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Research Article

Growth Performance and Mitochondrial Function in Juvenile Rainbow Trout (*Oncorhynchus mykiss*) Fed Graded Dietary Lipid Levels

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Abstract

A 2x3 factorial study was conducted to evaluate the effects of dietary lipid level on growth, feed utilization and mitochondrial enzyme activities in juvenile all-female rainbow trout (*Oncorhynchus mykiss*). Isonitrogenous diets with 400 g kg-1 crude protein, formulated to contain 100 (diet 40/10), 200 (diet 40/20) and 300 (40/30) g kg-1 dietary lipid, were fed to triplicate groups of high-feed efficient (F136) and low-feed efficient (F120) families with 217.66±2.24 g and 205.47±1.27 g initial average mass, respectively, for 90 days. At the end of the experiment, F120 fish fed diet 40/20 showed highest growth ($P<0.05$). Feed intake was not affected by the treatments ($P>0.05$). The genetic background (family effect) did not affect feed utilization ($P>0.05$). Dietary lipid level beyond 200 g kg-1 decreased feed efficiency and increased fat content in fish ($P<0.05$). The complex II activity in the liver, complex V activity in the intestine and the activities of complexes II and IV in the muscle were affected by the interaction diet x family; the diet and the genetic variation simultaneously affected the complex III activity in the muscle ($P<0.05$). The high feed efficiency is associated with high complex III activity in the intestine and the muscle. Results also suggest that genotype x diet interactions should be accounted for when considering strategies for using mitochondrial function as a criteria in rainbow trout selection programs for improved growth performance characteristics.

INTRODUCTION

Oxidative phosphorylation is the main process through which energy (ATP) is produced in the intermediary metabolism. This process occurs in mitochondria, which confers a central role to this organelle in nutrient utilization. The electron transport chain, which is located in the inner membrane of mitochondria, comprises five multi-subunit enzyme complexes, Complex I (NADH: ubiquinone oxidoreductase); Complex II (succinate: ubiquinone oxidoreductase); Complex III (ubiquinol: ferricytochrome c oxidoreductase); Complex IV (cytochrome c oxidase) and ATP synthase sometimes referred to as Complex V [1], as well as two mobile electron carriers, ubiquinone and cytochrome *c* [2-4]. It has been found that mitochondria generate about 90% of the energy in animal cells through the oxidation of proteins, carbohydrates and lipids [2]. Lipids are the main source of energy and essential fatty acids which are needed for normal growth and development of fish. In aquaculture, the main source of lipid used in aqua feed is fish oil, which is supplied at a dietary level generally ranging between 150 and 300 g kg^{-1} diet in intensive farming of salmonids, including rainbow trout *Oncorhynchus mykiss* [5-7], although fish also grow well when the dietary level is increased to up to 470 g kg^{-1} diet [8]. An important reason for the use of high-fat diet is the protein sparing effect of lipid [9-11], which increases farm profit as fish oil is cheaper than fish meal, and decreases phosphorus, nitrogen and solid compounds loads to the environment. Recently, the increasing fish oil and fish meal prices due to the global development of aquaculture during the last three decades, the limitation of the availability of these ingredients as a consequence of the overexploitation of seas and oceans, and the environmental concerns related to their production, has spurred scientific interest in 1) searching

for alternative to fish oil and fish meal through the partial or total use of ingredients from plant origin, and 2) in limiting the fish oil and fish meal content of aqua feed to the strict minimum needed for maximum growth and health [12].

When the fish oil and meal are reduced or their ratios in feed are changed, little is known about the effects on the molecular systems in fish that may impact its growth and health. Although many studies have been conducted on the effects of variation in dietary lipid levels on mitochondria function in terrestrial animals [13-18], only a few of such studies have been conducted in fish [19-23]. These studies on fish have shown that dietary lipid (and protein), affects the mitochondrial function, by inducing changes in the enzyme activities of the complexes of the electron transport chain and in the genes encoding these complexes. For instance, using a crude protein (CP) content of 420 g kg^{-1}, graded dietary lipid levels (100-300 g kg^{-1}) induced changes in the activities of complexes I-V [22] and in the genes encoding the expression of complexes I, IV-COX2 and V in the liver of rainbow trout [23]. In addition, it was found that a diet containing 420 g kg^{-1} CP and 200 g kg^{-1} crude lipid led to the highest growth and feed efficiency in rainbow trout, and these important zoo technical parameters were associated with high mitochondrial function [22]. Nevertheless, because of the constraints associated with the use of fish meal and fish oil in intensive aquaculture (see above), the present study was conducted to investigate the relation between growth, feed efficiency and mitochondrial function in low- and high-feed efficient (FE) families of juvenile rainbow trout (*Oncorhynchus mykiss*) fed graded dietary lipid levels and a fixed protein level at 40% of the diet.

MATERIALS AND METHODS

All the procedures used in this experiment were validated by the West Virginia State University Institutional Board and were in accordance with the guide for the care and use of animals in agricultural research and testing of the Federation of Animal Science Societies [24].

Fish and facility

Two different families of juvenile all-female rainbow trout, of the same age, designated F120 (high-feed efficient) and F136 (low-feed efficient) were obtained from the USDA-ARS National Center of Cool and Coldwater Aquaculture (NCCCWA) in Lee town, West Virginia, USA. The families were generated from sires and dams that were evaluated for feed efficiency under laboratory conditions as part of the NCCWA's growth improvement lines, and the FE potential of these families was based on the FE and growth performances of their parents. Out of the 96 families with different FE potentials that were produced by the NCCCWA, the parents used to generate F136 (high-FE) family showed the highest average parental body weight at 10 months post hatch (579.2 g and average breeding value for the body weight at 10 months post hatch was 57.65 g), while the parents used to generate the F120 (low-FE) family had 375.5 g average parental body weight at 10 months post hatch and average breeding value of -62.09 g for the body weight at 10 months post hatch. However, the fish used in this study had not previously been evaluated for either growth performance or feed efficiency traits. The fish were reared in an indoor flow-through system comprising glass tanks (152 L each) supplied with a continual flow of de chlorinated city water at a rate of 1.5 L min^{-1}. Water temperature was thermostatically controlled at 12±2°C and each tank was individually aerated to maintain a dissolved oxygen concentration above 6.5 mg L^{-1}. A 12 hr light and 12 hr dark photoperiod was simulated with electrically timed fluorescent lights in a temperature-controlled room. The fish were acclimated to the system for 14 days during which they were hand-fed the experimental diet containing 400 g crude protein and 100 crude lipid kg^{-1} diet, to apparent satiation twice daily. Then, fish from the same family were randomly distributed in 9 tanks, with a density of 10 fish per tank, for a total of 18 tanks. The initial weight ± SD of F120 fish was 217.66±2.24 g while that of F136 fish was 205.47±1.27 g.

Feed, feeding and data collection

Three experimental practical diets were formulated to contain a crude protein level fixed at 400 g kg^{-1} diet and crude lipid levels of 100 (diet 40/10), 200 (diet 40/20), and 300 g kg^{-1} diet (diet 40/30), respectively (Table 1). Each of these diets was randomly assigned to triplicate tanks per family, in a 3 x 2 factorial experimental design (3 diets x 2 families x 3 replications = 18 tanks or experimental units).

All ingredients were supplied by Rangen (Buhl, ID, USA). The ingredients were ground to less than 0.5 mm in a pin mill (Alpine, Hosokawa Micron Powder Systems, Summit, NJ, USA), sized with a screener (Rotex, Cincinnati, OH, USA), and weighed to produce 80-kg batches of each diet. Initially a portion of the diet ingredients were mixed with the vitamin and mineral premixes and additional supplements. The remaining diet ingredients were then added to the previous mixture and subsequently mixed for an additional 6 min (No. 4A Buffalo mixer, John E. Smith's Sons Co., Buffalo, NY, USA). Diets were extruded on a pilot-scale, single-screw extruder (Wenger X85, Wenger, Inc., Sabetha, KS, USA) to produce 4-mm pellets. Water (22-16 kg hr^{-1}) and steam (10.5-7.5 kg hr^{-1}) were injected into the pre-conditioner and water (5.7-4.2 kg hr^{-1}) and steam (6.4-6.1 kg hr^{-1}) were injected into the barrel during extrusion. Water and steam levels varied due to compositional differences among test diets. Pellets were dried in a variable speed circulation batch dryer (Proctor and Schwartz, Division of Wolverine (MA) Corp., Horsham, PA, USA), air cooled, and top-coated with lipid using a 22.7 kg capacity cement mixer (Kushland portable mixer, Kushlan Products, Inc., Goldendale, WA). The oil was added incrementally to each 22.7 kg batch of feed in the mixer.

The 400 g kg^{-1} crude protein level was selected because it is similar to the lowest level usually used in commercial trout production, while the three fat levels were selected to produce low-, medium- and high nutrient-dense diets. Diets were isonitrogenous and dietary energy varied with dietary lipid level (Table 1). During the experiment, fish were hand-fed to apparent satiation twice daily (7 days $week^{-1}$) at 08:00 and 16:00, for 90 days. Satiation feeding was achieved by allowing fish to eat until feeding activity stopped. Then, uneaten feed was collected by syphoning and stored at -20°C until dry matter analysis. This dry matter content of the uneaten feed was used to estimate the quantity of waste feed, which was in turn subtracted from the total quantity of feed served to obtain the daily feed consumption.

Table 1: Feed formulations and proximate composition of experimental diets fed to two families of rainbow trout (*Oncorhynchus mykiss*) during 90 days.

Ingredient (g kg^{-1} diet, as-fed basis)[2]	Experimental diets[1]		
	40/10	40/20	40/30
Menhaden fish meal (690 g kg^{-1} crude protein)	300.00	300.00	300.00
Soybean meal (470 g kg^{-1} crude protein)	150.00	150.00	150.00
Blood meal (880 g kg^{-1} crude protein)	30.00	50.00	60.00
Feather meal (840 g kg^{-1} crude protein)	50.00	50.00	50.00
Wheat flour (110 g kg^{-1} crude protein)	90.00	90.00	90.00
Brewer's yeast (460 g kg^{-1} crude protein)	20.00	20.00	20.00
Wheat midds (150 g kg^{-1} crude protein)	286.50	162.50	47.50
Vitamin premix	4.00	4.00	4.00
Mineral premix	1.00	1.00	1.00
Stay-C	1.40	1.40	1.40
Choline chloride	5.80	5.80	5.80
Dicalcium phosphate	4.00	4.00	4.00
Calcium propionate	1.30	1.30	1.30
Fish oil	56.00	160.0	265.00
Total	1000.00	1000.00	1000.00
Proximate composition			
Crude protein (g kg^{-1})	409.13	407.83	399.27
Fat (g kg^{-1})	100.23	200.41	301.94
Moisture (g kg^{-1})	78.47	52.87	70.31
Ash (g kg^{-1})	84.46	77.63	75.81
Gross energy (kJ g^{-1})	192.84	213.31	243.22

[1]Diets formulated to contain 400 g kg^{-1} protein and 100 g kg^{-1} fat (40/10), 400 g kg^{-1} protein and 200 g kg^{-1} fat (40/20) or 400 g kg^{-1} protein and 300 g kg^{-1} fat (40/30).
[2]All ingredients were supplied by Rangen (Buhl, ID, USA).

Fish in each aquarium were fasted for 24 hours prior to biweekly sampling and 48 hours at the final sampling. The fish were anaesthetized with amino benzoic acid ethyl ester (MS-222) by transferring them to water containing the anesthetic (8 mg L^{-1}), then they were individually weighed and counted biweekly (data not shown). When fish were removed for weighing, the aquaria were cleaned thoroughly and drained. Fish were observed daily for unusual behavior, morphological changes or mortality. At the end of the 90-day feeding trial, fish in each aquarium were killed (overdose of MS-222, 300 mg L^{-1}), individually weighed and counted. On three fish randomly chosen from each aquarium, a transverse slice of muscle (approximately 100 mg) located beneath the dorsal fin, the liver (~50-100 mg), and the intestine (~100 mg, proximal part of the intestine, just after the stomach) were collected for mitochondrial enzyme activity analysis. The whole viscera and visceral fat of these three fish were also weighed. The following parameters were determined for each treatment: Percent weight gain, WG = [(final weight – initial weight) (initial weight^{-1})] x100; Feed efficiency, FE = wet weight gain (dry feed fed^{-1}); Feed intake expressed as percent body weight (bw) per day, FI = dry weight of feed consumed over the 90-day study x [(initial weight + final weight) x 2^{-1}]$^{-1}$ x number of days^{-1} x 100; Protein efficiency ratio, PER = live weight gain (protein intake^{-1}); Protein productive value, PPV = [(final protein content–initial protein content) (protein consumed^{-1})]; Lipid productive value, LPV = [(final lipid content–initial lipid content) (lipid consumed^{-1})]; Visceral fat, VF = (mesenteric fat/ body weight)*100; Viscerosomatic index, VSI = (visceral weight / fish weight)*100; Hepatosomatic index, HSI = (liver weight / fish weight)*100; and Survival (%) = (final number of fish/initial number of fish)*100. Three fish from each tank were randomly chosen and homogenized for the determination of the whole body proximate composition.

ANALYTICAL PROCEDURES

a. Proximate Composition Analyses

Proximate composition of the experimental diets (Table 1) and whole body composition of the fish were measured at the conclusion of the experiment. Whole body proximate composition and energy were determined following AOAC [25]. Percent moisture was determined by drying to a constant weight in an oven at 80°C. Ash content was determined using a muffle furnace at 600°C for 3 h. Total nitrogen was measured using a Leco TruSpec N Nitrogen Determinator (LECO Corporation, St. Joseph, Michigan), and protein calculated as N x 6.25. Fat was determined by Chloroform-Methanol extraction procedure [25].

b. Extraction of mitochondria and measurements of enzyme activities

For enzyme activity analysis, the tissues selected in which the oxidative capacity may reflect growth rates of fish are: liver, which has a high metabolic capacity for oxidation and synthesis of numerous metabolites; digestive tract, which determines rates of nutrient assimilation; and the skeletal muscle, which has predominant role in the deposition of material during growth, locomotion and whole-body metabolic homeostasis. The extraction of mitochondria from liver tissues followed the procedure developed Suarez & Hochachka [26], while the extraction from on muscle and intestine tissues followed the procedure developed by Birch-Machin & Turnbull [27] and Kirby et al. [28] These procedures were described in details and successfully used by Eya et al. [19,20]. The mitochondrial suspensions were divided into aliquots, immediately frozen in liquid nitrogen and stored at -80°C for the spectrophotometric measurement of the activities of individual complexes I-V. Total protein concentration in the mitochondrial extracts was measured using the method of Lowry et al. [29] as adapted by Peterson [30].

The activities of the respiratory chain enzymes were determined at 28°C using Spectronic Genesys 2 spectrophotometer after rupture of the mitochondrial membrane by two freezing (in liquid nitrogen) and thawing (in ice cold water, 2-4°C) cycles. All assays were performed in duplicates in a Spectrosil quartz cuvette to a final volume of 1 mL. All enzyme activities were expressed in milliunits mg^{-1} mitochondrial protein with one unit of enzyme activity corresponding to the appearance of 1µmole of product, or consumption of 1µmole of substrate, per minute.

Complex I (NADH: Ubiquinone Oxidoreductase, EC 1.6.5.3), Complex II (Succinate: ubiquinone1 oxidoreductase, EC 1.3.5.1), Complex IV (cytochrome c oxidase, EC 1.9.3.1) and Complex V (F1-ATP synthase, EC 3.6.3.14) activities were assayed according to the procedure developed by Birch-Machin & Turnbull [27] and Kirby et al. [28] which was described in details and successfully used by Eya et al [19-23]. The complex III (ubiquinol: ferricytochrome *c* reductase, EC 1.10.2.2) was measured following the method developed by Jeejeebhoy [31], which was described in details and successfully used by Eya et al [19-23].

c. Respiratory Control Ratio Measurements (RCR)

Due to the fibrous nature of the intestine and muscle tissues, mitochondria could not be isolated consistently with the inner membrane intact in order to obtain reliable and repeatable results, despite the use of several different protocols. The integrity of the inner membrane, where the electron transport chain is imbedded, is needed for respiratory control measurements. Thus, the intestine and muscle were eliminated from this portion of the study. Oxygen consumption of hepatic mitochondria was measured polarographically according to Estabrook [32] with an Instech Model 110 Fiber Optic Oxygen Monitor (Instech Laboratories, Inc., Plymouth Meeting, PA) equipped with a magnetic stirrer and thermostatically-controlled chamber set to 12°C. Oxygen consumption rate was expressed as nmol of monomeric oxygen min^{-1} mg^{-1} of protein. The oxygen electrode system was calibrated according to Darley-Usmar et al. [33] using air-saturated distilled water and sodium dithionite ($Na_2S_2O_4$)-treated water as the high and low parameters, respectively. Equal amounts (0.5 mg mL^{-1}) of mitochondrial protein from each sample were added to the respiration chamber containing 250 µL of an assay medium (25 mM KH_2PO_4, 10 mM Tris, 100 mM KCl, and 2.7 mg mL^{-1} BSA, pH 7.4) and the rate of oxygen consumption was measured for one minute. Ten mM of sodium succinate hexahydrate (Alfa Aesar #33386) was then added and the rate measured for two minutes. Mitochondrial function was assessed with the addition of 0.4 mM of adenosine 5′-diphosphate sodium salt (ADP) (Sigma #A2754), which causes a sudden burst in oxygen consumption rate as it is being phosphorylated into ATP. The RCR was calculated by dividing the rate of state 3 respirations (in the presence of ADP) by the state 4 rates (resting respiration after all the ADP has been phosphorylated) according to Chance and Williams [34].

d. Statistical analysis

Preliminary data analysis using ANCOVA with initial body weight as covariate proved that responses were not influenced by the initial body weight of fish. Thus, data were re-analyzed using a two-way ANOVA and least significant difference procedure [35] using Statistical Analysis System version 9.0 software (SAS Institute Inc., Cary, North Carolina, USA). Each aquarium was used as an experimental unit (n=3) and a significant level of $P<0.05$ was used.

RESULTS

Fish growth and feed utilization

There was a significant interaction between the diet and fish family on growth (WG) ($P<0.05$; Table 2). The FI was not affected by the treatments and ranged between 1.39 and 1.56 % bw day^{-1} ($P>0.05$; Table 2).The effect of diet was significant on FE ($P<0.05$; Table 2). There was a significant effect of the diet on PER, PPV, LPV and LER ($P<0.05$; Table 3). The general trend of growth showed that. Numerically, F120 fish fed diet 40/20 had the highest growth followed by the group of F120 fish fed diet 40/10 and F136 fish fed diet 40/10 that had an intermediary growth, while the group of fish comprising F120 fish fed diet 40/30, F136 fish fed diet 40/20 and 40/30 had the lowest growth. With the same FI, fish fed diets 40/10 and 40/20 showed a similar FE, which was higher than that of those fed diet 40/30. Fish fed diet 40/20 showed higher PER than those fed diet 40/30, although no significant difference was found between fish fed diets 40/10 and 40/20 on the one hand, and between fish fed diets 40/10 and 40/30 on the other hand. Fish fed diets 40/10 and 40/20 had a similar PPV, which was higher than that of fish fed diet 40/30. Fish fed diets 40/20 and 40/30 had a similar LPV, which was lower than that of fish fed diet 40/10. Fish fed diet 40/10 had the highest LER, followed by those fed diet 40/20, while fish fed diet 40/30 had the lowest value.

Fish survival and body composition

Fish survival was not affected by the treatment ($P>0.05$) and ranged from 96 to 100 %. The diet effect was significant on protein, lipid and visceral fat contents of the fish as well as in the HSI ($P<0.05$; Table 4). The family effect was significant on lipid, moisture and visceral fat contents as well as on the

Table 2: Mean percent weight gain (WG), daily feed consumption (FC), and feed efficiency (FE) of two families of rainbow trout (*Oncorhynchus mykiss*) fed practical diets containing graded levels of dietary lipid for 90 days[1].

Families[2]	Practical diets[3]	Initial weight g	Final weight g	WG (% initial)	FI (% bw day^{-1})	FE (g gain g^{-1} feed)
Individual treatment means						
F120	40/10	214.873	545.827	154.023^{ab}	1.497	1.159
F120	40/20	218.827	619.693	183.189^{a}	1.398	1.084
F120	40/30	219.077	469.370	114.249^{c}	1.564	1.033
F136	40/10	205.566	521.155	153.522^{ab}	1.453	1.125
F136	40/20	207.733	480.299	131.210^{bc}	1.396	1.287
F136	40/30	205.607	484.770	135.775^{bc}	1.457	0.858
Pooled SEM				10.796	0.065	0.076
Main effect means						
F120				150.487	1.486	1.092
F136				140.169	1.435	1.090
	40/10			153.770	1.475	1.142^{a}
	40/20			157.200	1.397	1.186^{a}
	40/30			125.010	1.510	0.946^{b}
ANOVA: P values						
Family (F)				0.265	0.357	0.980
Diet (D)				0.022	0.242	0.019
Interaction (F x D)				0.015	0.725	0.080

[1]Means represent average values of three tanks. The LSD procedure was applied on individual means when the two-factor interaction was significant; the LSD procedure was applied on main effect means when the interaction was not significant. Individual treatment means and main effect means and main effect means within a column followed by different superscript letters were found to differ at 0.05 probability level.

[2] Families of fish selected experimentally for high feed efficiency (F136) and low feed efficiency (F120) by USDA-ARS National Center of Cool and Coldwater Aquaculture (NCCCWA) in Leetown, WV, USA.

[3] Diets formulated to contain 400 g kg^{-1} protein and 100 g kg^{-1} fat (40/10), 400 g kg^{-1} protein and 200 g kg^{-1} fat (40/20) or 400 g kg^{-1} protein and 300 g kg^{-1} fat (40/30).

Table 3: Mean values for protein efficiency ratio (PER), protein productive value (PPV), lipid productive value (LPV), and lipid efficiency ratio (LER) of two families of rainbow trout (*Oncorhynchus mykiss*) fed practical diets containing graded levels of dietary lipid for 90 days[1].

Families[2]	Practical Diets[3]	PER (g g^{-1})	PPV (g g^{-1})	LPV (g g^{-1})	LER (g g^{-1})
Individual treatment means					
F120	40/10	2.593	0.271	0.146	0.110
F120	40/20	3.048	0.227	0.114	0.063
F120	40/30	2.060	0.199	0.061	0.029
F136	40/10	2.665	0.283	0.196	0.114
F136	40/20	2.566	0.271	0.101	0.054
F136	40/30	2.494	0.207	0.108	0.035
Pooled SEM		0.180	0.025	0.018	0.005
Means of main effect					
F120		2.567	0.232	0.107	0.067
F136		2.575	0.254	0.135	0.068
	40/10	2.630^{ab}	0.277^{a}	0.171^{a}	0.112^{a}
	40/20	2.807^{a}	0.249^{ab}	0.108^{b}	0.059^{b}
	40/30	2.277^{b}	0.203^{c}	0.084^{b}	0.032^{c}
ANOVA: P values					
Family		0.957	0.313	0.086	0.908
Diet		0.035	0.036	0.001	<0.001
Interaction (F x D)		0.074	0.739	0.189	0.255

[1]Means represent average values of three tanks. The LSD procedure was only applied on main effect means because the two-factor interaction was not significant. Main effect means within a column followed by different superscript letters were found to differ at 0.05 probability level.

[2] Families of fish selected experimentally for high feed efficiency (F136) and low feed efficiency (F120) by USDA-ARS National Center of Cool and Coldwater Aquaculture (NCCCWA) in Leetown, WV, USA.

[3] Diets formulated to contain 400 g kg^{-1} protein and 100 g kg^{-1} fat (40/10), 400 g kg^{-1} protein and 200 g kg^{-1} fat (40/20) or 400 g kg^{-1} protein and 300 g kg^{-1} fat (40/30).

Table 4: Mean values for proximate composition (wet weight), visceral fat (VF), viscerosomatic index (VSI) and hepatosomatic index (HSI) of two families of rainbow trout (*Oncorhynchus mykiss*) fed practical diets containing graded levels of dietary lipid for 90 days[1].

Families[2]	Practical diets[3]	Proximate composition						
		Protein (g kg^{-1})	Lipid (g kg^{-1})	Moisture (g kg^{-1})	Ash (g kg^{-1})	VF (g kg^{-1})	VSI (g kg^{-1})	HSI (g kg^{-1})
Individual treatment means								
F120	40/10	194.33	48.81	740.54	16.74	36.69	98.89	12.54
F120	40/20	189.38	61.39	735.55	21.23	46.38	110.16	12.28
F120	40/30	184.04	65.78	736.55	17.39	54.08	119.64	11.07
F136	40/10	194.04	61.08	726.50	16.79	20.35	95.11	13.96
F136	40/20	189.44	64.84	731.75	16.32	28.06	97.18	11.34
F136	40/30	182.76	96.20	711.65	17.07	38.39	103.80	10.89
Pooled SEM		3.06	5.96	4.79	1.65	3.79	6.01	0.70
Means of main effect								
F120		189.25	58.66^{b}	737.54^{a}	18.45	45.72^{a}	109.57^{a}	11.96
F136		188.75	74.04^{a}	723.30^{b}	16.72	28.93^{b}	98.70^{b}	11.99
	40/10	194.19^{a}	54.94^{c}	733.52	16.76	28.52^{c}	97.00	13.25^{a}
	40/20	189.41^{ab}	63.12^{b}	733.64	18.77	37.22^{b}	103.67	11.81^{ab}
	40/30	183.40^{b}	80.99^{a}	724.10	17.22	46.23^{a}	111.71	10.88^{b}
ANOVA: P values								
Family (F)		0.843	0.008	0.003	0.223	<0.001	0.047	0.953
Diet (D)		0.0.14	0.003	0.114	0.465	0.002	0.088	0.017
Interaction (F x D)		0.975	0.110	0.130	0.282	0.937	0.592	0.249

[1]Means represent average values of three tanks. The LSD was only applied on for main effect means because the two-factor interaction was not significant. Main effect means within a column followed by different superscript letters were found to differ at 0.05 probability level.
[2] Families of fish selected experimentally for high feed efficiency (F136) and low feed efficiency (F120) by USDA-ARS National Center of Cool and Coldwater Aquaculture (NCCCWA) in Leetown, WV, USA.
[3] Diets formulated to contain 400 g kg^{-1} protein and 100 g kg^{-1} fat (40/10), 400 g kg^{-1} protein and 200 g kg^{-1} fat (40/20) or 400 g kg^{-1} protein and 300 g kg^{-1} fat (40/30).

Table 5: Mean values for respiratory control ratio (RCR) for glutamate, succinate, and pyruvate in the liver of two families of rainbow trout (*Oncorhynchus mykiss*) fed practical diets containing graded levels of dietary lipid for 90 days[1].

Families[2]	Practical Diets[3]	Glutamate	Succinate	Pyruvate
		(nmols of monomeric oxygen min^{-1} mg^{-1} of protein)		
Individual treatment means				
F120	40/10	4.390^{c}	4.013^{ab}	4.555
F120	40/20	4.380^{c}	3.860^{bc}	5.263
F120	40/30	4.402^{c}	3.873^{bc}	4.458
F136	40/10	4.530^{b}	3.962^{b}	4.700
F136	40/20	4.835^{a}	4.157^{a}	4.677
F136	40/30	4.448^{c}	3.750^{c}	4.645
Pooled SEM		0.022	0.057	0.284
Means of main effect				
F120		4.391	3.916	4.759
F136		4.604	3.956	4.674
	40/10	4.460	3.988	4.628
	40/20	4.608	4.008	4.970
	40/30	4.425	3.812	4.551
ANOVA: P values				
Family (F)		<.001	0.398	0.721
Diet (D)		<.001	0.009	0.327
Interaction (F x D)		<.001	0.007	0.343

[1]Means represent average values of three tanks. The LSD procedure was applied on individual means when the two-factor interaction was significant. The LSD procedure was applied on main effect means when the two-factor interaction was not significant. Individual treatment means and main effect means and main effect means within a column followed by different letters were found to differ at 0.05 probability level.
[2] Families of fish selected experimentally for high feed efficiency (F136) and low feed efficiency (F120) by USDA-ARS National Center of Cool and Coldwater Aquaculture (NCCCWA) in Leetown, WV, USA.
[3] Diets formulated to contain 400 g kg^{-1} protein and 100 g kg^{-1} fat (40/10), 400 g kg^{-1} protein and 200 g kg^{-1} fat (40/20) or 400 g kg^{-1} protein and 300 g kg^{-1} fat (40/30).

VSI index (P<0.05; Table 4). Fish fed diet 40/10 showed higher protein content than those fed diet 40/20, although no significant difference was found between fish fed diets 40/10 and 40/30 on the one hand, and between fish fed diets 40/20 and 40/30 on the other hand. Fish fed diet 40/30 had the highest lipid and VF contents, followed by those fed diet 40/20, while fish fed diet 40/10 had the lowest value. Fish fed diet 40/10 showed higher HSI than those fed diet 40/30, although no significant difference was found between fish fed diets 40/10 and 40/20 on the one hand, and between fish fed diets 40/20 and 40/30 on the other hand. F136 fish showed higher lipid content than F120 fish. F120 fish showed higher moisture and VF contents, as well as HSI than F136 fish.

Mitochondrial respiratory control ratio (RCR)

There was a highly significant interaction between the family and the diet in terms of RCR for glutamate and succinate (P<0.01; Table 5), while the RCR for pyruvate was not affected by the treatments and ranged from 4.458-4.700 nmols of monomeric oxygen min^{-1} mg^{-1} of protein (P>0.05; Table 5). When glutamate was used as substrate, RCR was highest in the liver of F136 fish fed diet 40/20, followed by F136 fish fed diet 40/10, and lowest in F136 fish fed diet 40/30 and all the other F120 fish. When succinate was used as substrate, RCR was highest in the liver of F136 fish fed diet 40/20, followed by F136 fish fed diet 40/10, and lowest in F136 fish fed diet 40/30, and there was not a significant difference between fish F136 fish fed diet 40/10 and all the F120 fish on the one and between F136 fish fed diet 40/30 and F120 fish fed diets 40/20 and 40/30.

Mitochondrial complex enzyme activities

Liver: The effect of the diet was significant on mitochondrial complex I activity, while that of the family was significant on the activity complex IV activity, and that of the interaction family x diet was significant on the activity complex II (P<0.05; Table 6). The dietary treatments did not affect the activities of complexes III, IV and V, which ranged from 119.61 to 145.38 milliunits mg^{-1} mitochondrial protein, 194.05 to 206.75, and from 29.20 to 31.54 milliunits mg^{-1} mitochondrial protein, respectively (P>0.05; Table 6). Fish fed diets 40/10 and 40/30 showed similar mitochondrial

Table 6: Degree of differential respiratory enzymatic (Complex I: NADH - Ubiquinone Oxidoreductase; Complex II: Succinate -Ubiquinone Oxidoreductase; Complex III: ubiquinol: ferricytochrome *c* reductase; Complex IV: Cytochrome *c* oxidase; and Complex V: F_1- ATP synthase) activities in the liver of two families of rainbow trout (*Oncorhynchus mykiss*) fed practical diets containing graded levels of dietary lipid for 90 days [1].

Families[2]	Practical diets[3]	Liver complexes				
		I	II	III	IV	V
		(milliunits mg^{-1} mitochondrial protein)[4]				
Individual treatment means						
F120	40/10	12.755	38.369^{ab}	143.076	147.388	29.320
F120	40/20	9.006	32.272^{bc}	130.602	153.967	31.670
F120	40/30	11.704	19.206^{e}	102.125	171.996	28.781
F136	40/10	11.996	29.093^{cd}	147.683	240.702	29.086
F136	40/20	10.092	42.390^{a}	146.844	245.396	31.412
F136	40/30	12.111	23.850^{de}	137.238	241.513	31.567
Pooled SEM		1.057	2.402	12.419	16.681	1.077
Means of main effect						
F120		11.155	29.949	125.268	157.784^{b}	29.924
F136		11.390	31.778	143.922	242.537^{a}	30.689
	40/10	12.361^{a}	33.731	145.379	194.045	29.203
	40/20	9.549^{b}	37.331	138.723	199.681	31.541
	40/30	11.907^{a}	21.528	119.681	206.754	30.174
ANOVA: P values						
Family		0.790	0.370	0.091	<0.001	0.402
Diet		0.044	<0.001	0.142	0.752	0.135
Interaction (F x D)		0.677	0.004	0.485	0.736	0.303

[1]Means represent average values of three tanks. The LSD procedure was applied on individual means when the two-factor interaction was significant. The LSD procedure was applied on main effect means when the two-factor interaction was not significant. Individual treatment means and main effect means and main effect means within a column followed by different letters were found to differ at 0.05 probability level.

[2] Families of fish selected experimentally for high feed efficiency (F136) and low feed efficiency (F120) by USDA-ARS National Center of Cool and Coldwater Aquaculture (NCCCWA) in Leetown, WV, USA.

[3] Diets formulated to contain 400 g kg^{-1} protein and 100 g kg^{-1} fat (40/10), 400 g kg^{-1} protein and 200 g kg^{-1} fat (40/20) or 400 g kg^{-1} protein and 300 g kg^{-1} fat (40/30).

[4] One unit of enzyme activity corresponds to the appearance of 1 µmole of product, or consumption of 1µmole of substrate per minute.

complex I activity, which was higher than that of fish fed diet 40/20. F136 fish showed higher complex IV activity than F120 fish. F136 fish fed diet 40/20 had the highest complex II activity, while the lowest value was obtained in F120 fish fed diet 40/30; there was no difference in complex II activity between F120 fish fed diets 40/10 and 40/20, between F120 fish fed diet 40/20 and F136 fish fed diet 40/10, between F136 fish fed diets 40/10 and 40/30, and between F120 fish and F136 fish fed diet 40/30.

Intestine: The effect of the diet was significant on mitochondrial complex III activity, while that of the family was significant on the activity of complexes I and IV, and that of the interaction family x diet was significant on the activity complex V ($P<0.05$; Table 7). The treatments did not affect complex II activity, which ranged from 25.80 to 33.16 milliunits mg^{-1} mitochondrial protein ($P>0.05$; Table 7). F136 fish showed higher complex I activity than F120 fish, while the opposite result was found with complex IV activity. Fish fed diets 40/20 and 40/30 showed similar mitochondrial complex III activity, which was higher than that of fish fed diet 40/10. F136 fish fed diet 40/30 had the highest complex V activity, followed by the group comprising F136 fish fed diet 40/10 and F120 fish fed diets 40/10 and 40/20, while the lowest value was obtained in F120 fish fed diet 40/30; there was no difference in complex V activity between diet F136 fish fed diets 40/20 and 40/30 and between F136 fish fed diet 40/20 and the group comprising F136 fish fed diet 40/10 and F120 fish fed diets 40/10 and 40/20.

Muscle: The effect of the diet was significant on the activities of mitochondrial complexes I and III, while that of the family was significant on the activity of complex III, and that of the interaction family x diet was significant on the activity of complexes II and IV ($P<0.05$; Table 8). The treatments did not affect complex V activity, which ranged from 52.76 to 62.75 milliunits mg^{-1} mitochondrial protein ($P>0.05$; Table 8). Fish fed diets 40/10 and 40/20 showed similar activity for the complexes I and III, which was lower than that of fish fed diet 40/30 for complex I, and which was higher than that of fish fed diet 40/30 for complex III. F120 fish showed higher complex I activity than F136 fish. F136 fish fed diet 40/10 had higher complex II activity and all the other treatment groups, which had a similar complex II activity. F136 fish fed diets 40/20 and 40/30 had a similar complex IV activity, which was the highest followed by the group comprising F120 fish fed diet 40/30 and F136 fish fed diet 40/10, while the

Table 7: Degree of differential respiratory enzymatic (Complex I: NADH - Ubiquinone Oxidoreductase; Complex II: Succinate -Ubiquinone Oxidoreductase; Complex III: ubiquinol: ferricytochrome *c* reductase; Complex IV: Cytochrome C oxidase; and Complex V: F_1- ATP synthase) activities in the intestine of two families of rainbow trout (*Oncorhynchus mykiss*) fed practical diets containing graded levels of dietary lipid for 90 days[1].

Families[2]	Practical diets[3]	Intestine complexes				
		I	II	III	IV	V
		(milliunits mg^{-1} mitochondrial protein)[4]				
Individual treatment means						
F120	40/10	12.743	25.5479	158.581	121.105	50.466[b]
F120	40/20	11.599	30.135	251.396	162.138	45.794[b]
F120	40/30	12.535	26.168	203.044	147.241	34.404[c]
F136	40/10	15.608	33.163	150.748	113.025	50.128[b]
F136	40/20	22.836	28.506	218.273	114.472	55.547[ab]
F136	40/30	19.799	25.801	222.427	120.548	61.929[a]
Pooled SEM		1.799	2.465	19.035	12.035	3.291
Means of main effect						
F120		12.292[b]	27.284	204.340	143.495[a]	43.555
F136		19.414[a]	29.157	197.149	116.015[b]	55.868
	40/10	14.174	29.355	154.664[b]	117.065	50.297
	40/20	17.218	29.320	234.834[a]	138.305	50.670
	40/30	16.167	25.984	212.735[a]	133.895	48.167
ANOVA: P values						
Family		0.004	0.370	0.652	0.016	0.006
Diet		0.267	0.326	0.003	0.218	0.721
Interaction (F x D)		0.107	0.169	0.413	0.295	0.004

[1]Means represent average values of three tanks. The LSD procedure was applied on individual means when the two-factor interaction was significant. The LSD procedure was applied on main effect means when the two-factor interaction was not significant. Individual treatment means and main effect means and main effect means within a column followed by different letters were found to differ at 0.05 probability level.

[2] Families of fish selected experimentally for high feed efficiency (F136) and low feed efficiency (F120) by USDA-ARS National Center of Cool and Coldwater Aquaculture (NCCCWA) in Leetown, WV, USA.

[3] Diets formulated to contain 400 g kg^{-1} protein and 100 g kg^{-1} fat (40/10), 400 g kg^{-1} protein and 200 g kg^{-1} fat (40/20) or 400 g kg^{-1} protein and 300 g kg^{-1} fat (40/30).

[4] One unit of enzyme activity corresponds to the appearance of 1 µmole of product, or consumption of 1µmole of substrate per minute.

Table 8: Degree of differential respiratory enzymatic (Complex I: NADH - Ubiquinone Oxidoreductase; Complex II: Succinate -Ubiquinone Oxidoreductase; Complex III: ubiquinol: ferricytochrome *c* reductase; Complex IV: Cytochrome *c* oxidase; and Complex V: F_1- ATP synthase) activities in the muscle from of two families of rainbow trout (*Oncorhynchus mykiss*) fed practical diets containing graded levels of dietary lipid for 90 days[1].

Families[2]	Practical diets[3]	Muscle complexes				
		I	II	III	IV	V
		(milliunits mg^{-1} mitochondrial protein)[4]				
Individual treatment means						
F120	40/10	17.949	26.153^{b}	131.466	37.831^{c}	61.893
F120	40/20	21.178	26.154^{b}	159.340	39.380^{c}	59.510
F120	40/30	23.785	20.939^{b}	100.878	58.931^{b}	52.764
F136	40/10	16.326	37.823^{a}	133.559	57.784^{b}	59.408
F136	40/20	16.930	20.346^{b}	111.693	80.966^{a}	55.514
F136	40/30	29.227	26.635^{b}	85.682	74.647^{a}	62.758
Pooled SEM		1.945	2.401	9.637	4.367	5.391
Means of main effect						
F120		20.971	28.268	130.561^{a}	45.380	58.056
F136		20.828	24.415	110.311^{b}	71.132	71.132
	40/10	17.138^{b}	31.988	132.512^{a}	47.807	60.651
	40/20	19.054^{b}	23.250	135.516^{a}	60.173	57.512
	40/30	26.506^{a}	23.787	93.280^{b}	66.789	57.761
ANOVA: P values						
Family		0.929	0.073	0.024	<0.001	0.795
Diet		0.001	0.005	0.001	0.003	0.814
Interaction (F x D)		0.071	0.010	0.066	0.026	0.392

[1]Means represent average values of three tanks. The LSD procedure was applied on individual means when the two-factor interaction was significant. The LSD procedure was applied on main effect means when the two-factor interaction was not significant. Individual treatment means and main effect means and main effect means within a column followed by different letters were found to differ at 0.05 probability level.

[2]Families of fish selected experimentally for high feed efficiency (F136) and low feed efficiency (F120) by USDA-ARS National Center of Cool and Coldwater Aquaculture (NCCCWA) in Leetown, WV, USA.

[3]Diets formulated to contain 400 g kg^{-1} protein and 100 g kg^{-1} fat (40/10), 400 g kg^{-1} protein and 200 g kg^{-1} fat (40/20) or 400 g kg^{-1} protein and 300 g kg^{-1} fat (40/30).

[4] One unit of enzyme activity corresponds to the appearance of 1 µmole of product, or consumption of 1µmole of substrate per minute.

lowest value was obtained in the group comprising F120 fish fed diets 40/10 and 40/20.

DISCUSSION

With the aim of reducing the dietary protein level in aquaculture feed, the objective of the present experiment was to study the relation between growth, feed efficiency and mitochondrial function in low- and high-feed efficient (FE) families of juvenile rainbow trout (*Oncorhynchus mykiss*) fed graded dietary lipid levels and a fixed protein level at 40% of the diet. The results showed that fish growth, respiratory control ration (RCR) for glutamate and succinate in fish liver, complex II activity un the liver, complex V activity in the intestine and the activities of complexes II and IV in the muscle were affected by the interaction diet x family; that the dietary lipid level solely affected feed utilization, crude protein content of the fish, complex I activity in the liver, complex III activity in the intestine, and complex I activity in the muscle; that the genetic variation (family effect) solely affected the moisture content of the fish, the VSI, the complex IV activity in the liver, and the activities of complexes I and IV in the intestine; and that the diet and the genetic variation simultaneously affected the lipid content of the fish, VF, and the complex III activity in the muscle.

High-FE fish (F136 family) fed diet 40/20 showed no difference in growth rate compared to the low-efficient family, which is inconsistent with the findings of Eya et al. [22] who observed that the maximum weight gain (WG) (487.51 % initial weight) of rainbow trout were reached with 200 g kg^{-1} dietary lipid, and no change in growth was obtained with a further increase in dietary lipid level. The insignificant difference in growth rate between the two families compared to the previous study could be several factors. First, the initial starting weight might have been a contributing factor and the fact that WG in the last study was more than the double of that observed in the present study is likely due to the combination of the facts that the initial weight of fish used in the former study (97.86-105.67 g) was twice as small as that used in the latter (present) study (205.46-217.89 g); the dietary protein level was higher in the former study (420 g kg^{-1} diet) than in the latter study (400 kg^{-1} diet); and the duration of the former study was longer (108 days) than that of the latter study (90 days). In the present study, the finding that an increase in the dietary lipid level from 100

to 300 g kg^{-1} did not affect FI is also inconsistent with previous works which showed a decrease in appetite caused by chronic exposure to high-fat diet, as a consequence of an attenuation of feedback signals that are related to feed intake by the abundance of dietary free fatty acids in the intestine in humans [36,37], and in rainbow trout fed a diet containing 420 g kg^{-1} crude protein [22]. In addition, it has been assumed that when a balanced diet is provided, fish adjust their FI so as to satisfy their energy requirements [38-40]. The fact that the increased in dietary lipid from 100 to 300 g kg^{-1} did not reduce feed intake (FI) in this study implies that rainbow trout rather adjusted its feed consumption in order to satisfy its protein requirement [41] as it was also the case in gilthead sea bream *Sparus aurata L.* [42], sea bass *Dicentrarchus labrax* [43,44], grass carp *Ctenopharyngodon idella* [45] and meager *Argyrosomus regius* [46] juveniles.

It is well documented that salmonids can perform well with diet containing up to 470 g kg^{-1} dietary lipid [8,47]. However, although rainbow trout fed a diet containing 400 g kg^{-1} crude protein does not seem to loose appetite when fed diet containing >200 g kg^{-1} dietary lipid, the FE, PER, PPV, LER were reduced when 300 g kg^{-1} dietary lipid was supplied. This reduction in nutrient utilization by feeding an excess dietary lipid is emphasized by the lowest fish growth observed in low-FE fish (F120) and high-FE fish (F136) fed diet 40/30 in this study. This result does not agree with previous work on salmonids which presented an improvement in feed utilization with an increase in dietary lipid level [48,49,9,50], however the result is in the line with the reduction in FE of juvenile sea bass *D. labrax* fed a diet containing 300 g kg^{-1} dietary lipid compared to those fed diets containing 120-240 g kg^{-1} dietary lipid [43]. An attempt to explain this finding is that in situation where excess dietary lipid is fed, an elevated proportion of lipid is used as energy to metabolize and store lipid in fish body [43]; this assumption is supported by the decrease in LER as dietary lipid increase in the present study, as it was the case in juvenile sea bass *D. labrax* [43], and also by the highest whole-body lipid content and VH recorded in fish fed diet 40/30 in the present study. Another explanation could be that a part of the excess dietary lipid has been excreted, as it is commonly accepted in fish nutrition that supplying dietary nutrients at levels higher than fish requirement leads to feed losses [51,47]. Excess dietary nutrient can also induce toxicity in fish [47], but because high mortality was not recorded rendering the limited data collected in this study insufficient to support this assumption. There was a positive correlation between the whole body lipid content and the dietary lipid level in the current study, which is in agreement with previous results that showed an increase in body lipid as a consequence of an increase in dietary lipid content in Salmonids [52,53,8,54-56]. However, excess body lipid content in food fish reduces its nutritional, organoleptic and commercial values [43].

All the information above, and the fact that PPV decreased as dietary lipid increase, indicate that there was no protein sparing effect of dietary lipid level higher than 200 g kg^{-1}. This suggests that the 400 g kg^{-1} crude protein level used in this study was sufficient to satisfy the protein requirement of rainbow trout, as the beneficial effects of a high dietary lipid level on protein utilization generally occurs in situation where dietary protein is limiting [11]. This assumption of satisfactory nutrient level by the diets used in this study is supported by the fact that the optimum dietary lipid level for maximum growth and feed efficiency for rainbow trout fed diet containing 350 g kg^{-1} crude protein has previously been estimated at 180 g kg^{-1} diet [48].

The environmental concerns about waste losses from aquaculture facilities and the rising cost of animal feed have spurred the development of animal breeding programs in order to select for families with improved feed utilization phenotype [4]. However, the results of the FE, PER, PPV, LPV and LER obtained in the present experiment suggests that the two experimental families selected on the basis of the improved growth rate of the dams and sires may not have genetic predisposition for improved feed efficiency compared with their parental lines. It has been observed in rainbow trout and other salmonids that the heritability values for feed efficiency is very low and ranges from 3±10% [57] and 6±10% [58].

Another aspect in this study was to link growth performance and feed utilization characteristics to mitochondrial respiratory control and enzymatic activities, which reflect mitochondrial energetic efficiency. This information is scarce for rainbow trout. Mitochondrial state 3 to state 4 respiratory ratio (RCR) was measured in the liver mitochondrial isolates to assess the metabolic activity of the whole tissue stimulated by NAD-and FAD-linked substrates. In this study the high hepatic RCR recorded (>3), when mitochondria were provided glutamate, succinate and pyruvate as energy source, indicated a high coupling of the electron transport chain and a good mitochondrial function [32] in all the fish groups. However, the liver mitochondria of rainbow trout oxidized substrate preferentially. Numerically, pyruvate was consistently the preferred as energy substrate, while glutamate was oxidized at higher rate than succinate. This was similar to the results obtained by Eya et al. [22]. This preferential substrate oxidation at mitochondrial level was previously noted by Guderley et al. [59]. Moreover, measurements of RCR showed an interactive effect of the family and diet with high FE designated rainbow trout fed a high-fat diet (42/30) exhibiting a significant decrease in RCR with both the NAD- and FAD-linked substrates (glutamate and succinate). The ATP synthesis rate is dependent on the genetic predisposition (family) of varying performance, rate and efficiency of substrate utilization, and parallels the coupling of oxidative phosphorylation. This observation, taken together with the LER and LPV data, suggest that excess lipid supplementation probably limits mitochondrial respiration, mainly through increase in energy expenditure that exceeds the obligatory cost of energy gain. This result implies that whole body energy expenditure and lipid oxidation in the liver are not coupled. This situation likely leads to a fall in hepatic mitochondrial energetic efficiency, with a subsequent wasteful increase in the oxidation of energetic substrates, such as fatty acids via NAD- and FAD-linked substrates. This is consistent with the findings of Iossa et al. [60] who reported a significant decrease in both state 3 and 4 respiratory capacities in liver and skeletal muscle of rats fed high-fat diet compared with the rats fed low-fat diet. It should also be noted that mitochondria from fish fed 42/30 diet showed a reduced preference for pyruvate oxidation at high lipid supplementation. The non-significant differences between fish families and among the diets for the rate of pyruvate oxidation suggests that pyruvate is unlikely to be the

major substrate for mitochondria oxidative action in rainbow trout liver at high lipid level, as a result of a probable shift in substrate preference toward alternate exogenous substrate such as free fatty acids.

Comparison between high-FE family and low-FE family showed that high FE fish had higher level of mitochondrial complex IV activity in the liver and muscle compared to low-FE. This may be due to increased energy production for higher anabolic activity in the liver and higher catabolic activity in the muscle. Because complex IV is recognized to play a major regulatory role in the respiratory chain as the last complex prior to the passage of electron to synthase, this complex has been established to reflect the nutritional state [61] and the aerobic metabolic rates [62] in several animal tissues. In contrast, higher IV activity in F120 fish intestinal tissue might reflect increased metabolic activity in this tissue, suggesting greater rates of splanchnic nutrient oxidation may negatively affect the amount of nutrients that reach the liver. Generally, there were tissue differences in mitochondrial respiratory enzyme activities and these findings are in concordance with studies that showed that mitochondria from different organ systems demonstrate morphological and functional differences [63] and there are examples of tissue-specific or organ-specific expression of mitochondrial proteins [64]. It is noteworthy that the high FE observed with dietary lipid level of 200 g kg^{-1} diet recorded seems to be associated to high activity of complex III in the intestine and in the muscle of rainbow trout. This observation is reinforced by the highest enzyme activity recorded with complex III, compared with the other respiratory chain complexes in these tissues (Tables 7 & 8). In fact, complex III, which is one of the five multi-protein enzyme complexes of the electron transfer chain, is responsible for electron transfer from ubiquinol to cytochrome c, and also links this electron transfer to proton pumping from the mitochondrial matrix into the inner membrane, creating a proton-motive force that leads to ATP synthesis when the protons are translocated back into the mitochondrial matrix through complex V [65,66,2-4]. This process through which complex III links electron transfer to proton translocation is known as proton motive Q cycle [67] and is considered to be one of the most important mechanisms of energy transduction in the cell [65,66]. In addition, complex III (together with complex I) is well-known as being the site of superoxide and hydrogen peroxide generation in mitochondrial respiratory chain [68-70]. Thus, a decrease in the activity of complex III will likely lead to the production of reactive oxygen species that impede mitochondrial function, as superoxide and hydrogen peroxide are associated to mitochondrial dysfunction, which is linked to some diseases and reduced FE phenotypic expression in animals [4]. In addition, the activity of complex III is well-known to affect that of other complexes in the mitochondrial respiratory chain. For instance, it has been demonstrated that respiratory Complex III is needed to maintain complex I activity in muscle mitochondria in mammalian systems [71]. In fish, only a few studies have related the high FE to increased mitochondrial function [72,19-23] but in other animals, such as broilers, genetic selection has also been conducted on the basis on the relation between mitochondrial function and FE phenotype [3, 4].

With regard to the regulation of mitochondrial complex enzyme activities by dietary factors in rainbow trout, our data showed a general reduction in complex II for all tissues in both families fed the 40/30 diet. A positive relationship was found between enzyme activities and nutrient utilization efficiencies, especially protein productive value, lipid productive value and lipid efficiency ratio. Since efficiency of nutrient utilization is higher for fat than for carbohydrates or protein [73], high fat diets tend to decrease rather increase energetic efficiency because of the wasteful increase in the oxidation of energetic substrates, such as fatty acids. It follows that in rainbow trout changes in nutrient utilization seem to parallel changes in diet composition and complex II activities of the mitochondrial oxidative phosphorylation chain, implying that greater growth of rainbow trout fed the 40/30 diet accompanied decreased nutrient utilization and may have been associated with decreased mitochondrial complex II enzyme activity. The decreased activity in the liver observed in fish fed the 40/30 diet could be a result of consuming more energy units per gram of feed, causing the mitochondria in their cells to increase their phosphorylation rate, a wasteful expenditure of fatty acids. This seems to be in agreement with a recent previous study by Iossa et al. [60], who observed that high fat feeding decreased the hepatic and muscle mitochondrial oxidative capacity. Because nutritional manipulation alters nutrient metabolism, it is likely that the effects on complex II activity is due to changes in mitochondrial protein synthesis, breakdown, or both.

Notwithstanding the higher whole body lipid deposition in both families of rainbow trout fed high lipid diet, the gross lipid retention efficiency was considerably lower in the high than the low lipid diet. This suggests that whilst economically desirable with regard to the development of nutrient-dense diets (high lipid supplementation) and feed inputs and to growth and body composition, higher dietary lipid level above 20% do not result in efficient use of lipids for sparing proteins.

CONCLUSION

In summary, the current study has demonstrated that when the dietary crude protein is fixed at 400 g kg^{-1} diet, the dietary lipid level beyond 200 g kg^{-1} diet decreased feed efficiency and increased fat content in rainbow trout, and that high feed efficiency is associated to high complex III activity in the intestine and the muscle in rainbow trout. It will be informative to conduct a further study on the genetic mechanism through which feed efficiency might be associated to complex III activity, through the analysis of the expression of the genes coding the complexes involved in electron transport chain.

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