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Optimization of dietary macronutrients for Atlantic salmon post-smolts using increasing ration levels

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ABSTRACT

Three fishmeal-based diets differing in macronutrient levels (crude protein/crude lipid (%) ratios of 40/26, 49/26 and 60/18) were studied in a requirement by ration level (RRL) trial with Atlantic salmon post-smolts. The diets were fed in excess (N = 6, 3, 3, respectively) and feed intake was estimated. Triplicate groups of fish were also fed each diet at ration levels of 20, 40 and 75% of the full ration. The fish were fed for 53 days.

In fish fed full rations, feed intake and growth rates were not significantly affected by dietary treatment, but feed efficiency was significantly higher in fish fed diets 49/26 or 60/18 compared with those fed diet 40/26. No significant effect of diet was found on utilization efficiency of digestible energy (DE) for energy gain above maintenance (pooled slope 0.82). DE maintenance was estimated at 38 kJ $kg^{-0.8}$ d^{-1}.

Linear regressions between digestible protein (DP) intake and protein gain were found for diets 49/26 or 60/18, but a second-order polynomial relationship was found for diet 40/26. Using the two highest ration levels for diet 40/26 (near satiety), the efficiency of utilization of DP for protein gain (k_{DP}) was 0.50. The k_{DP} values for diets 49/26 and 60/18 were 0.76 and 0.65, respectively ($P < 0.05$). Using data from the two lowest ration levels for diet 40/26 and the complete regressions for the other diets, the maintenance requirements for DP for the fish fed diets 40/26, 49/26 and 60/18 were 561, 542 and 651 mg $kg^{-0.7}$ d^{-1}, respectively.

The diminishing returns in protein growth as intake of diet 40/26 increased suggest that the dietary amino acid level was not high enough to elicit further protein gain. Diet 60/18 led to numerically higher growth rates, but diet 49/26 resulted in lower DP maintenance and higher efficiency of utilization of DP for growth above maintenance than diet 60/18. Thus, the results obtained with full-fed fish and those generated using the RRL technique, seem to indicate that diet 49/26 contains more optimal dietary nutrient and energy levels for post-smolts.

1. Introduction

The efficiency of utilization of nutrients and energy above maintenance may be determined by relating gradient intake levels of these components with growth, on a metabolic weight basis (Requirement by Ration Level, RRL). The efficiency of utilization of digestible protein (DP) on protein growth (k_{DP}) above maintenance has been reported to vary between 0.28 and 0.82, depending on fish species, diet production technique and chemical composition and, if the relationship is curvilinear, which part of the model was used to calculate the efficiency rate (Glencross, 2008; Glencross et al., 2007, 2008; Hatlen et al., 2007; Helland et al., 2010; Lupatsch et al., 1998). Hatlen et al. (2007) showed that a reduction in dietary protein from 65 to 54% and an increase in dietary lipid from 16 to 30% resulted in an increase in k_{DP} from 0.53 to 0.73 in Atlantic cod (*Gadus morhua*). This supports calculations made by Lupatsch et al. (2001) indicating increases in k_{DP} from about 0.35 to 0.60 when the digestible protein to digestible energy (DP/DE) ratio in diets fed to satiation to gilthead seabream (*Sparus aurata* L.) decreased from around 26 to 16 g MJ^{-1}. In salmonid diets, increasing concentrations of lupin kernel meal or lysine (Glencross et al., 2008; Grisdale-Helland et al., 2011a) did not affect k_{DP}, but k_{DP} was changed by the drying process of lupin protein concentrate (Glencross et al., 2007).

The efficiency of utilization of DE on energy growth (k_{DE}) above maintenance has been reported to vary between 0.31 and 0.88 (Booth and Allan, 2003; Grisdale-Helland et al., 2013; Huisman, 1976; Ohta and Watanabe, 1998; reviewed by Schrama et al., 2012). The efficiency of utilization of metabolizable energy (ME) on energy growth (k_{ME}) above maintenance has been shown to be greater for a high lipid diet compared with high protein or carbohydrate diets (Carter and Brafield, 1991; Schrama et al., 2012). Rodehutscord and Pfeffer (1999) summarized data for rainbow trout and found that k_{DE} was dependent on the dietary lipid level. In trials with gilthead seabream, Atlantic cod or yellowtail kingfish (*Seriola lalandi*) however, diet composition has not been found to affect k_{DE} (Booth et al., 2010; Hatlen et al., 2007; Lupatsch et al., 2001). Ohta and Watanabe

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(1998) and Glencross et al. (2007) showed though, that k_{DE} was affected by feed production technique.

The maintenance requirements for DP, DE or ME are estimated when gain of these components in the fish is zero. In many trials, fasted fish have been included in the relationships between intake and gain and thus, maintenance may be estimated where the regression crosses the x-axis. The linearity of the regression may not be affected by the inclusion of the fasted fish (Helland et al., 2010) or, as shown by Lupatsch et al. (1998), fasted fish may affect the estimation of maintenance when it is incorrectly assumed that the efficiency of utilization is equal below and above maintenance. When fasted fish are not included in the analysis, maintenance may be determined when the gain of the fish fed the lowest ration is less than or equal to zero, or by extrapolation to zero when the lowest intake level results in gain (Grisdale-Helland et al., 2011a; Hatlen et al., 2007; McGoogan and Gatlin, 1998; Ozório et al., 2009; Peres and Oliva-Teles, 2005; Pirozzi et al., 2010; Schrama et al., 2012). In the latter case, the y-intercepts may not be significantly different from zero and therefore, the maintenance requirement may not be estimable (Grisdale-Helland et al., 2011b). The maintenance requirements for DE and DP may be affected by temperature (Glencross and Bermudes, 2010; Lupatsch and Kissil, 2005; Pirozzi et al., 2010), but has not been shown to be affected by oxygenation (Glencross, 2009). Using linear regression between fasted fish and those fed the lowest ration level, Glencross et al. (2008) determined that there was no influence of lupin kernel meal inclusion level on the maintenance requirements for DE and DP in rainbow trout. Schrama et al. (2012) noted however, that the inclusion of the fasted fish equalizes the intercept of the regression lines, possibly biasing the estimated difference in the maintenance requirement for DE.

Growth trials with fish have indicated that replacing dietary protein with lipid improves feed utilization and reduces nitrogen waste (NRC, 2011). Protein may also be spared if replaced by carbohydrate (Grisdale-Helland and Helland, 1997; Hemre et al., 1995), but this is not always the case (Helland and Grisdale-Helland, 1998). The objective of this trial was to determine whether the RRL technique could aid in the evaluation of diets with different compositions on salmon growth and nutrient utilization.

2. Materials and methods

2.1. Diets

Three diets based on fish meal, fish oil and starch were formulated to contain different macronutrient compositions (dietary protein %/lipid % ratios of 40/26, 49/26 and 60/18; Table 1). The diets (3.0-mm pellets) were produced by extrusion by Nofima AS (Fyllingsdalen, Bergen, Norway). The diets contained yttrium oxide (Y_2O_3) as an inert marker for digestibility. The diets were analyzed for dry matter (DM) (105 °C, until constant weight), crude lipid (Soxtec HT6 after hydrolysis with HCl, Tecator, Höganäs, Sweden), nitrogen (crude protein (CP) = nitrogen × 6.25; Kjeltec Auto System, Tecator, Höganäs, Sweden) and ash (550 °C, overnight). Gross energy was measured using an adiabatic bomb calorimeter (Parr 6300, Parr Instrument Company, Moline, IL, USA). The amino acids (AA) in the diet were analyzed using a Biochrom 30 amino acid analyser (Cambridge, U.K.) following the EC Commission Directive 98/64/EC (1999), after hydrolysis in 6N HCl for 23 h at 110 °C. Tryptophan and tyrosine were analyzed after basic hydrolysis (Hugli and Moore, 1972). Yttrium was analyzed by inductivity-coupled plasma mass-spectroscopy (ICP) at AnalyCen (Moss, Norway).

Table 1
Formulation of experimental diets (g kg^{-1}).

Dietary protein %/lipid %	40/26	49/26	60/18
Fish meal[a]	524.8	662.8	793.7
NorSalmOil[b]	215.0	204.9	118.0
Pregeflo®M[c]	235.9	108.0	64.0
Wheat	10.0	10.0	10.0
Vitamin premix[d]	10.0	10.0	10.0
Mineral premix[d]	4.0	4.0	4.0
Carophyll Pink (10%)	0.2	0.2	0.2
Yttrium oxide	0.1	0.1	0.1

[a] Norse-LT 94, Norsildmel, Norway.
[b] NorSalmOil, Norsildmel, Norway.
[c] Pregelatinized native maize starch. Roquette Freres, Lestrem Cedex, France.
[d] As described by Mundheim et al. (2004).

2.2. Experimental setup

After a 2-day fast, groups of 19 Atlantic salmon (*Salmo salar*) post-smolts (Bolaks strain) grown at Nofima AS, Sunndalsøra, were weighed (initial weight, 105.4 ± 0.7 g; N = 39 tanks) and placed in 39 tanks (150 L) supplied with seawater (water flow, 5 L min^{-1}; 11.1 ± 0.7 °C) and constant 24-h light. The fish had been transferred to seawater one month prior to the start of the trial. Thirty fish were anesthetized (tricaine methanesulfonate, MS 222, Argent Chemical Laboratories Inc., Redmont, WA, USA), killed with a blow to the head, weighed and then the whole-bodies were stored at −20 °C until analysis.

The fish were fed from automatic feeders every 80 min. Excess rations (100% of satiation = full ration) of each diet were fed to triplicate groups of fish, except diet 40/26 that was fed to 6 groups. The effluent water of each tank was led into a wire mesh box to enable sieving of waste feed. To minimize leaching of the waste feed, the effluent water was directed to two different areas of the wire box using pinch valves on the water pipes, dependent on whether feeding was occurring. The feeding levels for each tank were adjusted every second day. The diets were also fed to triplicate groups at 20%, 40% and

Table 2
Chemical composition of experimental diets (g or MJ kg^{-1}).

Dietary protein %/lipid %	40/26	49/26	60/18
Dry matter (DM)	939	927	944
In DM			
Crude protein	400	488	597
Sum amino acids	314	387	466
Crude lipid	265	259	181
Ash	84	109	131
Carbohydrates[a]	251	144	91
Gross energy (GE)	24.3	24.5	22.9
Digestible energy (DE)[b]	21.6	22.5	21.0
DP/DE (g MJ^{-1})[c]	16.9	19.8	26.0
Amino acids[d]			
Ala	18.6	22.9	28.5
Arg	21.4	26.4	31.5
Asx	32.4	39.9	47.8
Cys	3.1	3.8	4.5
Glx	50.0	61.2	74.3
Gly	18.2	22.8	28.0
His	7.1	8.6	10.4
Ile	14.6	18.2	21.6
Leu	25.9	31.7	38.1
Lys	27.3	33.5	40.0
Met	10.2	12.4	14.9
Phe	13.8	17.1	20.2
Pro	13.6	16.8	20.0
Ser	13.5	16.6	20.2
Thr	13.9	17.2	20.8
Trp	3.3	4.3	5.0
Tyr	10.8	13.9	16.1
Val	16.4	20.2	24.6

[a] Carbohydrates calculated by difference; 100-crude protein–crude lipid–ash.
[b] Calculated using data for full-fed fish — see Table 3.
[c] DP/DE [Digestible protein (calculated using data for full-fed fish — see Table 3) to digestible energy ratio].
[d] Asx represents Asp and Asn (analyzed as Asp) and Glx represents Glu and Gln (analyzed as Glu).