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### Replacement of Fishmeal with Plant Protein Ingredients in Diets to Atlantic Salmon (Salmo salar) – Effects on Weight Gain and Accretion

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#### 1. Introduction

Farming of fish and especially farming of carnivorous fish depends on high protein diets. Atlantic salmon (Salmo salar) generally require 55-60% of dietary protein in juvenile stages thereafter the protein requirement declines (NRC, 1993). Historically the protein source has been fishmeal, produced from wild caught fish, while the lipid source has been the fish oil from the same source. Production of farmed Atlantic salmon in Norway has increased steadily during the last decade (from 440061 tons in year 2000 to 927876 metric tons in year 2010) while the wild fish catch has been stable during the same period of time (statistics from Directorate of Fisheries, Norway; www.fiskeridir.no). This also holds for the worldwide wild caught fish and aquaculture production (FAO; www.fao.org/fishery; Tacon, 1995; FAO, 2006; Tacon & Metian, 2008). Therefore if a continued increase of farmed fish is to occur, both alternative protein ingredients as well as lipid sources need to be assessed as feed ingredients. This is to be done by supporting both the growth and at the same time not compromising the health of the farmed fish. In future aquaculture these novel ingredients have to constitute the main protein and lipid sources in the fish diets. The main such sources are likely to be of plant origin but some animal- by-products and microbiologically produced proteins might be allowed by national and/or international legislation as well. Such animal-by-products may arrive from processing of poultry and swine (Sugiura et al., 1998; Yanik et al., 2003; Rahnema et al., 2005) and microbiological produced protein sources as single cell proteins (Storebakken et al., 2004; Ozyurt & Deveci, 2004; Berge et al., 2005). Plant proteins are the most likely candidates because of their abundance and relatively low cost. However, upon substituting the fishmeal with plant protein ingredients reduced growth performance generally occurs in Atlantic salmon (Olli et al., 1995; Refstie et al., 1998; 2000; Storebakken et al., 1998; Carter & Hauler, 2000; Krogdahl et al., 2003; Opstvedt et al., 2003) as well as in other fish species of commercial interest such as Atlantic cod, Gadus morhua (von der Decken & Lied, 1993; Hansen et al., 2007), sea bream, Sparus aurata (Robaina et al., 1995; Gòmez-Requeni et al., 2004), rainbow trout, Oncorhynchus mykiss (Pongmaneerat & Watanabe, 1992; Gomes et al., 1995; Kaushik et al., 1995) and turbot, Psetta maxima (Regost et al., 1999; Fournier et al., 2004). Increased feed conversion ratio (FCR) and reduced protein utilization are of major concern when fishmeal is replaced

with plant ingredients (Robaina et al., 1995; Refstie et al., 1998; 2000; Opstvedt et al., 2003). The reduced performance might be due to an imbalanced amino acid profile or simply due to lower voluntary feed intake in fish fed the diets based on plant ingredients.

Protein synthesis requires that all amino acids needed for the protein synthesis are present in the cell. If one amino acid is absent or present in lower concentrations than required for the synthesis to occur in any particular tissue, the protein cannot be synthesised and the constituent amino acids will be metabolised and used as fuel due to the simple fact that free amino acids are never stored in tissues (Geiger, 1947). The main amino acid depot is the muscle (Houlihan et al., 1995), but these amino acids are not available for protein metabolism until after the proteins are hydrolysed to free amino acids. Generally the free amino acids constitute less than 0.1% of the protein bound amino acids in animals (Njaa, 1990; Millward, 1988; 1999; Espe, 2008). Although the proteins are constantly turned over fish needs a continuous supply of indispensable amino acids (IAAs) as only about 50% of the protein bound amino acids are reutilised for protein synthesis (Houlihan et al., 1995). Recently, it was found that in addition to the IAAs, fish also seem to require the dispensable amino acids (DAA, Abbouti et al., 2009). Further, the delivery of adequate amounts of amino acids with a balanced profile is essential for maximal protein accretion and growth in fish (El-Mowafi et al., 2010). Animal derived protein ingredients have a balanced amino acid profile independent of being a beef muscle or a fish muscle and provide the indispensable amino acid profile required by the consumer or animal. For the formulation of diets this balanced amino acid profile is adopted by using the ideal protein concept in which the amount of each indispensable amino acid is presented relative to the amount of lysine (Wang & Fuller, 1987; 1989; Rollin et al., 2003). Further, any reduction in the apparent digestibility or presence of possible anti nutrients might add to the reduced absorption of nutrients in the fish fed the plant based diets and might add to the reduced performance. Both the reduced feed intake and the reduced absorption in fish fed such diets might change the availability of the balanced amino acid profile in tissue compartments. Any imbalances of amino acids in tissues may have a negative impact on the metabolism and significantly affect both the health and the protein and/or lipid deposition in the farmed fish and animals (Wu, 2009).

In fish as in domestic animals, the feed intake determines the weight gain. Traditionally feed intake has been of less concern when the diet fishmeal inclusion was high as fish had satisfactory voluntary feed intake to maximise the growth potential. But upon replacing the fishmeal with plant protein ingredients, the acceptability of the feed may reduce resulting in a reduced growth performance (Kaushik et al., 1995; Fournier et al., 2004; Glencross et al., 2004; Dias et al., 2005). Thus one of the greatest challenges in the formulation of high plant protein diets for farmed Atlantic salmon has been to secure that the fish accept the feed offered to them equally well as the fishmeal based diets. Therefore upon formulating the diets one needs to seek for ingredients that may help in maintaining the feed intake. Both fish protein hydrolysate (FPC) and stick water (the water soluble protein fraction obtained during fishmeal production) contain several non protein nitrogen compounds (NPN) that the plant ingredients contains in very different concentrations or are devoid of. Examples of NPN are taurine, betaine, trimethylamine oxide, spermine, spermidine among others (Espe & Lied, 1999; Liaset & Espe, 2008; historical data NIFES). As addition of both FPC (Espe et al., 1992a) and stick water (Laksesvela, 1958; 1960) improved the voluntary feed intake in poultry and FPC improved feed intake in rats (Espe et al., 1989; 1992b; Liaset et al., 2000)

and Atlantic salmon (Espe et al., 1999; Refstie et al., 2004; Hevrøy et al., 2005) both components seem to have the potential to help in maintaining the intake of fishmeal replacement diets for Atlantic salmon. Hydrolysed squid protein is known to contain quite high concentration of NPN compounds (~6%) of which about 1/6<sup>th</sup> is taurine (historical data NIFES) making it another possible candidate with the benefit of increasing dietary taurine concentration of the diets. Additionally squid contains quite high concentration of betaine (trimethylglycine, Treberg & Driedze, 2007). Betaine is consumed in the remethylation of homocysteine to regenerate methionine in the choline-betaine pathway and is crucial for the methylation capacity in animals (Finkelstein, 1990; Mato et al., 2002).

Therefore when we first formulated diets devoid of fishmeal (Espe et al., 2006) or containing low fishmeal (Espe et al., 2007) either stick water (5%) or hydrolysed squid (3%) were used with the aim to assess the effect on voluntary feed intake and growth. Additionally 5% FPC was included. Fish fed the total replacement diet consumed slightly less feed as compared to the fishmeal fed Atlantic salmon and those fish fed the diet containing hydrolysed squid consumed more feed than did the fish offered the diets containing the stick water. Fish fed diets not added any fishmeal grew less than the fish fed the reference diets in which most of the protein arrived from fishmeal. The reduced growth observed was due to a general reduced lipid gain (Espe et al., 2006). Addition of 5% fishmeal to the diets improved the lipid accretion to similar levels as the fish meal fed control (Espe et al., 2007). Therefore one probably should not formulate diets without any fishmeal for Atlantic salmon, but inclusion of low fishmeal (5%) together with the marine ingredients as squid or stick water and FPC seems to work well. It should be noted that Fournier et al. (2003) also added FPC in diets fed to turbot without any fishmeal and reported similar voluntary feed intake as compared to fish fed the fishmeal control, but in those experiments the growth was reduced. Experiments with different species fed total or high fishmeal replacement diets are summarised in Table 1, and generally total or high replacement of marine protein with plant proteins reduced the performance. As Atlantic salmon fed FPC and squid meal performed better than the fish fed the stick water (Espe et al., 2006; 2007), the test diets used by us in trials where Atlantic salmon were fed high fishmeal replacement diets were added FPC and hydrolyzed squid meal or krill meal and fishmeal was never less than 5%.

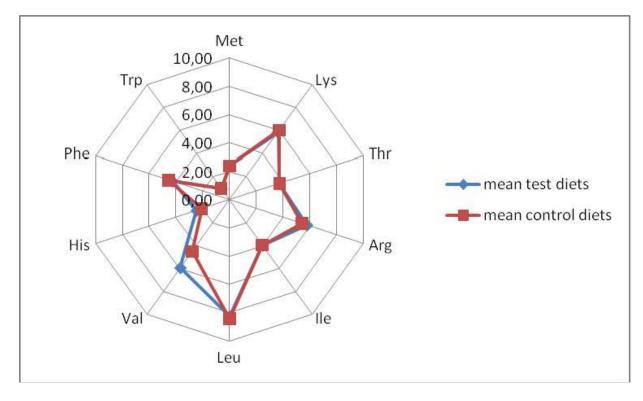
It should be noted that replacement of fish oil also depend on blends of plant oils to make sure the requirement of polyunsaturated fatty acids is met (Torstensen et al. 2004; 2005; 2008). However, in all our studies with Atlantic salmon, the lipid source was fish oil thus any discussion of alternative lipid sources in fish feed is not included in the current chapter. The effect of plant oil substitution for fish oil was recently reviewed by Leaver et al. (2009).

#### 2. The effect of fish meal replacement on growth and accretion

Six experiments in which high fishmeal replacement diets were fed to Atlantic salmon were compared for the wet weight gain, as well as protein and lipid gain. The diets tested contained balanced amino acid profiles (**Figure 1**), but were based on different plant protein ingredients (**Table 2**) and were supplemented with low concentration of crystalline amino acid to balance the amino acid profiles not to jeopardize growth (Espe & Njaa 1991; Espe et al., 1992a; Espe & Lied 1994; Berge et al., 1994; Cowey 1995; Dabrowski & Guderley 2002; Rollin et al., 2003). Daily voluntary feed intake was monitored carefully to make sure that

Table 1. The effect on voluntary feed intake, growth performance, protein accretion and feed utilisation in fish fed high plant protein diets compared to fishmeal control fed fish in some studies testing total or high fishmeal replacement diets. -No information given, Total fishmeal replacement trials are marked with bold letters

#### IAA (g/16g N):



#### DAA (g/16gN):

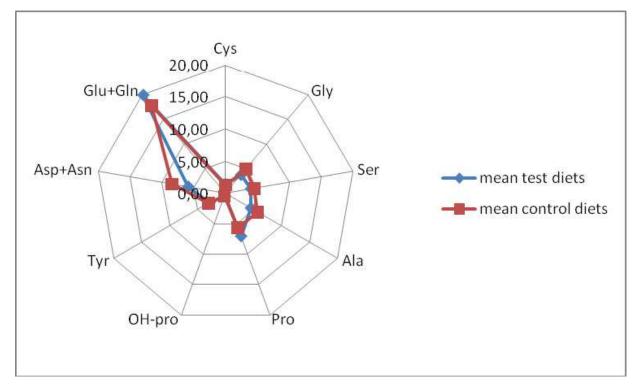


Fig. 1. The diets were balanced in both indispensable (IAAs) and dispensable (DAAs) amino acids towards the controls used in each trial and equalled the requirement of amino acids. Here is shown the mean values over all trials (g amino acid/16gN).

	I VI	н	50	59	150	0	0	C	0		130		155.9	30	50	293.9	4.4	42.1	360	367	25.8	9.4	18.6	
	Tria	ပ	50 50	0	50	0	0	144 4	0		120	,	0	0	0	312	0	13.6	373	373	24.7	50.9	50.9	
	Trial V	L	87.5	112.5	120	0	0	C	382		0		0	0	0	273	0	25	418	290	23.9	14.1	21.7	
	Tria	ပ	354	0	40	0	0	6	323		9		0	0	0	267	0	16	440	287	24.0	54.8	54.8	
	2	⊢	50	50	75.3	250	0	146 1	0		0		0	8	0	325	50	23.6	400	331	26.2	8.0	21.5	
	Tria	ပ	250 50	0	20	150	96	115.4	0		0		0	0	0	310	0	8.6	389	332	25.6	43.7	43.7	
		F	50	50	238	0	1	225.8	0		0		0	80	0	280	71.6	43.6	430	289	24.3	7.9	20.0	
	Tria	ပ	260 50	0	80	140	123	106.4	0		0		0	0	0	272	0	19.5	433	274	24.0	40.9	40.9	
											0		0	30	0	253	71	30.6	446	239	23.8	7.6	18 <u>.</u> 5	
	Trial II	ပ	400	0	0	170	35	156.4	0		0		0	0	0	230	0	8.6	424	241	23.7	64.1	64.0	
		F	50	50	151	222	0	1124	0		0		0	80	0	258	126.6	30.5	504	224	24.3	6.7	17.0	
	Trial	ပ	490	0	0	100	55	106.4	0		9	]	0	0	0	240	0	8.6	477	248	23.6	69.8	<u>69</u> .8	$\mathbb{C}$
		Diets	Fishmeal (Norse LT-94)	FPC (C.P.S.P.)	Wheat gluten	Corn gluten	Soy protein	wheat grain	Pea/ soy protein	mix	Pea protein conc.	50	Tapioca	Squid hydrolysates	Krill meal	Fish oil	AA mix	micronutrients*	Crude protein	Crude fat	Gross energy (MJ kg)	% fishmeal protein of diet protein	% marine protein of total diet protein	

\*crystalline amino acid to balance the dietary amino acids and vitamin + mineral mixtures to fulfil the requirement (for more details see Espe et al., 2007; 2008). Percentage of marine protein in the diets was calculated assuming fishmeal, krill meal and FPC contained 68% protein and squid 60% protein

Table 2. The compositions (g/kg) in the diets used in the different trials (I-VI). For each trial the control (C) contained more fishmeal, while the test (T) diets are based on different blends of plant proteins containing lower fishmeal inclusion.

any differences in feed intake should have any impacts on growth or accretion. Some of the general growth data of these trials have been published previously assessing the requirement of amino acids in Atlantic salmon (Espe et al., 2007; 2008) but here only fish fed the control and the test diets with similar amino acid profiles within each experiment are included. Additionally some unpublished experiments are included. The results were sorted after high protein low fishmeal (HPLF) and medium protein medium fishmeal (MPMF) replacement. Each response was modelled statistically with a mixed-effects model using the level of fishmeal (HPLF or MPMF) as a fixed effect and trial as a random effect.

To be able to compare the growth and accretion data obtained in the six experiments included, growth data was calculated g/kg average BW/day as both the duration of the experiments and the initial body weight of the fish varied. Likewise the protein and lipid gains were calculated as gain/kg average BW/day. The mean wet weight gain, the protein and lipid gains obtained in the six trials are listed in **Table 3**. The replacement ratios of both the fishmeal protein and the marine protein ingredients with plant proteins are summarised in **Table 2**. The test diets contained from 6.7 to 14.1% of dietary protein as fishmeal, while the control diets (MPMF) contained from 40.9 to 69.8% of the dietary protein as fishmeal. As the test diets were added FPC and squid or krill meal the marine protein content in test diets varied from 17.0 to 21.5 % of total protein while the medium fishmeal replacement diets contained from 40.9 to 69.8 % marine protein of the total dietary protein.

Weight gain varied from 6-12g/kg average BW/day in the trials included (Table 3), being highest in trials III and V. The protein gain varied from 0.6 to 1.8g/kg Average BW/day being highest in trials III and V (Table 3). The lipid gain varied from 0.8 to 3.2g/kg average BW/day, being highest in trials I, III and V (**Table 3**). There was a high probability (>99%) that lipid gain was reduced when high plant protein diets were fed to Atlantic salmon (Table 4). The 95% credible interval (CI) of this difference ranges from about -3 to -16%, i.e. it is clearly negative. Similarly, there was a high probability (>99%) that protein gain was reduced when fishmeal was replaced with plant ingredients (95% CI of the effects ranges from about -3 to -16%, Table 4). The estimated mean wet weight gain in the MPMF was 8.1g/kg BW/day and there was a high probability (>99%) that wet weight gain decreased with the HPLF fed groups (95% CI of the effect ranges from about -2 to -13%, Table 4). Although the effects on protein and lipid gain as well as the wet weight gain are clearly negative they are all less than 10% (i.e. effect of size median; Table 4). Any reduced performance of about 10% is however not devastating and show that probably the balancing of the dietary amino acids and addition of marine ingredients improved the performance of the fish fed the diets with HPLF.

Generally wet weight gain decrease when high plant protein diets are fed to fish (Gomes et al., 1995; Kaushik et al., 1995; 2004; Mambrini et al., 1999; Dias et al., 2005; Torstensen et al., 2008). This may of course be due to the fact that the diets do not contain a balanced amino acid profile, but even when the amino acid profile is balanced the performance declines as the plant ingredients increase (Fournier et al., 2003, 2004). However, when juvenile Senegalese sole were fed diets with very low fishmeal inclusion and balanced in all dietary amino acids and supplemented with taurine, the wet weight gain was equal to fish fed the diet containing high fishmeal inclusion (Silva et al., 2009). Also taurine supplementation in high fishmeal replacement diets fed to juvenile Atlantic salmon was found to reduce the whole body lipid to protein ratio (Espe et al., 2011). The higher the accretion of protein the

Trial VI		1441±25 1402±30 2435+88 2407+72	5.8±0.3 6.3±0.4		0.62±0.1 0.74±0.1	0.75±0.1 0.84±0.1
Trial V		371±11 383±6 1065+03 065+01			1.85±0.1 1.73±0.1	
Trial IV		1123±9 1128±55 1801+25 1028+77	6.0±0.3 6.0±0.8			1.30±0.1 1.24±0.3
Trial III		481±25 495±5 1235+04 1125+64			1.72±0.1 1.43±0.1	
Trial II		633±31 619±50 1108+23 086+48			1.14±0.1 0.84±0.1	
Trial I		333±4 327±8 763+37 735+15				320402
	Diets	Initial BW	Weight gain	ADC-N	Protein gain	

Arbitrary BW (ABW) is the mean wt during the trials (end BW+initial BW/2). In trials I to IV is mean of 3 tanks per treatment while trials V and VI is the mean of 4 tanks per treatment. Values are the tank means±standard deviation

Table 3. The mean initial and end body wt (BW, g), mean wet wt gain (g/kg ABW/day), mean apparent digestibility of nitrogen and mean protein and lipid gain (g/kg ABW/day) in the six trials included in the statistics to test the effects of high or medium fishmeal replacement diets fed to Atlantic salmon.

Replacement of Fishmeal with Plant Proteins

	MPMF	Diff with HPLF	SE diff	Effect size median	Effect size 95% CI low	Effect size 95% CI high	Probability HPLF <mpmf< td=""></mpmf<>	
		g/Kg ABW/day	g/Kg ABW/day	% of MPMF	% of MPMF	% of MPMF		
Wet wt gain	8.07	-0.59	0.23	-7.24	-13.14	-1.87	0.995	
Protein gain	1.19	-0.11	0.04	-9.47	-16.10	-2.77	0.998	
Lipid gain	1.64	-0.15	0.06	-9.47	-16.48	-3.01	0.997	

MPMF medium protein, medium fishmeal HPLF high protein, low fishmeal

Table 4. Statistical estimates of the mean wet weight gain, protein and lipid gain of the MPMF feeds together with the estimated differences of the HPLF feeds (g/Kg ABW/day). Median effect size and its 95% credible intervals are given as percentage of the MPMF estimate. The estimated probability that HPLF is smaller than MPMF is also given in the last column.

more cost effective is the feed. Also less nitrogen will be spilled to the ocean when accretion is high (Kaushik et al., 2004). Therefore also from an environmental aspect, the novel fishmeal replacement diets have to result in protein accretion close to what is the case in using the traditional fishmeal based diets. Upon feeding rainbow trout diets with high plant proteins the protein accretion declines (Fournier et al., 2003; Aksnes et al., 2006). This generally also is the case when any marine species is fed increasing amounts of plant proteins. Atlantic cod reduce protein accretion significantly when plant protein exceeds 50% of the dietary protein (Hansen et al., 2007) while the European seabass did not reduce protein accretion until plant protein inclusion exceeded 80% of the dietary protein (Kaushik et al., 2004). Also seabream shows reduction in protein gain when fishmeal was replaced with plant proteins (Robaina et al., 1995; Gomez-Requeni et al., 2004) and this was described to be due to an imbalanced amino acid profile. However, in turbot fed diets containing less than 20% fishmeal even though the amino acid profile in the diets was similar to the fishmeal based control, the protein accretion was less than the control fed turbot (Fournier et al., 2004).

All animals, man included, have a tremendous ability to adapt to a low protein intake (Millward, 1999). This adaption also seems to be the case in Atlantic salmon as protein gain was not affected even though the feed intake declined and lipid gain reduced when the fish was fed diets without any fishmeal (Espe et al., 2006).

The reduced lipid deposition also was reported by Dias et al. (2005) when European seabass was fed plant protein based diets as compared to fish fed a fishmeal diet. The protein source in the current diets was based on variable blends of plant proteins (**Table 2**), but the amino acid profiles were pretty similar within all trials (**Figure 1**) and well above the anticipated amino acid requirement. Any reduced availability of dietary amino acids cannot explain the reduced performance as the digestibility in all trials was high (**Table 3**). Therefore the

reduced performance when Atlantic salmon are fed plant protein based diets cannot be explained by any reduced availability of amino acids. However, the metabolic cost following high replacement diets might be increased due to the fact that NPN metabolites that are present in fishmeal either are not delivered or has to be synthesised by the fish.

Previously we found less fat in whole fish, muscle and liver of Atlantic salmon fed hydrolysed proteins as compared to fish fed diets containing the intact control protein (calculated from Espe et al., 1992c). As the fishmeal replacement diets compared in the current chapter contained 5-11.25% FPC and the control diet did not contain any FPC, the lesser fat deposition might have been due to the inclusion of the FPC in the test diets. Thus probably the reduced lipid deposition in total fishmeal replacement diets is due to metabolic changes or higher metabolic costs. How the fish sense the energy and choose either protein or fat as fuels needs to be focussed to understand how to formulate diets for Atlantic salmon that burn the lipid and deposit as much as possible of the dietary protein. Interestingly, we recently found that FPC affected viscera mass without affecting the dress out weight in Atlantic salmon fed diets containing medium fishmeal inclusion of which was replaced with FPC (Espe et al., In prep) supporting an altered deposition pattern in Atlantic salmon when FPC is added to the diets. Further, when both the protein and the lipid source were of plant origin more adipose tissue were present in Atlantic salmon (Torstensen et al., 2011) implying that the deposition pattern also should be addressed when the effects of high fishmeal replacement diets are assessed.

#### 3. Future aspects

Even though Atlantic salmon grew and utilise diets with total replacement of the fish meal with plant ingredients or very high replacement levels recently well, the compartmentalisation and lipid deposition may change even though the voluntary feed intakes are similar. This might be due to altered concentration of NPN in the replacement diets. In the current studies the NPN was higher in the replacement diets in trials III and VI (Figure 2), while the wet weight gain was best in trials III and V, thus NPN cannot alone explain the reduced performance. Atlantic salmon fed diets low in methionine had reduced concentration of taurine in both muscle and liver (Espe et al., 2008). The reduced methionine intake, and the lower liver taurine, resulted in higher accumulation of lipid in the liver (Espe et al., 2010) while lysine limitations increased the lipid concentration in the whole fish (Espe et al., 2007). Also in the rainbow trout the methionine and taurine was found to affect the lipid deposition, but methionine rather than taurine was responsible (Gaylord et al., 2007). In juvenile Atlantic salmon we reported that taurine supplementation to plant protein based diets reduced the whole body lipid deposition without affecting protein deposition (Espe et al., 2011). Thus the altered lipid deposition pattern following limitations in amino acids most probably is affected through interactions between metabolites when present in excess or in limited concentration in the diets. In the studies described on Atlantic salmon all diets were balanced in amino acids, but as amino acid requirements might change when fishmeal is replaced, the future research needs to focus metabolic consequences of feeding the Atlantic salmon total or almost total fish meal replacement diets and determine whether the NPN compounds are conditionally indispensable. Also the aspects of any increased requirement of indispensable amino acids and or dispensable amino acids should be addressed as synthesis of metabolites not present in the diets may increase the requirement of the precursor amino acids.

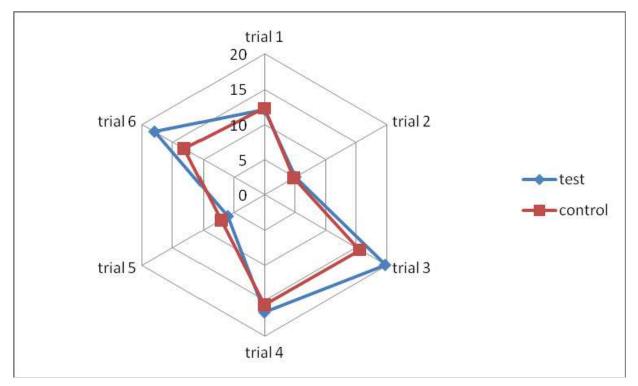


Fig. 2. The non amino acid nitrogen (NPN) differed between the diets in trial III and VI, otherwise the NPN was pretty similar. Here is shown the NPN in the relative high fishmeal diets (control) vs the NPN in the replacement diets.

As we previously reported that adding all protein as hydrolysed proteins, reduced lipid deposition in whole fish, fillet and liver (calculated from Espe et al., 1992c), the optimisation of FPC inclusion in producing a leaner fish might be a strategy if a leaner Atlantic salmon should be a goal in fish farming. Also in the rat the visceral lipid decreased upon offering them a diet in which the dietary protein source was FPC as compared to both casein and soy protein (Liaset et al., 2009). The results from the last years in replacing fishmeal by plant protein ingredients in diets to Atlantic salmon show clearly that a future sustainable aquaculture is possible. Meantime carefully use of the marine ingredients the fishmeal included to stimulate the voluntary feed intake and thus the growth, a normal healthy metabolism and the general health of the fish should be intensified to secure increased production of farmed fish without being too dependent on the limiting marine ingredients available.

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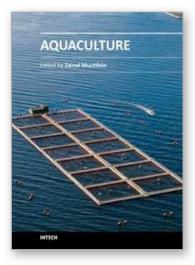
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This book provides an understanding on a large variety of aquaculture related topics. The book is organized in four sections. The first section discusses fish nutrition second section is considers the application of genetic in aquaculture; section three takes a look at current techniques for controlling lipid oxidation and melanosis in Aquaculture products. The last section is focused on culture techniques and management, ,which is the larger part of the book. The book chapters are written by leading experts in their respective areas. Therefore, I am quite confident that this book will be equally useful for students and professionals in aquaculture and biotechnology.

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