can be calculated as difference between intake of dietary P and deposition of P. This calculation is shown in Table 4. Trout fed the diets containing 1.81 or 2.75 g P/kg dry matter excreted the same amount of P as fish fed the basal diet without supplemental phosphate. This demon-

TABLE 4

Phosphorus excretion of trout, calculated as difference between intake and deposition of phosphorus¹

Dietary concentration of phosphorus	Phosphorus excretion in 53 d
g/kg dry matter	mg/fish
1.03	61
1.81	61
2.75	60
3.66	85
4.60	197
5.53	392
6.40	478
7.29	625
8.23	707
9.12	946
9.99	878
10.96	978

¹ Each value was determined in one group of 20 trout.

strates very clearly that P from dibasic sodium phosphate was completely available to the trout which confirms results from Frenzel and Pfeffer (1982) and Ogino et al. (1979). Phosphorus excretion of trout fed the basal diet may have resulted from two possible sources: endogenous losses of P and unavailable dietary P. Almost all P in this diet originated from wheat gluten and wheat starch, and nothing is known about availability of P contained in these components. Comparing P intake and P deposition of fish fed the basal diet shows a utilization of only 29%, which is at least partly due to endogenous P excretion. Surprisingly, P excretion of trout did not increase when diets containing 1.81 or 2.75 g P/kg dry matter were fed, although intake of P from the basal diet increased by about twofold due to increased feed intake. From this it must be concluded that P contained in the basal diet was also highly available and that ~60 mg P/fish was inevitably lost by trout during the experimental period, irrespective of body mass or feed intake.

With regard to the high availability of dietary P, conclusions from this study concerning the P requirement of trout must be expressed in terms of available P. From Table 3 it is clear that the required level of dietary P depends on the trait chosen and ranges between 2.4 and 5.9 g/kg dry matter for reaching 95% of the respective plateau values. If this 95% level is considered sufficient, it seems admissible to arrive at a requirement of 5 g available P/kg dry matter of the diet used here. This is substantially lower than the range of 7–8 g available P/kg diet determined by Ogino and Takeda (1978). This difference can be explained by the higher concentration of P and the much lower lipid concentration in the weight gain of the very young trout used by Ogino and Takeda (1978).

Concentration of DE grossly determines the gain/feed ratio and, therefore, the amount of P ingested per unit in weight gain. Because DE concentration may vary from 15 to 21 MJ/kg dry matter, concentrations of nutrients should, therefore, preferably be related to MJ DE than to kilograms of feed. From the results presented here, a value of 0.25 g available P/MJ DE may be recommended for trout weighing more than 50 g.

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