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Supplementation of maca (*Lepidium meyenii*) tuber meal in diets improves growth rate and survival of rainbow trout *Oncorhynchus mykiss* (Walbaum) alevins and juveniles

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Abstract

Maca tuber meal is used in fish diet formulations in Andean trout culture and knowledge of its effects on fish growth is paramount to healthy human food production. In the first experiment with rainbow trout alevins (0.096 ± 0.002 g), starter diets were offered from first feeding until 15 weeks. We formulated high protein content (~60%) semi-purified starter diets supplemented with 0%, 5%, 10%, or 15% maca tuber meal (control, M-5, M-10, and M-15 respectively). The second feeding trial was conducted with juveniles (1.56 ± 0.02 g) fed one of three diets (control, M-15, and commercial) for 8 weeks. In the first experiment, fish fed M-10 and M-15 diets exhibited significantly higher growth rates than the other dietary groups. Survival was significantly improved in the groups fed diets supplemented with maca tuber meal (60.0–69.2%) in comparison with the group fed a control diet (21.7%). The second experiment showed a higher growth rate in the M-15 group compared with the control and a commercial diet fed group. Leucocyte numbers were increased by dietary supplementation of maca tuber meal. The findings of the present study suggest that a maca tuber meal inclusion at least 5% improves growth rate, feed utilization, immunity by increased leucocyte number, and survival of rainbow trout alevins and juveniles.

Keywords: *Lepidium meyenii*, maca, rainbow trout, growth, survival

Introduction

Lepidium meyenii Walpers, known as 'maca' in South America, belongs to the plant family of Brassicaceae and has been cultivated for over 2000 years in the Andean Mountains (Leon 1964). The tuber of this plant has long been used as food and folk medicine (Zheng, He, Kim, Rogers, Shao, Huang, Lu, Yan, Qien & Zheng 2000; Li, Ammermann & Quirós 2001). Maca usually grows at a restricted area of high altitude (between 3500 and 5000 m), but it also can be cultivated in other conditions (Cicero, Bandieri & Arletti 2001). For medicinal human consumption, the tuber of this plant is dried and ground into a fine powder. Maca tuber meal has long been used as a remedy for the treatment of human male infertility in Peruvian rural communities (Quiros, Epperson, Hu & Holle 1996; Cicero *et al.* 2001; Gonzales, Cordova, Gonzales, Chung, Vega & Villena 2001a). Recently, oral administration of maca extracts was reported to enhance sexual behaviours in male and female mice, and to decrease erectile dysfunction in male rats (Zheng *et al.* 2000). In rats, aqueous extracts of maca roots increased weights of testis and epididymis, and activated spermatogenesis after 14 days of oral administration (Gonzales, Ruiz, Gonzales, Villega & Cordova 2001b). Cicero *et al.* (2001) also reported that the oral administration of maca pulverized root improved sexual performance of male rats.

It was found in our laboratory that some alkaloids and flavonoids, such as isopteropodin and quercetin,

are present in the maca tuber meal (unpublished data). Recently, Sandoval, Okuhama, Angeles, Melchor, Condezo, Lao & Miller (2002) demonstrated that maca has a high antioxidant activity related to catechins and epigallocatechins. Aqueous maca extract exhibited *in vitro* strong radical scavenging activities against peroxynitrite, 1,1-diphenyl-2-picrylhydrazyl, and peroxyls (Sandoval *et al.* 2002). Maca extract was shown to have protective roles in RAW 264.7 cells against peroxynitrite-induced apoptosis and deoxyribose degradation in the same study. It is also known that the tuber meal contains some phytosterols, such as campesterol, stigmasterol, β-sitosterol, and benzyl isothiocyanates (Zheng *et al.* 2000; Li *et al.* 2001). β-sitosterol has been known to have an effect on sex steroids, consequently affects reproductive events in mammals (Rosenblum, Stauber, Van Thiel, Campbell & Gavaler 1993) and fish (MacLatchy & Van Der Kraak 1995; Tremblay & Van Der Kraak 1998; Lehtinen, Mattsson, Tana, Engström, Lerche & Hemming 1999).

Nutritional information about this plant is scarce (Dini, Migliuolo, Rastrelli, Saturnino & Schettino 1994) and contradictory literature information is available in regard to the effects of its consumption on growth performance in animals (Obregón Vilches 1998; Canales, Aguilar, Prada, Marcelo, Huaman & Carbajal 2000). Maca tuber meal was found to have protein levels of 10–13% and highly hydrolysable carbohydrate levels of 59–68% on a dry-matter basis (Dini *et al.* 1994; Canales *et al.* 2000).

In the present study, rainbow trout alevins were fed experimental diets as the first exogenous feed. This stage of fish ontogeny poses a challenge for the investigator as a semi-purified (casein–gelatin based) diet is routinely used with larger salmonids (Halver 1957; Aoe, Masuda, Abe, Saito, Toyoda & Kitamura 1970). Therefore, a comparison of performance between alevins and larger juvenile rainbow trout provides additional information on ontogenic differences in diet utilization. An increased mortality of alevins offered exclusively a semi-purified diet would be anticipated whereas, juvenile fish, with 'adult-like' digestive tracts would be expected to show a dose–response relationship of nutrients and growth-enhancing substances on growth.

Two experiments were conducted to examine supplemental effects of maca tuber meal on growth, feed utilization, survival, and sex ratio in rainbow trout, *Oncorhynchus mykiss*, alevins (experiment I) at the first exogenous feeding and in juveniles (experiment II). Possible actions of maca tuber meal as a disease-resistance enhancing ingredient and steroidogenic substance (sex reversal effect) as well as a feed attractant were also investigated.

Material and methods

Experiment I

Four casein-based semi-purified diets were formulated to be isonitrogenous and isocaloric to contain four different levels of the maca tuber meal at the expense of wheat meal. The compositions of the four experimental diets and chemical composition of maca tuber meal used are shown in Table 1. Proximate and mineral compositions of the diets met the requirements of the nutrients for salmonids (Shearer 1988; National Research Council 1993), except for potassium and magnesium (Table 1). Maca tuber meal was incorporated into the diets as follows: no maca tuber meal (control), 5% maca tuber meal (M-5), 10% maca tuber meal (M-10), and 15% maca tuber meal (M-15). Maca tuber meal used in this study was provided from the National Agriculture University of La Molina (Lima, Peru). Five percent of fish protein concentrate (CPSP 90, Sopropeche S.A., Boulogne-Sur-Mer, France) was supplemented in the diets to enhance palatability of the semi-purified experimental diets. The diets were cold-pelleted into 2.0-mm diameter size with distilled water, then freeze-dried to have less than 5% moisture, crushed into desirable particle size (0.4–2.0 mm), and stored at -20 °C until use.

Rainbow trout alevins at the stage of first feeding (0.096 ± 0.002 g initial weight) were randomly distributed into groups of 40 fish per tank, three tanks per dietary treatment. Each experimental diet was fed to groups of fish with the feeding rates ranging from 6.0% of fish weight at the beginning to 3.0% at the end of the first feeding trial. The fish were fed four times per day, 7 days per week. The feeding trial was conducted for 15 weeks in 40-L glass aquariums, supplied with UV irradiated and filtered semi-circulated water at a flow rate of 1.0–1.5 L aquarium^{-1} min^{-1}. Supplemental aeration was also provided to maintain dissolved oxygen levels near saturation. The water contained 3.8 mg L^{-1} K, 11.1 mg L^{-1} Mg, 27.0 mg L^{-1} Ca, 16.1 mg L^{-1} Na and temperature gradually increased from 14.1 °C to 21.2 °C. Diurnal light:dark cycle was regulated at 13:00:11:00 hours. Total fish weight in each tank was measured every 2 weeks for growth and feeding rate adjustment. Feeding was stopped 18 h prior to weighing. All

Table 1 Ingredients and compositions of diets used for experiments I and II

	Diets			
	Control	**M-5**	**M-10**	**M-15**
Ingredients				
Casein*	40.00	40.00	40.00	40.00
Gelatin*	8.00	8.00	8.00	8.00
Dextrin*	6.25	6.25	6.25	6.25
Wheat meal†	15.00	10.00	5.00	0.00
Maca tuber meal‡	0.00	5.00	10.00	15.00
Fish protein concentrate§	5.00	5.00	5.00	5.00
Cod liver oil*	14.00	14.00	14.00	14.00
Vitamin mix¶	4.00	4.00	4.00	4.00
Mineral mix‖	3.00	3.00	3.00	3.00
Ascorbic acid#	0.05	0.05	0.05	0.05
Carboxymethylcellulose*	2.00	2.00	2.00	2.00
L-arginine*	0.50	0.50	0.50	0.50
L-methionine*	0.40	0.40	0.40	0.40
L-lysine*	0.80	0.80	0.80	0.80
Choline chloride*	1.00	1.00	1.00	1.00
Proximate composition (%)				
Protein	57.4	59.6	58.7	58.6
Lipid	16.9	16.6	16.8	16.2
Ash	5.43	5.48	5.45	5.45
Mineral composition				
P (%)	0.86	0.85	0.86	0.86
K (%)	0.26	0.34	0.42	0.50
Ca (%)	0.66	0.67	0.68	0.69
Na (%)	0.30	0.29	0.29	0.29
Mg (%)	0.02	0.03	0.03	0.03
Fe ($\mu g\,g^{-1}$)	54.5	53.9	55.1	57.9
Mn ($\mu g\,g^{-1}$)	99.2	98.1	97.8	97.5
Zn ($\mu g\,g^{-1}$)	30.4	33.6	33.6	39.2

*ICN Biomedicals (Costa Mesa, CA, USA).

†Wheat meal contained the following chemical composition (%): crude protein, 11.7; crude lipid, 1.2; ash, 0.4.

‡Maca tuber meal contained the following chemical composition (%): crude protein, 13.7; crude lipid, 5.22; ash, 5.57; moisture, 3.75; P, 0.34; K, 1.79; Ca, 0.44; Mg, 0.087; Fe, 0.0086; Na, 0.0235; Zn, 0.0043; and Mn, 0.0017.

§CPSP 90, Sopropeche S.A.

¶Roche performance premix composition per g of vitamin mixture: vitamin A, 2646 IU; vitamin D_3, 221 IU; vitamin E, 66.1 IU; vitamin B_{12}, 13 µg; riboflavin, 13.2 mg; niacin, 61.7 mg; D-pantothenic acid, 22.1 mg; menadione, 1.32 mg; folic acid, 1.76 mg; pyridoxine, 4.42 mg; thiamin, 7.95 mg; D-biotin, 0.31 mg (Hoffman-La Roche, Nutley, NJ, USA).

‖Five milligrams Se in the form of sodium selenite per kg Bernhart Tomarelli salt mixture (ICN).

#Mg-L-ascorbyl-2-phosphate (Showa Denko K.K., Tokyo, Japan).

procedures and handling of animals during both experiments were conducted in compliance with the guidelines of the Institutional Laboratory Animal Care and Use Committee, The Ohio State University.

At the end of the feeding trial, all fish were weighed and percent weight gain (body weight gain × 100/initial body weight), feed conversion ratio (FCR) (dry feed consumed/body weight gain), and cumulative survival were calculated. Blood was collected from the caudal vein of four fish randomly selected from each tank (total 12 fish per dietary treatment) with a heparinized syringe. Total haemoglobin was determined with Sigma Diagnostic Kits (procedure no. 525) by using human haemoglobin solution as standard. Livers were removed from the fish and weighed to calculate hepatosomatic index [HSI; (liver weight × 100)/body weight]. Five fish per tank (total 15 fish per dietary treatment) were killed for the whole-body proximate analysis and mineral composition. Analyses of proximate composition were performed by standard procedures (Association of Official Analytical Chemists 1995). Whole-body lipids were extracted according to the procedure of Foch, Lees & Standley (1957). Mineral composition of whole

body were determined by the inductively coupled plasma (ICP) emission spectrophotometric method using ARI-3560 Spectrometer (Applied Research Labs, Valencie, CA, USA) according to Watson & Isaac (1990).

For the determination of sex ratio, fish from control and M-15 groups were used. Gonads were removed from three fish per tank of control groups (total nine fish) and four fish per tank of M-15 groups (total 12 fish). Gonad tissues were preserved in Bouin's fixative, dehydrated through a graded series of alcohol and embedded in paraffin. Sections were cut at 5–7 μm, mounted on glass slides and stained routinely with haematoxylin and eosin. Total erythrocyte and leucocyte levels were studied using a microscopic counting technique as described by McCarthy, Stevenson & Robert (1973). For the white blood cell counting, blood films were prepared immediately after the blood collection. The films were fixed in methanol for 5 min, stained by May–Grünwald solution for 3 min followed by 50% May–Grünwald solution (diluted with 5-mM phosphate buffer, pH 7.0) for 1 min, and diluted with Giemsa solution (1:10 with 5-mM phosphate buffer) for 15 min. Slides were then rinsed with distilled water and air-dried. A total of 100 leucocytes were counted for each sample and the percentage of different types of leucocytes were calculated.

Experiment II

The second feeding trial was conducted to expand the findings with alevins into juveniles. Juvenile rainbow trout that had been fed with a commercial diet (1.56 ± 0.02 g initial weight) were randomly distributed into groups of 25, two groups per treatment. Fish were fed one of three experimental diets, commercial (Rangen, Buhl, ID, USA), control (casein–gelatin based; Table 1), and M-15 (Table 1) for 8 weeks with the feeding rates ranging from 3.5% of fish weight at the beginning to 3.0% at the end of the feeding trial (re-adjusted for the increased fish size). The culture conditions were similar to those reported in experiment I. The water temperature gradually increased from 20.0 °C to 22.7 °C.

A feed acceptance test (instantaneous feed intake) was conducted at the conclusion of the feeding trial to determine the palatability effect of maca tuber meal on diet consumption. Fish were fed *ad libitum* and the feed amounts that fish consumed were calculated as a percentage of fish weight. The test was conducted always in the morning after overnight fasting by feeding to apparent satiation, one meal a day, for 4 consecutive days. A different observer carried out each test and the mean (± SD) was calculated.

At the end of the experiment, fish were weighed and percent weight gain and FCR were calculated. Two fish were randomly selected per tank for the determinations of haematocrit and haemoglobin, and five fish per tank for whole-body proximate analysis and mineral composition. Haematocrit was determined by the microhaematocrit method (Brown 1980).

Statistical analysis

All data were subjected to one-way ANOVA test, and Tukey's multiple comparison test was used by the SPSS statistical package (Version 10.0, SPSS, Chicago, IL, USA). Percentages were arcsine transformed before analysis. Differences were considered significant at $P < 0.05$.

Results

Proximate composition of the diets (Table 1) revealed that diets were isonitrogenous and major essential minerals were at the required levels. The only exception were the concentrations of potassium showing suboptimal level in control, M-5 and M-10 diets (Shearer 1988).

In experiment I, growth rate was significantly increased in fish fed maca tuber meal-supplemented diets in comparison with the control groups (Fig. 1). The significantly different growth rate was evident from the eighth week to the end of the first experiment at 15 weeks. Among the maca tuber meal supplemented groups, the fish fed diets supplemented with 10% (M-10) and 15% (M-15) maca tuber meal exhibited significantly higher growth rates than fish fed a diet supplemented with 5% maca tuber meal (M-5). Feed efficiency, protein efficiency ratio (PER), and specific growth rate (SGR) were also significantly improved in the maca tuber meal-supplemented groups in comparison with control group (Table 2). No significant differences were found in HSI among treatment groups.

There were significant differences in cumulative survival of the alevins during experiment I (Fig. 1). Supplementation of maca tuber meal significantly increased survival of alevins. Major mortality was observed during the first 2 weeks of feeding

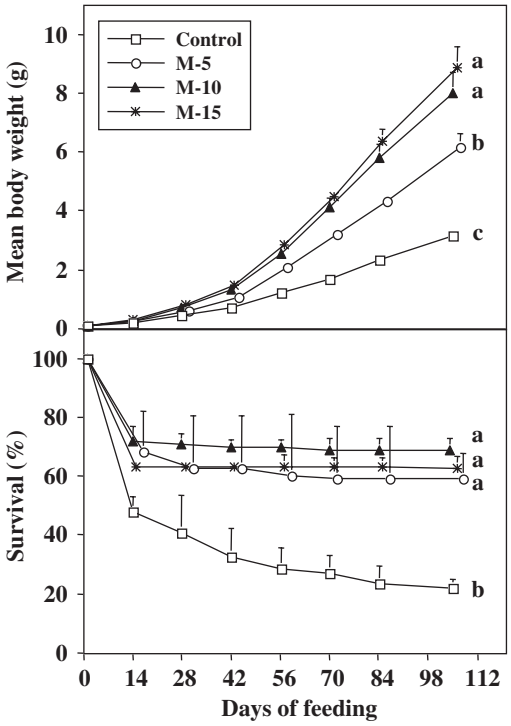

Figure 1 Mean body weight (top) and cumulative survival (bottom) of rainbow trout alevin fed four different experimental diets supplemented with different levels of maca tuber meal during 15 weeks. Experimental diets were designated as control, M-5, M-10, and M-15 for 0%, 5%, 10%, and 15% maca tuber meal inclusion respectively. Fish began to show significantly ($P<0.05$) different growth rates at the eighth week. Values ± SD having different letters are significantly different ($P<0.05$).

semi-purified experimental diets. No further significant mortality was observed in the groups fed maca tuber meal-supplemented diets after the first 2 weeks of feeding, whereas fish fed the control diet exhibited continuous steady mortality.

The period of sex differentiation is the most sensitive to possible action of phytochemicals with 'steroid activity'. However, there was no significant difference in sex ratios between the control and M-15 groups examined (data not shown). Regardless of the dietary treatment, histological analysis revealed that ovaries contained oocytes at the perinucleolar stage, while testes were filled with spermatogonia in both control and M-15 groups (Fig. 2).

We did not find significant differences in haemoglobin after 15 weeks of feeding (Table 2). No significant differences were found in percentage of each category of leucocytes, such as lymphocytes, neutrocytes, eosinophils, basophils, monocytes, and thrombocytes among treatments (data not shown). We did find, however, significantly increased total leucocyte counts in fish fed diets supplemented with maca tuber meal (Table 2).

In whole-body chemical compositions of rainbow trout (Table 3), there were no significant differences in crude protein and lipid among the treatments. Whole-body ash and water were significantly higher in the control group than in other dietary groups. In mineral compositions, significantly higher concentrations of phosphorus, calcium, and zinc were found in the control group than in other dietary groups,

Table 2 Weight gain, feed conversion ratio (FCR), protein efficiency ratio (PER), specific growth rate (SGR), hepatosomatic index (HSI), haemoglobin concentration, and leucocyte counts of fish fed four experimental diets supplemented with different levels of maca tuber meal after 15 weeks of experiment I*

	Diets			
	Control	**M-5**	**M-10**	**M-15**
Weight gain (%)†	3282 ± 163^{c}	6459 ± 457^{b}	8430 ± 743^{a}	9352 ± 743^{a}
FCR‡	1.22 ± 0.08^{a}	1.02 ± 0.06^{b}	1.00 ± 0.03^{b}	1.00 ± 0.04^{b}
PER§	1.46 ± 0.09^{b}	1.63 ± 0.09^{ab}	1.70 ± 0.05^{a}	1.71 ± 0.06^{a}
SGR (%)¶	3.35 ± 0.05^{c}	3.98 ± 0.07^{b}	4.23 ± 0.08^{a}	4.33 ± 0.07^{a}
HSI (%)‖	1.63 ± 0.37^{a}	1.20 ± 0.11^{a}	1.00 ± 0.05^{a}	1.02 ± 0.03^{a}
Haemoglobin (g dL^{-1})	8.21 ± 0.96^{a}	10.21 ± 0.40^{a}	9.81 ± 1.06^{a}	9.28 ± 0.81^{a}
Leucocyte count (thousand cm^{-2})	10.20 ± 3.10^{b}	18.20 ± 4.21^{a}	20.19 ± 3.18^{a}	17.67 ± 4.81^{a}

*Means of triplicate groups; values (±SD) having different superscripts in the same row are significantly different ($P<0.05$).
†Weight gain (%) = [(final weight − initial weight) × 100]/initial weight.
‡Feed conversion ratio = dry feed intake (g)/wet body weight gain (g).
§Protein efficiency ratio = body weight gain (g)/protein intake (g).
¶Specific growth rate (%) = [(ln final weight − ln initial weight) × 100]/days.
‖Hepatosomatic index (%) = [liver weight (g) × 100]/body weight (g).

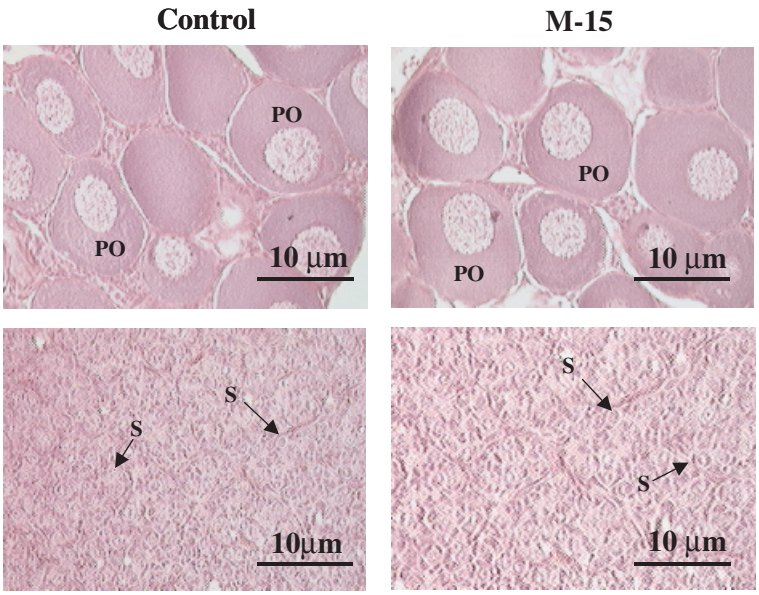

Figure 2 Cross-section of ovaries (top) and testis (bottom) of juvenile rainbow trout fed the control (left) and M-15 (right) diets after the first feeding trial. PO, perinucleolar oocyte; S, spermatogonia.

Table 3 Whole-body proximate and mineral composition of rainbow trout fed four experimental diets supplemented with different levels of maca tuber meal after experiment I*

	Diets			
	Control	**M-5**	**M-10**	**M-15**
Proximate composition (%)				
Protein	65.1 ± 3.13[a]	60.7 ± 2.88[a]	61.8 ± 3.22[a]	60.2 ± 4.41[a]
Lipid	37.0 ± 3.52[a]	44.1 ± 0.92[a]	43.9 ± 3.20[a]	40.9 ± 2.79[a]
Ash	11.7 ± 0.39[a]	10.4 ± 0.30[b]	9.88 ± 0.70[b]	9.88 ± 0.18[b]
Water	72.8 ± 0.81[a]	69.5 ± 0.32[b]	69.2 ± 0.21[b]	69.2 ± 0.56[b]
Mineral composition (%)				
Phosphorus	1.85 ± 0.10[a]	1.38 ± 0.33[b]	1.36 ± 0.05[b]	1.40 ± 0.06[b]
Potassium	0.87 ± 0.01[a]	0.72 ± 0.27[a]	0.84 ± 0.04[a]	0.84 ± 0.01[a]
Calcium	2.93 ± 0.18[a]	2.21 ± 0.41[b]	2.06 ± 0.16[b]	2.13 ± 0.10[b]
Magnesium	0.073 ± 0.003[a]	0.052 ± 0.019[a]	0.057 ± 0.002[a]	0.057 ± 0.003[a]
Zinc	0.019 ± 0.002[a]	0.012 ± 0.004[b]	0.013 ± 0.000[b]	0.012 ± 0.000[b]

*Values (± SD) are means of triplicate samples. Each sample was prepared by pooling five randomly selected fish per tank. Values having different superscripts in the same row are significantly different ($P < 0.05$).

whereas no significant differences were found in potassium and magnesium among the treatments.

In experiment II (Table 4), juvenile rainbow trout fed the diet supplemented with 15% maca tuber meal showed a significantly higher growth rate compared with other fish fed either control (no maca tuber meal) or a commercial trout diet. FCR and SGR followed the same trend as seen in growth rate, but PER was not significantly different between commercial and M-15 groups. The instantaneous feed intake (palatability test) indicated that supplementation of maca tuber meal up to 15% or more in the diet could increase the feed acceptance of diets for rainbow trout juveniles. There was a trend of increased palatability in M-15 groups in comparison with the control groups, even though there were no significant differences among the three dietary groups in the four separate times of the test. In experiment II, we did not find any mortality or any other body malformations during the 8-week trial. There were no significant

Table 4 Weight gain, feed conversion ratio (FCR), protein efficiency ratio (PER), specific growth rate (SGR), instantaneous feed intake (IFI), haematocrit and haemoglobin concentration of fish fed three test diets after 8 weeks of experiment II*

	Diets		
	Control	**Commercial**	**M-15**
Weight gain (%)†	236 ± 0.50[b]	245 ± 15.3[b]	303 ± 17.0[a]
FCR‡	1.28 ± 0.01[a]	1.24 ± 0.04[a]	1.08 ± 0.02[b]
PER§	1.39 ± 0.01[b]	1.53 ± 0.00[a]	1.52 ± 0.05[a]
SGR (%)¶	2.16 ± 0.00[b]	2.21 ± 0.08[b]	2.49 ± 0.08[a]
IFI (%)‖	2.28 ± 0.35[a]	2.14 ± 0.04[a]	2.50 ± 0.26[a]
Hematocrit (%)	31.5 ± 4.44[a]	31.0 ± 4.89[a]	32.8 ± 3.40[a]
Haemoglobin (g dL^{-1})	8.68 ± 1.25[a]	10.00 ± 1.28[a]	10.20 ± 0.48[a]

*Values (± SD) having different superscripts are significantly different ($P < 0.05$).
†Weight gain (%) = [(final weight − initial weight) × 100]/initial weight.
‡Feed conversion ratio = dry feed intake (g)/wet body weight gain (g).
§Protein efficiency ratio = body weight gain (g)/protein intake (g).
¶Specific growth rate (%) = [(ln final weight − ln initial weight) × 100]/days.
‖Instantaneous feed intake (%) = [dry feed intake (g) × 100]/body weight (g).

differences in haematocrit, haemoglobin, and whole-body proximate and mineral compositions in fish in experiment II.

Discussion

The growth results indicated that supplementation of maca tuber meal in feed can increase and/or accelerate growth rate of rainbow trout alevins at the first stage of exogenous feeding. FCRs were significantly improved in experimental treatments in comparison with control diet, both in alevins and juveniles of rainbow trout. The increased growth rate seemed to be attributable to the palatability of maca tuber meal supplemented in the semi-purified diets. However, quantitative data is required to compare the effect of maca tuber meal on feed acceptability in rainbow trout alevins. Another explanation could be that the presence of phytoestrogen (β-sitosterol) and other phytochemicals, such as quercetin and isothiocyanates (Li *et al.* 2001), in the maca tuber meal may stimulate growth hormone in fish. The oestrogenic effect of β-sitosterol has been reported in fish species, such as brown trout *Salmo trutta lacustris* (Lehtinen *et al.* 1999), goldfish *Carassius auratus* (MacLatchy & Van Der Kraak 1995), and rainbow trout (Mellanen, Petänen, Lehtimäki, Mäkelä, Bylund, Holmbom, Mannila, Oikari & Santti 1996; Tremblay & Van Der Kraak 1998). Studies have shown that oestrogen promotes the growth of yellow perch *Perca flavescens* (Malison, Best, Kayes & Amundson 1985) and the production of growth hormone in goldfish (Trudeau, Somoza, Nahorniak & Peter 1992; Zou, Trudeau, Cui, Brechin, Mackenzie, Zhu, Houlihan & Peter 1997). Also, testosterone was reported to have highly positive effect on growth hormone gene expression in goldfish (Huggard, Khakoo, Kassam & Habibi 1996). Taken together, the result of increased growth rate in the present study is very significant. It was, to our knowledge, the first study of maca supplementation in the semi-purified diets in rainbow trout alevins, which demonstrated the effect on survival and growth. Furthermore, the fish fed diet supplemented with maca tuber meal up to 15% in the present study showed growth rates comparable or higher (SGR, % growth rate day^{-1}, 4.33 ± 0.07) than those in other studies of rainbow trout alevins or juveniles (Springate & Bromage 1985; Blanc 2002).

Interestingly, we found that the survival of alevins can be improved by maca tuber meal supplementation (Fig. 1). The high mortality observed during the first 2 weeks was due to the low feed intake of semi-purified experimental diets at the first exogenous feeding. The delay of the first exogenous feeding has pronounced effects on success in growth and survival of salmonid alevins (Escaffre & Bergot 1985). Therefore, we expect that this result will stimulate a series of studies on the utilization of maca tuber meal in starter diets for fish.

The higher concentration of ash in the control group seemed to be due to the lower condition factors (robustness; not measured) of the fish fed the control diet. Potassium and magnesium concentrations in the experimental diets increased as maca tuber meal supplementation increased (Table 1), but no significant

differences were found in the whole body of fish fed the experimental diets for 15 weeks (Table 3). We conclude based on potassium concentrations in the body that in our experimental conditions this mineral, contrary to the suggestions made by Shearer (1988) about essentiality of dietary potassium, was not responsible for differences in growth.

Increased white blood cell numbers may be considered as a sign of improved immunity and disease resistance in rainbow trout alevins, and can be the result of maca tuber meal supplementation. However, this conclusion needs to be further examined, particularly as the differences in survival among treatments were significant (Fig. 1). A high antioxidant activity in maca meal (Sandoval *et al.* 2002) may be responsible for the increased immunity and resistance to pathological antigens in the alevins. Many medicinal plants have been shown to stimulate lymphocyte proliferation and white blood cell numbers, for instance, in rats treated with aqueous extracts of *Uncaria tomentosa* (Sheng, Bryngelsson & Pero 2000).

In experiment II, the growth rate of fish was also comparable or higher (SGR, % growth rate day^{-1}, 2.49 ± 0.08) than those in other studies of similar size rainbow trout juveniles (Watanabe, Pongmaneerat, Sato & Takeuchi 1993; Skonberg, Yogev, Hardy & Dong 1997). The reason for the lack of difference between PER for commercial and M-15 groups was possibly the different levels of crude protein in the two diets (51% and 56% in commercial and M-15 diets respectively).

Most of the existing literature on maca meal in mammals including humans (Zheng *et al.* 2000; Gonzales *et al.* 2001a, b) refers to its strong anabolic effects and increased fertilizing ability of sperm, further linked to a pharmacological action of macamides (Ganzera, Zhao, Muhammad & Khan 2002). However, in the present study, sex ratio and gonad development were not affected in rainbow trout, which were offered diets containing maca meal (up to 15%).

In conclusion, the findings in the present study suggest that a maca tuber meal inclusion, at least at 5% level in diets, could improve feed utilization, which will then result in higher growth rate in fish. The supplementation of the 5% maca tuber meal into diets increases white blood cells and potentially disease resistance. Also, 5% maca tuber meal incorporation in diets can increase survival of rainbow trout alevins at the first stage of exogenous feeding. Further studies are required to demonstrate the nature of phytochemicals and possible mechanisms of action in order to use maca tuber meal in fish diets to improve immune functions, thereby increasing disease and stress resistance.

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