AKVAFORSK

AKVAFORSK report 19/07

Nutritional requirements of fish with emphasis on Atlantic salmon and rainbow trout: A literature study by AKVAFORSK and NIFES

Edited by B. Grisdale-Helland

REPORT

AKVAFORSK
Institute of Aquaculture Research AS

Report type
Final report

Title
Nutritional requirements of fish with emphasis on Atlantic salmon and rainbow trout: A literature study by AKVAFORSK and NIFES

Employer
Fiskeri- og havbruksnæringens forskningsfond
Project number FHF: 511014

Report no.	19/07
Project no.	10-2464
No. of pages	110
Confidentiality	Open
Written by	B. Grisdale-Helland
Approved by	T. Åsgård
Approval date	07.05.2008

Objective of the project

Review the state of knowledge about the nutrient requirements of fish, with a special emphasis on Atlantic salmon and rainbow trout.

Summary

The growth rates of Atlantic salmon and rainbow trout in aquaculture are continuously increasing because of advances in husbandry, management, nutrition and genetic selection. Detailed evaluations of the nutrient requirements for these important species have not kept pace with these improvements in growth, however. In addition, many of the requirements determined may not be valid because the specialized diets used in these trials did not give growth rates in line with those obtained with a control diet or as expected under commercial conditions.

In this literature study, the nutrient requirements that have been determined for rainbow trout and Atlantic salmon are presented, with emphasis on the nutrients that most affect the growth, health and well-being of the fish and the quality of the final product. The contents of these nutrients in marine and different plant ingredients are discussed along with comments about any negative aspects these ingredients may have for fish performance or quality of the final product.

keywords

Atlantic salmon; rainbow trout; nutritional requirements

Use of the report:

Internal use by FHF.

Final report to

Fiskeri- og havbruksnæringens forskningsfond
Project number FHF: 511014

Nutritional requirements of fish with emphasis on Atlantic salmon and rainbow trout: A literature study by AKVAFORSK and NIFES

AKVAFORSK Report no. 19/07

Project no. 10-2464

07.05.2008

Contributors

Grete Baeverfjord
Senior Scientist
Nofima
6600 Sunndalsøra

Bjørn Bjerkeng
Senior Scientist
Nofima
6600 Sunndalsøra

Barbara Grisdale-Helland
Senior Scientist
Nofima and Aquaculture Protein Centre
6600 Sunndalsøra

Ståle J. Helland
Senior Scientist
Nofima and Leader, Amino Acid & Protein Metabolism Section, Aquaculture Protein Centre
6600 Sunndalsøra

Bente Ruyter
Senior Scientist
Nofima
1432 Ås

Bente E. Torstensen
Senior Scientist
NIFES
5817 Bergen

Rune Waagbø
Professor
NIFES
5817 Bergen

Trine Ytrestøyl
Scientist
Nofima
6600 Sunndalsøra

1. Introduction

Barbara Grisdale-Helland and Ståle J. Helland

The growth rates of Atlantic salmon and rainbow trout in aquaculture are continuously increasing because of advances in husbandry, management, nutrition and genetic selection. Detailed evaluations of the nutrient requirements for these important species have not kept pace with these improvements in growth, however. In addition, many of the requirements determined may not be valid because the specialized diets used in these trials did not give growth rates in line with those obtained with a control diet or as expected under commercial conditions. Because of the shortage of marine resources, fish meal and fish oil are currently being replaced by other protein and lipid sources. Many of these ingredients are not optimally balanced. In order to utilize these ingredients appropriately, either by blending them in the correct ratios or with the use of specific supplementations, the requirements of the fish must be known.

The requirements can be defined as: the combination of nutrients and energy that yields optimal performance. This will be reflected by growth rate, lack of nutrient deficiency symptoms, and an end product of optimal quality. This group of conditions is, naturally enough, difficult to fulfil at the same time, and a key phrase in this definition is: "the combination of nutrients". This implies not only that knowledge about the minimum amounts of nutrients is needed. There can equally well be a toxic effect of a nutrient and it is obvious that the balance between the various nutrients is very important. The consequence of this is that a nutrient requirement can be defined in several ways, all dependent on its intentional use. How then, should the requirements be used and in what framework? Nutrient modelling is a mathematical setup used to predict performance. The value of the output from any model is dependent on the quality of the input, and it would ease the situation if the requirements were established in a framework that is coordinated with the inner workings of the models used. A model also requires a set of correction factors that quantify the influence of the different biotic and abiotic factors on the model. Therefore, it is important to generate these correction factors in a setting that is as little influenced by other factors as possible, or, in other words, to generate a mathematical relationship that is as simple as possible. In classical animal energetic modelling, for example, the requirements are separated into two components. The first is the amount of energy that is needed to maintain zero energetic balance, or, as it is also called, the maintenance requirement for energy. The other component is the energy required

for growth above maintenance. In an ideal modelling situation, each of these would be a constant. The biological situation, however, is somewhat more complicated and influencing factors must be taken into account.

In this literature study, the nutrient requirements that have been determined for rainbow trout and Atlantic salmon will be presented, with emphasis on the nutrients that most affect the growth, health and well-being of the fish and the quality of the final product. The contents of these nutrients in marine and different plant ingredients will be discussed along with comments about any negative aspects these ingredients may have for fish performance or quality of the final product.

2. Energy

Ståle J. Helland

Energy is not defined as a nutrient. It is a fuel that is released by the break-up of chemical bonds when the three macronutrients (fat, protein and carbohydrates) undergo biological oxidation. A description of the central energy terms used is given below:

Gross energy (GE) - the energy released by total combustion of a biological sample in a bomb calorimeter.

Digestible energy (DE) - the dietary energy that remains when the energetic losses in the faecal material are deducted.

Metabolizable energy (ME) - the energy that remains after the energy in the branchial and urinary losses is subtracted from DE. For fish, this consists mostly of the energetic losses associated with the nitrogenous end products from the oxidation of dietary protein, that is, mainly urea and ammonia. The latter is the largest component (75-80%). The energetic value of the ammonia in fish, however, is low, and therefore, in fish ME is not much lower than DE.

Heat increment (HI) or *specific dynamic action* (SDA) - the energy required for the digestion and metabolism of the dietary nutrients.

Activity - the energy loss associated with locomotion and maintaining position in the water column.

Net energy (NE) - the dietary energy that is available for the fish. This is defined as: NE = ME - (HI + Activity). Traditionally, NE is split into that needed for maintenance (NE_m), for growth (NE_g) and for reproduction.

The majority of publications use DE_m, since this requires less complicated experimental setups than what is required for the determination of ME_m. In addition, as mentioned above, the numerical difference between DE and ME is small. The magnitude of experimental complications increases when going from ME_m to NE_m. HI, Activity and NE_m are all expressed in the form of chemically liberated energy (heat), and are often determined indirectly in respiration experiments through the measurement of oxygen consumption.

The size of the animals has often been a complicating factor in animal energetics. This problem has been circumvented by expressing the results on a Metabolic Body Weight basis (MBW), where the weight is raised to a certain power. The power used for energy is frequently 0.8 in fish. This factor may be different depending on the fish species.

The most frequently tabulated value for requirement is the minimum dietary inclusion level that is needed for optimal animal growth or the gain of a specific nutrient. The

experimental design used for establishing this type of requirement may be called Requirement by Dose Response (RDR). In this design, several diets are fed with only one nutrient is changed at a time. The result is a dilution of the other dietary components and thus, the nutrient in question is generally exchanged with another. It is important in such cases to maintain the required balance by exchanging the nutrient in question with another that has minimal impact on the desired response. The RDR design is not suitable for establishing the minimum energy required for optimal growth. This is because the diets needed for such a study have to be designed with confounding effects between energy and the macronutrients (protein, fat and carbohydrate).

Another design for establishing requirements can be termed Requirement by Ration Levels (RRL). This has also been termed the Factorial method. In this design, one diet is fed at different ration levels and the nutrient intake and growth response are expressed on a MBW basis. Both RDR and RRL can be used to determine the requirements for maintenance (DE_m) by extrapolating to zero gain. The RDR relationship is often curvilinear. The strength of the RRL-design is that it is frequently a linear relationship, possibly allowing a more correct extrapolation to zero gain. Additionally, the nature of the RRL design is such that data points can be generated that are closer to the DE_m than what is the case with the RDR design, thus requiring a smaller extrapolation. The slope of the linear relationship between energy intake and gain (k_{DEg}) describes the efficiency of utilization of digestible energy for growth above maintenance.

The major bulk of scientific information related to the needs of dietary energy is related to finding the optimal balance between the macronutrients and energy that gives highest growth. A problem with this pool of information is that it is comprised of data from different experimental conditions and the extraction of general conclusions from such data might be hampered by several non-quantified interactions from the different experiments. Rodehutscord and Pfeffer (1999) demonstrated this problem when looking at the maintenance requirement for energy in rainbow trout. They pooled data from 292 groups of fish from experiments conducted over 10 years, and re-evaluated the results by regression analysis. They found that the dietary fat content influenced both the efficiency of utilization of energy for growth and also DE_m. The authors concluded that "Maintenance can therefore, hardly be calculated by extrapolation of the regression line to zero energy retention. This can only be a rough estimate as it is not clear why maintenance requirement for digestible energy of a fish should depend on the fat level in the diet." The concept of RRL assumes that the maintenance requirement is fixed for that experiment. The traditional way to establish the maintenance

requirement in an RRL-design is to use only one diet fed at different ration levels, as used by Lofgreen (1965) for beef cattle. One explanation for the influence of dietary fat level on the estimate of the maintenance requirement reported by Rodehutscord and Pfeffer (1999) could be related to differences in substrates available or preferred for energy metabolism. This substrate can either come from the turnover of body tissues or from the diet. McKenzie et al. (1998) demonstrated with Atlantic salmon that the ratio of fish oil : rapeseed oil in the diet influenced the fish's swimming performance. With the same maximum oxygen consumption, increasing the fraction of rapeseed oil in the diet resulted in increased maximum swimming speed. It is suggested that increased levels of oleic and linoleic acid in the muscle, from the rapeseed oil, might influence the efficiency of aerobic energy production by mitochondria during exercise. This experiment indicates that the choice/availability of substrates for energy metabolism influences the energy balance, and could therefore, also influence the energy required for maintenance.

Several investigators that have used RRL to determine the DE_m and k_{DEg} have questioned whether it is valid to extrapolate the relationship between energy intake and gain to the maintenance level, or if special fasting processes set in at this low level of intake. Helland et al. (2006) used 100 g Atlantic salmon smolt in a single diet RRL-design and fed the fish at four levels (100%, 73%, 53% and 20%) or fasted. The full-fed groups had growth rates 140% of that given in Austreng et al. (1987) and similar to that found in the higher producing commercial salmon farms. In the relationship between digestible energy intake and energy gain, 100% of the variation was accounted for (R^2=1). The groups of fish that were fed at 20% of full-fed were at energy equilibrium (maintenance), giving evidence that the influence of any special energetic processes during low intake was negligible.

Extrapolation of regression lines outside the area covered by data should be approached with caution. Such exercises, however, give credit to a data set with little uncontrolled interactions if the maintenance estimates obtained by extrapolation are comparative to literature values. A maintenance requirement for rainbow trout of 137 kJ $kg^{-0.8}$ d^{-1} (Table 2.1) was reported by Rodehutscord and Pfeffer (1999). This is twice the value obtained by recalculating the trout data of Huisman (1976). Kaushik and Gomes (1988) fed 150 g rainbow trout a maintenance ration for 28 days and reported that DE_m was only 31 to 41 kJ $kg^{-0.8}$ d^{-1}, or close to a quarter of the DE_m reported by Rodehutscord and Pfeffer (1999). The DE_m values of Kaushik and Gomes (1998) are similar to those reported for the other species listed in Table 2.1 (32-50 kJ $kg^{-0.8}$ d^{-1}). These values are approximately 10% of the energy released by fasting

Table 2.1. Digestible energy for maintenance (DE_m) and efficiency of utilization of digestible energy for growth above maintenance (k_{Deg}) in various fish species[1]

	Start weight, g	DE_m, kJ MBW^{-1} d^{-1}	k_{DEg}	Temperature, ºC	R^2	Remarks	Highest growth rate, %[2]	Reference
Atlantic salmon, *Salmo salar*	100	31.5	0.80	10	1.00		~140	Helland et al., 2006
Rainbow trout, *Oncorhynchus mykiss*	14-113	137		15 (12-17.5)				Rodehutscord and Pfeffer, 1999
	13	-0.001	0.42	6		Recalculated[3]	~80	Azevedo et al., 1998
	13	-0.002	0.56	9		Recalculated	~80	Azevedo et al., 1998
	13	-0.0002	0.39	12		Recalculated	~70	Azevedo et al., 1998
	13	0.0006	0.60	15		Recalculated	~70	Azevedo et al., 1998
	13	0.0014	0.57	6-15		Recalculated		Azevedo et al., 1998
	70	57	0.57	15		Recalculated	~40	Huisman, 1976[4]
	140-150	31-41		18		Recalculated		Kaushik and Gomes, 1988
Atlantic cod, *Gadus morhua*	250	42.3	0.78	10	0.99			Hatlen et al., 2007
Gilthead seabream, *Sparus aurata*	17 - 32	43.8	0.50	21 - 24	0.94			Lupatsch et al., 2001a[5]
	30	46.0	0.65	19 - 27	0.97			Lupatsch et al., 2003
European sea bass, *Dicentrarchus labrax*	14 - 97	45.8	0.66	20-26	0.97			Lupatsch et al., 2001b[6]
White grouper, *Epinephelus aeneus*	10	33.2	0.69	19 - 27	0.99			Lupatsch et al., 2003
	13 - 90	32	0.64	22	0.98			Lupatsch and Kissil, 2005
		40	0.65	24	0.98			Lupatsch and Kissil, 2005
		50.3	0.68	27	0.98			Lupatsch and Kissil, 2005

[1]Exponent for metabolic body weight (MBW) is 0.8, unless otherwise indicated.

[2]Percentage of growth rates for salmon and trout given in Austreng et al. (1987).

[3]Recalculated: Used the authors' data and expressed the results on a DE basis using a MBW coefficient of 0.8.

[4]Have assumed an energy digestibility of 80% in this recalculation due to the high carbohydrate level in the diet.

[5]Used MBW coefficient of 0.83.

[6]Used MBW coefficient of 0.79.

homoeothermic animals (70 kcal $kg^{-0.75}$ d^{-1}; Kleiber, 1975). The maintenance values reported by Azevedo et al. (1998) were obtained by long extrapolations to zero, and seem to be unrealistic. The relatively high DE_m of Rodehutscord and Pfeffer (1999) could also be unrealistic and reflects an untraditional use of the RRL design. They did not use a single diet fed at several levels, but by pooled the uncontrolled variations obtained from a variety of experimental conditions and diets. The authors indicated that a larger database than the 292 experimental units was needed in order to separate out other effects than that of dietary fat level.

The requirements for DE above maintenance (k_{DEg} , Table 2.1) range from 0.39 to 0.80 kJ DE deposited per kJ consumed, with Atlantic salmon and Atlantic cod as the most efficient species. Apart from one observation on sea bream, rainbow trout seem to be the least efficient species depositing energy above maintenance. The recalculated values from Azevedo et al. (1998) change a fair amount. These authors state that temperature does not influence the k_{MEg}. This is in contrast to the results presented by Lupatsch and Kissil (2005), using white grouper. This discrepancy in conclusions between these papers, the large numerical spread in the k_{DEg} from the rainbow trout experiment, plus the unrealistic DE_m extrapolated from the data of Azevedo et al. (1998) warrants a re-evaluation of the effect of temperature on the growth and maintenance requirements of rainbow trout.

We have stated earlier that energy results obtained from nutrient and energy optimization experiments are of limited value when discussing the energy requirements in a general sense. The comparative slaughter technique, where the amount of consumed nutrients deposited or retained in the body is calculated, is a frequently used method in such studies. These values can also be defined as the amount of nutrients required for that particular level of production. A major reason why these values are of limited use in a general requirement discussion is that they are confounded with growth rate. With poorer growth, a higher proportion of the required energy is used to maintain energy balance (DE_m). Rodehutscord and Pfeffer (1999) reported that close to 30% of the DE intake was used for maintenance in poor-growing groups of fish, whereas this was reduced to 10% among the fast-growing groups. In agreement, Helland et al. (2006) found that the energy required for maintenance changed from 100% of the DE consumed for fish fed at the 20% level to 20% of the DE consumed for the full-fed groups.

In conclusion, for rainbow trout and Atlantic salmon, little data is available concerning energy utilization for maintenance and growth above maintenance. The ultimate comparison

of the energetic utilization between these two species in an RRL design has not yet been conducted.

2.1. References

Azevedo, P.A., Cho, C.Y., Leeson, S., Bureau, D.P., 1998. Effects of feeding level and water temperature on growth, nutrient and energy utilization and waste outputs of rainbow trout (*Oncorhynchus mykiss*). Aquat. Living Resour. 11, 227-238.

Hatlen, B., Helland, S.J., Grisdale-Helland, B., 2007. Energy and nitrogen partitioning in 250 g Atlantic cod (*Gadus morhua* L.) given graded levels of feed with different protein and energy content. Aquaculture 270, 167-177.

Helland, S.J., Hatlen, B., Grisdale-Helland, B., 2006. Changes in the utilization of energy and amino acids caused by feed intake level - implications for amino acid requirement studies. XIIth Int. Symp. Fish Nutrition & Feeding, Biarritz, France (O-45).

Huisman, E.A., 1976. Food conversion efficiencies at maintenance and production levels for carp *Cyprinus carpo* L., and rainbow trout, *Salmo gairdneri* Richardson. Aquaculture 9, 259-273.

Kaushik S.J., Gomes E.F., 1988. Effect of frequency of feeding on nitrogen and energy balance in rainbow trout under maintenance conditions. Aquaculture 73, 207-216.

Kleiber, M. 1975. The Fire of Life. An Introduction to Animal Energetics. 2nd Edition. Robert E. Kriegler Publishing Company, Huntington, New York.

Lofgreen, G.P., 1965. A comparative slaughter technique for determining net energy values for beef cattle. In: Blaxter, K.L. (Ed.), Energy Metabolism. Proc. 3rd Symp., Troon, Scotland, May 1964. EAAP Publication No. 11. Academic Press, London, pp. 309-317.

Lupatsch, I. Kissil, G.W., Sklan, D., Pfeffer, E., 2001a. Effects of varying dietary protein and energy supply on growth, body composition and protein utilization in gilthead seabream (*Sparus aurata* L.). Aquac. Nutr. 7, 71-80.

Lupatsch, I., Kissil, G.W., Sklan, D., 2001b. Optimalization of feeding regimes for European sea bass *Dicentrarchus labrax*: a factorial approach. Aquaculture 202, 289-302.

Lupatsch, I. Kissil, G.W., Sklan, D., 2003. Comparison of energy and protein efficiency among three fish species gilthead sea bream (*Sparus aurata*), European sea bass (*Dicentrarchus labrax*) and white grouper (*Epenephelus aeneus*): energy expenditure for protein and lipid deposition. Aquaculture 225, 175-189.

Lupatsch, I., Kissil, G.W., 2005. Feed formulations based on energy and protein demands in white grouper (*Epinephelus aeneus*). Aquaculture 248, 83-95.

McKenzie, D.J., Higgs, D.A., Dosanjh, B.S., Randall, D.J. 1998. Dietary fatty acid composition influences swimming performance in Atlantic salmon (*Salmo salar*) in seawater. Fish Physiol. Biochem. 19, 111-122.

Rodehutscord, M., Pfeffer, E., 1999. Maintenance requirement for digestible energy and efficiency of utilization of digestible energy for retention in rainbow rout, *Oncorhynchus mykiss*. Aquaculture 179, 95-107.

3. Protein and amino acids

Barbara Grisdale-Helland

3.1. Protein

Protein is a major component in fish diets, but there is no actual requirement for it. Rather, protein is consumed as a source of amino acids (AA). The amino acids are required for protein growth, as precursors of bioactive molecules such as hormones and neurotransmitters (D'Mello, 2003), or to replace these components in the body. Even if not in excess, protein will be used not only for growth, but also as a supply of energy. When protein is used for energy, ammonia is released from the amino acids and excreted, contributing to pollution of the environment. Thus, dietary formulations are generally designed to supply the minimum protein level for maximum growth along with an appropriate balance of the other nutrients to supply the required energy. When insufficient dietary protein is available to the fish, a reduction or lack of growth will generally be the result. Besides being species dependent, the demand for dietary protein, as summarized by Hardy (2002), may be affected by fish size, water temperature, activity level, reproductive status and dietary energy level.

Many trials with Atlantic salmon and rainbow trout have shown protein-sparing effects of lipids and/or carbohydrates (for example, Austreng, 1976a,b; Bergot, 1979; Pieper and Pfeffer, 1979; Watanabe et al., 1979; Kim and Kaushik, 1992; Johnsen et al., 1993; Hemre et al., 1995; Grisdale-Helland and Helland, 1997), and also possibly, nucleotides (Aas et al., 2006).

3.2. Amino acids

Ten specific AA have been found to be indispensable in the diet for all fish species studied so far, including rainbow trout (reviewed by Wilson, 1985, 2002). While specific studies have not been done to determine the essentiality in the diet of all of these 10 AA in Atlantic salmon, it is assumed, based on reports from the other fish species, that this species also requires these AA. Besides supplying the indispensable amino acids (IAA), dietary protein is also needed as a source of dispensable amino acids (DAA) or their precursors. The proportion of IAA and DAA in the diet affects growth and nitrogen (N) retention, and has been studied in several species (see section 3.5).

The IAA are: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. As described in Chapter 2, the minimum

dietary requirements for IAA have been determined using dose-response experiments. With this design, increasing increments of the AA under investigation are added to a basal diet deficient in that AA (D'Mello, 1982) and the AA concentration giving maximum response is assumed to be the requirement. The minimum IAA requirements may be affected by the age, life stage and genetics of the animal, dietary composition, feeding regime, growth rate and environmental variables. The response variable is usually either weight gain or protein gain, although other variables such as AA gain or feed efficiency may also be studied, depending on the production objectives. As seen in Table 3.1, the results of Rodehutscord et al. (1995b, 1997) show that the response variable greatly affects the estimated minimal AA requirements. In addition, the choice of mathematical model used to interpret the requirement may have a substantial effect on the result (Rodehutscord and Pack, 1999; Shearer, 2000; Encarnação et al., 2004). For rainbow trout, the minimal requirements for maximal weight gain have been determined using this method for all of the IAA. For Atlantic salmon, requirements by dose-response have been determined for arginine, histidine, lysine, methionine and threonine. Particularly for salmon, several of the requirements have only been published in abstracts or theses, but are considered valuable for the current report.

In dose-response trials, semi-synthetic diets have often been used in order to restrict the level of the AA under investigation. This has resulted in sub-optimal growth in comparison with current commercial conditions, and even the twenty-year old growth estimates of Austreng et al. (1987) (Tables 3.1 and 3.2). Recently, however, diets for use in AA requirement studies have been improved and growth rates have been achieved in rainbow trout (pers. comm., N. Bodin) and Atlantic salmon (Espe et al., 2007) at levels 90% or higher than those given by Austreng et al. (1987). The dietary lysine requirement for maximal protein growth determined by Espe et al. (2007) was 27-40% higher than that reported by Anderson et al. (1993) and Berge et al. (1998) (that had growth rates that were 35% and 60% respectively, of those given in Austreng et al. (1987)), but only 5% higher than that estimated by Rollin et al. (1994) [growth rate 60% of Austreng et al. (1987)], who used the diet-dilution method (see below). Han and Baker (1991) studied fast- and slow-growing broiler chicks and found that the amount of lysine required (consumed) per day was greater in the fast-growing poultry, but that the required dietary lysine concentration was not different for the two types. The authors noted that there was no difference in the protein concentration in the two chicken types. Interestingly, with rainbow trout, Chiu et al. (1988) showed that the dietary arginine requirement was lower when the fish were fed to satiation than when intake was restricted and that the daily intake of arginine per fish was not affected by the dietary arginine level. Thus,

Table 3.1. Dietary protein (N x 6.25) and indispensable amino acid (AA) requirements of rainbow trout for weight gain, protein gain or feed efficiency

Amino acid								Protein Source	Dietary protein (g kg^{-1} DM)	Highest growth rate (%)[§]	Evaluation method	Reference
Response	Weight gain	Protein gain	Weight gain	Protein gain	Weight gain	Protein gain	FER					
	g AA kg^{-1} dry diet*	g AA kg^{-1} dry diet*	g AA kg^{-1} dietary protein*	g AA kg^{-1} dietary protein*	g AA kg^{-1} gain*	g protein or AA kg^{-1} protein gain*	g AA kg^{-1} dry diet					
Protein						2280		CS	14-506	~75	Regression	Fournier et al., 2002
Arginine		14		35				FM, CS or egg	400		Protein gain	Ogino, 1980
			54-59					CG	470	~60	ANOVA	Ketola, 1983
	16		47					Milk, CG	330			Cho & Woodward, 1985[a]
	16-18		36-40					FM, Zein	450	~55	Broken line	Walton et al., 1986
	14 (full-fed); 17 (restricted feeding)		35 (full-fed); 42 (restricted feeding)					CS, GEL	400	TLS	Polynomial	Chiu et al., 1988
	14		42	42				Milk, CG	300	~75	ANOVA	Cho et al., 1992
			30					Milk, CG	300	~75	4-PL	Cho et al., 1992
	14.1		40					CS	350	~80	Broken line	Kim et al., 1992a
			38									Forster, 1993
	11.1	11.6	33	35			8.45	WG	335	~70	Exp.	Rodehutscord et al., 1995b
	14.1-28.1		32-63					CS	468-477	~35	ANOVA	Riley et al., 1996
						138		CS	14 - 508	~75	Regression	Fournier et al., 2002
		16.8		48				FM	350	~55	Ideal protein	Green and Hardy, 2002
Histidine		6.4		16				FM, CS or egg	400		Protein gain	Ogino, 1980
	5.2	5.8	15	17			4.0	WG	338	>50	Exp.	Rodehutscord et al., 1997
		6.4		18				FM	350	~55	Ideal protein	Green and Hardy, 2002
Isoleucine		9.6		24				FM, CS or egg			Protein gain	Ogino, 1980
	14.1	13.7	43	41			10.4	WG	331	>55	Exp.	Rodehutscord et al., 1997
		9.9		28				FM	350	~55	Ideal protein	Green and Hardy, 2002
Leucine		17.6		44					400		Protein gain	Ogino, 1980
	13.4	13.6	39	40			10.9	WG	344	>50	Exp.	Rodehutscord et al., 1997
		17.5		50				FM	350	~55	Ideal protein	Green and Hardy, 2002

[a] Cited by Wilson (1989).

Table 3.1. cont'd

Amino acid								Protein Source	Dietary protein (g kg^{-1} DM)	Highest growth rate (%)[§]	Evaluation method	Reference
Response	Weight gain	Protein gain	Weight gain	Protein gain	Weight gain	Protein gain	FER					
	g AA kg^{-1} dry diet*	g AA kg^{-1} dry diet*	g AA kg^{-1} dietary protein*	g AA kg^{-1} dietary protein*	g AA kg^{-1} gain*	g protein or AA kg^{-1} protein gain*	g AA kg^{-1} dry diet					
Lysine		21.2		53				FM, CS or egg	400		Protein gain	Ogino, 1980
			61					CG	470	~60	ANOVA	Ketola, 1983
	19.5		43		18.9∫			FM, gluten	450	~70	ANOVA	Walton et al., 1984a
	21.6		54		27.6∫				399		Broken line	Lanari et al., 1991
		17.5		37	17.7∫			WG	470	~75	Polynomial	Pfeffer et al., 1992
	13		37		15.7∫			CS	350	~85	Broken line	Kim et al., 1992a
	23.2	27.7	69	82	21.1∫		14.3	WG	338	>65	Exp.	Rodehutscord et al., 1997
		19.3		55				FM	350	~55	Ideal protein	Green and Hardy, 2002
	17.4-23.3		38-51		10.6 (16 MJ/kg diet), 6.0 (20 MJ/kg diet)	152 (16 MJ/kg diet), 129 (20 MJ/kg diet)		WG, FM, CG	460	>75	Broken line, 4-PL, Exp.	Encarnação et al., 2004
		15.2		56.9!		78		WG	DP, 286	~90!	Broken line	Bodin et al., 2006a
		20.5		53.8!		78		WG	DP, 430	~100!	Broken line	Bodin et al., 2006a
Methionine (Cysteine)		7.2 (3.6)		18 (9)				FM, CS or egg	400		Protein gain	Ogino, 1980
	10.1 (0) 5.1 (20.5)		20.2 (0) 10.2 (41)					CS, GEL	500	†	ANOVA	Walton et al., 1982
	5.5-7.5 (3)		16-21(8.6)					CS, GEL	350	<40	ANOVA	Rumsey et al., 1983
	11.3 (3.4)		29.7 (8.9)					SM	380	~60	Polynomial	Poston, 1986
	5.2 (3)		15 (8.6)					CS	350	~65	Broken line	Kim et al., 1992b
	6.0 (1.6)¶		15 (4)					FM, SM, WM	400	TLS	ANOVA	Cowey et al., 1992
	6.7 (3.0)	8.0 (3.0)						WG	340	~60	Exp.	Rodehutscord et al., 1995a
		8.1 (2.2)		23 (6)				FM	350	~55	Ideal protein	Green and Hardy, 2002
Phenylalanine (Tyrosine)		12.4 (8.4)		31 (21)				FM, CS or egg	400		Protein gain	Ogino, 1980
Phe + Tyr	15		43					CS, GEL	350	~65	Broken line	Kim, 1993
		13.7 (10.5)		39 (30)				FM	350	~55	Ideal protein	Green and Hardy, 2002
Threonine		13.6		34				FM, CS or egg	400		Protein gain	Ogino, 1980
	9.5	10.4	28	31			8.1	WG	335	~65	Exp.	Rodehutscord et al., 1995b
		12.3		35				FM	350	~55	Ideal protein	Green and Hardy, 2002
		10.5		27				WG	393	~100	Broken line	Bodin et al., 2006b

Table 3.1. cont'd

Amino acid								Protein Source	Dietary protein (g kg^{-1} DM)	Highest growth rate (%)§	Evaluation method	Reference
Response	Weight gain	Protein gain	Weight gain	Protein gain	Weight gain	Protein gain	FER					
	g AA kg^{-1} dry diet*	g AA kg^{-1} dry diet*	g AA kg^{-1} dietary protein*	g AA kg^{-1} dietary protein*	g AA kg^{-1} gain*	g protein or AA kg^{-1} protein gain*	g AA kg^{-1} dry diet					
Tryptophan		2		5				FM, CS or egg	400		Protein gain	Ogino, 1980
	2.3		5.8‡					GEL	420	~45	Regression	Poston & Rumsey, 1983
	2.5		4.5					FM, GEL	550	~45	Broken line	Walton et al., 1984b
	2-2.5		5.7-7.1									Kim et al., 1987
	2.1-2.6		3.8-4.6					GEL, CS	560	~65	ANOVA	Johnston et al., 1990
	1.9	2.0	5.7	6.0			1.8	WG	331	>55	Exp.	Rodehutscord et al., 1997
		2.1		6				FM	350	~55	Ideal protein	Green and Hardy, 2002
Valine		12.4		31				FM, CS or egg	400		Protein gain	Ogino, 1980
	15.5	15.7	45	46			7.8	WG	344	>70	Exp.	Rodehutscord et al., 1997
		11.2		32				FM	350	~55	Ideal protein	Green and Hardy, 2002

Abbreviations: CG, corn gluten; CS, casein; DP, digestible protein; Exp., exponential model; FER, feed efficiency ratio; FM, fish meal; GEL, gelatine; TLS, too large change in weights for comparison with table in Austreng et al. (1987); SM, soybean meal; WG, wheat gluten; WM, wheat middlings; 4-PL, 4-parameter logistic equation.

§Percentage of growth rates for salmon and trout given in Austreng et al. (1987).

*If not presented, then calculated from data given in the article.

†Water temperature not given.

∫From Hauler and Carter (2001b).

!Pers. comm., N. Bodin.

¶6 g Met/kg diet and 3.6 g Cys/kg diet required for prevention of lens abnormality.

‡6.3 g Trp/kg dietary protein required for prevention of pathology.

Table 3.2. Dietary protein (N x 6.25) and indispensable amino acid (AA) requirements of Atlantic salmon for weight gain or protein gain

Amino acid							Protein source	Dietary protein (g kg^{-1} DM)	Highest growth rate (%)[§]	Evaluation method	Reference
Response	Weight gain	Protein gain	Weight gain	Protein gain	Weight gain	Protein gain					
	g AA kg^{-1} dry diet*	g AA kg^{-1} dry diet*	g AA kg^{-1} dietary protein*	g AA kg^{-1} dietary protein*	g AA kg^{-1} gain*	g protein or AA kg^{-1} protein gain*					
Protein						3050	FM	14-541	~55	Regression	Abboudi et al., 2006b
						1560	FM	540	~140	Regression	Helland et al., 2006
Arginine	16		41				CS, CG	400	~65	Broken line	Lall et al., 1994
	21.2	21.6	50	51			Zein	420	~35	Asymptotic	Berge et al., 1997
	18.2		40				FM	450	~60	Ideal protein	Rollin et al., 2003
						95	FM	540	~140	Regression	Helland et al., 2006
Histidine	8.1		20				CS, CG	460	~70	Broken line	Scott, 1998
	6.7		15				FM	450	~60	Ideal protein	Rollin et al., 2003
						31	FM	540	~140	Regression	Helland et al., 2006
Isoleucine						52	FM	540	~140	Regression	Helland et al., 2006
Leucine						98	FM	540	~140	Regression	Helland et al., 2006
Lysine	19.9		40		21.3∫		FM, WG	500	~35	Broken line	Anderson et al., 1993
	18		42		15.4∫		Zein	427	~60	Asymtotic	Berge et al., 1998
					19.3		Zein, FM	430	~40	Regression	Hauler and Carter, 2001a
	23.9		53				FM	450	~60	Ideal protein	Rollin et al., 2003
					22.5	152	FM	14-382	~55	Regression	Abboudi et al., 2006b
						105	FM	540	~140	Regression	Helland et al., 2006
		25.2		50.4			FM, WG, CG	500	~90	Exponential	Espe et al., 2007
Methionine (Cysteine)	11		24				CS	450		Broken line	Rollin et al., 1994
	11.4 (3.6)		24.2 (8)				FM, SM, WM	470	~60	Broken line	Scott, 1998
	8.7 (≥2.6)		20.3 (6)				FM, SM	410	100	Regression	Sveier et al., 2001
						40	FM	540	~140	Regression	Helland et al., 2006
Met & Cys	15.4		34				FM	450	~55	Ideal protein	Rollin et al., 2003
Phenylalanine & Tyrosine	25.1		56				FM	450	~55	Ideal protein	Rollin et al., 2003
Phenylalanine						51	FM	540	~140	Regression	Helland et al., 2006
Threonine	12.1		27				FM	450	~65	Ideal protein	Rollin et al., 2003
	11.4		28		9.3	66	WG	397	~80	Regression	Rollin et al., 2006
		10.6		27			WG	393	~85	Broken line	Bodin et al., 2006b
						55	FM	540	~140	Regression	Helland et al., 2006
Tryptophan	3.3		7				FM	450	~60	Ideal protein	Rollin et al., 2003
						19	FM	540	~140	Regression	Helland et al., 2006
Valine	14.1		31				FM	450	~60	Ideal protein	Rollin et al., 2003
						64	FM	540	~140	Regression	Helland et al., 2006

Abbreviations: CAS, casein; CG, corn gluten; CS, casein; FER, feed efficiency ratio; FM, fish meal; SM, soybean meal; WG, wheat gluten; WM, wheat middlings.

[§]Percentage of growth rates for salmon and trout given in Austreng et al. (1987).

*If not presented, then calculated from data given.

∫From Hauler and Carter (2001b).

the effect of growth rate on minimal AA requirements of fish has still not been resolved.

Several other methods have been used to determine AA requirements. Ogino (1980) used the daily increase in IAA in rainbow trout fed a 40% crude protein diet for estimating the requirement of all of the IAA. In calculating the requirements, Ogino (1980) reported a feed intake level of 3% body weight per day, but Kim et al. (1992b) suggests that this level is too low according to the data presented, resulting in an overestimation of the requirements (28% overestimation for methionine). Walton et al. (1986) also noted that Ogino (1980) did not take into account maintenance losses of AA and assumed that there were no oxidation or transformation losses of the dietary IAA.

Green and Hardy (2002) and Rollin et al. (2003) used the diet-dilution method to determine the optimum dietary IAA pattern in rainbow trout and Atlantic salmon. The diet-dilution method assumes that the reduction of a non-limiting IAA has no effect on N gain. When a single AA is limiting, the rate of body protein accretion is directly related to the level of that AA. The change in N gain measured on removal of a proportion of the dietary content of an IAA is used to calculate the appropriate AA pattern. The pattern was determined for all of the IAA in the study of Green and Hardy (2002) and all of the IAA except leucine and isoleucine in the study of Rollin et al. (2003). For the latter two IAA, no decrease in N gain was registered when either of these was diluted in the diet. Both groups used the IAA pattern to establish the dietary requirement for the IAA.

During the last decade, the factorial approach, or the requirement by ration level method (RRL; described in Chapter 2) has been used to determine the requirements for growth and maintenance for crude protein and energy in fish species such as African catfish, carp, European sea bass, gilthead seabream, rainbow trout, silver perch, white grouper, Atlantic cod (cited by Hatlen et al., 2007), brown trout (Mambrini et al., 2004) and Atlantic salmon (Helland et al., 2006). The method is dependent on accurate measurements of feed intake, digestibility and nutrient accretion. Usually, only one diet is used and it is offered at different levels of feeding or ration levels (Fig. 3.1). In the relationship between the intake of a nutrient and its deposition, or the deposition of a related nutrient (per unit of weight), the maintenance requirement is the amount of nutrient consumed (or absorbed) when gain is zero. This value may be estimated from a measured point of zero nutrient gain, or through an extrapolation to zero gain from higher intake levels. As defined by Beck and Gropp (1995), an approximate maintenance requirement reflects the loss of the nutrient during starvation, and is a value correlated with, but lower than the maintenance requirement. For crude protein and AA, the maintenance requirement includes AA losses from the skin and gastrointestinal

tract, ammonia loss from oxidation of amino acids in body protein, AA used for synthesis of other bioactive molecules, irreversible alteration of an AA (proline to hydroxyproline and lysine to hydroxylysine in protein), and the loss of AA in the urine (Moughan and Fuller, 2003). According to Boisen et al. (2000), the maintenance requirement for amino acids is difficult to determine because at N-equilibrium redistribution of tissue protein will occur. Muscle protein is degraded at low feed intake to compensate for the endogenous loss of amino acids. The methionine, cystine and threonine contents in muscle protein are lower than those in the endogenous losses, thus making the other AA in excess when used for replacing the endogenous losses. The excess of the other AA in this redistribution may result in an underestimation of the required amounts of these AA for maintenance (Boisen et al., 2000). Nevertheless, the dose-response method (Rodehutscord et al., 1997) has been used to estimate the maintenance requirements for the AA for trout. We are currently evaluating the suitability of the RRL method for estimating AA maintenance and growth requirements (Helland et al., 2006). The requirement for growth reflects the efficiency of deposition of the nutrient in the body per unit intake above that required for maintenance.

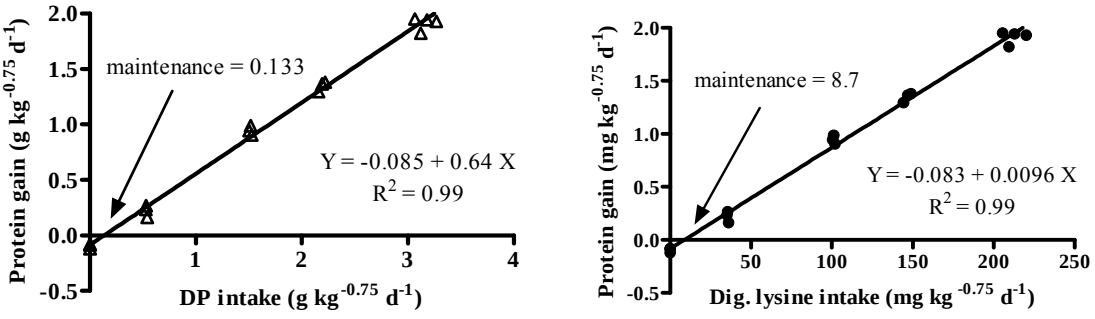

Figure 3.1. The relationships between digestible protein (DP) and digestible lysine intake on protein gain in Atlantic salmon post-smolt fed different rations (Helland et al., 2006).

3.3. Amino acid deficiency

Feed intake is generally reduced when there is a severe amino acid deficiency. Fish may compensate though, when the deficiency is not too severe and maintain feed intake. As reviewed by D'Mello (2003), acute amino acid deficiency, in animals fed to *ad libitum*, may result in decreased protein content of the liver, muscle and pancreas, increased free amino acid levels in the plasma, increased protein synthesis in the liver and blood, and decreased

protein synthesis in the muscle. In dose-response trials with trout, Rodehutscord et al. (1995a; 1995b; 1997) found that deficiencies of arginine, isoleucine, lysine, leucine, methionine, threonine or tryptophan resulted in increased lipid deposition, valine deficiency resulted in reduced lipid deposition, whereas no change in lipid deposition was found with histidine deficiency.

3.4. Amino acid requirements

In the following summaries of AA requirements, priority has been given to the use of *g AA kg^{-1} dietary protein* as the independent variable and *protein gain* as the dependent or response variable.

3.4.1. Lysine

Most commonly, the first-limiting AA in protein sources used in fish diets is lysine. As a result, the lysine requirement has been evaluated most often. Hauler and Carter (2001b) reviewed the lysine requirements that have been determined in dose-response trials in different fish species. They concluded that when the requirement is expressed as a percentage of the diet, variation between the results, within species, could not be explained by experimental factors such as dietary formulation, growth rate, statistical model or response variable. In contrast, Hauler and Carter (2001b) found that if the requirement was expressed relative to weight gain (as in the factorial method), less variation between experiments was evident. Their calculations showed that for salmon (Table 3.2), the lowest requirement for lysine was 15.4 g kg^{-1} weight gain from the study of Berge et al. (1998), where 0.5 kg fish were studied. For 2 g salmon, Abboudi et al. (2006b) reported the highest value measured (22.5 g lysine kg^{-1} weight gain) and showed a strong negative correlation (r=0.99) between the lysine requirement (g kg^{-1} weight gain) and log body weight in the four available studies with Atlantic salmon. Hauler and Carter (2001b) also summarized data for rainbow trout and found that 15.7 to 21.1 g lysine was required per kg gain (the data of Lanari et al. (1991) was ignored because of overestimated feed intake). Later data with trout shows though, that only 6 to 11 g lysine were required per kg weight gain (Encarnação et al., 2004). Hauler and Carter (2001b) noted that this expression would overestimate AA requirements in fish depositing adipose tissue (such as in the study of Berge et al., 1998) and that the most appropriate responses would be protein or AA gain, instead of live weight gain. Using this response, Abboudi et al. (2006b) reported a lysine requirement of 152 g kg^{-1} protein gain in

salmon fry, whereas the data of Helland et al. (2006), with on-growing salmon, shows a lysine requirement of 105 g kg^{-1} protein gain.

Encarnação et al. (2004) reported that there was no effect of the dietary energy level on the minimal dietary lysine requirement for trout and concluded that there was no reason to express the AA requirements in relation to the dietary digestible energy content. In contrast, the dietary energy level has a negative effect on the lysine requirement for protein gain in trout. A lysine requirement of 152 g kg^{-1} protein gain was found when trout were fed a diet containing 16 MJ kg^{-1} and 129 g kg^{-1} protein gain was required when trout were fed a diet containing 20 MJ kg^{-1} (Encarnação et al., 2004). The data of Bodin et al. (2006a) shows that in rainbow trout fry fed a diet containing an even higher level of energy (22-23 MJ kg^{-1}), the lysine requirement was about 78 g kg^{-1} protein gain. This suggests increasing AA-sparing through the catabolism of greater amounts of dietary non-protein energy sources. The effect of dietary protein level on the utilization efficiency of lysine in trout is not clear. Rodehutscord et al. (2000) reported a positive effect, whereas Bodin et al. (2006a) found no effect. Bodin et al. (2006a) showed though, that an increase in the digestible protein concentration from 286 to 430 g kg^{-1} diet increased the dietary lysine requirement of rainbow trout from 15 to 20 g kg^{-1} diet, and decreased the required lysine concentration in the dietary protein (pers. comm., N. Bodin).

Rodehutscord et al. (2000) showed significant, but slight, effects of dietary lysine concentration on organic matter and energy digestibility in trout. Encarnação et al. (2004) also found an effect of dietary lysine level on the digestibility of carbohydrates in trout, but noted that this may have resulted from experimental error because the carbohydrates were calculated by difference from the other organic diet materials. This also seems to be the case in the trial of Rodehutscord et al. (2000).

The minimum lysine requirements determined for rainbow trout vary from 37 to 82 g kg^{-1} dietary protein, although most values are in the range of 40-60 g kg^{-1} dietary protein (Table 3.1). The lysine requirement has not been determined as often for Atlantic salmon as for rainbow trout, but the four trials from which the salmon requirement can be expressed in relation to the dietary protein level, gave results in the range of 40-53 g kg^{-1} dietary protein (Table 3.2), or quite similar to the values for rainbow trout. The lysine requirement values for Pacific salmon are also in this range (38-50 g kg^{-1} dietary protein) (reviewed by Wilson, 2003).

The lysine maintenance requirement reported for rainbow trout (11.2 mg $kg^{-0.75}$ d^{-1}) (Rodehutscord et al., 1997) is between the levels that have been estimated for Atlantic salmon

by Helland et al. (2006) (8.7 mg $kg^{-0.75}$ d^{-1}) and Abboudi et al. (2006b) (20 mg $kg^{-0.75}$ d^{-1}) (Tables 3.3 and 3.4).

3.4.2. Arginine

In rainbow trout, arginine deficiency causes reduced growth, high mortality and fin erosion (Ketola, 1983). Based on the increase in free arginine levels in the blood and muscle when the dietary arginine intake was met, Kaushik (1979) concluded that the arginine requirement of freshwater trout was 12 g kg^{-1} diet, but this was not confirmed in an accompanying growth trial. The requirement level estimated by Kaushik (1979) is, however, only slightly lower than the requirement levels subsequently determined, when weight gain was used as the response variable (14-28 g kg^{-1} diet; Table 3.1), except that of Rodehutscord et al. (1995b) (11 g kg^{-1} diet). Furthermore, when expressed in terms of the dietary protein level, the arginine requirement given by Kaushik (1979) (33 g kg^{-1} dietary protein[b]) was in the range of that determined in most of the later trials (30-40 g kg^{-1} dietary protein; Table 3.1). On a dietary basis, the arginine requirements estimated for Atlantic salmon (16-21 g kg^{-1} diet) are similar to those found for trout, whereas on a protein basis (40-50 g kg^{-1} dietary protein) these are a little higher. The arginine requirements reported for salmon species from the Pacific Ocean (chinook, chum and coho salmon) range from 32 to 65 g kg^{-1} dietary protein (reviewed by Wilson, 2003).

The maintenance requirement for arginine has been studied in two trials with rainbow trout (Table 3.3). Rodehutscord et al. (1997) reported an arginine requirement of 15.5 mg $kg^{-0.75}$ d^{-1}, whereas Fournier et al. (2002) could find little or no maintenance needs for arginine in rainbow trout, turbot or gilthead seabream. The arginine requirement for maintenance for Atlantic salmon (Table 3.4) determined by Helland et al. (2006) was 8 mg $kg^{-0.75}$ d^{-1}, approximately half of that determined by Rodehutscord et al. (1997) for trout. In the neonatal and immediate post-weaning phases of the pig, endogenous synthesis of arginine from glutamate/glutamine and proline occurs, covering about 50% of the daily requirement (Wu et al., 1997). It has been suggested that *de novo* synthesis of arginine may also occur in fish (Buentello and Gatlin, 2000, 2001), but this has not been unequivocally shown based on the presence of the necessary enzymes.

[b] The dietary protein content was not given. This value is cited by Wilson (2003).

Table 3.3. Protein and indispensable amino acid (AA) requirements of rainbow trout for maintenance and growth

Amino acid	Maintenance requirement	Growth requirement	Protein source	Dietary protein (g kg^{-1})	Evaluation method	Reference
Response	Zero protein gain (mg protein or AA $kg^{-.75}$ d^{-1})	For max. protein or weight gain (mg AA kg^{-1} d^{-1})				
Protein	774					Kaushik et al., 1981
	325					Mambrini and Kaushik, 1995 (cited by Abboudi et al, 2006b)
	236		CS	14-506	Regression	Fournier et al., 2002
Arginine		210†	Zein, FM		Graphic	Kaushik, 1979
	15.5		WG	344	Exponential	Rodehutscord et al., 1997
	0		CS	14-506	Regression	Fournier et al., 2002
Histidine	6.0		WG	344	Exponential	Rodehutscord et al., 1997
Isoleucine	5.1		WG	344	Exponential	Rodehutscord et al., 1997
Leucine	47.8		WG	344	Exponential	Rodehutscord et al., 1997
Lysine	11.2		WG	344	Exponential	Rodehutscord et al., 1997
		640	WG	DP, 286 or 430	Regression	Bodin et al., 2006a
Methionine	9.0		WG	344	Exponential	Rodehutscord et al., 1997
Threonine	18.8		WG	344	Exponential	Rodehutscord et al., 1997
Tryptophan	6.0		WG	344	Exponential	Rodehutscord et al., 1997
		50	FM, GEL	550	Broken line	Walton et al., 1984
Valine	16.7		WG	344	Exponential	Rodehutscord et al., 1997

Abbreviations: CS, casein; DP, digestible protein; FM, fish meal; GEL, gelatine; WG, wheat gluten.

† Based on increase in free arginine in blood and muscle.

Table 3.4. Protein and indispensable amino acid (AA) requirements of Atlantic salmon for maintenance

Amino acid	Maintenance requirement	Protein source	Dietary protein (g kg^{-1})	Evaluation method	Reference
Response	Zero protein gain (mg protein or AA $kg^{-.75}$ d^{-1})				
Protein	337	FM	14-541	Regression	Abboudi et al., 2006b
	133	FM	540	Regression	Helland et al., 2006
Arginine	7.9	FM	540	Regression	Helland et al., 2006
Histidine	2.6	FM	540	Regression	Helland et al., 2006
Isoleucine	4.5	FM	540	Regression	Helland et al., 2006
Leucine	8.3	FM	540	Regression	Helland et al., 2006
Lysine	20	FM	14-382	Regression	Abboudi et al., 2006b
	8.7	FM	540	Regression	Helland et al., 2006
Methionine	3.3	FM	540	Regression	Helland et al., 2006
Phenylalanine	4.4	FM	540	Regression	Helland et al., 2006
Threonine	6	FM, WG	398	Regression	Rollin et al., 2006
	4.7	FM	540	Regression	Helland et al., 2006
	9.9	FM		Regression	Abboudi et al., 2006a
	41.2	Acc. CAA†	398	Regression	Abboudi et al., 2006a
	5.4	WG	398	Regression	Abboudi et al., 2007
Tryptophan	1.7	FM	540	Regression	Helland et al., 2006
Valine	5.4	FM	540	Regression	Helland et al., 2006

Abbreviations: FM, fish meal; WG, wheat gluten.

†Pre-accustomed for 14 days to a crystalline AA diet.

3.4.3. Histidine

There is good agreement between the histidine requirements for Atlantic salmon and rainbow trout (15-20 g kg^{-1} dietary protein) and these are also in line with most of the histidine requirements determined for other salmonid species (16-18 g kg^{-1} dietary protein), except the 9 g kg^{-1} dietary protein for coho salmon estimated by Arai and Ogata (1993) (reviewed by Wilson, 2003).

Breck et al. (2003) reported that elevated levels of dietary histidine (from 8 to 15 g kg^{-1} diet) and/or Fe in the diet had an alleviating effect on cataract development in Atlantic salmon, rather than correcting a nutritional deficiency. Compared with the minimal requirement determined for maximum growth (7-8 g kg^{1} diet; Scott, 1998; Rollin et al., 2003), these results may indicate a higher requirement for normal eye development. In a later study, Breck et al. (2005a) found that the supplementation of a fishmeal-based diet with crystalline histidine (increasing the dietary histidine level from 12.6 to 19.0 g kg^{-1} diet) resulted in a reduction in the severity of cataract scores, but no effect on the frequency of cataract development. The greatest effect of supplementary histidine was found when it was fed in the saltwater period, although some effect was also seen in the freshwater period. A reduction in cataract scores in salmon fed a diet containing 15.3 g histidine kg^{-1} compared with those fed a control diet (9.6 g histidine kg^{-1}) was also seen in the study of Breck et al. (2005b). Breck et al. (2005a) observed that despite 10% lower growth in the salmon fed the histidine-supplemented diet for six weeks in fresh water, growth was better in the fish fed the supplemented diet after a total of 26 weeks (including 17 weeks in saltwater), compared with those fed the control diet. The fish fed the histidine-supplemented diet also had better feed conversion than those fed the control diet.

As with arginine, the maintenance requirement for histidine in Atlantic salmon (2.6 mg $kg^{-0.75}$ d^{-1}; Helland et al., 2006) was about half of that found for rainbow trout (6.0 mg $kg^{-0.75}$ d^{-1}; Rodehutscord et al., 1997) (Table 3.3 and 3.4).

3.4.4. Isoleucine

The isoleucine requirements for rainbow trout determined by Ogino (1980) and Green and Hardy (2002) were 24 and 28 g kg^{-1} dietary protein, respectively, whereas that estimated by Rodehutscord et al. (1997) was 41 g kg^{-1} dietary protein. No dose-response study has been done to determine the isoleucine requirement for Atlantic salmon. As noted above, Rollin et al. (2003) used the diet-dilution method to estimate the IAA requirements in Atlantic salmon, but this was not possible for isoleucine and leucine, since no decrease in N gain was

registered when either of these AA was diluted in the diet. As reviewed by Wilson (2003), the isoleucine requirements for Pacific salmon and lake trout (12-26 g kg^{-1} dietary protein) are similar to the lowest value determined for rainbow trout.

The isoleucine maintenance requirements are similar in Atlantic salmon (4.5 mg $kg^{-0.75}$ d^{-1}; Helland et al., 2006) and rainbow trout (5.1 mg $kg^{-0.75}$ d^{-1}; Rodehutscord et al., 1997) (Tables 3.3 and 3.4).

3.4.5. Leucine

The leucine requirements determined for rainbow trout vary from 40 to 50 g kg^{-1} dietary protein, and are in the range of those determined for Pacific salmon and lake trout (35-46 g kg^{-1} dietary protein) (reviewed by Wilson, 2003). As noted above, the leucine requirement for Atlantic salmon has not been determined.

The maintenance requirement for Atlantic salmon is 8.3 mg $kg^{-0.75}$ d^{-1} (Helland et al., 2006) (Table 3.4). For rainbow trout, Rodehutscord et al. (1997) reported a value of 47.8 mg $kg^{-0.75}$ d^{-1}, which, the authors noted, appeared surprisingly high compared to the level for the other AA (Table 3.3).

3.4.6. Methionine

The requirements for the sulfur-containing AA, methionine and cysteine, for rainbow trout are in the range 19-39 g kg^{-1} dietary protein, with most values varying from 19 to 30 g kg^{-1} dietary protein (Table 3.1). Trials with Atlantic salmon juveniles and smolt have shown that the methionine requirement for maximal growth is 24 g kg^{-1} dietary protein (Rollin et al., 1994; Scott, 1998), whereas Sveier et al. (2001) reported that for 1 kg salmon, the methionine requirement was 20 g kg^{-1} dietary protein. The total amount of the sulfuric AA in these diets was about 26-34 g kg^{-1} dietary protein. For Pacific salmon, the total requirement for these AA (27 to 50 g kg^{-1} dietary protein; reviewed by Wilson, 2002) is similar or higher than the results for Atlantic salmon and rainbow trout.

Methionine deficiency causes bilateral cataracts in trout (Poston et al., 1977), probably as a result of oxidation of the methionine in the lens (reviewed by Cowey et al., 1992). Cowey et al. (1992) found that trout fed a methionine-deficient diet exhibited cataracts and increased focal length variability in the lenses compared with normal fish. Although reductions in the number of cataracts occurred when the methionine level was increased in steps up to 19 g kg^{-1} diet, an increase in the dietary cystine level to 3.6 g kg^{-1}, at a methionine level of 6 g kg^{-1}, was necessary before a well-defined focal point with little evidence of

spherical aberration was found in the trout (Cowey et al., 1992). Rodehutscord et al. (1995a) found no effect of increasing the dietary cystine level from 3.0 to 5.8 g kg^{-1} (dry matter) on the growth and protein deposition of rainbow trout, both when the methionine supply was deficient or adequate. As shown by Kim et al. (1992b), cystine may be able to replace some of the methionine when the cystine level is much lower. Rodehutscord et al. (1995a) concluded that since the cystine level in trout feeds based on usual ingredients should not normally be lower than 3 g kg^{-1} diet, recommendations for sulfur-containing AA should be specified in terms of methionine.

The methionine maintenance requirement has been estimated to be 9.0 mg $kg^{-0.75}$ d^{-1} for rainbow trout (Rodehutscord et al., 1997) and 3.3 mg $kg^{-0.75}$ d^{-1} for Atlantic salmon (Helland et al., 2006) (Table 3.3 and 3.4).

Increasing the dietary methionine content in trout diets from about 8 g kg^{-1} up to 12 or 16 g kg^{-1} (plus ~4 g kg^{-1} cysteine) resulted in a tendency for decreased feed intake and significantly poorer gain (Gaylord et al., 2007).

3.4.7. Phenylalanine

Phenylalanine and tyrosine are aromatic amino acids. Tyrosine is a DAA because it can be synthesized from phenylalanine. Growth studies indicate that tyrosine can replace or spare about 48% of the phenylalanine requirement in rainbow trout (Kim, 1993).

The requirement for phenylalanine and tyrosine for rainbow trout is in the range of 43 (Kim, 1993) to 69 g kg^{-1} dietary protein (Green and Hardy, 2002). The value for Atlantic salmon, determined by Rollin et al. (2003), is between these values (56 g kg^{-1} dietary protein). The requirement values for phenylalanine and tyrosine for Pacific salmon are in a similar range (45-63 g kg^{-1} dietary protein) (reviewed by Wilson, 2002).

The phenylalanine requirement for maintenance for Atlantic salmon is 4.4 mg $kg^{-0.75}$ d^{-1} (Helland et al., 2006) (Table 3.4). This requirement has not been determined for rainbow trout.

3.4.8. Threonine

Only small differences have been reported in the threonine requirement for rainbow trout determined in four trials (27-35 g kg^{-1} dietary protein; Table 3.1). The requirement for threonine for Atlantic salmon, determined in three trials, has showed little variation (27-28 g kg^{-1} dietary protein; Table 3.2). These values are similar to those determined for Pacific salmon (20-30 g kg^{-1} dietary protein) (reviewed by Wilson, 2002).

The maintenance requirement for threonine in rainbow trout (Table 3.3) has been reported to be 18.8 mg $kg^{-0.75}$ d^{-1} for rainbow trout (Rodehutscord et al., 1997). The maintenance requirement for threonine in Atlantic salmon (Table 3.4) has been determined using three of the methods described above. The dose-response (Rollin et al., 2006; Abboudi et al., 2006a) and diet-dilution methods (Abboudi et al., 2007) have been used for estimating the requirement in salmon fry, whereas Helland et al. (2006) used the factorial method with on-growing salmon. Together, these studies show that when protein-bound AA are used in the diet the threonine maintenance values range from 4.7 to 9.9 mg $kg^{-0.75}$ d^{-1} (Rollin et al., 2006; Helland et al., 2006; Abboudi et al., 2006a). When salmon are first accustomed to a crystalline AA before the start of the trial, however, the threonine maintenance was increased to 41 mg $kg^{-0.75}$ d^{-1} (Abboudi et al., 2006a). The reason for this difference has not been determined.

3.4.9. Tryptophan

Tryptophan deficiency may result in low feed intake and growth, scoliosis, lordosis, fin erosion, cataracts and mortality in rainbow trout (reviewed by Poston and Rumsey, 1983). Poston and Rumsey (1983) found that 5.8 g tryptophan kg^{-1} dietary protein resulted in maximum weight gain (95% confidence intervals), whereas 6.3 g kg^{-1} dietary protein was required for maximum predicted health score. The tryptophan requirement for Atlantic salmon (7 g kg^{-1} dietary protein; Table 3.2) is at the upper end of the requirement values determined in various trials for rainbow trout (3.8-7.1 g kg^{-1} dietary protein; Table 3.1). The tryptophan requirement values for Pacific salmon are similar to those for the other salmonids (5-7 g kg^{-1} dietary protein; reviewed by Wilson, 2002).

The maintenance requirement for tryptophan is considerably higher in rainbow trout than in Atlantic salmon (6.0 and 1.7 mg $kg^{-0.75}$ d^{-1}, respectively) (Rodehutscord et al., 1997; Helland et al., 2006) (Tables 3.3 and 3.4).

3.4.10. Valine

The valine requirement for Atlantic salmon (31 g kg^{-1} dietary protein) (Rollin et al., 2003) is very similar to that determined in two trials with rainbow trout (31-32 g kg^{-1} dietary protein; Ogino, 1980; Green and Hardy, 2002). Somewhat higher was valine requirement value for rainbow trout determined by Rodehutscord et al. (1997), 46 g kg^{-1} dietary protein.

Rainbow trout seem to require a higher level of valine for maintenance than Atlantic

salmon (16.7 and 5.4 mg $kg^{-0.75}$ d^{-1}, respectively) (Rodehutscord et al., 1997; Helland et al., 2006) (Tables 3.3 and 3.4).

3.4.11. Effect of salinity on protein and arginine needs

Zeitoun et al. (1973) found that the minimum protein levels for optimum growth in rainbow trout fingerlings were 400 and 450 g kg^{-1} when the salinity was 10 and 20 ppt, respectively. In contrast, based on free arginine levels in the blood and muscle, Kaushik (1979) concluded that the arginine requirement was decreased from 12 g kg^{-1} diet for trout held in fresh water to 8 to 12 g kg^{-1} at 20 ppt salinity and 8 g kg^{-1} in sea water. Krogdahl et al. (2004) compared feed utilization in fresh water and sea water by rainbow trout and Atlantic salmon. The authors found that the protein concentration in the body of the trout was 0.5% higher in fish maintained in sea water compared with those maintained in fresh water, although this difference may have been related to the body lipid level. No differences in protein or lipid concentrations were found in the salmon. For the two species together, feed efficiency increased from 1.23 to 1.30 and the retention of digestible protein in the body increased by almost 5% when the fish were held in sea water compared with fresh water. Except for a slight decrease in protein digestibility in sea water compared with fresh water, these results do not seem to support a higher dietary protein need in sea water.

3.4.12. Validation of IAA minimum requirements

In an interesting study, Green and Hardy (2002) compared the growth and N retention of rainbow trout fed diets containing IAA patterns generated using different techniques. The IAA patterns used included 1) one that they themselves determined with the diet-dilution method (Green and Hardy, 2002), 2) the pattern reflecting the IAA composition of rainbow trout whole-body protein from Wilson and Cowey (1985), 3) the requirements published by the National Research Council (NRC, 1993), or 4) the requirements determined in the studies of Rodehutscord et al. (1995a; 1995b; 1997). The fish were fed to the same level (near-satiation for the fish with the least intake) in quadruplicate groups. The results showed that both the growth rate and N retention were significantly greater in the fish fed the NRC requirement pattern compared with those fed the Rodehutscord pattern. The other two diets yielded differences that were intermediate to the others. Green and Hardy (2002) concluded that the NRC pattern, derived from a large number of studies, was best matched to the optimum IAA pattern for rainbow trout, even though it was not statistically better than that derived from whole-body protein or determined with the diet-dilution method. Unfortunately,

the best growth rate in this comparison trial was under 50% of that estimated by Austreng et al. (1987) for the fish size and water temperature used. The pattern of IAA needed for maintenance is not the same as that for growth, and thus, it is not certain that the same conclusion would be obtained with higher growth rates.

3.4.13. Comparison of maintenance requirements for Atlantic salmon and rainbow trout

Seven of the nine IAA maintenance requirement values determined for trout by Rodehutscord et al. (1997), are two to six times higher than those found for salmon by Helland et al. (2006). The exceptions are for isoleucine and lysine that were only 10 and 30%, respectively, higher for trout than for salmon. Differences between these trials include the feed efficiency ratio achieved by each species and the digestible energy level of the diets used. The feed efficiency ratios in the dose-response trials for each AA for the trout were all about 1, whereas in the salmon trial, this ratio varied from 1.2 to 1.8, depending on the ration level. As seen in Fig. 3.2, greater gain with the same intake will result in a greater slope and a lower maintenance level. In addition, in the salmon trial, the digestible energy level of the diet was almost 24 MJ kg^{-1}, whereas in the trout trials the DE levels were about 20 MJ kg^{-1}. An increase in the dietary digestible energy content may spare protein (as, for example, shown in Grisdale-Helland and Helland, 1997), again suggesting a greater efficiency slope and lower maintenance level for the IAA. Direct comparisons between Atlantic salmon and rainbow trout show that salmon retain greater amounts of consumed or digested protein in the body than trout do (Refstie et al., 2000; Azevedo et al., 2004; Grisdale-Helland et al., 2007).

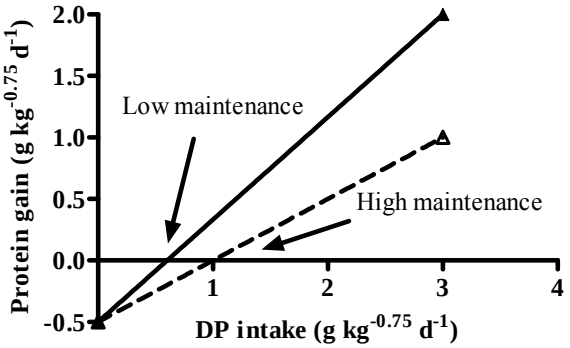

Figure 3.2. Higher feed efficiency (gain/intake) results in lower maintenance.

3.4.14. Amino acid requirements for growth determined using the requirement by ration level (RRL) method

The RRL method can be used to determine the AA requirements for maintenance and for AA or protein gain in fish (Helland et al., 2006; Table 3.2). This method does not give the minimum dietary requirement for an AA, but rather the efficiency of utilization of the AA for growth. Together with the maintenance requirement for the AA, the reciprocal of the efficiency coefficient can be used to determine the total requirement of the AA per kg growth. From Table 3.2, it can be seen that much less lysine and total protein were required per kg protein gain with post-smolt salmon (Helland et al., 2006) than with fry (Abboudi et al., 2006b). The data for the latter trial were determined using diets with different N and lysine levels and the best growth rate was about 55% of that estimated by Austreng et al. (1987). The AA requirement for growth above maintenance determined with the RRL method is, of course, independent of the growth rate of the fish. At the same time, the diet used in the study of Helland et al. (2006) seemed to be well balanced and led to excellent growth in the full-fed groups of fish. Our results with an RRL study with Atlantic cod (Hatlen et al., 2007) suggest that the utilization efficiency of the AAs will be negatively affected by an increase in the dietary protein level. This does not, however, seem to be the reason for the difference between the results in the two salmon studies described above. The protein levels in the diets used in the study of Abboudi et al. (2006b) ranged from 14 to 54%, whereas the dietary protein level used in the trial of Helland et al. (2006) was 54%.

To accurately determine the AA requirements for maintenance and growth of fish, the effects of both biotic and abiotic factors need to be evaluated. Fish size, stage of growth and genetics, diet ingredients and composition, and the environment are all assumed to be important in influencing these requirements. It is clear from this review of AA requirements that much research is necessary to generate data that can be used in modelling to predict the AA levels required in diets for fast-growing fish. In this context, the RRL-method is very interesting since it predicts both the requirements for maintenance and growth above maintenance for all the essential amino acids in one trial and, at the same time, permits an evaluation of the interaction between energy and amino acid metabolism.

3.5. Dietary indispensable : nondispensable AA ratio

NAA can be obtained from the diet or synthesized in the body. Diets lacking NAA, however, result in low growth of trout (Schuhmacher et al., 1995). These authors found that the growth rate of trout was equal when they were fed diets containing IAA:NAA ratios of

40:60 or 60:40. Nile tilapia (*Oreochromis niloticus*) had greater body and N growth when fed a diet with an IAA:NAA ratio of 40:60 compared with a diet with an IAA:NAA ratio of 25:75 (Mambrini and Kaushik, 1994). In a more detailed trial, Green et al. (2002) found that N retention in trout responded in a quadratic manner to an increasing dietary IAA:NAA ratio (from 23:77 to 66:34) and that 95% of the maximum N retention was found with a ratio of 46:54.

3.6. Amino acid antagonisms and toxicities

In many animals, excess lysine impairs the utilization of arginine, whereas excess leucine reduces the utilization of the other two branched-chain AA, isoleucine and valine, even when they are not limiting in the diet. Reversal of these effects may be obtained by supplementation of the AAs that were negatively affected. The antagonism between lysine and arginine has not been demonstrated with growth studies in channel catfish, blue tilapia, rainbow trout, hybrid striped bass or yellow perch (reviewed by Wilson, 2002). In chicks fed excess lysine, an enhanced activity of arginase may occur, resulting in increased catabolism of arginine (Austic, 1986). In salmonids, some evidence indicates metabolic changes when excess levels of lysine or arginine are fed.

Excess branched-chain amino acids may reduce the brain pool of other amino acids, particularly phenylalanine and tryptophan, which are the precursors of the neurotransmitters (D'Mello, 1994). This may be the reason for the adverse effect of excess leucine on food intake in fish, as often seen when corn gluten is used in the diet. As reviewed by Wilson (2002), the interactions among the branched-chain AA seem to be different in the various species of fish.

3.7. Alternative protein sources

Due to the increased demand in aquaculture for the restricted supplies of fish meal, plant feedstuffs are being increasingly used in diet production. An excellent overview of the utilization of plant products in diets for fish has recently been published (Gatlin et al., 2007). Important aspects from that review in relation to the present topic are included here.

Fish meal is an excellent dietary ingredient for aquafeeds because of its high protein content, balanced AA profile, high digestibility, lack of anti-nutrients and availability. Plant protein sources are all at some disadvantage to fish meal for their use in aquafeeds. As stated by Gatlin et al. (2007), the nutritional characteristics of candidate ingredients must include "low levels of fibre, starch, especially non-soluble carbohydrates and anti-nutrients, plus have

a relatively high protein content, favourable amino acid profile, high nutrient digestibility and reasonable palatability."

The AA profile of diets can be adjusted by combining different plant sources, such as soybean meal and corn gluten meal for balancing the lysine content (Table 3.5), and/or supplementing with crystalline AA. Some plant products, however, have anti-nutritional factors that may or may not be possible to remove through processing. Much research has been done on the use of soybean meal in fish diets, but it is still not clear why salmonids have limited tolerance to this ingredient. As reviewed by Gatlin et al. (2007), impaired utilization of soybean meal in fish diets may be related to protein toxicity, as well as an immunological intolerance, exhibited as skin lesions, gastrointestinal tract alterations and excess mucus excretion in the faeces. Lectins, anti-nutritional factors present in soybean meal or other legumes, may bind with the intestinal wall, interfere with nutrient absorption and precipitate immune reactions causing cell death.

Although peas and lupins have high potential for use in aquafeed, as their protein digestibility is high, the low energy digestibility as a result of the high carbohydrate content is a negative factor.

Concerning the supplementation of crystalline AA to diets containing imbalanced AA profiles, a preliminary report indicates that the source of plant protein may affect the retention efficiency of supplemented lysine in diets for rainbow trout (Tran et al., 2006). The lysine retention efficiency in a diet containing wheat gluten was significantly higher than that in a diet containing both wheat gluten and sesame oil cake, or a diet containing maize gluten.

Table 3.5. Crude protein concentration (%) and amino acid profile [% of crude protein (CP)] in various protein sources[1, 2]

	Fish meal, Norway	Casein	Gelatine	Wheat gluten meal	Corn gluten meal, 60% CP	Soybean meal, 48% CP	Lupin seed, Australia	Field peas
Crude protein	70.5	87.2	74.2	75.9	61.1	47.6	*31.0*	*21.7*
Lysine	7.4	8.0	*4.3*	*1.5*	*1.6*	6.1	*4.6*	7.1
Methionine	2.7	2.9	*1.0*	*1.5*	2.4	*1.3*	*0.6*	*0.9*
Cystine	0.9	*0.5*	0.3	**2.1**	1.7	1.5	1.3	1.4
Met+Cys	3.6	3.4	*1.3*	3.5	4.1	2.8	1.9	2.3
Threonine	4.1	4.3	*2.2*	*2.4*	3.3	3.9	3.4	3.7
Tryptophan	1.0	1.3	*0.2*	0.9	*0.5*	1.4	0.8	0.9
Arginine	6.1	3.5	7.9	3.3	3.2	7.3	**10.6**	8.4
Isoleucine	4.0	5.2	*1.7*	3.5	3.9	4.5	4.0	4.1
Leucine	7.1	9.5	*3.8*	6.7	**16.1**	7.6	6.6	7.1
Valine	4.8	6.6	2.8	3.8	4.5	4.7	3.9	4.6
Histidine	2.2	3.0	1.9	2.1	2.1	2.7	2.7	2.5
Phenylalanine	3.9	5.1	2.5	5.0	6.2	5.0	3.8	4.8
Tyrosine						3.2	5.8	3.7

[1]From AminoDat, v. 3.0, Degussa AG, Hanau-Wolfgang, Germany.

[2]Compared with fish meal, amino acids with low concentrations are shown in italics and high concentrations in bold.

3.8. References

Aas, T., Grisdale-Helland, B., Terjesen, B.F., Helland, S.J., 2006. Improved growth and nutrient utilisation in Atlantic salmon (*Salmo salar*) fed diets containing a bacterial protein meal. Aquaculture 259, 365-376.

Abboudi, T., Bodin, N., Larondelle, Y., Rollin, X., 2006a. The effect of past nutritional history on threonine requirement for maintenance in Atlantic salmon fry. XIIth Int. Symp. Fish Nutrition & Feeding, Biarritz, France (P-113).

Abboudi, T., Mambrini, M., Ooghe, W., Larondelle, Y., Rollin, X., 2006b. Protein and lysine requirements for maintenance and for tissue accretion in Atlantic salmon (*Salmo salar*) fry. Aquaculture 261, 369-383.

Abboudi, T., Ooghe, W., Larondelle, Y., Rollin, X., 2007. Determination of the threonine requirement for maintenance in Atlantic salmon (*Salmo salar* L.) fry with the diet dilution procedure. Aquac. Nutr. 13, 281-290.

Anderson, J.S., Lall, S.P., Anderson, D.M., McNiven, M.A., 1993. Quantitative dietary lysine requirement of Atlantic salmon (*Salmo salar*) fingerlings. Can. J. Aquat. Sci. 50, 316-322.

Arai, S., Ogata, H., 1993. Quantitative amino acid requirmenets of fingerline coho salmon (*Oncorhynchus kisutch*). In: Collie, M.R., McVey, J.P. (Eds.), Proc. Twentieth U.S.-Japan Symp. Aquaculture Nutrition. Newport, Oregon, Oct. 1991, pp. 19-28. (Cited by Wilson, 2003.)

Austic, R.E., 1986. Biochemical description of nutritional effects. In: Fisher, C., Boorman, K.N. (Eds.), Nutritent Requirements of Poultry and Nutritional Research. Butterworths, London, pp. 59-77.

Austreng, E., 1976a. Fat and protein in diets for salmonoid fishes. I. Fat content in dry diets for salmon parr (*Salmo salar* L.). Meld. Norg. LandbrHøgsk. 55(5), 16 pp.

Austreng, E., 1976b. Fat and protein in diets for salmonoid fishes. II. Fat content in dry diets for rainbow trout (*Salmo gairdneri*, Richardson). Meld. Norg. LandbrHøgsk. 55(6), 14 pp.

Austreng, E., Storebakken, T., Åsgård, T., 1987. Growth rate estimates for cultured Atlantic salmon and rainbow trout. Aquaculture 60, 157-160.

Azevedo, P.A., Leeson, S., Cho, C.Y., Bureau, D.P., 2004. Growth and feed utilization of large size rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*) reared in freshwater: diet and species effects, and responses over time. Aquac. Nutr. 10, 401-411.

Beck, F., Gropp, J., 1995. Estimation of the starvation losses of nitrogen and energy in the rainbow trout (*Oncorhynchus mykiss*) with special regard to protein and energy maintenance requirements. J. Appl. Ichthyol. 11, 263-275.

Berge, G.E., Lied, E., Sveier, H., 1997. Nutrition of Atlantic salmon (*Salmo salar*): the requirement and metabolism of arginine. Comp. Biochem. Physiol. 117A, 201-509.

Berge, G.E., Sveier, H., Lied, E., 1998. Nutrition of Atlantic salmon (*Salmo salar*); the requirement and metabolic effect of lysine. Comp. Biochem. Physiol. 120A, 477-485.

Bergot, F., 1979. Carbohydrate in rainbow trout diets: Effects of the level and source of carbohydrate and the number of meals on growth and body composition. Aquaculture 18, 157-167.

Bodin, N., Tran, T.N.T., Detavernier, C., De Saeger, S., Larondelle, Y., Rollin, X., 2006a. Dietary protein content affects lysine requirement but not lysine utilization efficiency in rainbow trout. XIIth Int. Symp. Fish Nutrition & Feeding, Biarritz, France (P-119).

Bodin, N., Wauters, J.-B., Wilfried, O., Larondelle, Y., Rollin, X., 2006b. Comparison of threonine requirement and retention efficiency in a fast growing and a slow growing salmonid species at the fry stage. XIIth Int. Symp. Fish Nutrition & Feeding, Biarritz, France (P-116).

Boisen, S., Hvelplund, T., Weisbjerg, M.R., 2000. Ideal amino acid profiles as a basis for feed protein evaluation. Livest. Prod. Sci. 64, 239-251.

Breck, O., Bjerkås, E., Campbell, P., Arnesen, P., Haldorsen, P., Waagbø, R., 2003. Cataract preventative role of mammalian blood meal, histidine, iron and zinc in diets for Atlantic salmon (*Salmo salar* L.) of different strains. Aquac. Nutr. 9, 341-350.

Breck, O., Bjerkås, E., Campbell, P., Rhodes, J.D., Sanderson, J., Waagbø, R., 2005a. Histidine nutrition and genotype affect cataract development in Atlantic salmon, *Salmo salar* L.. J. Fish Dis. 28, 357-371.

Breck, O., Bjerkås, E., Sanderson, J., Waagbø, R., Campbell, P., 2005b. Dietary histidine affects lens protein turnover and synthesis of N-acetylhistidine in Atlantic salmon (*Salmo salar* L.) undergoing parr-smolt transformation. Aquac. Nutr. 11, 321-332.

Buentello, J.A., Gatlin III, D.M., 2000. The dietary arginine requirement of channel catfish (*Ictalurus punctatus*) is influenced by endogenous synthesis of arginine from glutamic acid. Aquaculture 188, 311-321.

Buentello, J.A., Gatlin III, D.M., 2001. Plasma citrulline and arginine kinetics in juvenile channel catfish, *Ictalurus punctatus*, given oral gabaculine. Fish Physiol. Biochem. 24, 105-112.

Chiu, Y.N., Austic, R.W., Rumsey, G.L., 1988. Effect of feeding level and dietary electrolytes on the arginine requirement of rainbow trout (*Salmo gairdneri*). Aquaculture 69, 79-91.

Cho, C.Y., Woodward, B., 1985. IUNS Satellite Symposium, XIII International congress of Nutrition, Biological Assessment of Nutrient Requirements and Availability in fish. Brighton, England (Abstr.). (Cited by Wilson, 1989.)

Cho, C.Y., Kaushik, S., Woodward, B., 1992. Dietary arginine requirement of young rainbow trout (*Oncorhynchus mykiss*). Comp. Biochem. Physiol. 102A, 211-216.

Cowey, C.B., Cho, C.Y., Sivak, J.G., Weerheim, J.A., Stuart, D.D., 1992. Methionine intake in rainbow trout (*Oncorhynchus mykiss*), relationship to cataract formation and the metabolism of methionine. J. Nutr. 122, 1154-1163.

D'Mello, J.P.F., 1982. A comparison of two empirical methods of determining amino acid requirements. World Poult. Sci. J. 38, 114-119.

D'Mello, J.P.F., 1994. Amino acid imbalances, antagonisms and toxicities. In: D'Mello, J.P.F. (Ed.), Amino Acids in Farm Animal Nutrition. CAB International Publishing, Oxon, pp. 63-97.

D'Mello, J.P.F., 2003. Amino acids as multifunctional molecules. In: D'Mello, J.P.F. (Ed.), Amino Acids in Animal Nutrition, 2nd ed. CAB International Publishing, Oxon, pp. 1-14.

Encarnacao, P., de Lange, C., Rodehutscord, M., Hoehler, D., Bureau, W., Bureau, D.P., 2004. Diet digestible energy content affects lysine utilization, but not dietary lysine requirements of rainbow trout (*Oncorhynchus mykiss*) for maximum growth. Aquaculture 235, 569-586.

Espe, M., Lemme, A., Petri, A., El-Mowafi, A., 2007. Assessment of lysine requirement for maximal protein accretion in Atlantic salmon using plant protein diets. Aquaculture 263, 168-178.

Forster, I.O., 1993. Arginine requirement of juvenile rainbow trout (Oncorhynchus mykiss). Ph.D. dissertation, University of Washington, Seattle, Washington. (Cited by Wilson, 2003.)

Fournier, V., Gouillou-Coustans, M.F., Métailler, R., Vachot, C., Guedes, M.J., Tulli, F., Oliva-Teles, A., Tibaldi, E., Kaushik, S.J., 2002. Protein and arginine requirements for maintenance and nitrogen gain in four teleosts. Br. J. Nutr. 87, 459-469.

Gatlin III, D.M., Barrows, R.T., Brown, P., Dabrowski, K., Gaylord, T.G., Hardy, R.W., Herman, E., Hu, G., Krogdahl, Å., Nelson, R., Overturf, K., Rust, M., Sealey, W., Skonberg, D., Souza, E.J., Stone, D.,

Wilson, R., Wurtele, E., 2007. Expanding the utilization of sustainable plant products in aquafeeds: a review. Aquac. Res. 38, 551-579.

Gaylord, T.G., Barrows, F.T., Teague, A.M., Johansen, K.A., Overturf, K.E., Shepherd, B., 2007. Supplementation of taurine and methionine to all-plant protein diets for rainbow trout (*Oncorhynchus mykiss*). Aquaculture 269, 514-524.

Green, J.A., Hardy, R.W., 2002. The optimum dietary essential amino acid pattern for rainbow trout (*Oncorhynchus mykiss*), to maximize nitrogen retention and minimize nitrogen excretion. Fish Physiol. Biochem. 27, 97-108.

Green, J.A., Hardy, R.W., Brannon, E.L., 2002. The optimum dietary essential : nonessential amino acid ratio for rainbow trout (*Oncorhynchus mykiss*), which maximizes nitrogen retention and minimizes nitrogen excretion. Fish Physiol. Biochem. 27, 109-115.

Grisdale-Helland, B., Helland, S.J., 1997. Replacement of protein by fat and carbohydrate in diets for atlantic salmon (*Salmo salar*) at the end of the freshwater stage. Aquaculture 152, 167-180.

Grisdale-Helland, B., Shearer, K.D., Helland, S.J., 2007. Energy and nutrient utilization of Atlantic cod, Atlantic salmon and rainbow trout fed diets differing in energy content. Aquac. Nutr. 13, 321-334.

Han, Y., Baker, D.H., 1991. Lysine requirements of fast- and slow-growing broiler chicks. Poult. Sci. 70, 2108-2114.

Hardy, R.W., 2002. Rainbow trout, *Oncorhynchus mykiss*. In: Webster, C.D., Lim, C. (Eds.), Nutrient Requirements and Feeding of Finfish for Aquaculture. CAB International, Oxon, UK, pp. 184-202.

Hatlen, B., Helland, S.J., Grisdale-Helland, B., 2007. Energy and nitrogen partitioning in 250 g Atlantic cod (*Gadus morhua* L.) given graded levels of feed with different protein and lipid content. Aquaculture 270, 167-177.

Hauler, R.C., Carter, C.G., 2001a. Lysine deposition responds linearly to marginal lysine intake in Atlantic salmon (*Salmo salar* L.) parr. Aquac. Res. 32, 147-156.

Hauler, R.C., Carter, C.G., 2001b. Reevaluation of the quantitative dietary lysine requirements of fish. Rev. Fish. Sci. 9, 133-163.

Helland, S.J., Hatlen, B., Grisdale-Helland, B., 2006. Changes in the utilization of energy and amino acids caused by feed intake level - implications for amino acid requirement studies. XIIth Int. Symp. Fish Nutrition & Feeding, Biarritz, France (O-45).

Hemre, G.-I., Sandnes, K., Lie, Ø., Torrissen, O., Waagbø, R., 1995. Carbohydrate nutrition in Atlantic salmon, *Salmo salar* L.: growth and feed utilization. Aquac. Res. 26, 149-154.

Johnsen, F., Hillestad, M., Austreng, E., 1993. High energy diets for Atlantic salmon. Effects on pollution. In: Kaushik, S.J., Luquet, P. (Eds.), Fish Nutrition in Practice. INRA, Paris, pp. 391-401.

Johnston, W.L., Atkinson, J.L., Hilton, J.W., Were, K.E., 1990. Effect of dietary tryptophan on plasma and brain tryptophan, brain serotonin, and brain 5-hydroxyindoleacetic acid in rainbow trout. J. Nut. Biochem. 1, 49-54.

Kaushik, S.J., 1979. Application of a biochemical method for the estimation of amino acid needs in fish: quantitative arginine requirements of rainbow trout in different salinities. In: Halver, J.E., Tiews, K. (Eds.), Proc. World Symp. Finfish Nutrition and Fishfeed Technology, Vol. 1. Heenemann, Berlin, pp. 197-207.

Kaushik, S.J., Luquet, P., Blanc, D., 1981. Usefulness of feeding protein and non-protein calories apart in studies on energy-protein interrelationships in rainbow trout. Ann. Zootech. 30, 3-11.

Ketola, H.G., 1983. Requirement for dietary lysine and arginine by fry of rainbow trout. J. Anim. Sci. 56, 101-107.

Kim, K.-I., 1993. Requirement for phenylalanine and replacement value of tyrosine for phenylalanine in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 113, 243-250.

Kim, J.D., Kaushik, S.J., 1992. Contribution of digestible energy from carbohydrates and estimation of protein/energy requirements for growth of rainbow trout (*Oncorhynchus mykiss*). Aquaculture 106, 161-169.

Kim, K.-I., Kayes, T.B., Amundson, C.H., 1987. Effects of dietary tryptophan levels on growth, feed/gain, carcass composition and liver glutamate dehydrogenase activity in rainbow trout (*Salmo gairdneri*). Comp. Biochem. Physiol. 88B, 737-741.

Kim, K.-I., Kayes, T.B., Amundson, C.H., 1992a. Requirements for lysine and arginine by rainbow trout (*Oncorhynchus mykiss*). Aquaculture 106, 333-344.

Kim, K.-I., Kayes, T.B., Amundson, C.H., 1992b. Requirements for sulfur amino acids and utilization of D-methionine by rainbow trout (*Oncorhynchus mykiss*). Aquaculture 101, 95-103.

Krogdahl, Å., Sundby, A., Olli, J.J., 2004. Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) digest and metabolize nutrients differently. Effects of water salinity and dietary starch level. Aquaculture 229, 335-360.

Lall, S.P., Anderson, S., 2005. Amino acid nutrition of salmonids: dietary requirements and bioavailability. Cahiers Options Méditerranéennes 63, 73-90.

Lall, S.P., Kaushik, S.J., Le Bail, P.Y., Keith, R., Anderson, J.S., Plisetskaya, E., 1994. Quantitative arginine requirement of Atlantic salmon (*Salmo salar*) reared in sea water. Aquaculture 124, 13-25.

Lanari, D., Tibaldi, E., Ballestrazzi, R., Tulli, F., 1991. Impiego di diete a moderato contenuto di farina di pesce per la trota iridea. Rivalutazione del fabbisogno di lisina della specie ed effetti sulle acque reflue. In: Atti IX Congresso Nazionale ASPA (Ministero dell' Agricoltura e Foreste, ed.), vol. 2, pp. 1089-1098. ISMEA-Agricoltura Ricerca, Rome, Italy. (Cited by Rodehutscord et al, 1997.)

Mambrini, M., Kaushik, S.J., 1994. Partial replacement of dietary protein nitrogen with dispensable amino acids in diets of Nile tilapia, *Oreochromis niloticus*. Comp. Biochem. Physiol. 109A, 469-477.

Mambrini, M., Kaushik, S.J., 1995. Effect of temperature on sulphur amino acid requirements for maintenance and growth of juvenile rainbow trout. In: Costa, J.P., Ribeiro, J.R. (Eds.), Protein Metabolism and Nutrition. INIA, AF Nunes, AV Portugal, Portugal, pp. 117-122.

Mambrini, M., Medale, F., Sanchez, M.-P., Recalde, B., Chevassus, B., Labbe, L., Quillet, E., Boujard, T., 2004. Selection for growth in brown trout increases feed intake capacity without affecting maintenance and growth requirements. J. Anim. Sci. 82, 2865-2875.

Moughan, P.J., Fuller, M.F., 2003. Modelling amino acid metabolism and the estimation of amino acid requirements. In: D'Mello, J.P.F. (Ed.), Amino Acids in Animal Nutrition, 2nd ed. CAB International, Oxon, UK, pp. 187-202.

NRC (National Research Council), 1993. Nutrient Requirements of Fish. National Academy Press, Washington D.C., U.S.A.

Ogino, 1980. Requirements of carp and rainbow trout for essential amino acids. Bull. Jpn. Soc. Sci. Fish. 46, 171-174.

Pfeffer, E., Al-Sabty, H., haverkamp, R., 1992. Studies on lysine requirements of rainbow trout (*Oncorhynchus mykiss*) fed wheat gluten as only source of dietary protein. J. Anim. Physiol. a. Anim. Nutr. 67, 74-82.

Pieper, A., Pfeffer, E., 1979. Carbohydrates as possible sources of dietary energy for rainbow trout (*Salmo gairdneri*, Richardson). In: Halver, J.E., Tiews, K. (Eds.), Proc. World Symp. Finfish Nutrition and Fishfeed Technology, Vol. 1. Heenemann, Berlin, pp. 209-219.

Poston, H.A., 1986. Response of rainbow trout to source and level of supplemental dietary methionine. Comp. Biochem. Physio. 83A, 739-744.

Poston, H.A., Riis, R.C., Rumsey, G.L., Ketola, H.G., 1977. The effect of supplemental dietary amino acids, minerals and vitamins on salmonids fed cataractogenic diets. Cornell Vet. 67, 472-509. (Cited by Cowey et al., 1992.)

Poston, H.A., Rumsey, G.L., 1983. Factors affecting dietary requirement and deficiency signs of L-tryptophan in rainbow trout. J. Nutr. 113, 2568-2577.

Refstie, S., Korsøen, Ø.J., Storebakken, T., Baeverfjord, G., Lein, I., Roem, A.J., 2000. Differing nutritional responses to dietary soybean meal in rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*). Aquaculture 190, 49-63.

Riley, Jr., W.W., Higgs, D.A., Dosanjh, B.S., Eales, J.G., 1996. Influence of dietary arginine and glycine content on thyroid function and growth of juvenile rainbow trout, *Oncorhynchus mykiss* (Walbaum). Aquac. Nutr. 2, 235-242.

Rodehutscord, M., Pack, M., 1999. Estimates of essential amino acid requirements from dose-response studies with rainbow trout and broiler chicken: effect of mathematical model. Arch. Anim. Nutr. 52, 223-244.

Rodehutscord, M., Jacobs, S., Pack, M., Pfeffer, E., 1995a. Response of rainbow trout (*Oncorhynchus mykiss*) growing from 50 to 150 g to supplements of DL-methionine in a semipurified diet containing low or high levels of cystine. J. Nutr. 125, 964-969.

Rodehutscord, M., Jacobs, S., Pack, M., Pfeffer, E., 1995b. Response of rainbow trout (*Oncorhynchus mykiss*) growing from 50 to 170 g to supplements of either L-arginine or L-threonine in a semipurified diet. J. Nutr. 125, 970-975.

Rodehutscord, M., Becker, A., Pack, M., Pfeffer, E., 1997. Response of rainbow trout (*Oncorhychus mykiss*) to supplements of individual essential amino acids in a semipurified diet, including an estimate of the maintenance requirement for essential amino acids. J. Nutr. 127, 1166-1175.

Rodehutscord, M., Borchert, F., Gregus, Z., Pack, M., Pfeffer, E., 2000. Availability and utilisation of free lysine in rainbow trout (*Oncorhynchus mykiss*) 1. Effect of dietary crude protein level. Aquaculture 187, 163-176.

Rollin, X., Hidalgo, Y., Valdez, M., Teller, E., Vanbelle, M., 1994. Quantitative methionine requirement of Atlantic salmon (*Salmo salar* L.) juveniles. Aquaculture 124, 61 (Abstract).

Rollin, X., Mambrini, M., Abboudi, T., Larondelle, Y., Kaushik, S.J., 2003. The optimum dietary amino acid pattern for growing Atlantic salmon (*Salmo salar* L.) fry. Br. J. Nutr. 90, 865-876.

Rollin, X., Wauters, J.-B., Bodin, N., Larondelle, Y., Ooghe, W., Wathelet, B., Abboudi, T., 2006. Maintenance threonine requirement and efficiency of it use for accretion of whole-body threonine and protein in Atlantic salmon (*Salmo salar* L.) fry. Br. J. Nutr. 95, 234-245.

Rumsey, G.L., Page, J.W., Scott, M.L., 1983. Methionine and cystine requirements of rainbow trout. Prog. Fish-Cult. 45, 139-143.

Schuhmacher, A., Münch, M., Gropp, J.M., 1995. Non-essential amino acid sources in crystalline amino acid diets for trout (*Oncorhynchus mykiss*). J. Appl. Ichthyol. 11, 317-321.

Scott, L., 1998. Essential amino acid requirements of Atlantic salmon (*Salmo salar*) reared in seawater with emphasis on histidine and methionine. M.Sc. thesis, Dalhousie University, Canada. (Cited by Lall and Anderson, 2005).

Shearer, K.D., 2000. Experimental design, statistical analysis and modelling of dietary nutrient requirement studies for fish: a critical review. Aqua. Nutr. 6, 91-102.

Sveier, H., Nordås, H., Berge, G.E., Lied, E., 2001. Dietary inclusion of crystalline D- and L-methionine: effects on growth, feed and protein utilization, and digestibility in small and large Atlantic salmon (*Salmo salar* L.). Aquac. Nutr. 7, 169-181.

Tran, T.N.T., Parkouda, C., Larondelle, Y., Rollin, X., 2006. Comparison of the lysine utilization efficiency in different vegetal protein sources supplemented with L-Lysine.HCl in rainbow trout fry. XIIth Int. Symp. Fish Nutrition & Feeding, Biarritz, France (P-051).

Walton, M.J., Coloso, R.M., Cowey, C.B., Adron, J.W., Knox,, D., 1984a. The effects of dietary tryptophan levels on growth and metabolism of rainbow trout (*Salmo gairdneri*). Br. J. Nutr. 51, 279-287.

Walton, M.J., Cowey, C.B., Adron, J.W., 1982. Methionine metabolism in rainbow trout fed diets of differing methionine and cystine content. J. Nutr. 112, 1525-1535.

Walton, M.J., Cowey, C.B., Adron, J.W., 1984b. The effect of dietary lysine levels on growth and metabolism of rainbow trout (*Salmo gairdneri*). Br. J. Nutr. 52, 115-122.

Walton, M.J., Cowey, C.B., Coloso, R.M., Adron, J.W., 1986. Dietary requirements of rainbow trout for tryptophan, lysine and arginine determined by growth and biochemical measurements. Fish Phyiol. Biochem. 2, 161-169.

Watanabe, T., Takeuchi, T., Ogino, C., 1979. Studies on the sparing effect of lipids on dietary protein in rainbow trout (Salmo gairdneri). In: Halver, J.E., Tiews, K. (Eds.), Proc. World Symp. Finfish Nutrition and Fishfeed Technology, Vol. 1. Heenemann, Berlin, pp. 113-125.

Wilson, R.P., 1985. Amino acid and protein requirements of fish. In: Cowey, C.B., Mackie, A.M., Bell, J.G. (Eds.), Nutrition and Feeding in Fish. Academic Press, London, pp. 1-16.

Wilson, R.P., 1989. Amino acids and proteins. In: Halver, J.E. (Ed.), Fish Nutrition, 2nd ed. Academic Press, London, pp. 111-151.

Wilson, R.P., 2002. Amino acids and proteins. In: Halver, J.E., Hardy, R.W. (Eds.), Fish Nutrition, 3rd ed. Academic Press, London, pp. 143-179.

Wilson, R.P., 2003. Amino acid requirements of finfish and crustaceans. In: D'Mello, J.P.F. (Ed.), Amino Acids in Animal Nutrition, 2nd ed. CAB International, Oxon, UK, pp. 427-447.

Wilson, R.P., Cowey, C.B., 1985. Amino acid composition of whole body tissue of rainbow trout and Atlantic salmon. Aquaculture 48, 373-376.

Wu, G., Davis, P.K., Flynn, N.E., Knabe, D.A., Davidson, J.T., 1997. Endogenous synthesis of arginine plays an important role in maintaining arginine homeostasis in postweaning growing pigs. J. Nutr. 127, 2342-2349.

Zeitoun, I.H., Halver, J.E., Ullrey, D.E., Tack, P.I., 1973. Influence of salinity on protein requirements of rainbow trout (*Salmo gairdneri*) fingerlings. J. Fish Res. Board Can. 30, 1867-1873.

4. Fatty acids and lipids

Bente Ruyter and Bente E. Torstensen

4.1. Introduction

The fatty acid composition of most fish species in the wild is characterised by high concentrations of the n-3 fatty acids 20:5 and 22:6, and low concentrations of the fatty acids of the n-6 series (Ackman 1982, Robinson, 1992, Henderson and Tocher 1987). The proportion of n-3 polyunsaturated fatty acids (PUFA) relative to the proportion of n-6 fatty acids in fish is naturally much higher than in land mammals, where n-6 fatty acids, particularly 18:2 n-6 dominate (Bourre et al., 1990). This difference may be due to different fatty acid composition in the respective diets. Humans consume large quantities of saturated fatty acids from ruminants and 18:2 n-6 from plant seed oils, whereas fish consume marine food sources rich in the very long chain n-3 PUFAs, 20:5 n-3 and 22:6 n-3 (reviewed by Sargent 1997).

The fatty acid composition of body lipids, however, is not the same as that of the diet. Regardless of whether they are assimilated from the diet, or formed endogenously, fatty acids can be subjected to the various processes outlined in Figure 1 (Henderson 1996). They can, for example, be esterified into phospholipids, the structural lipid of biomembranes, or they may be incorporated into triacylglycerols, the neutral reserve lipid. The degradation of fatty acids via β-oxidation can also be used to provide energy. In addition, fatty acids can also be subjected to various modification processes such as elongation or desaturation. Polyunsaturated fatty acids can also be converted to eicosanoids.

4.2. Essential fatty acids

Lipid requirements of fish came under investigation in the 1960s, following the growth in the aquaculture industry. Although n-3 polyunsaturated fatty acids are abundant in natural fish oils, early fish nutritionists assumed that the essential fatty acid requirement of fish was the same as that of land animals. They used plant oils rich in 18:2 n-6 as dietary lipids for farmed trout (Owen et al., 1972). They found that the use of plant oils rich in n-6 polyunsaturated fatty acids, however, produced a number of adverse effects on the growth and

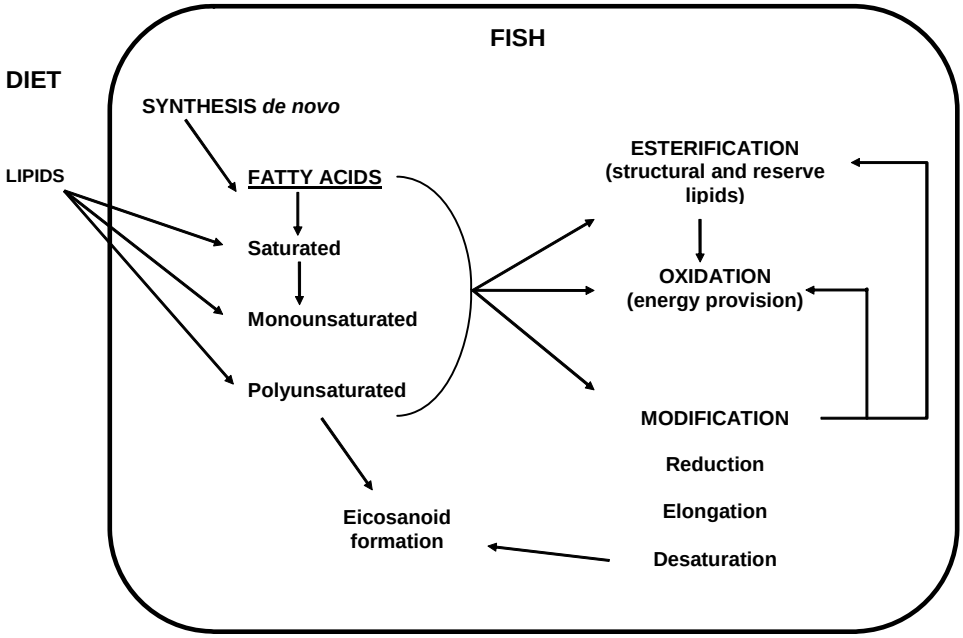

Figure 4.1. Schematic representation of fatty acid metabolism in freshwater fish (Henderson 1996).

behaviour of fish. Fingerling rainbow trout fed corn oil or soybean oil for 12 weeks grew poorly and had only 75% survival. They showed signs of essential fatty acid deficiency, such as fin erosion, swollen pale livers, heart myopathy and shock syndrome. Trout fed a diet which included n-3 fatty acids grew faster, with better feed efficiency and lower mortality (Lee et al., 1967). Similar studies were later performed on Atlantic salmon (Ruyter et al., 2000a,b), where fry were fed for 4 months on semi-synthetic diets containing fatty acid methyl esters of either 18:2 n-6, 18:3 n-3 or a mixture of equal amounts of 20:5 n-3 and 22:6 n-3. Increasing levels of dietary n-3 fatty acids up to 1% gave faster growth rates, and fish fed the mixture of 20:5 n-3 and 22:6 n-3 grew faster than fish fed only 18:3 n-3. Fish fed only 18:2 n-6 had the lowest growth rate and highest mortality. All studies so far have shown that fish require the n-3 PUFAs in their diet, but it is still not known whether the n-6 PUFAs are essential in some salmonids like rainbow trout (Castell et al., 1972a, b), cherry salmon (Thongrod et al., 1990) and Atlantic salmon (Ruyter et. al. 2000 a,b). If so, they are probably needed only in small amounts, since pure 18:2 n-6 deficiency (with n-3 PUFA available in the diet) has little effect on growth in these fish species (Sargent et al., 1995; Ruyter et al., 2000a). It is shown though, that chum salmon (Takeuchi et al., 1979) and coho salmon (Yu

and Sinnhuber 1979) require dietary supplementation of n-6 fatty acids in order to achieve good growth.

The early investigations about the relative importance of n-3 and n-6 fatty acids were conducted without much consideration of the chain length of the fatty acids in the dietary lipid. Once it was clearly established that n-3 fatty acids were required, research emphasis shifted to longer chain polyunsaturated fatty acids and the processes of desaturation and elongation of fatty acids. Arachidonate (20:4 n-6) and docosahexaenoate (22:6 n-3) were classified as essential fatty acids (EFAs). This classification was made even though these fatty acids could normally be synthesised from 18:2 n-6 and 18:3 n-3. These longer chain polyunsaturated fatty acids became interesting particularly after it was shown that they are important membrane constituents of fish, and that metabolites from 20:4 n-6 and 20:5 n-3 are prostaglandins. These prostaglandins have more or less the same important biological functions in fish as in mammals (Bell et al., 1991b). Generally, prostaglandins and leukotrienes constitute a group of extracellular mediator molecules that are part of an organism's defence system. Prostaglandins and leukotrienes are formed during the inflammatory process, and if the inflammation is caused by invading bacteria, the formation of prostaglandins and leukotrienes will stimulate macrophages and other leukocytes to begin the process of destroying the bacteria. Prostaglandins can also act as hormones (reviewed by Hansen 1994).

4.3. Desaturation, elongation and β-oxidation of fatty acids

The ability of tissues or organs to synthesise carbon-20 and carbon-22 PUFAs from carbon-18 fatty acids depends on the presence of active desaturase and elongase enzymes. The relative activities of these enzymes determine the relative amounts of products formed.

Three types of desaturases are distinguished in animals based on their desaturation site in the fatty acids: Δ9, Δ6 and Δ5, respectively (Wakil et al., 1961, Nugteren et al., 1962, Jeffcoat et al., 1979, Sprecher 1981) (Figure 4.2). Δ9-desaturase acts only on saturated fatty acids which it desaturates specifically between carbon-9 and carbon-10. 18:0 is the main substrate for this enzyme (Blomfield and Block 1960, Wakil 1961). Δ6-desaturase introduces an additional ethylene bond between carbon-6 and carbon-7 of poly- or mono-unsaturated fatty acids. Three fatty acids, 18:1 n-9, 18:2 n-6 and 18:3 n-3, compete for the same Δ6-desaturase (Brenner 1977). Which fatty acid becomes desaturated depends on affinities of the fatty acids for Δ6-desaturase, and on their relative proportions. Nevertheless, the affinity for the enzyme varies in the order 18:3 > 18:2 > 18:1, which illustrates the importance of a

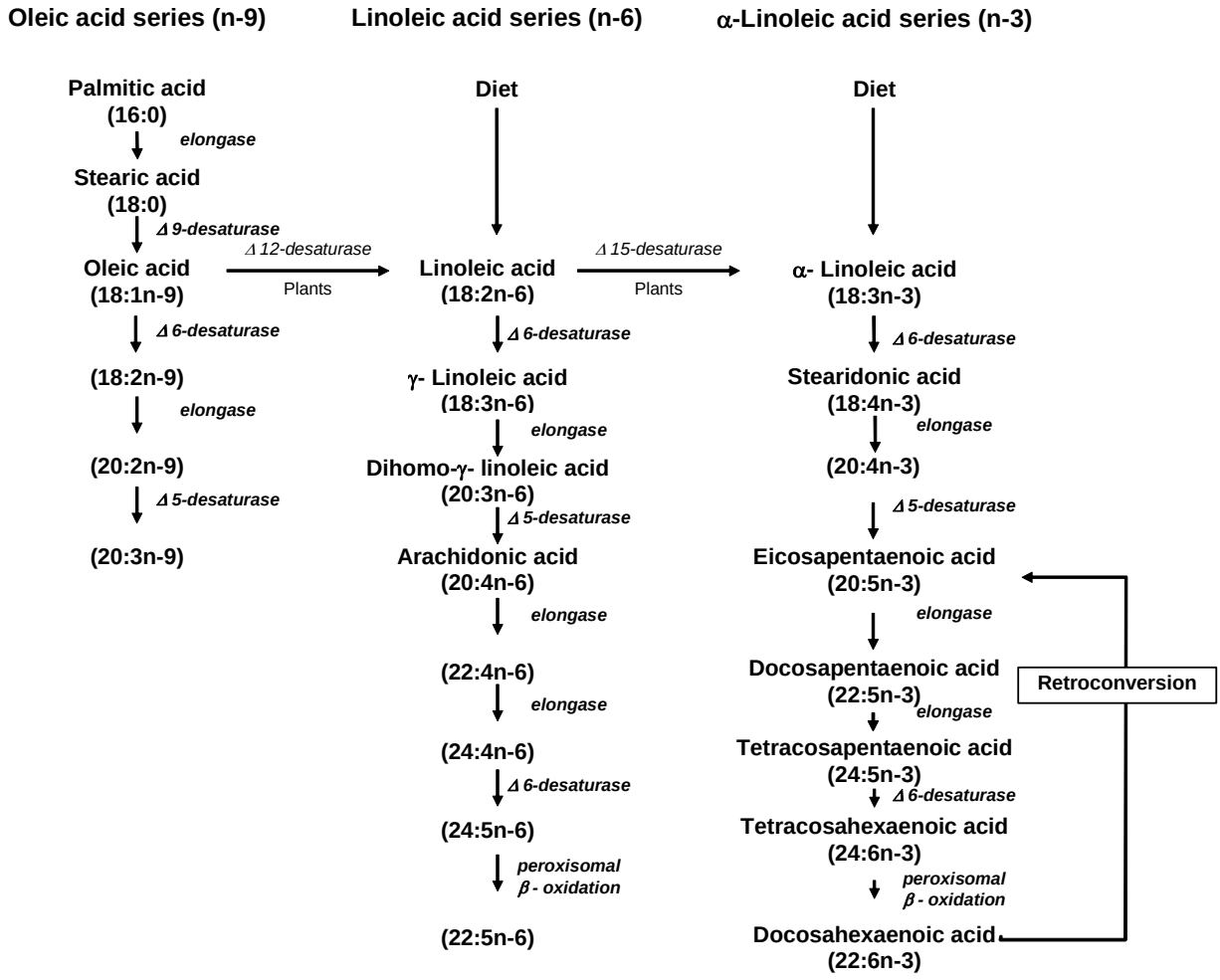

Figure 4.2. A simplified scheme showing the biosynthesis of mono- and polyunsaturated fatty acids, indicating pathways of elongation, desaturation and retroconversion (Modified from Pan et al., 1994).

balanced proportion of these three fatty acids in the feed. Only then can the essential carbon-20 and carbon-22 PUFAs of the n-6 and n-3 families be synthesised (Cook and Emken 1990).

The factors which influence desaturase activity can be classified into nutritional factors, environmental factors (temperature), hormonal factors and physiological factors (such as age). These factors are not independent and, in some cases, the effects of nutritional factors may be explained by changes in the hormonal status.

Atlantic salmon is an anadromous fish which begins life in fresh water and migrates to the sea after 1-2 years. It returns to fresh water to spawn after spending 1-3 years at sea. Atlantic salmon may not be able to desaturate and elongate 18-carbon EFAs during smoltification (Ackman 1986), but this inability is not apparent during the parr stage in fresh

water and the post-smolt seawater phase of the salmon life cycle (Bell et al., 1989). Atlantic salmon post-smolts in sea water are able to convert 18:2 n-6 and 18:3 n-3 to their higher homologs (Bell *et. al,* 1993, 1997). High levels of DHA in the diets and in the different tissue lipids may, however, partly inhibit the conversion of 18:3 n-3 to EPA and DHA, which means that a fish that has a relatively low tissue concentration of DHA (like a parr in fresh water may show higher capacity to convert 18:3 n-3 to DHA than a post–smolt in sea water with higher tissue concentrations of DHA (Bell et al., 1993). Furthermore, 22:6 n-3 is shown to inhibit the Δ6-desaturase in rainbow trout, and thereby preventing the desaturation of both 18:2 n-6 and 18:3 n-3 (Leger et al., 1981).

The efficiency of the conversion of carbon-18 PUFAs to carbon-20 and carbon-22 PUFAs may differ between freshwater species as shown for different mammalian species. Rats, for instance, have a very efficient conversion, whereas man has a very slow rate (Blond et al., 1981). Species that have a relative low efficiency of the conversion of 18:3 n-3 to EPA and DHA, must be supplemented with carbon-20 and carbon-22 PUFAs in their diets. In Atlantic salmon for instance (Ruyter et al*.,* 2000), very low dietary doses of 18:3 n-3 resulted in a moderate increase in the percentage of 22:6 n-3 in the liver. Higher dietary doses of 18:3 n-3 resulted mainly in accumulation of 20:5 n-3, and did not result in a further increase in the percentage of 22:6 n-3. Similarly, 22:5 n-6 was not detected in fish groups fed increasing dietary levels of 18:2 n-6, even though an accumulation of 20:4 n-6 was observed. The low conversion to carbon-22 fatty acids described above may be due to competition between 18:3 n-3 and 24:5 n-3 for the Δ6-desaturase. High dietary levels of carbon-18 PUFAs, as for instance from linseed oil, may inhibit the desaturation of carbon-24 fatty acids to 22:6 n-3. This means that Atlantic salmon cannot be fed a diet with 18:3 n-3 as the only EFA source (as from instance from linseed oil), but need also a dietary supplementation of the longer chain EPA and DHA.

Most marine fish lack or have a very low activity of Δ5-desaturase, and so they need a supply of carbon-20 and carbon-22 PUFAs in the diet. In contrast, the n-3 EFA requirement of pure freshwater fish can generally be met by 18:3 n-3 (Sargent 1997). Since 20:5 n-3 and 22:6 n-3 are the biologically active forms of n-3 EFA in vertebrates, we can conclude that 18:3 n-3 can be converted to 20:5 n-3 and 22:6 n-3 in freshwater fish, but not in marine fish. This is not, however, a general rule because at least one freshwater species, mature pike (*Essox lucius*), is known to be incapable of converting 18:3 n-3 to 22:6 n-3 (Henderson et al., 1995). It was recently reported that Atlantic cod express the Δ6-desaturase gene (Tocher et

al., 2007). The enzyme was barely functional, however, and the authors concluded that further studies are needed in order to understand this problem.

β-oxidation of EFAs has not been as extensively investigated as their desaturation and chain-elongation pathway. Even though salmonids can oxidise EFAs, however, Sargent et al., (1989) postulated that they are only used as energy sources in fish when they are abundant and when their role as EFAs has been satisfied [for further details about how different dietary oils affect β-oxidation of EFAs, see sections about oils sources (4.7) and body composition (4.9)] .

4.4. Dietary requirement for n-3 and n-6 PUFAs

The precise nutritional requirements of any animal species for n-6 and n-3 polyunsaturated fatty acids are not known with certainty (reviewed by Bezard et al., 1994). Moreover, the situation with fish is particularly complex since there is a wide variety of species and a wide range of natural habitats and diets. Not only do we need to determine the absolute requirements of the total amount of each series of PUFAs for a given species, and as an extension of this, to determine the optimal dietary n-6 to n-3 ratio. We also need to know the optimal balance between individual carbon-18, carbon-20 and carbon-22 fatty acids within a given PUFA series. In addition, the methods used for determination of the EFA requirement of a species are seldom optimal. The use of semi-synthetic diets with unbalanced lipid composition and the state of the fatty acid reserves in the body of the fish may influence the results.

Rainbow trout (Castell et al., 1972c, Yu and Sinnhuber, 1972, Rinchard et al., 2007) and Arctic charr (Yang and Dick 1993) require from 0.5 to 1% of n-3 PUFA in the feed to attain good growth, whereas dietary inclusion of 18:2 n-6 does not improve growth. This is different from coho salmon (Yu and Sinnhuber 1979) and chum salmon (Takeuchi et al., 1979) where 18:2 n-6 enhances growth significantly.

Atlantic salmon seemed to grow faster and have lower mortality with substantially lower dietary doses of 20:5 n-3 and 22:6 n-3 than with 18:3 n-3 (Ruyter et al., 2000a). The same effects are seen in rainbow trout, where 20:5 n-3 and 22:6 n-3 had higher EFA efficiency than did 18:3 n-3 (Takeuchi and Watanabe, 1979). Atlantic salmon fed diets supplemented with only 18:2 n-6 had lower growth rates than fish fed n-3 supplemented diets (Ruyter et al., 2000a). Thus, Atlantic salmon fry seem to have an EFA requirement similar to that of rainbow trout (Castell et al., 1972c, Yu and Sinnhuber, 1972) and Arctic charr (Yang and Dick 1993).

The requirement of Atlantic salmon for n-3 EFAs cannot, however, be directly transferred to fish in production. Firstly, the diet used in the EFA-deficient trials were semi-synthetic (Ruyter et al., 2000a,b), which may result in slower growth rates than commercial diets (Lochman and Gatlin 1993). Secondly, the EFA requirement probably changes during the life cycle of Atlantic salmon, and may also be influenced by other dietary fatty acids. Thirdly, the requirement may increase with increasing dietary lipid levels. Takeuchi and Watanabe (1977) reported that the 18:3 n-3 requirements of trout increased with increasing dietary lipid levels. Thus, the EFA requirement of farmed salmon, fed diets containing 30-40% fat, is probably different from that for salmon fed an 8% fat diet, as used in the trials to establish the EFA requirement (Ruyter et al., 2000 a,b). Fourthly, the ratio between the different n-6 and n-3 PUFAs is probably important in Atlantic salmon, as it is in rainbow trout (Bell et al., 1991b). In this study, rainbow trout were fed a sunflower oil diet which contained a considerable excess of n-6 polyunsaturated fatty acids compared to n-3 fatty acids. So although the diet was not deficient in n-3 PUFAs in an absolute sense, the ratio of n-3 to n-6 was low. Fish fed the sunflower oil diet had levels of 20:3 n-6 and 20:4 n-6 which were higher, and a 20:5 n-3 level which was lower, than the corresponding levels in fish fed a fish oil diet. Fish fed the sunflower oil diet developed severe heart lesions, and were also susceptible to transportation shock resulting in 30% mortality. This phenomenon is very similar to the fainting or shock syndrome described by Castell et al. (1972b), in trout fed a diet deficient in n-3 PUFA. A number of later studies, however, have shown no negative effects with 75-100% replacement of fish oil with a plant oil mix, through the whole production cycle (reviewed in section 4.7).

The activities of desaturases and elongases in salmonids are also affected by water temperature (Ruyter et al., 2003; Tocher et al., 2004). Fatty acids in phospholipids (PLs) are less saturated in fish exposed to low temperatures than in fish exposed to high temperatures, such that the membranes keep their fluidity (Sellner and Hazel, 1982; Cossins, 1983, 1987). This phenomenon is known as homeoviscous adaptation, and it ensures that the function of the membranes is unaltered during changes in water temperature (Sinesky, 1974). The increase in the degree of FA unsaturation at low temperature may be due to changes in both the desaturase and elongase capacities (Schuenke and Wodtke, 1983; Hagar and Hazel, 1985; Trueman et al., 2000; Ruyter et al., 2003).

4.5. Concluding remarks to EFA requirement

Atlantic salmon and rainbow trout fry require at least of 0.5 to 1% n-3 PUFA in the feed to attain good growth. These requirement results cannot, however, be directly transferred to fish in general. One must consider that the EFA requirement may change during the life cycle, as well as with the fatty acid composition and fat percentage of the diet. Atlantic salmon and rainbow trout are able to convert dietary 18:3 n-3 and 18:2 n-6 to C-20 and C-22 PUFAs, thus indicating that only the 18-carbon forms of these fatty acids are essential in the diet. High dietary levels of 18:3 n-3 inhibited, however, its conversion to 22:6 n-3, and salmonids may therefore, become deficient in this essential, long chain PUFA if 18:3 n-3 is the only n-3 PUFA supplied in the diet. Atlantic salmon seem to need a dietary supplementation of the longer chain n-3 PUFAs, EPA and DHA, in addition to 18:3 n-3. The ratio between different fatty acids may be more important than the actual level of each fatty acid, in securing the production of longer chain fatty acids. Future work on the aspects of Atlantic salmon dietary requirement for certain fatty acids must focus on finding the most optimal ratio between the different EFAs of different chain lengths in today's high fat diets.

It is important to define some early symptoms of EFA deficiency, since sub-deficiency states can result in increased health risks, even when not manifested by overt symptoms. This is particularly important under suboptimal conditions, like low water temperatures, stress, infections etc.

4.6. Fatty acid peroxidation

Fish are generally regarded as PUFA-rich organisms providing a virtually unique source of the very long chain n-3 fatty acids EPA and DHA. These EFAs, which are vital constituents of cell membrane structure and function, are very susceptible to attack by oxygen and other organic radicals. Resulting damage to PUFA in membrane phospholipids can have damaging consequences for cell membrane structure and fluidity (muscle quality), with potential pathological effects on cells and tissues (Sies et al., 1991). An efficient antioxidant protection system is essential for the physiological well-being of the animals. When parts of the antioxidant protective mechanism are placed under stress, however, perhaps due to dietary deficiencies of essential antioxidant nutrients or intake of oxidized (rancid) foodstuffs, then pathological consequences can result. Reduced growth, loss of appetite, decreased feed efficiency, reduced pigmentation and increased mortality have all been reported in Atlantic salmon (Ketola et al., 1989) and rainbow trout (Cowey et al., 1984) when fed oxidised lipids.

A number of histological lesions due to lipid peroxidation have been identified, including myopathy of skeletal muscle (Cowey et al., 1984). We have recently shown that very high levels of EPA and DHA in salmon diets may result in intracellular peroxidation inducing cell death both in liver, muscle and adipose tissue (unpublished results).

4.7. Oil sources – Effects on body fatty acid composition

Atlantic salmon and rainbow trout in aquaculture are increasingly being fed diets based on plant oils (PO) as a replacement for fish oil. In contrast to fish oil, PO do not contain fatty acids longer than 18 carbons and three double bonds and thus, lack the long chain n-3 PUFAs such as EPA (20:5 n-3) and DHA (22:6 n-3). PO for fish should contain a low level of 18:2 n-6. Furthermore, a high level of 18:1 n-9 is desirable, whereas the level of 18:3 n-3 should possibly be moderate. These criteria are currently best met by rapeseed oil, although even this oil contains 16-25% 18:2 n-6.

A number of studies have shown that complete or partial replacement of fish oil with a single PO, such as rapeseed oil, palm oil, linseed oil, or soybean oil, in parts of the salmon production cycle, and 75-100% replacement of fish oil with a PO mix, through the whole production cycle, does not affect growth negatively, but does affect the fatty acid composition of the edible portion (Bell et al., 2002; Bell et al., 2001; Bell et al., 2003a; Bell et al., 2003b; Regost et al., 2004; Rosenlund et al., 2001; Torstensen, 2000; Torstensen et al., 2005; Torstensen et al., 2004b; Waagbø et al., 1991; Waagbø et al., 1993). Furthermore, dietary fatty acid composition is also mirrored by the fish's organs and lipid stores (Bell et al., 2001; Bell et al., 2003b; Caballero et al., 2002; Nanton et al., 2007; Olsen et al., 1999; Stubhaug et al., 2007; Thomassen & Røsjø, 1989; Tocher et al., 2003; Torstensen et al., 2005; Torstensen et al., 2004a; Torstensen et al., 2004b; Torstensen et al., 2000; Waagbø et al., 1991). Thus, replacement of fish oil with PO and thereby increasing dietary 18:2 n-6, 18:1 n-9 and 18:3 n-3 and the marine n-3 fatty acids EPA and DHA, will be reflected in the organs and flesh of the fish. The magnitude, however, of fatty acid changes depends on the type of tissue (Bell et al., 2001; Bell et al., 2003a; Torstensen et al., 2004a; Grisdale Helland et al., 2002; Moya-Falcon et al., 2005) and the amount of phospholipids relative to neutral lipids in the tissue, since neutral lipids are reported to be more influenced by dietary fatty acid composition than polar lipids (Brodtkorb et al., 1997; Olsen & Henderson, 1997, Moya Falcon et al., 2005, Ruyter et al., 2006).

Fish fatty acid composition also depends on factors other than dietary fatty acid composition. Digestibility (Torstensen et al., 2000; Sigurgisladottir et al., 1992), transport and

uptake, elongation and desaturation processes (Bell et al., 2001; Bell et al., 2002) and β-oxidation of fatty acids (Frøyland et al., 2000; Torstensen et al., 2000) will affect the fatty acid composition in the membranes (polar lipids) and storage depots (neutral lipids).

Lipid digestibility in rainbow trout is fairly constant (≈ 90%) for lipid sources with melting points <10°C, but is dramatically reduced for sources with higher melting points (Austreng et al. 1979). A practical consequence is that fats with low melting points (oils) are preferred in diets for salmonids and probably other coldwater fishes as well. This favours PO from oilseeds, which usually have melting points well below 0°C.

Studies of β-oxidation in fish suggest that there exist substrate preferences for saturated and monounsaturated fatty acids over polyunsaturated fatty acids (Kiessling & Kiessling, 1993; Henderson, 1996). A recent report from Stubhaug et al. (2007) showed that Atlantic salmon fed low levels of marine n-3 fatty acids selectively stored these in the tissue. Fish-oil fed fish stored about 20% EPA and 30% DHA, whereas PO-fed fish stored 70% EPA and 80% DHA during the fast growth period in sea water (Stubhaug et al., 2007). Thus, these results suggest a switch in fatty acid substrate used for β-oxidation when dietary levels of EPA and DHA are low; these PUFA are preferentially stored in membranes instead of being used as an energy substrate in PO-fed fish.

By introducing a finishing-diet period when fish are being fed 100% FO diet, the levels of marine n-3 PUFA increase whereas the typical PO fatty acids decrease to levels comparable with fish oil fed fish. Studies have shown that a period long enough for the fish to double its weight is necessary for obtaining fatty acid compositions comparable to fish oil fed fish (Bell et al., 2003a; Bell et al., 2003b; Torstensen et al., 2004b; Torstensen et al., 2005).

4.8. Oil sources and effects on colour

Flesh pigmentation is an important factor in perception of flesh quality in salmonids (Bell et al., 1998; Refsgaard et al., 1998). The flesh levels of lipid soluble nutrients, such as astaxanthin are dependent on the dietary composition (Lie, 2001), and it has been shown that both total lipid level and type of oil can affect carotenoid absorption in rodents (Clark & Furr, 2001; Clark et al., 2000) and colour characteristics in raw and smoked Atlantic salmon fillets (Regost et al., 2004). The majority of reports show no effects on flesh astaxanthin levels of using up to 100% PO (Bell et al., 2001; Bell et al., 2002; Torstensen et al., 2004b; Torstensen et al., 2005), however in none of these studies was fish oil replaced with soybean oil. As

reported by Regost et al (2004), dietary soybean oil resulted in reduced flesh astaxanthin, cantaxanthin and visual colour, especially after four months frozen storage.

4.9. Effects of high fat diets on body lipid composition

Depending on the species, fish are able to accumulate fat in different tissues such as liver for Atlantic cod and abdomen and flesh for Atlantic salmon (see figure to the left) and rainbow trout. Fat stores may supply energy in periods of low feed availability and more specifically for use in periods of particularly high energy demand such as reproduction and smoltification. Since most fish species have a limited capacity to utilize carbohydrates, a common way of reducing feed cost is to replace as much protein as possible with fat. Increased energy levels improve growth and feed utilization in most fish species. Increased dietary energy levels though, also increase fat deposition in the fish's fat storage organs and particularly increased visceral fat deposition reduces harvest yields (Hillestad et al., 1998). By preventing excessive fat deposition in cultivated fish, sustainability is strengthened, more marine fat is available for food production, feed efficiency is increased, and cost of fish production is reduced.

The use of high-lipid feed for cultured fish may also affect fish flesh quality by increasing the percentage of lipids stored in the edible muscle (Watanabe, 1982; Hemre & Sandnes, 1999; Arzel et al., 1993; Arzel et al., 1994; Bendiksen et al., 2003). Generally, increased dietary lipid also results in increased muscle lipid levels, however this is reported to level off so that increasing dietary lipid from 38% to 47% did not increase muscle total lipid levels beyond 16% (Hemre & Sandnes, 1999). Contradicting results are reported on the impact of dietary fatty acid composition on body composition. In Atlantic salmon, changing dietary fatty acid composition by replacing fish oil with a PO blend during both fresh- and seawater stages did not change the body lipid stores in any major way (Nanton et al., 2007). There was a trend towards decreased triacylglycerol (TAG)/PL ratio, however, in both visceral lipid stores and myosepta in 100% PO-fed Atlantic salmon. Muscle lipid and protein levels have been reported not to be affected by dietary fatty acid composition (Torstensen et al., 2005; Bendiksen et al., 2003).

Reports on the effects of changes in the dietary fatty acid composition through replacement of fish oil with PO show contradictory results regarding hepatic lipid storage in fish. By replacing fish oil with oleic acid enriched-sunflower oil and rapeseed oil, a slight increase in hepatic total lipid storage was reported (Torstensen et al., 2000; Bell et al., 2001), whereas no effects on hepatic lipid were found when replacing with palm oil (Bell et al., 2002; Torstensen et al., 2000) or a 1:1 rapeseed oil: linseed oil mix (Tocher et al., 2001). Further, Ruyter and co-workers (2006) have reported increased liver lipid when replacing fish oil with 100% soybean oil at 5˚C, but not at 12˚C. A long term feeding experiment reported increased hepatic lipid stores, especially at low water temperatures, in Atlantic salmon fed a PO blend (Jordal et al., 2007). Increased liver TAG stores are reported as one of the indicators of essential fatty acid deficiency in salmonids (Castell et al., 1972b; Takeuchi et al., 1979). Increased n-3 PUFA requirement during very high growth and/or low water temperatures in Atlantic salmon may be indicated by the increased liver TAG results when decreasing n-3/n-6 ratio and decreasing dietary marine n-3 fatty acids, however this needs further evaluation.

The digestibility of fatty acids has been reported to be affected by dietary fatty acid composition; specifically the amount of dietary saturated fatty acids influences the digestibility of lipids, especially at low water temperatures (Caballero et al., 2002; Ng et al., 2004; Olsen et al., 1999; Torstensen et al., 2000). As a consequence, at low water temperatures, the replacement of capelin oil with rapeseed oil leads to less saturated fatty acids in the diet, and increased monounsaturated fatty acids increase lipid digestibility, in both rainbow trout and Atlantic salmon (Caballero et al., 2002; Karalazos et al., 2007). Thus, changes observed in total liver lipids, especially at low water temperatures, may be related to increased lipid uptake from the diet. Also, increased protein retention has been reported at low temperatures when replacing dietary fish oil with PO (Bendiksen et al., 2003; Torstensen et al., 2005). If the increased protein retention was due to increased fatty acid digestibility or increased fatty acid β-oxidation capacity at low water temperatures remains to be elucidated. In conclusion, the impacts of dietary fatty acid composition on total body composition seem to be highly influenced by water temperature, both for Atlantic salmon and rainbow trout.

4.10. Fish health and welfare

In the growing fish farming industry there are always new challenges concerning fish health and fish diseases. Still, diseases and health problems lead to substantial losses every year. Fish farming has changed quickly, and fish growth has increased significantly throughout the last decade. This always brings new aspects of health and welfare for the fish,

where several of the diseases and production related disorders are proved to be preventable by correct nutrition.

Dietary fat and polyunsaturated fatty acids are important regulators of numerous cellular functions, including those related to inflammation and immunity. Although little is known for fish, there is convincing evidence from studies with mammals that both the type and level of fat in the diet have major impacts on several aspects of immune function and heart health. A large number of experiments have shown that a relatively high intake of high fat plant-oil diets under normal production conditions does not affect the growth and feed utilisation of the salmon to any notable degree. It still remains though, to find out how much and how long the fish can be fed different types of plant oils and high fat diets without it affecting fish health negatively, especially under suboptimal conditions such as fluctuating farming conditions, stress and lowered health condition. Combined with a low level of exercise, fish in aquaculture consume high energy diets, resulting in large lipid stores especially around the viscera. Importantly, high inclusion levels of plant ingredients in fish diets lead to reduced levels of the health-promoting, marine n-3 fatty acids. Together, these factors may push the system towards a state similar to metabolic syndrome in humans and the lifestyle diseases that follow. Epidemiologic human studies have shown that higher aerobic fitness is associated with lower incidence of inflammatory illnesses (Milne et al., 2008). In aquaculture, fish are kept in net pens during the grow-out phase where the degree of exercise depends on the local currents at the fish farm and, in most cases, these are low. This phenomenon may lead to low cardio-respiratory fitness and eventually lead to development of obesity related metabolic disorders. High mortality in slaughter size Atlantic salmon, due to handling stress, has in fact been reported as frequently occurring in aquaculture farms (Poppe et al, 2004).

Further, obesity has been associated with reduced antioxidant defense mechanisms in humans, including decreased antioxidant enzyme activity, giving rise to increased oxidative stress in the body (reviewed by Milne et al., 2008). Although not known, a reduced antioxidant defense system in obese fish may have major implications also for the ability to recover and survive acute stresses such as handling or crowding. Stress and obesity are linked in several ways, chronic stress with hyper-activation of the hypothalamic-pituitary-adrenal (HPA) axis favors accumulation of visceral fat and vice versa (Balkan et al., 1993). Obese rats demonstrated an elevated response to mild stress compared to lean rats measured as plasma epinephrine and cortisol (Kyrou and Tsigos, 2007). Similarly in fish, the energy status significantly affected the cod's ability to recover from handling stress (Hemre et al., 1991).

A high fat level in the blood has proved to be a serious risk factor for the development of life style diseases, as for example heart diseases and diabetes II in humans. Several studies in humans have shown that dietary EPA and DHA decrease plasma TAG (Harris et al., 1983; Nestel, 1990) and protect against coronary heart diseases (Bang et al., 1971; Seierstad et al., 2005b). If this also is a risk factor in fish, is still uncertain, but it has been shown by using *in vitro* experiments that the lipoprotein secretion from liver cells is significantly higher in salmon fed with PO than salmon fed with fish oil (Vegusdal et al., 2005). Some of the most frequently used plant oils in salmon diets contain high levels of 18:1n-9 which has been shown in different cell systems (Ranheim et al., 1994; Vegusdal et al., 2005) and rats (Halvorsen et al., 2001) to affect liver lipid and lipoprotein metabolism. Further, both EPA and DHA are reported to affect hepatic triacylglycerol (TAG) metabolism and β-oxidation capacity (Berge et al., 1999; Madsen et al., 1999; Nossen et al., 1986; Willumsen et al., 1996) with especially EPA having a plasma lipid lowering effect in rats (Frøyland et al., 1997; Frøyland et al., 1996).

It is important to be conscious about possible heart health implications when increasing amounts of the fat in salmon feed are replaced with plant oils which have less of the healthy n-3 fatty acids. Studies on effects of reduced n-3 HUFA and increased n-6 PUFA on salmonid heart histology are, however, so far contradictory (Bell et al., 1993; Grisdale-Helland et al., 2002; Seierstad et al., 2005a) and predominantly show no negative effects of significantly reduced n-3 polyunsaturated fatty acids (PUFA) (EPA and DHA) in the diet (Seierstad et al., 2007). The dietary levels of phytosterols, however, provided from the plant oils used, were not considered in any of these studies. Phytosterols are reported to reduce intestinal cholesterol uptake, reduce plasma LDL-cholesterol and protect against cardiovascular disease in mammals (reviewed by Orzechowski et al., 2002). The amount and type of phytosterol varies depending on the type of plant oil and the degree of refinement (Weihrauch & Gardner, 1978), and to ensure that cardiovascular disease is not developed when different oils are used, the impact of dietary phytosterols needs to be evaluated in salmonids.

Examination of the literature shows that changing the concentration of dietary n-3 PUFA in fish feeds can have both beneficial and, in some instances, detrimental effects on disease resistance. When challenged with *Aeromonas salmonicida* and *Vibrio anguillarum* Atlantic salmon fed plant oils were less resistant to infection compared to fish oil fed fish (Thompson et al., 1996). In contrast, Erdal et al., (1991) found that increasing the amount of

dietary n-3 PUFA (by increasing 20:5 n-3 and 22:6 n-3) from 13 to 24% of total fatty acids actually had an immuno-suppressive effect on Atlantic salmon, and resulted in higher rates of mortality against *Yersinia ruckeri.* In line with results from Erdal et al., (1991), high n-3 PUFA has been reported to result in decreased response by the unspecific immune system in Atlantic salmon (Waagboe et al., 1993). In a study by Bransden et al. (2003) resistance to *V. anguillarum* was significantly impaired in salmon supplied feeds where fish oil was replaced by sunflower oil at different inclusion levels, although it remains unclear as to why some diets improved disease resistance in salmon, whereas others did not. This suggests an optimal dietary n-3/n-6 ratio probably exists for disease resistance, although not yet identified.

Head kidney tissue of fish has a high prevalence of macrophages and is hence, essential in the immune system of salmon. One mechanism by which macrophages remove invading microorganisms is through phagocytosis and subsequent presentation of antigens on major histocompatibility complex (MHC) molecules for other cells of the immune system. Macrophages also secrete interferons (IFNs) upon stimulation. Experiments with Atlantic salmon have shown that changes in the composition of fatty acids can affect the immune system through changes in the phagocytotic capacity of macrophages. These effects were especially clear at low water temperatures (5°C) (Gjøen et al., 2007). Another important macrophage function is to modulate the immune system by producing eicosanoids. Eicosanoids are a group of lipid mediators of inflammation that are derived mainly from arachidonic acid (20:4 n-6). After its release from membrane phospholipids by the action of enzymes like phospholipase A_2 (PLA_2), it has been shown that dietary lipids may influence the eicosanoid production in both mammals (Brouard and Pascaud 1990) and fish (Bell et al., 1996; Gjøen et al., 2004). Furthermore, by feeding plant oils, the tissue n-6 levels increase and thus, possibly the amount of eicosanoids produced from arachidonic acid (ARA) compared to EPA increase (Gjøen et al., 2004; Gjøen et al., 2007). This is thought to have an impact on a number of responses such as stress response and smoltification (Bell et al., 1993; Bell et al., 1996; Bell et al., 1991a; Bell & Raynard, 1990; Bell et al., 1991b; Bell et al., 1992; Bell et al., 1997).

It has also been shown that fast growing salmon that had 75 to 100% plant oils (fish meal as protein source) in the feed, from start feeding and onwards, developed the eye disorder cataract two-fold greater compared to fish fed a diet containing 100% fish oil (Waagboe et al., in prep).

4.11. Future Challenges within lipid nutrition of salmonids

- **Decrease obesity of cultured fish:**
  - It is necessary with increased knowledge about the mechanisms underlying obesity in fish in order to pave the way for new methods for obesity control and thus contribute to increase fish flesh quality and decrease the production losses due to fat offals.
  - It is necessary with increased knowledge about the impact of high lipid storage depots on fish health and stress tolerance.

- **Safe usage of plant ingredients in fish diets:**
  - When not only fish oil but also fish meal is replaced by plant oils and plant protein sources in combination, it is necessary with more knowledge on how these components in combination will affect the fish growth, health, metabolism and final quality.
  - The following questions need to be answered: What is the n-3 PUFA requirement for salmonids at different life stages and at different water temperatures? What is the impact of n-3/n-6 ratio on fish health, particularly under sub-optimal conditions?
  - The impact and function of bioactive components present in plant raw materials and the impact on fish metabolism and health need to be evaluated.
  - Studies on how to secure the nutritional quality of the fish, particularly related to the level of the healthy omega-3 fatty acids.

4.12. References

Ackman, R.G., 1982. Fatty acid composition of fish oils. In Nutritional evaluation of long-chain fatty acids in fish oils. Edited by S.M. Barlow and M-E. Stansby. Academic Press Inc., London. pp. 25-88.

Ackman, R.G. and Takeuchi, T. 1986. Comparison of fatty acids and lipids of smolting hatchery-fed and wild Atlantic salmon (*Salmo salar*). Lipids. 21, 117-120.

Armitage, J.A. 2004. Developmental programming of the metabolic syndrome by maternal nutritional imbalance: how strong is the evidence from experimental models in mammals? J. Physiol. 561, 355-377.

Arzel, J., Cardinal, M., Cornet, J., Metailler, R. & Guillaume, J.C. 1993. Nutrition of brown trout (*Salmo trutta*) reared in seawater, effect of dietary lipid on growth performances, body composition and fillet quality. Spec. Publ. Eur. Aquacult. Soc. 19**,** 309.

Arzel, J., Martinez Lopez, F.X., Metailler, R., Stephan, G., Viau, M., Gandemer, G. & Guillaume, J. 1994. Effect of dietary lipid on growth performance and body composition of brown trout (*Salmo trutta*) reared in seawater. Aquaculture, 123**,** 361-375.

Austreng, E., Skrede, A., Eldegaard, Å. 1979. Effect of dietary fat source on the digestibility of fat and fatty acids in rainbow trout and mink. Acta Agric. Scand. 29, 119-126.

Balkan, B., Strubbe, J.H., Bruggink, J.E. & Steffens, A.B. 1993. Overfeeding-induced obesity in rats: insulin sensitivity and autonomic regulation of metabolism. Metabolism, 42, 1509-1518.

Bang, H.O., Dyerberg, J. & Nielsen, A.B. 1971. Plasma lipid and lipoprotein pattern in Greenlandic west-coast eskimos. Lancet, 1**,** 1143-1145.

Bell, J.G, Raynard, R.S. and Sargent, J.R. 1991. The effect of dietary linoleic acid on the fatty acid composition of individual phospholipids and lipoxygenase products from gills and leukocytes of Atlantic salmon (*Salmo salar*). Lipids 26, 445-450.

Bell, J.G., Ashton, I., Secombes, C.J., Weitzel, B.R., Dick, J.R., Sargent, J.R. 1996. Dietary lipid affects phospholipid fatty acid compositions, eicosanoid production and immune function in Atlantic salmon (*Salmo salar*). Prostag. Leukotr. Ess. 54, 173-182.

Bell, J.G. & Raynard, R.S. 1990. Effects of a high linolic acid diet on the fatty acid compsition of leucocytes from Atlantic salmon (*Salmo salar*). Transactions. Biochemical Society. 18**,** 911-912.

Bell, J.G., Dick, J.R., MC Vicar, A.H., Sargent, J.R. and Thompson, K.D. 1993. Dietary sunflower, linseed and fish oils affect phospholipid fatty acid composition, development of cardiac lesions, phospholipase activity and eicosanoid production in Atlantic salmon. Prostag. Leukotr. and Ess. 49, 665-673.

Bell, J.G., Farndale, B.M., Dick, J.R. & Sargent, J.R. 1996. Modification of membrane fatty acid composition, eicosanoid production, and phospholipase A activity in Atlantic salmon (*Salmo salar*) gill and kidney by dietary lipid. Lipids. 31**,** 1163-1171.

Bell, J.G., Henderson, R.J., Tocher, D.R., McGhee, F., Dick, J.R., Porter, A., Smullen, R.P. & Sargent, J.R. 2002. Substituting fish oil with crude palm oil in the diet of Atlantic salmon (*Salmo salar*) affects muscle fatty acid composition and hepatic fatty acid metabolism. Journal of Nutrition. 132, 222-230.

Bell, J.G., Mc Vicar, A.H., Park, M.T. & Sargent, J.R. 1991a. High dietary linoleic acid affects the fatty acid compositions of individual phospholipids from tissues of Atlantic salmon (*Salmo salar*): Association with stress susceptibility and cardiac lesions. J. Nutr. Biochem, 121**.** 1163-1172.

Bell, J.G., McEvoy, J., Tocher, D.R., McGhee, F., Campbell, P.J. & Sargent, J.R. 2001. Replacement of fish oil with rapeseed oil in diets of Atlantic salmon (*Salmo salar*) affects tissue lipid compositions and hepatocyte fatty acid metabolism. J. Nutr. 131**,** 1535-1543.

Bell, J.G., McEvoy, J., Webster, J.L., McGhee, F., Millar, R.M. & Sargent, J.R. 1998. Flesh lipid and carotenoid composition of Scottish farmed Atlantic salmon (*Salmo salar*). J. Agric. Food Chem. 46**,** 119-127.

Bell, J.G., McGhee, F., Campbell, P.J. & Sargent, J.R. 2003a. Rapeseed oil as an alternative to marine fish oil in diets of post-smolt Atlantic salmon (*Salmo salar*): changes in flesh fatty acid composition and effectiveness of subsequent fish oil "wash out". Aquaculture. 218**,** 515-528.

Bell, J.G., Raynard, R.S. & Sargent, J.R. 1991b. The effect of dietary linoleic acid on the fatty acid composition of individual phospholipids and lipoxygenase products from gills and leucocytes of Atlantic salmon (*Salmo salar*). Lipids. 26, 445-450.

Bell, J.G., Tocher, D., Farndale, B.M., Cox, D.I., McKinney, R.W. & Sargent, J.R. 1997. The effect of dietary lipid on polyunsaturated fatty acid metabolism in Atlantic salmon (*Salmo salar*) undergoing parr-smolt transformation. Lipids. 32**,** 515-525.

Bell, J.G., Tocher, D., Henderson, R.J., Dick, J.R. & Crampton, V.O. 2003b. Altered fatty acid compositions in Atlantic salmon (*Salmo salar*) fed diets containing linseed and rapeseed oils can be partially restored by a subsequent fish oil finishing diet. J. Nutr. 133**,** 2793-2801.

Bell, J.G., Youngson, A., Mitchell, A.I. and Cowey, C.B. 1989. The effect of enhanced intake of linoleic acid on the fatty acid composition of tissue polar lipids of post-smolt Atlantic salmon (*Salmo salar*). Lipids. 24, 240-242.

Brouard, C., Pascaud, M. 1990. Effects of moderate dietary supplementations with n -- 3 fatty acids on macrophage and lymphocyte phospholipids and macrophage eicosanoid synthesis in the rat. BBA - Lipids and Lipid Metabolism. 1047, 19-28.

Bendiksen, E.Å., Berg, O.K., Jobling, M., Arnesen, A.M. & Måsøval, K. 2003. Digestibility, growth and nutrient utilisation of Atlantic salmon (*Salmo salar L.*) in relation to temperature, feed fat content and oil source. Aquaculture. 224**,** 283-299.

Berge, R.K., Madsen, L., Vaagenes, H., Tronstad, K.J., Göttlicher, M. & Rustan, A.C. 1999. In contrast with docosahexaenoic acid, eicosapentaenoic acid and hypolipidaemic derivatives decrease hepatic synthesis and secretion of triacylglycerol acyltransferase activity and stimulation of fatty acid oxidation. The Biochemical Journal. 343**,** 191-197.

Bézard, J., Blond, J.P., Bernard, A. and Clouet, P. 1994. The metabolism and availability of essential fatty acids in animal and human tissues. Reprod. Nutr. Dev. 34, 539-568.

Blond, J.P., Lemarchal, P., Spielman, D. 1981. Désaturation compareé des acides linoléique et dihomo-gamma-linolénique par des homogénats de foie humain in vitro. CR Acad. Sci. Paris. 292, 911-914.

Bloomfield, D. K. and Block, K. 1960. The formation of Δ9-unsaturated fatty acids. J. Biol. Chem. 235: 337-345.

Bourre, J.M., Dumont, D., Pascal, G. and Durand, G. 1990. Dietary linoleic acid and polyunsaturated fatty acids in rat brain and other organs. Minimal requirements of linoleic acid. Lipids. 25, 465-472.

Bransden, M.P., Carter, C.G. & Nichols, P.D. .2003. Replacement of fish oil with sunflower oil in feeds for Atlantic salmon (*Salmo salar L.*): effect on growth performance, tissue fatty acid composition and disease resistance Comp. Biochem. Physiol. B. 135**,** 611-625.

Brenner, R.R. .1977. Regulatory function of Δ6 desaturase: a key enzyme of polyunsaturated fatty acid synthesis. Adv. Exp. Med. Biol. 83, 85-101.

Brodtkorb, B.T., Rosenlund, G. & Lie, Ø..1997. Effects of 20:5n-3 and 22:6n-3 on tissue lipid composition in juvenile Atlantic salmon, *Salmo salar*, - with emphasis on brain and eye. Aquac. Nutr. **3,** 175-187.

Buzzi, M., Henderson, R.J and Sargent, J.R. .1997. Biosynthesis of docosahexaenoic acid in trout hepatocytes proceeds via 24-carbon intermediates. Comp. Biochem. Physiol. 116B, 263-267.

Caballero, M.J., Obach, A., Rosenlund, G., Montero, D., Gisvold, M. & Izquierdo, M. 2002. Impact of different dietary lipid sources on growth, lipid digestibility, tissue fatty acid composition and histology of rainbow trout, *Onchorhynchus mykiss*. Aquaculture. 214**,** 253-271.

Castell, J.D., Lee, D.J. and Sinnhuber, R.O. 1972a. Essential fatty acids in the diet of rainbow trout (*Salmo gairdneri*): Lipid metabolism and fatty acid composition. J. Nutr. 102, 93-100.

Castell, J.D., Sinnhuber, R.O., Lee, D.J. and Wales, J.H. .1972b. Essential fatty acids in the diet of rainbow trout (*Salmo gairdneri*): physiological symptoms of EFA deficiency. J. Nutr. 102, 87-97.

Castell, J.D., Sinnhuber, R.O., Wales, J.H. and Lee, D.J. .1972c. Essential fatty acids in the diet of rainbow trout (*Salmo gairdneri*): growth, feed conversion and some gross deficiency symptoms. J. Nutr. 102, 77-86.

Clark, R.M. & Furr, H.C. .2001. Absorption of Canthaxanthin by the rat is influenced by total lipid in the intestinal lumen. Lipids. 36, 473-475.

Clark, R.M., Yao, L., She, L. & Furr, H.C. 2000. A comparison of Lycopene and Astaxanthin absorption from corn oil and olive oil emulsions. Lipids. 35, 803-806.

Cook, H.W. and Emken, E.A. 1990. Geometric and positional fatty acid isomers interact differently with desaturation and elongation of linoleic and linolenic acids in cultured glioma cells. Biochem. Cell. Biol. 68, 653-660.

Cowey, C.B., Degener, E., Tacon, A.G.J., Youngson, A., and Bell J.G. 1984. Comp. Biochem. Physiol. 53B, 399.

Erdal, J.I., Evensen, O., Kaurstad, O.K., Lillehaug, A., Solbakken, R. & Thorud, K. 1991. Relationship between diet and immune response in Atlantic salmon (*Salmo salar L.*) after feeding various levels of ascorbic acid and omega-3 fatty acids. Aquaculture. 98, 363-379.

Frøyland, L., Lie, Ø. & Berge, R.K. 2000. Mitochondrial and peroxisomal beta-oxidation capacities in various tissues from Atlantic salmon Salmon salar. Aquaculture Nutrition, 6, 85-89.

Frøyland, L., Madsen, L., Vaagenes, H., Totland, G.K., Auwerx, J., Kryvi, H., Staels, B. & Berge, R.K. .1997. Mitochondrion is the principal target for nutritional and pharmacological control of triglyceride metabolism. J. Lipid. Res. 38, 1851-1858.

Frøyland, L., Vaagenes, H., Asiedu, D.K., Garras, A., Lie, Ø. & Berge, R.K. 1996. Chronic Administration of Eicosapentaenoic Acid and Docosahexaenoic Acid as Ethyl Esters Reduced Plasma Cholestrol and Changed the Fatty Acid Composition in Rat Blood and Organs. Lipids. 31, 169-178.

Grisdale-Helland, B., Ruyter, B., Rosenlund, G, Obach, A., Helland, S.J., Sandberg, M.G., Standal, H. and Røsjø, C. 2002. Influence of high contents of dietary soybean oil on growth, feed utilization, tissue fatty acid composition, heart histology and standard oxygen consumption of Atlantic salmon (*Salmo salar*) raised at two temperatures. Aquaculture. 207, 311-329.

Gjøen, T., Obach, A., Røsjø, C., Helland, B.G., Rosenlund, G., Hvattum, E. and Ruyter, B. .2004. Effect of dietary lipids on macrophage function, stress susceptibility and disease resistance in Atlantic salmon (*Salmo salar*). Fish Physiol. Biochem. 30, 149-161.

Gjøen, T. Kleveland, E.J., Moya-Falcón, C., Frøystad, M.K., Vegusdal, A, Hvattum, E., Berge, R.K., and Ruyter B. 2007. Effects of dietary thia fatty acids on lipid composition, morphology and macrophage function of Atlantic salmon (*Salmo salar L.*) kidney. Comp. Biochem. Physiol. B.

Grisdale-Helland, B., Ruyter, B., Rosenlund, G., Obach, A., Helland, S.J., Sandberg, M.G., Standal, H. & Roesjoe, C. 2002. Influence of high contents of dietary soybean oil on growth, feed utilization, tissue fatty acid composition, heart histology and standard oxygen consumption of Atlantic salmon (Salmo salar) raised at two temperatures. Aquaculture. 207, 311-329.

Hagar, A. F., Hazel, J. R., 1985. Changes in desaturase activity and the fatty acid composition of microsomal membranes from liver tissue of thermally-acclimating rainbow trout. J. Comp. Physiol. B. 156, 35-42.

Halvorsen, B., Rustan, A.C., Madsen, L., Reseland, J., Berge, R.K., Sletnes, P. & Christiansen, E.N. 2001. Effects of long-chain monounsaturated and n-3 fatty acids on fatty acid oxidation and lipid composition in rats. Ann. Nutr. Metab. 45, 30-37.

Hansen, H. S. 1994. New biological roles for the n-6 and n-3 fatty acids. Nutr. Rev. 52, 162-167.

Harris, W.S., Connor, W.E. & McMurphy, M.P. 1983. The comparative reductions of the plasma lipids and lipoproteins by dietary polyunsaturated fats: salmon oil versus vegetable oils. Metabolism. 32, 179-184.
Hemre, G.I. & Sandnes, K. 1999. Effect of dietary lipid level on muscle composition in Atlantic salmon, *Salmo salar*. Aquacult. Nutr. 5, 9-16.
Hemre, G.-I., Lambertsen, G. & Lie, Ø. 1991. The effect of dietary carbohydrate on the stress response in cod (Gadus morhua). Aquaculture. 95, 319-328.
Henderson, R.J. 1996. Fatty acid metabolism in freshwater fish with particular reference to polyunsaturated fatty acids. Arch. Anim. Nutr. 49, 5-22.
Henderson, R.J. 1996. Fatty acid Metabolism in Freshwater Fish with particular reference to Polyunsaturated Fatty acids. Arch. Anim. Nutr. 49, 5-22.
Henderson, R.J. and Tocher, D.R. .1987. The lipid composition and biochemistry of freshwater fish. Prog. Lipid Res. 26, 281-347.
Henderson, R.J., Park, M.T. and Sargent, J.R. 1995. The desaturation and elongation of ^{14}C-labelled polyunsaturated fatty acids by pike (Esox lucius L.) in vivo. Fish Physiol. Biochem. 14, 223-235.
Hillestad, M., Johnsen, F., Austreng, E. and Asgaard, T. 1998. Aquacult. Nutr. 4, 89.
Austreng, E., Skrede, A., Eldegaard, Å. 1979. Effect of dietary fat source on the digestibility of fat and fatty acids in rainbow trout and mink. Acta Agric. Scand. 29, 119-126.
Jeffcoat, R. 1969. The biosynthesis of unsaturated fatty acids and its control in mammalian liver. Essays Biochem. 15, 1-36.
Jordal, A.-E.O., Lie, Ø. & Torstensen, B.E. 2007. Complete replacement of dietary fish oil with a vegetable oil blend affect liver lipid and plasma lipoprotein levels in Atlantic salmon (Salmo salar L.). Aqua. Nutr. 13, 114-130.
Ketola, H.G., Smith, C.E., and Kindschi, G.A. 1989. Aquaculture. 79, 417.
Karalazos, V., Bendiksen, E.Å., Dick, J.R. & Bell, J.G. 2007. Effects of dietary protein, and fat level and rapeseed oil on growth and tissue fatty acid composition and metabolism in Atlantic salmon (*Salmo salar L.*) reared at low water temperatures. Aquaculture Nutrition, 13, 256-265.
Kiessling, K.-H. & Kiessling, A. 1993. Selective Utilization of Fatty acids in Rainbow trout (*Onchorhychus mykiss Walbaum*) Red muscle Mitochondria. Canadian J. Zool. 71, 248-251.
Kyrou, I. & Tsigos, C. 2007. Stress mechanisms and metabolic complications. Horm. Metab. Res., 39, 430-438.
Lang, C.A. & Davis, R.A. 1990. Fish oil fatty acids impair VLDL assembly and/or secretion by cultured rat hepatocytes. J. Lipid Res. 31, 2079-2086.
Lee, D.J. and Roehm, J.J., Yu, T.C. and Sinnhuber, R.O. 1967. Effects of ω-3 fatty acids on the growth rate of rainbow trout (Salmo gairdneri) J.Nutr. 92, 92-98.
Leger, C. and Fremont, L. 1981. Fatty acid composition of lipids in the trout (Salmo gairdneri) Comp. Biochem. Biophys. 69B, 99-105.
Lie, Ø. .2001. Flesh quality - the role of nutrition. Aquacult. Res., 32, 341-348.
Lochman, R.T. and Gatlin III, D.M. 1993. Essential fatty acidrequirement of juvenile red drum (Sciaenops ocellatus). Fish Physiol. Biochem. 12, 221-235.
Madsen, L., Rustan, A.C., Vaagenes, H., Berge, K., Dyrøy, E. & Berge, R.K. 1999. Eicosapentaenoic and docosahexaenoic acid affect mitochondrial and peroxisomal fatty acid oxidation in relation to substrate preference. Lipids. 34, 951-963.
Makrides, M., Neumann, M., Simmer, K., Pater, J. and Gibson, R. 1995. Are long chain polyunsaturated fatty acids essential nutrients in infancy? Lancet. 345, 1463-1468.
Milne, G.L., Gao, L., Brooks, J.D. & Morrow, J.D. 2008. The role of oxidative stress indiseases associated with overweight and obesity In Oxidative stress and inflammatory mechanisms in obesity, diabetes, and the metabolic syndrome (Packer, L. & Sies, H. eds.), pp. 33-46. CRC Press, Boca Raton.
Moya-Falcón, C., Thomassen, M.S., Jakobsen, J.V.and Ruyter B. 2005. Effects of supplementation of rapeseed oil on metabolism of [1-^{14}C] 18:1 n-9, [1-^{14}C] 20:3 n-6 and [1-^{14}C] 20:4 n-3 in Atlantic salmon hepatocytes. Lipids. 40,709-717.
Nanton, D.A., Vegusdal, A., Benze-Røraa, A.M., Ruyter, B., Baeverfjord, G. & Torstensen, B.E. 2007. Muscle lipid storage pattern, composition, and adipocyte distribution in different parts of Atlantic salmon (*Salmo salar*) fed fish oil and vegetable oil. Aquaculture. 265, 230-243.
Nestel, P.J. 1990. Effects of n-3 fatty acids on lipid metabolism. Annu. Rev. Nutr. 10, 149-167.
Ng, W.-K., Sigholt, T. & Bell, J.G. 2004. The influence of environmental temperature on the apparent nutrient and fatty acid digestibility in Atlantic salmon (*Salmo salar L.*) fed finishing diets containing different blends of fish oil, rapeseed oil and palm oil. Aquacult. Res. 35, 1228-1237.
Nossen, J.Ø., Rustan, A.C., Gloppestad, S.H., Målbakken, S. & Drevon, C.A. 1986. Eicosapentaenoic acid inhibits synthesis and secretion of triacylglycerols by cultured rat hepatocytes. Biochim. Biophys. Acta., 879, 56-65.

Nugteren, D.H. 1962. Conversion in vitro of linoleic acid into γ-linolenic acid by rat liver enzymes. Biochim. Biophys. Acta. 60, 656-657.

Olsen, R.E. & Henderson, R.J. 1997. Muscle fatty acid composition and oxidative stress indices of Arctic charr, *Salvelinus alpinus (L.)*, in relation to dietary polyunsaturated fatty acid levels and temperature. Aquac. Nutr. 3, 227-238.

Olsen, R.E., Henderson, R.J. and Ringø, E. 1991. Lipids in Arctic charr, *Salvenius alpinus (L)*. I. Dietary induced changes in lipid class and fatty acid composition. Fish. Physiol. Biochem. 9, No.2, 151-164.

Olsen, R.E., Løvaas, E. & Lie, Ø. 1999. The influence of temperature, dietary polyunsaturated fatty acids, alfa-tocopherol and spermine on fatty acid compostion and indices of oxidative stress in juvenile Artic char, *Salvelinus alpinus (L.)*. Fish Physiol. Biochem. 20, 13-29.

Orzechowski, A., Ostaszewski, P., Jank, M. & Berwid, S.J. 2002. Bioactive substances of plant origin in food - impact on genomics. Reprod. Nutr. Dev. 42, 461-477.

Owen, J.M., Adron, J.A., Sargent, J.R. and Cowey, C.B. 1972. Mar. Biol. 13, 160-166.

Owen, J.M., Adron, J.W., Middleton, C. and Cowey, C.B. 1975. Elongation and desaturation of dietary fatty acids in turbot (*Scophthalmus maximus L.*), and rainbow trout (*Salmo gairdneri*). Rich. Lipids. 10, 528-531.

Pan, D. A., Hulbert, A. J. and Storlien, L. H. 1994. Dietary fats, membrane phospholipids and obesity. J. Nutr. 124, 1555-1565.

Poppe, T., Johansen, R., Sverdrup, A., Helle, K.B., Kongtorp, R.T., Krogdahl, Å., Storset, A.k., Ottesen, N., Østvik, A. & Brun, E. 2004. Heart report (in Norwegian). 1-69.

Ranheim, T., Gedde-Dahl, A., Rustan, A.C. & Drevon, C.A. 1994. Effect of chronic incubation of CaCo-2 cells with eicosapentaenoic acid (20:5n-3) and oleic acid (18:1n-9) on triacylglycerol production. The Biochem. J. 303, 155-161.

Refsgaard, H.H.F., Brockhoff, P.B. & Jensen, B.H. 1998. Biological variation of lipid constituents and distribution of tocopherols and astaxanthin in farmed Atlantic salmon (*Salmo salar*). J. Agric. Food Chem. 46, 808-812.

Regost, C., Jakobsen, J.V. & Rørå, A.M.B. 2004. Flesh quality of raw and smoked fillets of Atlantic salmon as influenced by dietary oil sources and frozen storage. Food Res. Int. 37, 259-271.

Rinchard, J., Czesny, S. and Dabrowski, K. 2007. Influence of lipid class and fatty acid deficiency on survival. growth and fatty acid composition in rainbow trout juveniles. Aquaculture. 264, 363-371.

Robinson, E.H. .1992. Recent advances in aquaculture nutrition research. Proc. Ann. Pfizer Res. Conf. 40, 243-276.

Rørå, A.M.B., Ruyter, B., Skorve, J., Berge, R.K. & Slinning, K-E. 2005. Influence of high content of dietary soybean oil on quality of market sized raw, smoked and frozen Atlantic salmon (*Salmo salar*). Aquacult. Int. 13, 217-231.

Rosenlund, G., Obach, A., Sandberg, M.G., Standal, H. & Tveit, K. 2001. Effect of alternative lipid sources on long-term growth performance and quality of Atlantic salmon (Salmo salar L.). Aquacult. Res., 32, 323-328.

Ruyter, B., Røsjø, C., Måsøval. K., Einen, O. and Thomassen, M.S. 2000. Influence of dietary n-3 fatty acids on the desaturation and elongation of [1-^{14}C] 18:2 n-6 and [1-^{14}C] 18:3 n-3 in Atlantic salmon hepatocytes. Fish Physiology and Biochemistry. 23, 151-158.

Ruyter, B., Røsjø, C., Einen, O. and Thomassen, M.S. 2000a. Essential fatty acids in Atlantic Salmon: effects of increasing dietary doses of n-6 and n-3 fatty acids on growth, survival and fatty acid composition of liver, blood and carcass. Aquaculture Nutrition. 6, 119-127.

Ruyter, B., Røsjø, C., Einen, O. and Thomassen, M.S. 2000b. Essential fatty acids in Atlantic Salmon: time course of changes in fatty acid composition of liver, blood and carcass induced by a diet deficient in n-3 and n-6 polyunsaturated fatty acids. Aquacult. Nutr. 6, 109-117.

Ruyter, B., Røsjø, C., Grisdale-Helland, B. Rosenlund, G., Obach, A. and Thomassen M.S. 2003. Influence of temperature and high dietary linoleic acid content on the elongation and desaturation of polyunsaturated fatty acids in Atlantic salmon hepatocytes. Lipids. 38, 833-839

Ruyter, B., Moya-Falcon, C., Rosenlund, G and Vegusdal, A. 2006. Fat content and morphology of liver and intestine of Atlantic salmon (*Salmo salar*). Effects of temperature and dietary soybean oil. Aquaculture. 252, 441-452.

Sargent, J.R. 1997. Fish oils and human diet. Br. J. Nutr. 78, suppl.1, 5-13.

Sargent, J.R., Bell, J.G., Bell, M.V., Henderson, R.J. and Tocher, D.R. 1995. Requirement criteria for essential fatty acids. J. Appl. Ichtyol. 11, 183-198.

Sargent, J.R., Henderson, R.J. and Tocher, D.R. 1989. In: (Ed.) Halver, J.E. The lipids, Fish Nutrition. Academic Press, London. 153-218.

Schuenke, M., Wodtke, E. 1983. Cold-induced increase of Δ^9 and Δ^6 desaturase activities in endoplasmatic membranes of carp liver. Bioche. Biochys. Acta. 734, 70-75.

Seierstad, S.L., Poppe, T.T., Koppang, E.O., Svindland, A., Rosenlund, G., Frøyland, L. & Larsen, S. 2005a. Influence of dietary lipid composition on cardiac pathology in farmed Atlantic salmon, *Salmo salar L.* J. Fish Dis. 28, 677-690.

Seierstad, S.L., Seljeflot, I., Johansen, O., Hansen, R., Haugen, M., Rosenlund, G., Frøyland, L. & Arnesen, H. 2005b. Dietary intake of differently fed salmon; the influence on markers of human atherosclerosis. Eur. J. Clin. Invest. 35, 52-59.

Seierstad, S.L., Svindland, A., Larsen, S., Rosenlund, G., Torstensen, B.E. & Evensen, Ø. 2007. Temporal and spatial development of intimal thickening of coronary vessels in Atlantic salmon (*Salmo salar L.*) correlates with heart and fish weight while dietary lipid sources have no influence. J. Fish. Dis. Accepted.

Sellner, P.A., Hazel, J. R. 1982. Incorporation of polyunsaturated fatty acids into lipids of rainbow trout hepatocytes. Am. J. Physiol. 243, 223-228.

Sies, H. 1991. Oxidative stress: Oxidants and Antioxidants. Academic Press, New York.

Sigurgisladottir, S., Lall, S.P., Parrish, C.C. & Ackman, R.G. 1992. Cholestane as a digestability marker in the absorbtion of polyunsaturated fatty acid ethyl esters in Atlantic salmon. Lipids. 27, 418-424.

Sinesky, M., 1974. Homeoviscous adaptation: a homeostatic process that regulates the viscosity of membrane lipids in Escherichia coli. Proc. Nat. Sci. USA. 71, 522-525.

Sprecher, H. 1981. Biochemistry of essential fatty acids. Prog. Lipid. Res., 20, 13-22.

Stubhaug, I., Lie, Ø. & Torstensen, B.E. 2007. Fatty acid productive value and β-oxidation capacity in Atlantic salmon tissues (*Salmo salar L.*) fed on different lipid sources along the whole growth period. Aquacult. Nutr. 13, 145-155.

Takeuchi, T. and Watanabe, T. 1977. Requirements of carp for essential fatty acids. Bull. Jap. Soc. Sci. Fish. 43, 541-551.

Takeuchi, T. and Watanabe, T. 1979. Effects of excess amounts of essential fatty acids on growth of rainbow trout. Bull. Japan. Soc. Scient. Fish. 45, No. 12, 1517-1519.

Takeuchi, T., Watanabe,T. and Nose, T. 1979. Requirement for essential fatty acids of chum salmon (*Oncorhychus keta*) in freshwater environment. Bull. Japan. Soc. Sci. Fish. 45, 1319-1323.

Thomassen, M.S. & Røsjø, C. 1989. Different Fats in Feed for Salmon: Influence on Sensory Parameters, Growth Rate and Fatty Acids in Muscle and Heart. Aquaculture. 79, 129-135.

Thompson, K.D., Tatner, M.F. & Henderson, R.J. 1996. Effects of dietary (n-3) and (n-6) polyunsaturated fatty acid ratio on the immune response of Atlantic salmon, *Salmo salar L.* Aquaculture Nutrition, 2, 21-31.

Thongrod, S., Takeuchi, T., Satoh, S. and Watanabe, T. 1990. Requirement of Yamane (*Onchorhynchus masou*) for essential fatty acids. Nippon Suisan Gakkaishi. 56, 1255-1262.

Tocher, D.R., Bell, J.G., Dick, J.R. & Crampton, V.O. 2003. Effects of dietary vegetable oil on Atlantic salmon hepatocyte fatty acid desaturation and liver fatty acid compositions. Lipids. 38, 723-732.

Tocher, D.R., Bell, J.G., MacGlaughlin, P., McGhee, F. & Dick, J.R. 2001. Hepatocyte fatty acid desaturation and polyunsaturated fatty acid composition of liver in salmonids: effects of dietary vegetable oil. Comp. Biochem. Physiol. B. 130, 257-270.

Tocher, D., Fonseca-Madrigal, J., Dick, J.R., Ng, W-K., Bell, J.G., Campbell, J. 2004. Effects of water temperature and diets containing palm oil and fatty acid desaturation and oxidation in hepatocytes and intestinal enterocytes of rainbow trout (*onchorynchus mykiss*). Comp. Biochem. Biophys. B. 137, 49-63.

Torstensen, B.E. 2000. Transport and metabolism of lipids in Atlantic salmon (*Salmo salar L.*). Thesis for the degree of doctor scientiarum, University of Bergen, Norway. 5-43.

Torstensen, B.E., Bell, J.G., Rosenlund, G., Henderson, R.J., Graff, I.E., Tocher, D.R., Lie, Ø. & Sargent, J.R. 2005. Tailoring of Atlantic salmon (Salmo salar L.) flesh lipid composition and sensory quality by replacing fish oil with a vegetable oil blend. J. Agric. Food Chem. 53, 10166-10178.

Torstensen, B.E., Frøyland, L. & Lie, Ø. 2004a. Replacing dietary fish oil with increasing levels of rapeseed oil and olive oil - Effects on Atlantic salmon (*Salmo salar*) tissue and lipoprotein composition and lipogenic enzyme activities. Aqua. Nutr. 10, 175-192.

Torstensen, B.E., Frøyland, L., Ørnsrud, R. & Lie, Ø. 2004b. Tailoring of a cardioprotective fillet fatty acid composition of Atlantic salmon (*Salmo salar*) fed vegetable oils. Food Chemistry. 87, 567-580.

Torstensen, B.E., Lie, Ø. & Frøyland, L. 2000. Lipid metabolism and tissue composition in Atlantic salmon (Salmo salar L.) - Effects of capelin oil, palm oil, and oleic acid-enriched sunflower oil as dietary lipid sources. Lipids. 35, 653-664.

Trueman, R. J., Tiku, P. E., Caddick, M. X., Cossins, A. R. 2000. Thermal thresholds of lipid restructuring and Δ^9 desaturase expression in the liver of carp (*Cyprinus carpio L*). J. Exp. Biol. 203, 641-650.

Vegusdal, A., Gjøen, T., Berge, R.K., Thomassen, M.S. & Ruyter, B. 2005. Effect of 18:1n-9, 20:5n-3 and 22:6n-3 on lipid accumulation and secretion by Atlantic salmon hepatocytes. Lipids. 40, 477-486.

Waagbø, R., Sandnes, K., Sandvin, A. & Lie, Ø. 1991. Feeding three levels of n-3 polyunsaturated fatty acids at two levels of vitamin E to Atlantic salmon (*Salmo salar*). Growth and chemical composition. Fisk. Dir. Skr., Ser. Ernaering. 4, 51-63.

Waagbø, R., Sandnes, K., Torrisen, O.J., Sandvin, A. & Lie, Ø. 1993. Chemical and sensory evaluation of fillets from Atlantic salmon (*Salmo salar*) fed three levels of n-3 polyunsaturated fatty acids at two levels of vitamin E. Food Chemistry. 46, 361-366.

Waagboe, R., Sandnes, K., Lie, Ø. & Nilsen, E.R. 1993. Health aspects of dietary lipid sources and vitamin E in Atlantic salmon (*Salmo salar L*). 1. Erythrocyte total lipid fatty acid composition, haematology and humoral immune response. Fisk. Dir. Skr. Ser. Ernaering. 6, 47-62.

Wakil, S.J. 1961. Mechanism of fatty acid synthesis. J. Lipid. Res. 2, 1-4.

Watanabe, T. 1982. Lipid nutrition in fish. Comparative Biochemistry and Physiology, 73B, 3-15.

Watanabe, T. 1982. Lipid nutrition in fish. Comp. Biochem. Physiol. 73B, 3-15.

Weihrauch, J.L. & Gardner, J.M. 1978. Sterol content of foods of plant origin. J. Am. Diet. Ass., 73, 39-47.

Willumsen, N., Vaagenes, H., Asiedu, D., Lie, Ø., Rustan, A.C. & Berge, R. 1996. Eicosapentaenoic acid but not docosahexaenoic acid (both as ethyl esters) increases mitochondrial fatty acid oxidation and upregulates 2,4-dienoyl-CoA reductase gene expression. A potential mechanism for the hypolipidemic action of fish oil in rats. Lipids. 31, 579-592.

Yang, X. and Dick, T.A. 1993. Effects of dietary fatty acids on growth, feed efficiency and liver RNA and DNA content of Arctic charr, (*Salvelinus alpinus L*). Aquaculture. 116, 57-70.

Yu, T.C. and Sinnhuber, R.O. 1972. Effect of dietary linolenic acid and docosahexaenoic acid on growth and fatty acid composition of rainbow trout (*Salmo gairdneri*). Lipids. 7, 450-454.

Yu, T.C. and Sinnhuber, R.O. 1976. Growth response of rainbow trout to dietary ω3 and ω6 fatty acids. Aquaculture. 8, 309-317.

Yu, T.C. and Sinnhuber, R.O. 1979. Effect of dietary ω3 and ω6 fatty acids on growth and feed conversion efficiency of Coho salmon (*Onchorhynchus Kisutch*). Aquaculture. 16, 31-38.

Yu, T.C., Sinnhuber, R.O. and Hendricks, J.D. 1979. Reproduction and survival of rainbow trout (*Salmo gairdneri*) fed linolenic acid as the only source of essential fatty acids. Lipids. 14, 572-575.

5. Vitamins

Rune Waagbø

5.1. Introduction

Most vitamins defined in the literature have also been demonstrated to be essential for fish. A status of vitamin minimum requirements was published by National Research Council, USA (NRC 1993), and revisions were published by (Waagbø et al. 2001a; Halver 2002; Hardy 2002; Storebakken 2002). Changes in genetics, husbandry and management routines, and overall dietary composition, as well as increased feed utilization and use of novel feed ingredients in intensive fish farming has urged the need for revised recommendations on vitamin supplementation. We may assume that the minimum requirements, as well as situation dependent requirements have changed along with the other advances made in the aquaculture industry, including the development of modern diets. Micronutrient deficiencies are among the common category of diseases observed in aquaculture, and also the easiest to correct if they are identified (Hardy 2001).

In the present chapter, vitamin minimum requirements and recommendations are covered. The former expresses the vitamin level requirement needed to prevent overt signs of deficiency (NRC 1993), while the latter is the suggested vitamin level required for "optimum health and productivity" under practical conditions. Dietary vitamin recommendations are often several fold the minimum requirement, for safety. One has to remember that the minimum requirements often are established in juveniles and performed with suboptimal semi-synthetic diets ("pure ingredients") that seldom supported optimal growth.

It is still natural to divide the vitamins into lipid soluble and water soluble vitamins, especially since these are inherent in the lipid rich and protein rich feed ingredients, respectively. It is important to consider both the vitamin contents and possible anti-nutrients and anti-metabolites in novel feed ingredients, as compared to the traditional marine feedstuffs, since these may interfere with the natural and supplemented vitamins in the feed (Table 5.1).

Table 5.1. Aspects to consider for vitamin requirements and recommendations for fish

Aspect	**Cause**
Processing and storage stability	Inherent chemical vitamin forms Additives with vitamins Vitamin derivatives Heat destruction Oxidation
Bioavailability	Inherent chemical vitamin forms Additives with vitamins Vitamin derivatives
Alternative feed resources	Inherent chemical vitamin forms Anti-nutrients Anti-metabolites Thiaminase; avidin
Nutrient interactions	Energy content Macronutrient content Oxidized lipids Minerals Antioxidants
Environmental interactions	Water temperature O_2 Salinity
Beyond minimum requirement during periods of production cycle	Health implications (prevention/repair) Smoltification Reproduction Larvae nutrition

The content of micronutrients in the feed varies with the type, treatment and quantity of feed ingredients (Hertrampf and Piedad-Pascual 2000), as well as the vitamin supplementation levels. The introduction of novel and sustainable feed resources calls for more attention to both inherent or natural vitamin contents and potential anti-nutrients. The latter has to be overcome by proper pre-treatment to destroy the adverse component or by vitamin over-fortification of the feed. In addition, strong biochemical interactions both within micronutrients (vitamin-vitamin; vitamin-mineral) and between micro- and macronutrients may influence the requirement of the vitamins. As examples, lipid utilization for energy purposes seems to be affected in Atlantic salmon parr fed high energy diets (30 % lipid) with riboflavin content close to the requirement (6 mg kg^{-1}), as compared to the same diet supplemented with 20 mg riboflavin/kg (Brønstad et al. 2002). Similarly, a variety of micronutrient interactions affect the requirement of vitamin C in fish (Waagbø et al. 2001b).

Recently, there has been an increasing focus on how changes in environment and husbandry management may affect vitamin requirements in farmed fish, for example related to changes in antioxidant protection [oxygenation; (Lygren 1999)], detoxification [pollutants; (Norrgren et al. 2001)] and disease resistance (Waagbø 2006). This focus also includes promising preventive measures by dietary vitamin regimes, of which vitamin C has been by far the most studied (Dabrowski 2001; Li and Robinson 2001), while vitamin E have been examined as a protective measure against outbreak of bacterial disease (Salte et al. 1988; Waagbø et al. 1993b).

The requirements for vitamins are normally given as mg vitamin kg^{-1} dry feed. Since many of the vitamins participate in the intermediary metabolism, questions have been raised if the requirement should be given on an energy basis, relative to growth or as a daily requirement intake (mg vitamin kg^{-1} fish day^{-1}) like in human recommendations (Woodward 1994; Kaushik et al. 1998; Masumoto 2002). Due to missing updated information on requirement studies for many vitamins, the requirements are given here as mg kg^{-1} dry feed.

5.2. Lipid soluble vitamins

The term lipid soluble vitamins covers vitamins A, D, E and K, and suggested minimum requirements are available for salmonid species (Table 5.2), including symptoms of vitamin deficiences (Hardy 2001; Halver 2002). In general, this group of vitamins follows the lipids in the digestion, intestinal absorption, transport, storage and excretion in the fish body, although specialized extracellular and intracellular proteins may be involved in transport and storage of the single vitamin. Consequently, any changes in diet or husbandry that impact

Table 5.2. Main functions, minimum requirements and recommendations of lipid soluble vitamins (mg kg^{-1} dry feed), including suggested or defined legal (vitamin D only) safe upper limits. Care should be taken to correct for vitamin purity of the supplemented products.

Vitamin (common name) Main vitamin function	**Minimum requirement (mg kg^{-1}) [1]**	**Updates for salmonids (mg kg^{-1})**	**Recommended target level (mg kg^{-1})**	**Suggested safe upper limit (mg kg^{-1})**
Vitamin A (Retinol) Growth, cell differentiation, vision	0.75	1.5 [12]	1.5 mg kg^{-1}	> 37 [2] <122 [3]
Vitamin D_3 (Cholecalciferol) Calcium regulation	0.06	0.013 [13] 0.06 [12] 0.05 [14]	0.1	0.075 (feed legislation > 57 [4] (no effect) <29 [5] (no effect) <6.25 [6] (growth reduction) 1.25 [4] (consumer safety)
Vitamin E (α-Tocopherol) Antioxidant	30-50	30 [12]- 60 [8]	100	700
Vitamin K (Menadione) Coagulation, bone composition	10	0.2 [9] 40 [11] 0.45 [12]	2.5 [9]	10 [9, 2]

[1](Halver 2002); [2](Grisdale-Helland et al. 1991); [3](Ørnsrud et al. 2002); [4] (Graff et al. 2002b); [6](Vielma et al. 1998); [7] [8] (Hamre and Lie 1995); [9] (Grahl-Madsen and Lie 1997) in Atlantic cod (*Gadus morhua*); [11] (Storebakken 2002) – practical addition; [12](Woodward 1994); [13](Horvli and Lie 1994b); [14] (Barnett et al. 1982);

(Vitamin A IU=0.3 μg all-trans retinol or 1 mg retinol kg^{-1} = 3333 IU kg^{-1});

(Vitamin D IU= 0.025 μg cholecalciferol or 1 mg cholecalciferol =40 000 IU)

lipid metabolism in a broad sense, may also influence the supply and need for these vitamins. Deficient or suboptimal dietary vitamin E is among the most commonly observed deficiencies in aquaculture, related to rancid diets and a general lack of antioxidants.

The safety levels of dietary vitamins are generally high, while dietary vitamins A and D are among the few vitamins that have been suspected to cause hypervitaminosis and toxicological symptoms in farmed salmonids (Graff 2002; Ørnsrud 2003). Bioaccumulation and magnification of these vitamins in the food web may result in extremely high levels of these vitamins in fish species used for fish meal and fish feed (Grisdale-Helland et al. 1991; Julshamn et al. 2003), which subsequently may result in exceeding the upper tolerable limits for these vitamins in feed for rapidly growing salmonids.

5.2.1. Vitamin A

The standing minimum requirement of vitamin A for salmonids (NRC 1993) is 0.75 mg retinol kg^{-1} (2500 IU kg^{-1}), based on a study on rainbow trout recording growth and mortality (Kitamura et al. 1967) (Table 5.2). A feeding study on Atlantic salmon fry using a mixture of retinyl-acetate and retinyl-palmitate (1:1) in a semi-synthetic diet suggests that this recommendation may represent a low estimate (Christiansen et al. 1994). The latter may however, reflect reduced bioavailability of retinyl palmitate in salmon fry intestine, as observed for other vitamin palmitate derivatives (Albrektsen et al. 1988).

The inherent natural content of vitamin A in marine resources depends on fish species and catch season. Thus, the vitamin A content in fish meal may vary from approx. 1 to 70 mg vitamin A kg^{-1}, while fish oils based on different fish species range from 6 to 690 mg vitamin A kg^{-1} (Table 5.3). Based on the natural content in the marine resources there is no need for further vitamin A supplementation in fish feeds. Indeed, the concentration of retinol in commercial feed samples produced in Norway during the years 2002-2006 varied from 4 to 121 mg retinol kg^{-1}; NIFES, Feed Surveillance Program, 2006). These surveys indicate that vitamin A deficiency in salmonids mediated through commercial feeds based on fish meal and oil is unlikely, while vitaminosis A is more likely. A change in dietary vitamin A levels relative to changes in major feed ingredients is not known (Table 5.3), however, there is less inherent vitamin A in the applied plant ingredients. Further, contributions of vitamin A that may arise from astaxanthin, as a provitamin A compound, is not known, but indications of approx. 10% vitamin activity have been suggested (Al-Khalifa and Simpson 1988). Astaxanthin, however, may only be important as a vitamin A source under vitamin A deprivation (Christiansen et al. 1994). The typical inclusion level of astaxanthin ranges

Table 5.3. Concentration range of fat soluble vitamins (mg kg^{-1} dry feed) in fish meal, soybean meal, fish oil and linseed oil, illustrating need for attention

Vitamin	**Fish meal (mg kg^{-1})**	**Soybean meal, extracted (mg kg^{-1})**	**Fish oil (mg kg^{-1})**	**Soybean oil (mg kg^{-1})**	**Linseed oil (mg kg^{-1})**
Vitamin A (Retinol)	1-70 [7]	0.12-0.24 [2]	6-690 [5] Pro vitamin A pigments [6]	-	-
Vitamin D (Cholecalciferol)	0.006-0.179 [1]	-	2.5-5.0 (liver oils) [3]	-	-
Vitamin E (α-Tocopherol)	3-10 [2] 5-22 [4]	3-9 [2]	-	27-95	75
Vitamin K (Menakinone)	2.4 [2]	-	-	5.4	-

[1](Horvli and Lie 1994a); [2](Hertrampf and Piedad-Pascual 2000); [3](DTU 2007); [4](NRC 1993); [5] (Opstvedt et al. 1997); [6] assumed approx. 10 % retinol equiv. (Al-Khalifa and Simpson 1988); [7](Ørnsrud 2003)

between 30 and 60 mg kg^{-1}, with an legally given upper limit of 100 mg kg^{-1} in feed for salmonids beyond 6 months of age (LD/FD 2002).

Toxic levels of vitamin A result in mortality, reduced growth, and disturbances in development of fish fry, including backbone deformities (Primbs et al. 1971; Hilton 1983; Ørnsrud et al. 2002). The sensitivity for vitamin A intoxication may depend on stage of development, with juveniles as more sensitive (Cahu et al. 2003). In general, salmonids seem to tolerate high dietary levels of vitamin A. In rainbow trout diets, (Hilton 1983) found 270 mg kg^{-1} (900 000 IU kg^{-1}) to be the upper limit, using the retinyl palmitate form. In Atlantic salmon fry, Ørnsrud et al. (Ørnsrud et al. 2002) found that a dietary level of 122 mg kg^{-1} (407 000 IU kg^{-1}) retinyl acetate fed for 14 weeks reduced the growth and the fish showed

symptoms of metabolic stress. Thus, the upper limit for vitamin A in Atlantic salmon feed seems to be between 37 (Grisdale-Helland et al. 1991) and 122 mg kg^{-1}.

5.2.2. Vitamin D

Vitamin D deficiency was described in amago salmon fed a vitamin D free diet, as compared to a diet containing 0.5 mg kg^{-1} (Taveekijakarn et al. 1996a). The minimum dietary requirement for vitamin D in salmonid feeds is set at 0.06 mg kg^{-1} (2400 IU kg^{-1}) (NRC 1993) (Table 5.2). Based on the natural content in marine resources there is no need for further vitamin D supplementation in marine based fish feeds (Table 5.3), while use of plant oils and meals do not support any vitamin D to the diet. The concentration of vitamin D in commercial feed samples produced in Norway during the years 2002-2006 varied from 0.1 to 1.04 mg vitamin D kg^{-1} (NIFES feed reports, Feed Surveillance Program, 2006). The variation in vitamin D recorded in fish meals and fish oils varied 50 and 32 fold, respectively (Horvli and Lie 1994a; Opstvedt et al. 1997), indicating that fish diets based on traditional marine ingredients may be exposed to extremely high levels of vitamin D, including the active vitamin D metabolites.

To present knowledge, megadoses of vitamin D_3 supplementation are not harmful for fish. Feeding experiments with vitamin D doses of 57.5 mg kg^{-1} did not affect growth and health in Atlantic salmon (Graff et al. 2002b). According to the Norwegian feed legislation (LD/FD 2002) vitamin D_3 supplementation should be below 0.075 mg kg^{-1} (3000 IU kg^{-1}), while inherent vitamin D is not included in the upper limit unless a supplementation is performed. An upper limit of vitamin D of 1.25 mg kg^{-1} is suggested in Table 5.2, despite no harmful impacts on fish health observed far above this level. This limit reflects, however, more an aspect of seafood safety for humans. Salmon retain between 10-20 % of feed vitamin D in fillet. By feeding Atlantic salmon 1.25 vitamin D kg^{-1} feed, the fillet will contain between 0.13-0.25 mg kg^{-1}, and consumption of 200 g fillet will contain 50 µg vitamin D. While clinical vitamin D toxicity has been observed at daily intakes of 10-20 times this dose, selected parts of the population like children and elderly are more sensitive to toxicity.

5.2.3. Vitamin E

A major essential role of vitamin E (tocopherols/tocotrienols) is to protect body lipids and biomembranes towards oxidation or rancidity (Hamre 1995). Thus, a state of vitamin E deficiency is characterized by anemia and accumulation of lipid oxidation products in the liver with liver degeneration, exudative diathesis and exophthalmia (Hamre et al. 1994).

Muscle degeneration is also observed, however mostly in dual vitamin E and selenium deficiency (Hamre 1995; Halver 2002). Vitamin E deficiency was commonly observed in early aquaculture history with low quality rancid diets, not protected with synthetic antioxidants nor added vitamin E. Estimated minimum requirement in coldwater species is 50-60 mg kg^{-1} dry feed (NRC 1993; Hamre and Lie 1995). The requirement may, however, vary between 5 and 120 mg kg^{-1}, depending on fish species and farming conditions (Cowey et al. 1981; Hamre and Lie 1995). Increasing levels of dietary polyunsaturated fatty acids (PUFA) increases the need for antioxidants and vitamin E (Watanabe et al. 1981). This includes increased requirement at lower water temperatures, since the fish adapts its biomembranes to lower temperatures by replacing saturated fatty acids with higher proportions of PUFA (Cowey et al. 1984). Most studies of vitamin E requirement are performed with low lipid levels (<10%) in feed to juvenile fish, while modern salmonid feeds contain 25-40 % lipid. Due to its protective role as antioxidant, recommended levels of vitamin E should be adjusted according to dietary lipid level. Further, micronutrient interactions may increase the requirement of vitamin E, including low dietary vitamin C levels (Hamre et al. 1997), low selenium levels (Poston et al. 1976) and oxidized lipid (Cowey et al. 1984). Environmental conditions, like water oxygenation, as well as infectious diseases, expose the fish to increased oxidative stress, which may be alleviated with elevated supplementation of vitamin E or tissue vitamin E status (Lygren et al. 2000). Based on established minimum requirements, and nutritional and environmental interactions, it is recommended to target 100 mg kg^{-1} of vitamin E (α-tocopherol) in practical diets for salmonids.

The muscle vitamin E level reflects the dietary vitamin E supplementation in a linear way, and this can be used to tailor the vitamin E content in the final product by use of finishing diets. This allows the producer to both increase the nutritional value of the produce, as well as improve the protection against oxidation during storage and processing (Frigg et al. 1990; Waagbo et al. 1993; Sigurgisladottir et al. 1994a; Sigurgisladottir et al. 1994b; Hamre et al. 1998). The finishing diet should be used for at least three months prior to slaughter (Hamre et al. 1998).

Processed marine feed ingredients are protected towards oxidation by synthetic antioxidants and normally contain low inherent levels of vitamin E. Plant oils and meals contain considerable concentrations of inherent vitamin E (Table 5.3). However, changes towards will also increase the relative contribution of plant specific tocopherols (β-, γ- and δ-tocopherols) with adequate antioxidant properties, but with considerably lower biological

vitamin E activity (Hamre et al. 1993; Sigurgisladottir et al. 1994a; Hamre et al. 1998). Vitamin E toxicity is not observed in farmed fish, however elevated levels (> 700 mg kg^{-1}) should be avoided for extended feeding periods, since high doses of vitamin E may be pro-oxidative in antioxidant imbalances (Hamre et al. 1997).

5.2.4. Vitamin K

Vitamin K plays vital roles in blood coagulation and bone mineralization (Udagawa 2000). The minimum requirement is suggested to be 2.5 mg kg^{-1} using menadione (vitamin K_3) as vitamin K source. A study on amago salmon, *Oncorhyncus rhodurus,* fed a vitamin K deficient, purified diet developed deficiency symptoms like mortality, anemia, increased blood clotting time and histopathological findings in liver and gills, which was recovered after feeding a diet containing vitamin K (Taveekijakarn et al. 1996b). Feeding 10 mg kg^{-1} of menadione sodium bisulphite (MSB; vitamin K_3) increased the active form menakinone (MK-4; vitamin K_2) in the Atlantic salmon liver compared to no supplementation (0.05 mg phyllokinones kg^{-1}; vitamin K_1), while the latter diet did not result in vitamin K deficiency (Graff et al. 2002a). The MSB vitamin K form used in fish feed is unstable during feed processing and storage (Marchetti et al. 1999), and the dietary content may under unfavorable production and storage conditions reach critical low levels. Menadione nicotinamide bisulphite (MNB) has been suggested as a more stable and less toxic alternative for vitamin K supplementation (Marchetti et al. 1995). Recent unpublished data however, questions the availability of this form of vitamin K in feed for salmonids (Krossoy 2007). While a minimum requirement is probably <1 and recommended target level are 2.5 mg vitamin K kg^{-1} (Table 5.2), there are analytical difficulties to correctly assessing inherent (phyllokinones) and supplemented forms (MSB or MNB) of vitamin K for exact determinations. Relevant feed ingredients of plant origin may cover the requirement for vitamin K (Table 5.3).

5.3. Water soluble vitamins

The expression water soluble vitamins covers eight B vitamins (Table 5.4) and vitamin C. Additionally, inositol and choline are often included as vitamin-like compounds since salmonids seem to require supplementations during juvenile stages with high growth rates. The minimum requirements have been estimated for most of the vitamins in salmonid species, although not always including Atlantic salmon and rainbow trout [(Halver 2002; Hardy 2002; Storebakken 2002); Table 5.5]. Although (Halver 2002) represents a recent publication on

Table 5.4. Names, importance in metabolism, sensitive organ at deficiency, and main biochemical function of the single B-vitamins

Vitamin	**Familiar name**	**Essential for**	**Sensitive organs**	**Biochemical function**
Vitamin B_1	Thiamin	Carbohydrates	Nerve system, muscle tissue	Carboxylation, decarboxylation
Vitamin B_2	Riboflavin	Lipid, protein, carbohydrate, energy	Skin, epithelium, nerves, eye tissues	Redox-reactions
Niacin	Nicotinic acid, Vitamin B_3	Lipid, protein, carbohydrate, energy	Skin, epithelium, nerves, muscle cell	Redox-reactions
Vitamin B_6	Pyridoxine	Protein, amino acids, lipid	Muscle cell, nerves, blood cell	Transaminations/ deaminations
Biotin	Vitamin H	Lipid, carbohydrates	Skin, gill, liver tissues	Carboxylation, decarboxylations
Pantothenic acid	Vitamin B_5	Carbohydrates, proteins, fatty acids	Gills, skin, nerve system	Acetylations, acylations
Folate	Folic acid	Amino acids, nucleic acids	Blood cells, nerves, skin, cell division	1C- transfers
Vitamin B_{12}	Cyano-cobalamin	Amino acids, nucleic acids, fatty acids	Blood cells, nerves, cell division	Methylations

Table 5.5. Minimum requirements, updates and recommended supplementation to target levels in commercial salmonid diets (mg kg^{-1} dry feed). Water soluble vitamins are regarded safe at high dietary doses (minimum requirement x 100) and no current legal upper limits exist.

Vitamin	**Minimum requirement (mg kg^{-1}) [1]**	**Updates for fish species (mg kg^{-1}) [2]**	**Recommended target level (mg kg^{-1})**
Vitamin C	20	10	100
Thiamin	10-15	1	10
Riboflavin	20-30	4-6 [5,6]	15
Niacin	88-200[7]	10 [8]	100
Pantothenic acid	40-50	19	50
Pyridoxine	10-20	2-8 [9]	10 [2]
Biotin	1.0-1.5	0.1-0.3 [3,4]	0.5
Folic acid	6-10	0.6 3.6 [3]	10
Vitamin B_{12}	0.015-0.02	0.007 0.014	0.03
Vitamin- like compounds			
Inositol	200-400	250	500
Choline	2000-4000	430-4000	2000

[1] (Halver 2002); [2](Woodward 1994); [3](Koppe 1993) ; [4](Mæland et al. 1998); [5](Serrini et al. 1996); [6](Brønstad et al. 2002); [7] (Chuang 1991); [8](Poston and Wolfe 1985); [9] (Albrektsen et al. 1993)

water soluble vitamins, virtually no new information on this area is included since the previous 1989 book edition (Halver 1989).

Lack of B-vitamins is not normally considered as an obvious reason for deficiency in salmonid farming, and potential single vitamin deficiencies are relatively easily discovered (Hardy 2001). There seems to be even less chance for toxic conditions, with safety factors for humans over 100 times the RDA (recommended daily allowances) for most water soluble vitamins, and 50 times the RDA for folate (Marks 1989).

Basic roles and deficiency symptoms of the single vitamins are reported in detail by (NRC 1993; Halver 2002). All the B-vitamins participate in biochemical reactions in the intermediate energy metabolism, and high concentrations of the vitamins are found in metabolically active fish tissues [(Brækkan 1959); (Table 5.4)]. The roles of the single B-vitamins in the fish body are not fully known, and there is a large gap between historical requirement experiments and modern intensive farming conditions. Uncertainty is also illustrated by wide ranges in the suggested minimum requirements (Table 5.5). In human nutrition, beyond deficiency concepts are considered, and often include other roles and recently discovered novel essential functions of single vitamins. For B-vitamins, such functions include, for example, the role of folate derivatives in regulation of tissue differentiation and growth. Likewise, novel roles of pyridoxine in steroid hormone regulation in stress, smoltification and reproduction has been examined in Atlantic salmon (Albrektsen 1994). Symptoms of suboptimal vitamin supplies may be expressed under intensive farming conditions, like elevated water temperatures, manipulation of light regimes and extreme water gas regimes. Own unpublished data show up to three-fold fluctuations in liver and muscle status of selected B-vitamins in Atlantic salmon smolts reared at three different temperatures while given the same diet. This is probably related to differences in feed intake, metabolism and requirements for the vitamins. Research is needed to examine if B-vitamins introduce biological limitations in intensive farming of adult salmonids.

Changes in feed ingredients from marine towards plant ingredients may introduce changes both in dietary vitamin levels, their relative bio-availabilities and their content of anti-vitamins and anti-metabolites (Table 5.6). Vitamins are supplemented in different chemical forms, often protected towards harsh production conditions. The stability of crystalline and coated forms of water soluble vitamins during feed production (pelleting or extrusion) and storage identified greater loss of unstable vitamins during modern extrusion

Table 5.6. Concentrations (range) of water-soluble vitamins in fish meal, soybean meal and corn gluten meal (mg kg^{-1}dry ingredient). In cases of major fish meal replacement by plant ingredients, care should be taken to fulfill vitamin recommendations.

Vitamin	**Fish meal types (mg kg^{-1})** [1,2]	**Defatted soybean meal (mg kg^{-1})** [1,2]	**Corn gluten meal (60% protein) (mg kg^{-1})** [1,2]
Vitamin C	-	-	-
Thiamin	0.1-2	3-7	0.3
Riboflavin	5-10	3	2
Niacin	18-144 [6]	22-30	60
Pantothenic acid	8-30	15	4
Pyridoxin	3-6	5-7	7
Biotin	0.1-0.8 [4]	0.3 [5]	0.2
Folic acid	0.1-0.8 [7]	0.7-2.8	0.3
Vitamin B_{12}	0.07-0.43 [7]	-	-
Vitamin- like compounds			
Inositol	700-800 [3]	-	-
Choline	3000-5300	2 700-2 800	350

[1](Hertrampf and Piedad-Pascual 2000); [2](NRC 1993); [3](Waagbø et al. 1998); [4](Mæland and Sandnes 1999); [5](Lovell and Buston 1984); [6] (Blum 1991); [7] (Sandnes and Mæland 1994)

production than yesterdays pelleting production procedure, and subsequent storage (Gadient and Fenster 1994; Gabaudan and Hardy 2000). This holds especially for crystalline vitamin C, thiamine, pyridoxine, vitamin B_{12} and folate.

5.3.1. Vitamin C

Vitamin C or ascorbic acid deficiency has probably been the most widespread nutritional disease in aquaculture history due the extreme instability of the vitamin during processing and storage (Sandnes 1991; Gadient and Fenster 1994; Gabaudan and Hardy 2000). Several protected vitamin compounds and derivatives have been examined for their stability, bioavailability and efficacy in salmonids, of which the phosphate derivatives seem to be superior (Waagbø et al. 1991; Sandnes and Waagbø 1991b; Sandnes et al. 1992). There is an extensive body of literature on vitamin C (ascorbic acid) qualitative (Mæland and Waagbø 1998) and quantitative requirements in fish species (Sandnes 1991; Dabrowski 2001), including the use of excess dietary vitamin C under stressful and unfavourable farming conditions. Severe vitamin C deficiency is characterized by bone deformities, anemia and massive mortalities. Detailed histopathological and descriptive deficiency symptoms are reviewed by (Meier and Wahli 1990; Dabrowski 2001). No vitamin C is normally found in processed feed materials due to its instability during feed manufacture (Table 5.6). The minimum requirement in Atlantic salmon is covered by 20 mg ascorbic acid kg^{-1}, using stable phosphate derivatives or target levels of crystalline ascorbic acid [(Sandnes et al. 1992); Table 5.5]. The vitamin C status of fish easily reflects the dietary vitamin C concentration. At the same dietary level, however, fluctuations in vitamin C status have been observed over time, probably related to factors such as feed intake, rate of excretion or metabolism of ascorbic acid (Waagbø et al. 1993a). On this basis, 100 mg ascorbic acid equivalents kg^{-1} is recommended (Table 5.5). Feed levels used to improve stress and disease resistance are most often in the order of magnitude > 1000 mg kg^{-1} for limited feeding periods (Lim et al. 2001).

5.3.2. Thiamin

Thiamin (vitamin B_1) is a coenzyme vitamin (active form is thiamin pyrophosphate) participating in several biochemical pathways in the metabolism of carbohydrates and lipids. Thiamin deficiency is described in several salmonid and carp species, with heavy mortalities following initial behavioral and homeostatic disturbances (Morito et al. 1986; NRC 1993; Woodward 1994; Halver 2002). Juveniles are more susceptible to deficiency due to rapid metabolism and lack of storage capacity, while larger fish show more general and obscure

symptoms. Development of thiamin deficiency is accelerated at elevated temperatures relative to increased metabolism, and by increased dietary carbohydrates. Thiamin deficiency may be explored by studying activity of key thiamin-depending enzymes, like α-ketoglutarate dehydrogenase and transketolase, in selected tissues. The M74 syndrome, a reproduction disorder of Baltic salmon manifested as death of developing yolk-sac fry, seems to be related to thiamin deficiency (Amcoff et al. 2002). A similar disorder, "Cayuga syndrome", has been described in the US (Fisher et al. 1995). Low salmon egg thiamin concentration is probably mediated through imbalanced broodstock nutrition or increased degradation of thiamin by thiaminase from the wild prey in the brood fish intestine. Consequently, brood fish have been injected with thiamin to improve egg thiamin status (Fitzsimons et al. 2005), while M74 affected fry have successfully recovered after a thiamin bath treatment (Bylund and Lerche 1995). Smolt mortalities shortly after seawater transfer has been associated with "energy deficiency" and a disturbed thiamin metabolism (Salte 1993). The thiamin contribution from marine ingredients is variable, probably depending on thiaminase activity in the minced fish raw material during meal production prior to heating. The dietary thiamin requirement is uncertain, but is suggested to be considerably lower than 10 mg kg^{-1}, earlier suggested by NRC (NRC 1993). Woodward suggested 1 mg kg^{-1}, while Morris & Davis estimated a thiamin requirement in excess of 5 mg kg^{-1} for gilthead seabream (*Sparus aurata* L.) fed a diet with moderately elevated lipid content (Woodward 1994; Morris and Davies 1995b). Besides variable inherent thiamin contents (Table 5.6), plant ingredients may contain heat stable antithiamin factors, like tannins and polyphenols (Tanphaichitr 1999). Thus, the suggested dietary thiamin concentration for modern salmonid diets should be targeted to be 10 mg kg^{-1} (Table 5.4).

5.3.3. Riboflavin

For rainbow trout (Takeuchi et al. 1980; Hughes et al. 1981; Woodward 1985; Amezaga and Knox 1990), blue tilapia, *Oreochromis aureus* (Soliman and Wilson 1992), red hybrid tilapia (Lim et al. 1993) and channel catfish, *Ictalurus punctatus* (Serrini et al. 1996), levels of 3-6 mg riboflavin kg^{-1} diet seemed to be sufficient for maximal growth and feed efficiency. Besides unspecific deficiency symptoms like growth depression, reduced feed conversion and dark pigmentation, cataract is a classical symptom of riboflavin deficiency (Hughes et al. 1981; Lim et al. 1993; Bjerkås et al. 2006). This led to increased focus on riboflavin during outbreaks of severe cataracts in Atlantic salmon farms during the nineties (Bjerkås et al. 1996; Waagbø et al. 1996; Midtlyng et al. 1999). Despite adequate inherent

riboflavin content in practical feeds with 50% fish meal (6-8 mg kg^{-1}), Brønstad *et al.* found that riboflavin supplementation (20 mg kg^{-1}) affected lipid metabolism in smolting Atlantic salmon fed a practical high energy diet (Brønstad et al. 2002). Similar conditions may also be valid for the feed and energy utilisation in adult salmon, where even higher lipid concentrations are used.

Variable amounts of bioavailable forms of riboflavin are found in most plant and animal feed ingredients (Table 5.6). For optimal growth, lipid metabolism and health in salmonid farming, a target level of 15 mg riboflavin kg^{-1} should be achieved.

5.3.4. Biotin

Biotin functions as a coenzyme for carboxylases in metabolism of carbohydrates, lipids and some amino acids (Dakshinamurti and Cauhan 1989). Biotin deficiency symptoms in fish are reduced growth rate and increased mortality, as well as more specific symptoms such as abnormalities in skin, intestine and gill tissue (including "blue slime patch disease") and reduced activity of biotin dependent enzymes in metabolically active tissues (Phillips et al. 1950; Lovell and Buston 1984; Woodward and Frigg 1989; Koppe 1993). One of the biotin dependent enzymes, pyruvate carboxylase (PC), converts pyruvic acid to oxaloacetic acid in glycolysis and this enzyme has been showed to be a sensitive indicator of biotin status in fish (Woodward and Frigg 1989). Among the proteins in raw egg white, avidin binds strongly to biotin and makes the vitamin inaccessible for absorption in the intestine (Mock 1999). Biotin deficiency due to intake of raw egg white is a part of the history on the discovery of biotin as a vitamin [reviewed by (Mock 1999)]. Supplementation of raw egg white or avidin to diets induces biotin deficiency symptoms in several fish species (Poston 1976; Casteldine et al. 1978; Lovell and Buston 1984; Mæland et al. 1998). The biotin requirement in Atlantic salmon has not been established. The minimum dietary requirement for biotin for optimal growth in other salmonids, such as rainbow trout and lake trout, *Salvelinus namaycush,* have been estimated to be 0.14 mg kg^{-1} and 0.10 mg kg^{-1}, respectively (Poston 1976; Woodward and Frigg 1989). Koppe (Koppe 1993) demonstrated that the biotin requirement was met in young rainbow trout fed a practical diet without any supplementation of biotin (0.11 mg biotin kg^{-1} diet), while somewhat higher levels were needed for optimal lysozyme levels in serum and mucus (0.15 mg biotin kg^{-1} diet). The biotin concentration in Norwegian fish meal ranges between 0.4 and 0.8 mg kg^{-1} (Mæland and Sandnes 1999), depending on fish species and season. Practical feeds based on marine raw materials covered

the requirement for biotin for Atlantic salmon juveniles (Mæland et al. 1998). Similarly, practical feeds for channel catfish, based on dehulled soybean meal (75%) seem to contain enough biotin (0.35 mg biotin kg^{-1}) to meet the requirement (Lovell and Buston 1984). Elevated biotin levels, up to 1 mg kg^{-1}, may be favourable during salmon smoltification and seawater transfer (Waagbø et al. 1994). The bioavailability of biotin in feedstuffs varies greatly, between 0-100% in animal and plant ingredients (Frigg 1976; 1984). Considering variable bioavailability of biotin also in fish meals, a target level of 0.5 mg biotin kg^{-1} is suggested for salmonids (Table 5.5).

5.3.5. Pantothenic acid

Pantothenic acid is a part of coenzyme A, essential in transfer of acetyl- and acyl-groups as substrates in energy production, the synthesis of the neurotransmitter acetylcholine, acetylation of aromatic amines and synthesis of cholesterol. Mitochondria rich tissues like the kidney and gills, are specially sensitive to pantothenic acid deficiency (NRC 1993; Halver and Hardy 2002). The NRC requirement is estimated to be in the range of 10 - 50 mg kg^{-1} (NRC 1993), while Smith et al. suggested 10-15 mg kg^{-1} in their review on comparative requirement of pantothenic acid (Smith and Song 1996). In blue tilapia (*Tilapia aurea*), requirement was estimated to be 10 mg kg^{-1} based on growth and lack of pathology (Roem et al. 1991). Fish meal contains between 8 - 30 mg kg^{-1}, while plant materials contain somewhat less (Table 5.6). Pantothenic acid is added in the form of a stable calcium salt. Due to its role in lipid and energy metabolism, a conservative target feed level of 50 mg kg^{-1} is suggested.

5.3.6. Niacin

Niacin deficiency symptoms in fish include reduced growth, appetite, and feed utilization, dark pigmentation, skin lesions, muscular weakness, behavioral changes, edemas and mortalities (Chuang 1991; Halver 2002). Since niacin participates in plentiful enzymatic reactions (Table 5.4), deficiency may develop relative rapidly. Requirements in salmonids vary between 10 - 175 mg kg^{-1}, reflecting the degree of uncertainty in the estimates. A requirement level of 63-83 mg kg^{-1} was calculated for gilthead seabream using a semi-purified diet (Morris and Davies 1995a), while channel catfish required 7.4 mg kg^{-1} (Ng et al. 1997) and rainbow trout 10 mg kg^{-1} (Poston and Wolfe 1985). The given wide range in requirements among species suggests that the niacin requirement may vary according to dietary composition, like the lipid content and lipid metabolism. In contrast to land-living animals,

tryptophan have been shown to be inefficient as a niacin precursor in channel catfish (Ng et al. 1997). A major part of the niacin in cereals and oil seed (Table 5.6) is poorly utilized, probably due to bindings to indigestible components in the plant or seed (Blum 1991). According to these considerations, the requirement for energy rich salmonid diets should target a conservative level of 100 mg niacin kg^{-1} feed (Table 5.5).

5.3.7. Folate and vitamin B_{12}

Vitamin B_{12} and folate are involved as coenzymes in a series of biochemical reactions in the intermediary metabolism, mainly as donors of one carbon units, and are essential for growth and health [(NRC 1993; Halver 2002); Table 5.4]. Folate deficiency symptoms such as impaired haematopoiesis, reduced growth, survival and resistance to infectious diseases have been observed in several fish species (NRC 1993; Waagbø et al. 2001a; Halver 2002; Hardy 2002), including coho salmon, *Oncorhynchus kisutch* (Smith 1968), rainbow trout (Cowey & Woodward 1993), channel catfish (Duncan *et al.* 1993) and Atlantic salmon (Waagbø et al. 2001a). Haematological effects of folate deficiency in teleosts include macrocytic anaemia with abnormal cell nucleus segmentation of the blood cells (Smith 1968; Waagbø et al. 2001a). The quantitative requirement of folate has been established in channel catfish (Duncan et al. 1993; Robinson and Li 2002) and rainbow trout (Cowey and Woodward 1993). In the latter study, the authors suggested that the dietary requirement of folate in rainbow trout was between 0.6-1.1 mg kg^{-1}, depending on response criteria used like reduced concentration of folate in liver, kidney and whole blood (Cowey and Woodward 1993).

For vitamin B_{12}, qualitative requirement studies exist for Japanese eel, *Anguilla japonica* and red sea bream, *Pagrus major* (Koshio 2002), yellowtail, *Seriola quinqueradiata* (Hosokawa 1999), cited by (Masumoto 2002), and European sea bass, *Dicentrarchus labrax* (Kaushik et al. 1998). Difficulties in determining quantitative requirements have been related to intestinal microbial vitamin synthesis (Sugita et al. 1990). Despite differences in chemical structure and biochemical actions of vitamin B_{12} and folate, a close functional relationship exists between the two vitamins, especially in cell division. Requirements and interactions between vitamin B_{12} and folate, and bioavailability of these vitamins from practical diets were studied in two experiments with Atlantic salmon (Sandnes and Mæland 1994). Feeding a purified diet without supplementation of folic acid showed growth reduction, reduced levels of folate in liver and muscle tissue, and anemia characterized by reduced blood hemoglobin concentration, enlarged immature erythrocytes with fragmented nuclei and reduced erythrocyte hemoglobin content (MCH). Vitamin B_{12} deficient fish also showed anemia with

immature erythrocytes. Most severely, anemia was observed in fish fed a diet without both vitamin B_{12} and folate supplementation. Dietary supplementation of 0.014 mg vitamin B_{12} kg^{-1} and 3.6 mg folate kg^{-1} in purified diets prevented vitamin deficiency signs. By feeding a practical fish meal based diet without supplementation of both vitamins, reduced organ levels of folic acid, but not vitamin B_{12} were demonstrated. All organs responded rapidly to a folate supplementation according to NRC recommendation (10 mg folate kg^{-1}), while only plasma vitamin B_{12} reflected extra supplementation of vitamin B_{12} (0.2 mg kg^{-1} vitamin B_{12}). Fish meals contain marginal levels of folate, 0.3-1.0 mg kg^{-1}, whereas the concentration of vitamin B_{12} in fish meal from Norway has been found to be 0.2-0.4 mg kg^{-1} [(Waagbø et al. 2001a) reported from (Sandnes and Mæland 1994)] which is ten times higher than the recommendation of NRC (0.02 mg kg^{-1} diet). These data suggest that it is necessary to supplement folic acid in practical salmon diets based on marine ingredients, whereas the natural content of vitamin B_{12} is high enough to cover its dietary requirement. Vitamin B_{12} occurs in animal tissue, while not in plant ingredients (Table 5.6). Folates are ubiquitous in nature and are present in nearly all feed items in varying amounts. However, both folate and vitamin B_{12} are among the most sensitive vitamins in fish feed and considerable losses occur during ingredient processing, fish feed production (50-65 %) and storage (Gabaudan and Hardy 2000). Again conservative target levels are suggested; 0.03 mg kg^{-1} for vitamin B_{12} and 10 mg kg^{-1} for folate (Table 5.5).

5.4. Vitamin-like compounds

5.4.1. Inositol (myo-inositol)

Myo-inositol is a structural component in living tissues, as well as an important participant in transmembrane signal transfer in the phospholipid (PL) form, phosphatidyl inositol (Aukema and Holub 1994). Inositol is classified as a vitamin-like nutrient and is often supplemented to fish diets. Fish and other vertebrates, or their intestinal microbial flora, may synthesize inositol (Burtle and Lovell 1989; Aukema and Holub 1994). Pathological conditions including fatty liver and muscle degeneration have been reported in some fish species due to inositol deficiency, as well as more general signs of poor appetite, anemia, growth reduction, fin erosion and skin lesions (NRC 1993). In carp (*Cyprinus carpio* L.), deficiency symptoms occurred after three to five weeks on an inositol deficient diet, including hemorrhages, skin and fin lesions and reduced growth and protein and energy retention (Meyer-Burgdorff et al. 1986). According to NRC (1993) the general requirements of inositol for fish seem to be in the range of 250 to 500 mg kg^{-1}. In fatty fish species, lipid metabolism

may be affected by inositol deficient diets, as compared to lean species (Burtle and Lovell 1989; Waagbø et al. 1998). The latter authors showed that inositol concentrations in whole fish and liver were affected by dietary inositol inclusion level. The natural content in marine feed ingredients may fully meet the requirement reported by NRC (1993) for several fish species. A study on Atlantic salmon fry indicated that the requirement for inositol is covered through the natural content of inositol in practical feed ingredients at around 300 mg inositol kg^{-1} (Waagbø et al. 1998). The authors advised to supplement starter diets with moderate amounts of inositol, around 200 mg kg^{-1}, to compensate for fluctuations in inositol concentrations in natural ingredients, leaching loss of inositol from the diet and for any potential increased inositol requirement in salmon fry. By using plant ingredients, inositol may be supplied in the phytate form (phosphorylated inositol), considered an anti-nutrient (Francis et al. 2001). Enzymatic pre-treatment of plant feed ingredients or inclusion of phytase in plant based diets may reduce the negative impact of phytate and supply inositol.

5.4.2. Choline

Like inositol, choline is involved in the synthesis of phospholipids (PL), in form of the major membrane and transport PL, phosphatidyl choline. Besides roles in structural lipids, choline is also a component in synthesis of the neurotransmitter acetylcholine. It participates in one carbon transfer reactions, among others the methylation of homocysteine to methionine and the synthesis of betaine in the mitochondria. Marine ingredients normally cover the "requirement" for choline (Table 5.6), suggested to be between 2 and 4 g kg^{-1}. Soy products also contain considerable amount of choline in form of lecithin (Hertrampf and Piedad-Pascual 2000). Since choline and PL also participate in digestion and metabolism of lipids, symptoms of deficiency have been related to malfunctions in lipid metabolism and organ hemorrhages (Halver 2002). Similarly, the required amount of choline depends on the dietary lipid content, age of the fish, and the rearing water temperature (Hertrampf and Piedad-Pascual 2000). In marine larvae feeding, focus has been placed on PL more than choline since problems seem to be related to the role of PL in intestinal micelle formation, lipid absorption and subsequently lipoprotein transport of lipids (Hamre et al. 2007). From its nature as a strong basic compound, choline may chemically react with other vitamins in feed mixtures (vitamins E and K). Betaine is also used as an additive in fish feed to improve osmoregulatory capacity, and betaine may replace choline since it is more chemically stable.

5.5. Summary

Vitamin requirements and recommendations must be seen in relation to fish species, stages of life cycle, overall feed composition, and ambient farming conditions. The single vitamins fulfill known and unknown roles in the integrated metabolism, like the oil in the body machinery. Clearly, care should be taken to be sure that the micronutrients are not limiting productivity and hazarding welfare in farmed fish. The present report suggests target levels of vitamins rather than supplementations, to minimize the risk for deficiencies from qualitative and quantitative changes in feed composition and from vitamin product purities. Still, there are uncertainties relative to vitamin bioavailability arising from inherent and supplemented vitamins and possible contents of antinutrients in novel ingredients. This means an increased focus and awareness on these aspects rather than passively adding a "safe" vitamin recommendation mixture.

5.6. References

Al-Khalifa, A.S., Simpson, K.L., 1988. Metabolism of astaxanthin in the rainbow trout (*Salmo gairdneri*). Comparative Biochemistry and Physiology 91B, 563-568.

Albrektsen, S., Lie, Ø., Sandnes, K., 1988. Ascorbyl palmitate as a dietary vitamin C source for rainbow trout (*Salmo gairdneri*). Aquaculture 71, 359-368.

Albrektsen, S., Waagbø, R., Sandnes, K., 1993. Tissue vitamin B_6 concentrations and aspartate aminotransferase (AspT) activity in Atlantic salmon (*Salmo salar*) fed graded dietary levels of vitamin B_6. Fiskeridirektoratets Skrifter, Serie Ernæring 6, 21-34.

Albrektsen, S., 1994. Studies on vitamin B6 nutrition in Atlantic salmon (*Salmo salar*), Department of Fisheries and Marine Biology. University of Bergen, Bergen, pp. 35 (+ 35 publications).

Amcoff, P., Akerman, G., Tjarnlund, U., Borjeson, H., Norrgren, L., Balk, L., 2002. Physiological, biochemical and morphological studies of Baltic salmon yolk-sac fry with an experimental thiamine deficiency: relations to the M74 syndrome. Aquatic Toxicology 61, 15-33.

Amezaga, M.R., Knox, D., 1990. Riboflavin requirements in on-growing rainbow trout, *Oncorhynchus mykiss*. Aquaculture 88, 87-98.

Aukema, H.M., Holub, B.J., 1994. Inositol and pyrroloquinoline quinone. In: Shils, M.E., Olson, J.A., Shike, M. (Eds.), Modern nutrition in health and disease. Lea & Febiger, Malvern, PA, pp. 466-472.

Barnett, B.J., Cho, C.Y., Slinger, S.J., 1982. Relative biopotency of dietary ergocalciferol and cholecalciferol and the role of and requirement for vitamin D in rainbow trout (*Salmo gairdneri*). J. Nutr. 112, 2011-2019.

Bjerkås, E., Waagbø, R., Sveier, H., Breck, O., Bjerkås, I., Bjørnestad, E., Maage, A., 1996. Cataract development in Atlantic salmon (*Salmo salar* L) in fresh water. Acta vet. scand. 37, 351-360.

Bjerkås, E., Breck, O., Waagbø, R., 2006. The role of nutrition in cataract formation in farmed fish, CAB Reviews. Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources 1, 1-16.

Blum, R.A., 1991. 6. Niacin in Feedstuffs. In: Fenster, R., Blum, R.A. (Eds.), Niacin in Animal Nutrition. F. Hoffmann-La Roche Ltd, Basel, Switzerland, pp. 38-39.

Brækkan, O.R., 1959. Comparative studies of vitamins in fishes. John Griegs Boktrykkeri Bergen, Norway, 7 publications pp.

Brønstad, I., Bjerkås, I., Waagbø, R., 2002. The need for riboflavin supplementation in high and low energy diets for Atlantic salmon *Salmo salar* L. parr. Aquaculture Nutrition 8, 209-220.

Burtle, G.J., Lovell, R.T., 1989. Lack of response of channel catfish (*Ictalurus punctatus*) to dietary myo-inositol. Canadian Journal of Fisheries and Aquatic Sciences 46, 218-222.

Bylund, G., Lerche, O., 1995. Thiamine therapy of M 74 affected fry of Atlantic salmon *Salmo salar*. Bull. Eur. Ass. Fish Pathol. 15, 93-97.

Cahu, C., Infante, J.Z., Takeuchi, T., 2003. Nutritional components affecting skeletal development in fish larvae. Aquaculture 227, 245-258.

Casteldine, A.J., Cho, C.Y., Slinger, S.J., Hicks, B., Bayley, H.S., 1978. Influence of dietary biotin level on growth, metabolism and pathology of rainbow trout. Journal of Nutrition 108, 698-711.
Christiansen, R., Lie, Ø., Torrisen, O.J., 1994. Effect of astaxanthin and vitamin A on growth and survival during first feeding of Atlantic salmon, *Salmo salar* L. Aquaculture and Fisheries Management 25, 903-914.
Chuang, J.L., 1991. 5.7 Fish and shrimp. In: Fenster, R., Blum, R.A. (Eds.), Niacin in Animal Nutrition. F. Hoffmann-La Roche Ltd, Basel, Switzerland, pp. 34-37.
Cowey, C.B., Adron, J.W., Walton, M.J., Murray, J., Youngson, A., Knox, D., 1981. Tissue distribution, uptake and requirement for α-tocopherol of rainbow trout (*Salmo gairdneri*) fed diets with a minimal content of unsaturated fatty acids. J. Nutr. 111.
Cowey, C.B., Degener, E., Tacon, A.G.J., Youngson, A., Bell, J.G., 1984. The effect of vitamin E and oxidized fish oil on the nutrition of rainbow trout (*Salmo gairdneri*) grown on natural varying water temperatures. Br. J. Nutr. 51.
Cowey, C.B., Woodward, B., 1993. The dietary requirement of young rainbow trout (*Oncorhynchus mykiss*) for folic acid. Journal of Nutrition 123, 1594-1600.
Dabrowski, K., 2001. Ascorbic Acid in Aquatic Organisms - Status and perspectives. CRC Press, Boca Raton, 288 pp.
Dakshinamurti, K., Cauhan, J., 1989. Biotin. Vitamines and Hormones???, 337-384.
DTU, N.F.I., 2007. Danish Food Composition Databank. DTU, National Food Institute, Department of Nutrition, Søborg, Denmark
Duncan, P.L., Lovell, R.T., Butterworth, C.E., Freeberg, L.E., Tamura, T., 1993. Dietary folate requirement determined for channel catfish, *Ictalurus punctatus*. Journal of Nutrition 123, 1888-1897.
Fisher, J.P., Spitsbergen, J.M., Iamonte, T., Little, E., DeLonay, A., 1995. Pathological and behavioral manifestations of the 'Cayuga syndrome' a thiamine deficiency in larval landlocked Atlantic salmon. Journal of Aquatic Animal Health 7, 269-282.
Fitzsimons, J.D., Williston, B., Amcoff, P., Balk, L., Pecor, C., Ketola, H.G., Hinterkopf, J.P., Honeyfield, D.C., 2005. The effect of thiamine injection on upstream migration, survival, and thiamine status of putative thiamine-deficient Coho salmon. Journal of Aquatic Animal Health 17, 48-58.
Francis, G., Makkar, H.P.S., Becker, K., 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture 199, 197-227.
Frigg, M., 1976. Bio-availability of biotin in cereals. Poultry Science 55, 2310-2318.
Frigg, M., 1984. Available biotin content of various feed ingredients. Poultry Science 63, 750-753.
Frigg, M., Prabucki, A.L., Ruhdel, E.U., 1990. Effect of dietary vitamin E levels on oxidative stability of trout fillets. Aquaculture 84, 145-158.
Gabaudan, J., Hardy, R.W., 2000. Vitamin sources for fish feed. In: Stickney, R.R. (Ed.), Encyclopedia of Aquaculture. John Wiley & Sons, New York, USA, pp. 961-964.
Gadient, M., Fenster, R., 1994. Stability of ascorbic acid and other vitamins in extruded fish feeds. Aquaculture 124, 207-211.
Graff, I., Waagbø, R., Fivelstad, S., Vermeer, C., Lie, Ø., Lundebye, A., 2002a. A multivariate study on the effects of dietary vitamin K, vitamin D3 and calcium, and dissolved carbon dioxide on growth, bone minerals, vitamin status and health performance in smolting Atlantic salmon Salmo salar L. Journal of Fish Diseases 25, 599-614.
Graff, I.E., 2002. Vitamin D3 in Atlantic salmon (*Salmo salar* L.): metabolism, toxicity and function in bone mineralisation, Department of Fisheries and Marine Biology. University of Bergen, Bergen, Norway, pp. 52 (& 54 publications).
Graff, I.E., Hoie, S., Totland, G.K., Lie, O., 2002b. Three different levels of dietary vitamin D-3 fed to first-feeding fry of Atlantic salmon (*Salmo salar* L.): effect on growth, mortality, calcium content and bone formation. Aquaculture Nutrition 8, 103-111.
Grahl-Madsen, E., Lie, Ø., 1997. Effects of different levels of vitamin K in diets for cod (*Gadus morhua*). Aquaculture 151, 269-274.
Grisdale-Helland, B., Helland, S., Åsgård, T., 1991. Problems associated with the present use of menadione sodium bisulfite and vitamin A in diets for Atlantic salmon. Aquaculture 92, 351-358.
Halver, J.E., 1989. The vitamins. In: Halver, J.E. (Ed.), Fish Nutrition. Academic Press, New York, pp. 31-109.
Halver, J.E., 2002. The Vitamins. In: Halver, J.E., Hardy, R.W. (Eds.), Fish Nutrition. Academic Press, London, pp. 61-141.
Halver, J.E., Hardy, R.W., 2002. Fish Nutrition. Academic Press, London, 824 pp.
Hamre, K., Berge, R.K., Lie, 1993. Turnover of alpha-, gamma- and delta-tocopherol and distribution in subcellular and lipoprotein fractions indicate presence of an hepatic tocopherol binding protein in Atlantic salmon (Salmo salar, L.). Fish Physiol. Biochem. Submitted.

Hamre, K., Hjeltnes, B., Kryvi, H., Sandberg, S., Lorentzen, M., Lie, Ø., 1994. Decreased concentration of hemoglobin, accumulation of lipid oxidation products and unchanged skeletal muscle in Atlantic salmon (*Salmo salar*) fed low dietary vitamin E. Fish Physiol. Biochem. 12, 421-429.

Hamre, K., 1995. Metabolism, interactions and requirement of vitamin E in Atlantic salmon (*Salmo salar*, L.), Department of Fisheries and Marine Biology. University of Bergen, Bergen, Norway, pp. 57 (incl. 54 publications).

Hamre, K., Lie, Ø., 1995. Minimum requirement of vitamin E for Atlantic salmon *Salmo salar* L., at first feeding. Aquaculture Research 26, 175-184.

Hamre, K., Waagbø, R., Berge, R.K., Lie, Ø., 1997. Vitamins C and E interact in juvenile Atlantic salmon (*Salmo salar*, L.). Free Rad. Biol. Med. 22, 137-149.

Hamre, K., Berge, R., Lie, Ø., 1998. Oxidative stability of Atlantic salmon (*Salmo salar*, L.) fillet enriched with a-, g-, and d-tocopherol through dietary supplementation. Food Chemistry 62, 173-178.

Hamre, K., Rønnestad, I., Rainuzzo, J.R., Barr, Y., Harboe, T., 2007. Nutritional Aspects – Marine Fish Larvae. In: Thomassen, M., Gudding, R., Norberg, B., Jørgensen, L. (Eds.), Aquaculture Research: From Cage to Consumption. The Research Council of Norway, Oslo, Norway, pp. 217-234.

Hardy, R.W., 2001. Nutritional deficiencies in commercial aquaculture: Likelihood, onset, and identification. In: Lim, C., Webster, C.D. (Eds.), Nutrition and Fish Health. Food Products Press, Birminghamton, NY, pp. 131-147.

Hardy, R.W., 2002. Rainbow trout, *Oncorhynchus mykiss*. In: Webster, C.D., Lim, C. (Eds.), Nutrient requirements and feeding of finfish for aquaculture. CABI Publishing, Oxon, pp. 184-202.

Hertrampf, J.W., Piedad-Pascual, F., 2000. Handbook on Ingredients for Aquaculture Feeds. Kluwer Academic Publishers, Dordrecht, The Netherlands, 573 pp.

Hilton, J.W., 1983. Hypervitaminosis A in rainbow trout (*Salmo gairdneri*): Toxicity signs and maximum tolerable level. Journal of Nutrition 113, 1737-1745.

Horvli, O., Lie, Ø., 1994a. Determination of vitamin D_3 in fish meals by HPLC. Fiskeridirektoratets Skrifter, Serie Ernæring 6, 163-175.

Horvli, O., Lie, Ø., 1994b. Vitamin D -betydning for folk og laks. Norsk Fiskeoppdrett 2, 26-27.

Hosokawa, H., 1999. Studies on the vitamin nutrition in yellowtail, United Graduate School of Agriculture Sciences. Ehime University, Ehime, Japan.

Hughes, S.G., Riis, R.C., Nickum, J.G., Rumsey, G.L., 1981. Biomicroscopic and histologic pathology of the eye in riboflavin deficient rainbow trout (*Salmo gairdneri*). Cornell Veterinarian 71, 269-279.

Julshamn, K., Lunestad, B.T., Haldorsen, A.K., Berntssen, M.H.G., Waagbø, R., 2003. Overvåkningsprogram for fiskefôr - årsrapport 2002, NIFES Report. NIFES, Bergen, Norway, pp. 28.

Kaushik, S.J., Gouillou-Coustans, M.F., Cho, C.Y., 1998. Application of the recommendations on vitamin requirements of finfish by NRC (1993) to salmonids and sea bass using practical and purified diets. Aquaculture 161, 463-474.

Kitamura, S., Suwa, T., Ohara, S., Nakagawa, K., 1967. Studies on vitamin requirements of rainbow trout. III. Requirements for vitamin A and deficiency symptoms. Bulletin of the Japanese Society of Scientific Fisheries 33, 1126-1131.

Koppe, W., 1993. Auswirkungen einer unterschiedlichen biotinversorgung auf wachstum, biotinstatus sowie parameter der immun- und stressrespons bei regenbogenforellen (*Oncorhynchus mykiss*), Institut für Tierphysiologie und Tierernährung. Georg-August-Universität zu Göttingen, Göttingen, pp. 123.

Koshio, S., 2002. Red Sea Bream, *Pagrus major*. In: Webster, C.D., Lim, C.E. (Eds.), Nutrient Requirements and Feeding of Finfish for Aquaculture, Wallingford, Oxon, UK, pp. 51-63.

Krossoy, C., 2007. Vitamin K in feed to Atlantic salmon (*Salmo salar* L.) parr. In prep.

LD/FD, 2002. Forskrift om fôrvarer av 7. november 2002. In: fiskeridepartement, D.k.l.D.k. (Ed.). Informasjonsforvatning (in Norwegian), pp. 256.

Li, M.H., Robinson, E.H., 2001. Dietary ascorbic acid requirement for growth and health in fish. In: Lim, C., Webster, C.D. (Eds.), Nutrition and Fish Health. Food Products Press, NY, pp. 163-187.

Lim, C., Leamaster, B., Brock, J.A., 1993. Riboflavin requirement of fingerling red hybrid tilapia grown in seawater. In: Kaushik, S.J., Luquet, P. (Eds.), Fish nutrition and practice. Proceedings of the IV International Symposium on Fish Nutrition and Feeding 1991. INRA Editions, Paris, pp. 743-752.

Lim, C., Shoemaker, C.A., Klesius, P.H., 2001. The effect of ascorbic acid on the immune response in fish. In: Dabrowski, K. (Ed.), Ascorbic Acid in Aquatic Organisms - Status and perspectives. CRC Press, Boca Raton, Florida, pp. 149-166.

Lovell, R.T., Buston, J.C., 1984. Biotin supplementation of practical diets for channel catfish. Journal of Nutrition 114, 1092-1096.

Lygren, B., 1999. Dietary pro- and antioxidants: effects on immune functions, disease resistance and antioxidant status in Atlantic salmon, *Salmo salar* L., Department of Fisheries and Marine Biology. University of Bergen, Norway, Bergen, pp. 57 (& 55 publications).

Lygren, B., Hamre, K., Waagbø, R., 2000. Effect of induced hyperoxia on the antioxidant status of Atlantic salmon *Salmo salar* L. fed three different levels of vitamin E. Aquaculture Research 31, 401-407.
Marchetti, M., Tassinari, M., Bauce, G., 1995. Tolerance of high dietary levels of menadione bisulfite-nicotinamide by rainbow trout, *Onchorynchus mykiss*. Aquaculture 134, 137-142.
Marchetti, M., Tossani, N., Marchetti, S., Bauce, G., 1999. Stability of crystalline and coated vitamins during manufacture and storage of fish feeds. Aquaculture Nutrition 5, 115-120.
Marks, J., 1989. Vitamin Safety. Vitamin Information Roche, Basle, pp. 28.
Masumoto, T., 2002. Yellowtail, *Seriola quinqueradiata*. In: Webster, C.D., Lim, C. (Eds.), Nutrient requirements and feeding of finfish for aquaculture. CABI Publishing, Oxon, pp. 131-146.
Meier, W., Wahli, T., 1990. Vitamin C deficiency in the rainbow trout, Roche Animal Nutrition Events (ANE). F. Hoffmann-La Roche Ltd, Basel, Switzerland, pp. 1-18.
Meyer-Burgdorff, K.H., Becker, K., Guenther, K.D., 1986. Inositol: Deficiency symptoms and requirements of growing mirror carp (*Cyprinus carpio* L.). Journal of Animal Physiology and Animal Nutrition 56, 232-241.
Midtlyng, P.J., Ahrend, M., Bjerkås, E., Waagbø, R., Wall, T., 1999. Current research on cataracts in fish. Bull. Eur. Ass. Fish Pathol. 19, 299-301.
Mock, D.M., 1999. Biotin. In: Shils, M.E., Olson, J.A., Shike, M., Ross, A.C. (Eds.), Modern Nutrition in Health and Disease. Williams & Wilkins, Baltimore, Maryland, US, pp. 459-466.
Morito, C.L.H., Conrad, D.H., Hilton, J.W., 1986. The thiamin deficiency signs and requirement of rainbow trout (*Salmo gairdneri*, Richardson). Fish Physiol Biochem 1, 93-104.
Morris, P.C., Davies, S.J., 1995a. The requirement of the gilthead sea bream (*Sparus aurata* L.) for nicotinic acid. Animal Science 61, 437-443.
Morris, P.C., Davies, S.J., 1995b. Thiamin suplementation of diets containing varied lipid: carbohydrate ration given to gilthead seabream (*Sparus aurata* L.). Animal Science 61, 597-603.
Mæland, A., Sandnes, K., Hjeltnes, B., Waagbø, R., 1998. Biotin in practical fish-meal based diet for Atlantic salmon, *Salmo salar* L. fry. Aquaculture Nutrition 4, 241-247.
Mæland, A., Waagbø, R., 1998. Examination of the qualitative ability of some cold water marine teleosts to synthesise ascorbic acid. Comparative Biochemistry and Physiology 121A, 249-255.
Mæland, A., Sandnes, K., 1999. Determination of biotin in low-temperature fish-meal processed from different species by means of a microbiological method using *Lactobacillus plantarum* as test organism. Journal of the Science of Food and Agriculture 79, 1298-1300.
Ng, W.-K., Serrini, G., Zhang, Z., Wilson, R.P., 1997. Niacin requirement and inability of tryptophan to act as a precursor of NAD^+ in channel catfish, *Ictalurus punctatus*. Aquaculture 152, 273-285.
Norrgren, L., Börjeson, H., Förlin, L., Åkerblom, N., 2001. The role of ascorbic acid and its derivatives in resistance to environmental and dietary toxicity of aquatic organisms. In: Dabrowski, K. (Ed.), Ascorbic Acid in Aquatic Organisms - Status and perspectives. CRC Press, Boca Raton, pp. 133-147.
NRC, 1993. Nutrient Requirements of Fish. In: Committee on animal nutrition, B.o.a. (Ed.), National Research Council. National Academy Press, Washington DC, USA, pp. 114.
Opstvedt, J., Knudsen, G., Asbjørnsen, B., 1997. Innhold av fettløselige vitaminer i fiskemel og fiskeolje produsert fra sild, lodde, tobis, kolmule og brisling (In Norwegian). Sildeolje- og sildemelindustriens forskningsinstitutt C-295, 10.
Phillips, A.M., Brockway, D.R., Kolb, A.J.J., Maxwell, J.M., 1950. Further studies upon biotin and trout diets. Fisheries Research Bulletin 19, 9-12.
Poston, H.A., 1976. Optimum level of dietary biotin for growth, feed utilization, and swimming stamina of fingerling lake trout (*Salvelinus namaycush*). J. Fish. Res. Bd. Canada 33, 1803-1806.
Poston, H.A., Combs, G.F., Leibovitz, L., 1976. Vitamin E and Selenium interrelations in the diet of Atlantic salmon (*Salmo salar*): Gross, histological and biochemical deficiency signs. J. Nutr. 106.
Poston, H.A., Wolfe, M.J., 1985. Niacin requirement for optimum growth, feed conversion and protection of rainbow trout, Salmo gairdneri Richardson, from ultraviolet-B irradiation. Journal of Fish Diseases 8, 451-460.
Primbs, E.R.J., Sinnhuber, R.O., Warren, C.E., 1971. Hypervitaminosis A in the rainbow trout: Counteraction by vitamin C. International Journal of Vitamin and Nutrition Research 41, 331-338.
Robinson, E.H., Li, M.H., 2002. Channel Catfish, *Ictalurus punctatus*. In: Webster, C.D., Lim, C.E. (Eds.), Nutrient Requirements and Feeding of Finfish for Aquaculture. CABI Publishing, Wallingford, Oxon, UK, pp. 293-318.
Roem, A.J., Stickney, R.R., Kohler, C.C., 1991. Dietary Pantothenic-Acid Requirement of the Blue Tilapia. Progressive Fish-Culturist 53, 216-219.
Salte, R., Åsgård, T., Liestøl, K., 1988. Vitamin E and selenium prophylaxis against 'Hitra Disease' in farmed Atlantic salmon - a survival study. Aquaculture 75, 45-55.

Salte, R., 1993. Betinget vitamin B1-mangel og smoltdød kort tid etter sjøutsett (in Norwegian). AKVAFORSK, Ås, Norway, pp. 5.

Sandnes, K., 1991. Vitamin C in fish nutrition - a review. Fiskeridirektoratets Skrifter, Serie ernæring 4, 3-32.

Sandnes, K., Waagbø, R., 1991b. Enzymatic hydrolysis of ascorbate-2-monophosphate and ascorbate-2-sulphate in vitro, and bioactivity of ascorbate-2- monophosphate in Atlantic salmon (*Salmo salar*). Fiskeridirektoratets Skrifter, Serie ernæring IV, 33-39.

Sandnes, K., Torrissen, O.J., Waagbø, R., 1992. The minimum dietary requirement of vitamin C in Atlantic salmon (*Salmo salar*) fry using Ca ascorbate-2-monophosphate as dietary source. Fish Physiology and Biochemistry 10, 315-319.

Sandnes, K., Mæland, A., 1994. Vannløselige vitaminer - Folinsyre og vitamin B12 i fôr til laks. Norwegian Research Council Research Report, Institute of Nutrition, Directorate of Fisheries, Bergen, Norway, pp. 5.

Serrini, G., Zhang, Z., Wilson, R.P., 1996. Dietary riboflavin requirement of fingerling channel catfish (*Ictalurus punctatus*). Aquaculture 139, 285-290.

Sigurgisladottir, S., Parrish, C.C., Ackman, R.G., Lall, S.P., 1994a. Tocopherol deposition in the muscle of Atlantic salmon (Salmo salar). J. Food Sci. 59, 256-259.

Sigurgisladottir, S., Parrish, C.C., Lall, S.P., Ackman, R.G., 1994b. Effects of feeding natural tocopherols and astaxanthin on Atlantic salmon (Salmo salar) fillet quality. Food Research International 27, 23-32.

Smith, C.E., 1968. Haematological changes in coho salmon fed a folic acid deficient diet. J. Fish. Res. Bd. Canada 25, 151-156.

Smith, C.M., Song, W.O., 1996. Comparative nutrition of pantothenic acid. The Journal of Nutritional Biochemistry 7, 312-321.

Soliman, A.K., Wilson, R.P., 1992. Water-soluble vitamin requirements of tilapia. 2. Riboflavin requirements of blue tilapia, *Oreochromis aureus*. Aquaculture 104, 309-314.

Storebakken, T., 2002. Atlantic salmon, *Salmo salar*. In: Webster, C.D., Lim, C.E. (Eds.), Nutrient Requirements and Feeding of Finfish for Aquaculture. CABI Publishing, Oxon, pp. 79-112.

Sugita, H., Miyajiama, C., Deguchi, Y., 1990. The vitamin B_{12}-producing ability of intestinal bacteria isolated from tilapia and channel catfish. Nippon Suisan Gakkaishi 56, 701.

Takeuchi, L., Takeuchi, T., Ogino, C., 1980. Riboflavin requirements in carp and rainbow trout. Bulletin of the Japanese Society of Scientific Fisheries 46, 733-737.

Tanphaichitr, V., 1999. Thiamin. In: Shils, M., Olson, J., Shike, M., Ross, A. (Eds.), Modern Nutrition in Health and Disease. Lippincott Williams & Wilkins, Maryland, USA, pp. 381-389.

Taveekijakarn, P., Miyazaki, T., Matsumoto, M., Arai, S., 1996a. Histopathological and haematological changes in amago salmon, *Oncorhynchus rhodurus* (Jordan & McGregor), fed a vitamin -D-free diet. Journal of Fish Diseases 19, 289-294.

Taveekijakarn, P., Miyazaki, T., Matsumoto, M., Arai, S., 1996b. Studies on vitamin K deficiency in amago salmon, *Oncorhyncus rhodurus* (Jordan & McGregor). Journal of Fish Diseases 19, 209-214.

Udagawa, M., 2000. Physiological Role of Vitamin K in Fish - Review Japan Agricultural Research Quaterly 34, 279-284.

Vielma, J., Lall, S.P., Koskela, J., Schöner, F.J., Mattila, P., 1998. Effects of dietary phytase and cholecalciferol on phosphorus bioavailability in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 163, 309-323.

Watanabe, T., Takeuchi, T., Wada, M., 1981. Dietary lipid levels and the α-Tocopherol requirement of carp. Bull. Jap. Soc. Scie. Fish. 47, 1585-1590.

Woodward, B., 1985. Riboflavin requirement for growth, tissue saturation and maximal flavin-dependent enzyme activity in young rainbow trout (*Salmo gairdneri*) at two temperatures. . J. Nutr. 115, 78-84.

Woodward, B., Frigg, M., 1989. Dietary biotin requirements of young rainbow trout (*Salmo gairdneri*) determined by weight gain, hepatic biotin concentration and maximal biotin-dependent enzyme activities in liver and white muscle. Journal of Nutrition 119, 54-60.

Woodward, B., 1994. Dietary vitamin requirements of cultured young fish, with emphasis on quantitative estimates for salmonids. Aquaculture 124, 133-168.

Waagbo, R., Sandnes, K., Torrissen, O.J., Sandvin, A., Lie, O., 1993. Chemical and sensory evaluation of fillets from Atlantic salmon (*Salmo salar*) fed three levels of N-3 polyunsaturated fatty acids at two levels of vitamin E. Food Chemistry 46, 361-366.

Waagbø, R., Øines, S., Sandnes, K., 1991. The stability and biological availability of different forms of vitamin C in feed for Atlantic salmon (*Salmo salar*). Fiskeridirektoratets Skrifter, Serie ernæring IV, 95-101.

Waagbø, R., Glette, J., Raa-Nilsen, E., Sandnes, K., 1993a. Dietary vitamin C, immunity and disease resitance in Atlantic salmon (*Salmo salar*). Fish Physiology and Biochemistry 12, 61-73.

Waagbø, R., Sandnes, K., Jørgensen, J., Engstad, R., Glette, J., Lie, Ø., 1993b. Health sapect of dietry lipid sources and vitamin E in Atlantic salmon (*Salmo salar*). II. Spleen and erythrocyte phospholipid fatty acid composition, nonspecific immunity and disease reistance. Fiskeridirektoratets Skrifter, Serie Ernæring 6, 63-80.

Waagbø, R., Sandnes, K., Mæland, A., 1994. Biotinbehov hos smolt. Norsk Fiskeoppdrett 2A, 24-25.
Waagbø, R., Bjerkås, E., Sveier, H., Breck, O., Bjørnestad, E., Maage, A., 1996. Nutritional status assessed in groups of smolting Atlantic salmon, *Salmo salar* L., developing cataracts. Journal of Fish Diseases 19, 365-373.
Waagbø, R., Sandnes, K., Lie, Ø., Roem, A., 1998. Effects of inositol supplementation on growth, chemical composition and blood chemistry in Atlantic salmon, *Salmo salar* L., fry. Aquaculture Nutrition 4, 53-59.
Waagbø, R., Graff, I.E., Hamre, K., 2001a. Vitaminer. In: Waagbø, R., Espe, M., Hamre, K., Lie, Ø. (Eds.), Fiskeernæring. Kystnæringen, Bergen, pp. 93-124.
Waagbø, R., Hamre, K., Maage, A., 2001b. Review: The impact of micronutrients on the requirement of ascorbic acid in crustaceans and fish. In Ascorbic Acid in Aquatic Organisms - Status and perspectives. In: Dabrowski, K. (Ed.), Boca Raton. CRC Press, pp. 105-131.
Waagbø, R., 2006. Feeding and disease resistance in fish. In: Mosenthin, J., Zentek, R., Zebrowska, T. (Eds.), Biology of growing animal. Elsevier Limited, pp. 387-415.
Ørnsrud, R., Graff, I.E., Høie, S., Totland, G., Hemre, G.-I., 2002. Hypervitaminosis A in first-feeding fry of the Atlantic salmon (*Salmo salar* L.). Aquaculture Nutrition 8, 7-13.
Ørnsrud, R., 2003. Retinoids in salmonid aquaculture with special emphasis on developmental deformities, Department of Fisheries and Marine Biology. University of Bergen, Bergen, Norway, pp. 58 (& 54 publications).

6. Dietary mineral requirements and skeletal deformities

Grete Baeverfjord

6.1. Introduction

The role of minerals in skeletal pathology was recently reviewed by Lall and Lewis-McCrea (2007), and where the impact of dietary calcium (Ca) and phosphorus (P) was addressed. In the present summary, current knowledge on mineral requirements in compound diets will be discussed in view of the relation between mineral deficiencies and skeletal development. In addition to Ca and P, magnesium (Mg) and zinc (Zn) are included. These two elements have shown variable and low values in whole-body analyses done on farmed Atlantic salmon within the last years, and have previously been associated with skeletal malformations.

6.2. Minerals of known relevance to skeletal development

Calcium and phosphorus are closely related to the development and maintenance of the skeletal system, and the stability of the vertebrae and other skeletal structures is maintained by solid phases of calcium phosphates, hydroxyapatites (Lall and Lewis-McCrea, 2007). Fish and other aquatic organisms absorb Ca and P from water, and their Ca requirement is met partly by their ability to absorb Ca directly from water. The concentration of P is low in both freshwater and seawater and diet is the main source of P (Lall, 2002). Also, according to Lall (2002), the Ca:P ratio in the diet is less important in fish than in terrestrial animals. Of particular relevance is the fact that without available P, Ca cannot be deposited. P deficiency will lead to an accompanying decrease in whole-body Ca levels (Baeverfjord et al., 1998; Baeverfjord, 2005). In fish with proper mineralization of bone, whole-body levels of Ca and P are ≥ 4000 mg kg^{-1}, and the Ca:P ratio is ≥ 1 (Shearer et al., 1994; Baeverfjord et al., 1998). When P supply is insufficient, Ca and P levels are < 4000 mg kg^{-1} and Ca:P < 1.

Most of the magnesium in fish is located in bone. Magnesium is also an essential cofactor in many enzymatic reactions in intermediary metabolism (Lall, 2002). Freshwater salmonids derive Mg ions by active uptake from water and from dietary sources. The uptake from water can partially meet the requirement for Mg in freshwater, depending on the water Mg concentration (El-Mowafi and Maage, 1998; Shearer and Åsgård, 1992). In seawater, Mg is abundant and fish obtain Mg by drinking; surplus Mg is excreted over the kidneys (Lall, 2002). Reference values (whole-body analyses) indicate that the normal level for both

rainbow trout and salmon is > 300 mg kg^{-1} (Shearer, 1984; Shearer et al., 1994; Shearer and Åsgård, 1992).

Zinc is important for metabolic processes as part of metalloenzymes (Lall, 2002), and contributes to a high number of metabolic processes. Some of the deficiency signs observed in fish may relate to disturbances of nucleic acid and protein metabolism. Zinc deficiency is associated with shortening of the spine, "short body dwarfism", in some flatfish species and, as reported in Baeverfjord (2005), an aggravation of phosphorus-induced deformities was observed when dietary zinc levels were restricted. Reference values for whole-body content of Zn given by Shearer for rainbow trout (Shearer, 1984) were 20-30 mg kg^{-1}. Similar values for Atlantic salmon were 40-60 mg kg^{-1} (Shearer et al., 1994).

6.3. Dietary mineral supply and skeletal deformities

The relation between reduced mineralization and skeletal deformities was established for Atlantic salmon both in freshwater and seawater (Baeverfjord et al., 1998; Baeverfjord, 2005; Fjelldal et al., 2007). Corresponding studies in rainbow trout are currently in progress. In Baeverfjord (2005), it was demonstrated that suboptimal dietary phosphorus supply resulted in poor mineralization of bone as an acute response, whereas the long term effects were deformities of jaw and tail and a prominent shortening of the spinal column in a significant number of fish. The condition was aggravated by restricted dietary zinc levels. Growth was little affected. The gravity of skeletal defects increased with the duration of suboptimal mineral supply, but deformities in harvest-size fish were also observed in response to a transient period of undermineralization in the freshwater stage, in particular in the period from first feeding to 20 g size when the growth rates are extremely high (Baeverfjord, 2005). In a study from 1983, Satoh et al. reported shortened spine, or "short body dwarfism", in response to zinc deficiency in rainbow trout. Likewise, magnesium deficiency in rainbow trout was associated with vertebrae deformity in a study with purified diets from 1978 (Ogino et al., 1978).

6.4. Reference values for whole-body contents

Reference values for whole-body content of elements in various life stages of Atlantic salmon and rainbow trout were given by Shearer et al. (1994) and Shearer (1984) (Table 6.1). The Atlantic salmon values cited are for fish sizes from 1 g to 4 kg. For Ca, the levels seem to be somewhat lower and more variable in seawater than in freshwater. For rainbow trout, the values cited are for analyses of fish from 1 g to 1 kg reared in freshwater. The whole-body Zn

levels for rainbow trout seem inexplicably low. In Hardy and Shearer (1985), all corresponding values were > 40 mg kg^{-1}.

Table 6.1. Reference values for whole-body content of calcium, phosphorus, magnesium and zinc in Atlantic salmon and rainbow trout. Data for Atlantic salmon were extracted from Figures 2 and 3 in Shearer et al. (1994), and for rainbow trout from Figure 3 in Shearer (1984).

	Ca (mg kg^{-1})	P (mg kg^{-1})	Mg (mg kg^{-1})	Zn (mg kg^{-1})
Atlantic salmon	4000-5200 (FW[a]) 3000-4500 (SW[b])	4000-5200	380-480	40-60
Rainbow trout	4000-6000	4000-5000	300-400	20-30

[a]Freshwater.

[b]Saltwater.

6.5. Current requirement estimates

The following dietary mineral requirements were suggested by Lall (2002) (Table 6.2).

Table 6.2. Requirement estimates for some elements (extracted from Lall, 2002). The estimates are given as amount per kg feed. Requirements were determined using purified or semipurified diets.

	Ca	P (mg kg^{-1})	Mg (mg kg^{-1})[a]	Zn (mg kg^{-1})
Atlantic salmon	not applicable	6000	500	15-30
Rainbow trout	not applicable	6000	400	37-67

[a]Freshwater.

The suggested dietary P requirement estimates were 6000 mg kg^{-1}, or 0.6% of the diet for both Atlantic salmon and rainbow trout. The estimates were based on experiments with purified or semipurified diets with highly available P sources, and Lall comments that factors affecting bioavailability must be considered when formulating fish feeds. The P requirement of juvenile Atlantic salmon was estimated to approximately 1.0-1.1 % of the diet, or 10-11 000 mg kg^{-1}, by Åsgård and Shearer (1997) in a study using semipurified diets, when whole-body levels of P was used as a criterion. In the study, P availability was calculated to be 86% and feed conversion ratio (FCR, feed given/weight gained) was 0.7. This study was

done with 1-5 g fish. This would correspond to a requirement of available P of 6000-6600 mg kg^{-1} weight gain. A different approach was chosen by Vielma and Lall (1998), who estimated a P requirement of 0.28 mg available P per kJ digestible energy. Expressed as dietary concentration, the requirement estimated by Vielma and Lall (1998) was 0.83-0.86 % (8300-8600 mg kg^{-1}) of the diet, when using a highly available P source.

The Zn requirement of Atlantic salmon seems to be severely underestimated by Lall (2002). A Zn requirement of 57-97 mg Zn kg^{-1} dry feed was estimated by Maage et al. (1991) in order to sustain normal whole-body zinc levels in the period following first feeding. In Baeverfjord (2005), a dietary level of 50-80 mg Zn kg^{-1} feed resulted in a severe depletion of whole-body zinc levels.

6.6. Monitoring of mineral status

The difficulties associated with establishing mineral requirements in fish were reviewed by Lall (2002). Test methods that monitor specific biological functions are sparse in fish, and future development of methods that allow for more refined evaluation of mineral homeostasis are anticipated. Some studies related to expression of genes involved in P uptake have been presented (Sugiura et al., 2007), and this field is expected to expand rapidly.

In previous studies on mineral requirements, the recommendations were based on a range of different criteria (Rodehutscord, 1996; Vielma and Lall, 1998; Maage et al., 2001). Also, in view of the restrictions on P outlet from freshwater fish farms in some countries, it is reasonable to suggest that interpretation of some of the data may be considered restrictive, i.e. recommendations are in the lower range of what is indicated by the data. In view of recent results that identify the link between suboptimal mineralization of bone and deformities, the response variables used to monitor mineral status should include some measure of mineral contents of bone.

The most relevant and reliable measure for mineral content of fish appears to be whole-body mineral content (El-Mowafi et al., 1997). The methodology related to sampling and analysis contains few sources of error, and gives data that can be compared across sampling points and fish sizes. For Atlantic salmon and rainbow trout, relatively complete sets of reference values for different stages of the life cycle exist (Shearer, 1984; Shearer et al., 1994).

A range of other response variables have been used to describe mineral status in experiments. Plasma concentrations of elements describe the immediate effects related to absorption (Vielma and Lall, 1998), but are of little use for evaluation of long-term effects.

Bone mineral content is a highly relevant and much used variable, and is mentioned by Lall (2002) as the most sensitive criterion for evaluating P utilization. It is a problem that analytical values seem to vary inexplicably between experiments. Also, the data are reported in different ways, such as on a fat-free or a dry-weight basis. It is likely that sampling and preparation methods can be better standardized, but the method is still labour consuming and with a significant risk of unintended variation or misinterpretation. Fjelldal et al. (2007) and Albrektsen (2005) use the ash content of the bone, relative to the average content within the experiment, as a measure of mineralization, and link it with mechanical properties and morphology (Fjelldal et al., 2007). Without any reference to absolute values however, these data are of limited value.

In summary, based on the literature, whole-body elemental concentration is suggested as the standard response parameter. There is a strong correlation between mineral content in bone and whole-body levels (Baeverfjord et al., 1998), there are absolute reference values to compare with, and the methods for sampling and analysis are relatively foolproof. The value given should be mineral content in wet weight, as it was demonstrated by Shearer (1984) that values given on a dry-weight basis are too much influenced by variation in lipid content. The main weakness is that the values will be influenced by the fat and muscle content of the fish, but in commercially farmed salmonids, this variation is not expected to be very high.

6.7. Fish welfare versus environmental concerns

Analyses done in 2006 and 2007 of whole-body mineral levels in farmed, freshwater salmon from commercial operations demonstrate that mineral levels vary widely (data from Nofima Marine). Some groups of fish have whole-body calcium and phosphorus concentrations that are comparable to reference values (Shearer et al., 1994), but more than half of the samples have concentrations that are lower than these values (Fig 6.1).

Similarly, many of the values for zinc and magnesium were low. The mean whole-body concentration of magnesium in the 25 samples shown in Figure 1 was 263 mg kg^{-1} (SD 25.8, min 225, max 320), compared to reference values for Atlantic salmon of 380-480 mg kg^{-1}. The mean whole-body zinc concentration in these samples was 32.7 mg kg^{-1} (SD 4.1, min 23.2, max 41.1), compared to reference values of 40-60 mg kg^{-1}. These data indicate that the mineralization status in Atlantic salmon juveniles in commercial production is far from satisfactory.

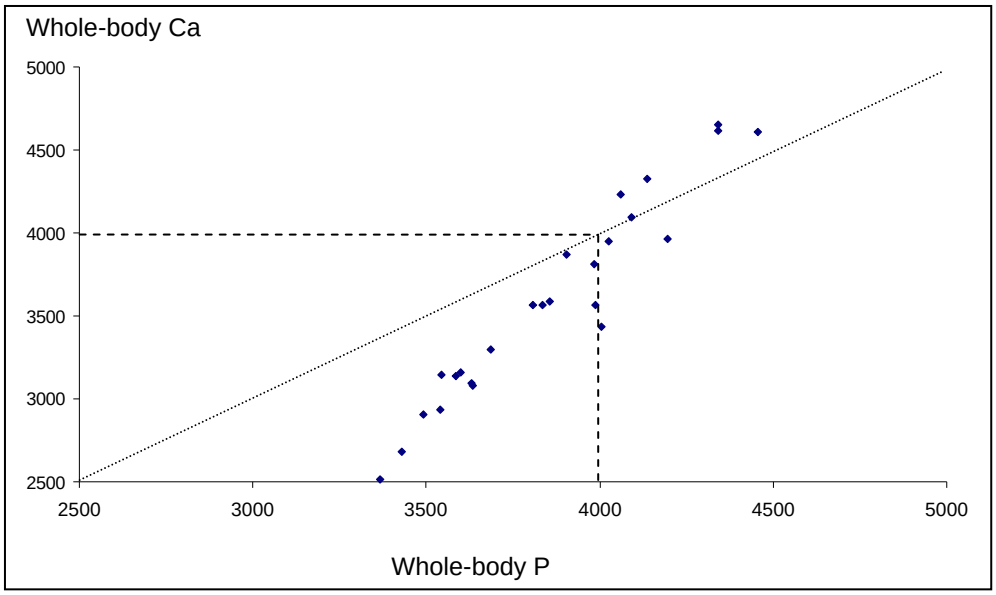

Figure 6.1. Whole-body concentrations of Ca and P in 25 samples of fish fed commercial diets in 2006 and 2007. Values are given as mg kg^{-1} wet weight, in pooled samples of 10 fish per fish group. Fish weights were 20-60 g. The diagonal line represents Ca:P 1:1. Reference values for fish with normal mineralization of bone are Ca and P > 4000 (dotted lines) and Ca ≥ P. Data from Nofima Marine.

Phosphorus output from freshwater production sites is a major concern, and restrictions on P discharge from freshwater sites are in existence in several of the countries that produce rainbow trout in freshwater. Strategies to minimize P output were reviewed by Lall (2002), and recommendations include reductions in dietary P levels (without compromising the fish), as well as increasing P availability through different approaches. In the current situation, there is no reason to activate self-imposed restrictions on P discharge in freshwater effluents in Norway, and considering the observations of unsatisfactory mineralization presented above, such restrictions would represent a potential fish welfare risk.

6.8. Factorial model for determination of dietary requirements of elements

Shearer (1995) suggested a factorial model for determination of dietary requirements of essential elements in fishes. A simplified version of the model was suggested by Åsgård and Shearer (1997) for minerals where feed is the major source and endogenous loss is low:

$$C=(G/A)/F \quad \text{or} \quad G=C*F*A,$$

where G: Elemental concentration in fish, cfr reference values

C: Elemental concentration in feed

F: Expected feed conversion ratio, kg feed/kg weight gain

A: Predicted bioavailability of element in feed.

The model demonstrated that a requirement estimate given as concentration in the diet (C, e.g. mg P kg^{-1} feed) is of little value, unless feed conversion ratio (F) and bioavailability (A) are specified. A range of other methods have been used to establish requirements, leading up to the authoritative recommendations given in NRC (1993) and Lall (2002). Some studies include comments or footnotes that specify that the requirement estimates refer to available amounts, others do not. Also, few, if any, of the studies acknowledge the importance of growth rate and feed efficiency for the relevance of the estimates. None of the estimates discuss safety margins.

6.9. Growth rates and feed efficiency – impact on requirement estimates

In intensive rearing of salmonids, utilization of nutrients is extremely efficient. The two most common measures are feed efficiency (FE, weight gained/feed given) or the inverse value feed conversion ratio (FCR, feed given/weight gained).

FCR may be extremely low, especially in small fish in freshwater. Mean FCR values of 0.6 and lower are not uncommon in juvenile fish, at the stages where growth rate is highest. Expected growth rates for freshwater juveniles of Atlantic salmon and parr at 12 °C are between 3 and 5% per day, and at 16°C between 4 and 6% per day (Tvenning, 1998). In seawater, a close correlation between FE and thermal growth coefficient (TGC) ($r=0.79$, $P<0.001$) was observed by Thodesen et al. (2001b), and in consequence, a corresponding negative correlation exists between growth rate and FCR: High growth rates → low FCR.

A FCR value of 0.6 implies that no more than 600 g of feed is transformed to 1 kg of fish. Thus, the element concentration in the feed formulated to support such efficient growth must be relatively higher than when FCR is low. Requirement estimates should include this aspect. A different approach to this was chosen by Rodehutscord (1996) and Vielma and Lall (1998), who calculated P requirement in relation to kJ available energy. This approach may be equally correct, and relating mineral requirements to dietary energy content may give more precise estimates than using FCR. FCR, on the other hand, is a commonly recorded parameter, and represents a more practical choice.

In the context of dietary mineral supply versus growth rate, it is worth noting that growth rates of farmed salmonids are continuously high throughout the life cycle. As a result

of selective breeding, growth potential of farmed stocks continues to increase. This represents a desired development, and is part of the foundation for profitability in the production. Considering the data from Thodesen et al. (2001b) cited above, further increase in TGC is likely to be associated with a corresponding decrease in FCR. No data exist so far to determine whether there is an upper limit for growth rate at which normal development of skeletal tissues can be obtained.

6.10. Mineral bioavailability

The common fish meals and vegetable meals used in fish diets contain minerals in significant amounts (NRC, 1993). There is, however, no corresponding authoritative database available over P digestibility.

Two studies refer to variation in uptake of elements due to biological factors, both done in seawater. Thodesen et al. (2001a) demonstrates a genetic variation in mineral absorption among family groups of large seawater-adapted salmon (Table 6.3). Rydland (1998) presents individual digestibility of minerals, examined by repeated sampling of faeces in 15 large, seawater-adapted Atlantic salmon (Table 6.4).

Table 6.3. Apparent digestibility coefficients (ADC, %) for some elements in 82 full-sib family groups of Atlantic salmon (average weight 4.6 kg). Data are given as mean values (n=82), standard deviation (SD), coefficient of variation (CV) and minimum and maximum values. (Modified after Thodesen et al., 2001a)

Element	Mean ADC	SD	CV	Min	Max
Ca	-11.7	14.5	-123	-91.8	11.1
P	40.4	6.4	15.8	23.9	54.8
Mg	-260	91	-35.0	-768	-119
Zn	37.9	6.7	17.7	18.3	51.3

Table 6.4. Apparent digestibility coefficients (ADC, %) for some elements in 15 individual Atlantic salmon (average weight 1.7 kg). Analyses were done in 3 to 6 repeated samples per individual fish. Data are given as mean values (n=15), standard deviation (SD), coefficient of variation (CV) and minimum and maximum values. (Modified from Rydland, 1998)

Element	Mean ADC	SD	CV	Min	Max
Ca	-0.5	9.9	-1980	-20.9	20.7
P	38.9	5.8	14.9	30.4	49.2
Mg	-325	79	-24.0	-483	-251
Zn	44.7	7.8	17.4	32	57.7

The P digestibility coefficients of approximately 40% were low compared to some of the earlier studies. It should also be noted that Zn digestibility coefficients were at the same level, around 40%. The coefficients of variation for P and Zn digestibility are similar in these two studies, 15.8 and 14.9 for P, and 17.7 and 17.4 for Zn. In Thodesen et al. (2001a), the best family has a P digestibility coefficient of 54.8%, the worst 23.9%. The range from the best to the worst individual in Rydland (1998) was almost of the same magnitude. These values indicate that there should be some consideration of safety margins when applying mineral requirement estimates in feed formulation. The corresponding values for Ca and Mg are of little interest, since these were seawater-adapted fish.

Albrektsen (2005) presented data on the low availability of phosphorus in fish meals produced from blue whiting, thus confirming previous findings on the unpredictable availability of P from different fish meals. Also, availability of minerals may be severely impaired in diets containing vegetable ingredients, e.g. soybean meal (Storebakken et al., 1998). Thus, prediction of actual digestibility of P in formulated diets requires knowledge about feed ingredients and expected availability of P for each batch produced. The data presented above on zinc availability indicate that it may be necessary to do the same considerations also for zinc.

6.11. Recommendations

The following recommendations are suggested:

- New studies should be done to determine dietary mineral requirements using current fish stocks with growth potential, feed intake and feed efficiencies which are relevant for today's aquaculture
- Requirement estimates should include safety margins based on knowledge about within-population variation in digestibility and utilization
- In addition to P, Zn should have high priority
- Studies on development of bone quality in response to deficiency conditions are recommended, in order to characterize bone formed during deficient periods. This is of importance for monitoring of mineral status and evaluation of dietary history of fish
- Interrelation with other metabolic functions and requirements should be clarified
- Development of non-invasive indicators, including molecular markers, for mineral metabolism, should be given a high priority
- Updated requirement estimates should be given as available amount per unit of weight gain, in order to include consideration related to growth rate, feed conversion ratio and digestibility of elements
- Further development of methods for estimating digestibility of minerals in commercial feeds should be done
- Procedures for improving mineral availability from major feed ingredients should be further developed.

6.12. References

Albrektsen, S., 2005. Impaired mineralisation in Atlantic salmon. Project summary. Outlook on aquaculture 1-2005, Fiskeriforskning, Tromsø. 1p.

Åsgård, T., Shearer, K.D., 1997. Dietary phosphorus requirement of juvenile Atlantic salmon, *Salmo salar* L. Aquac. Nutr. 3, 17-23.

Baeverfjord, G., 2005. Rygg- og kjevedeformiteter hos laks: Genetisk disposisjon, mineralernæring og produksjonstemperatur (Deformities of the spinal column and lower jaw in Atlantic salmon: Genetic predisposition, mineral nutrition and production temperature). Report to the Norwegian Research Council, 22 p.

Baeverfjord, G., Åsgård, T., Shearer, K.D., 1998. Development and detection of phosphorus deficiency in Atlantic salmon parr and post smolt. Aquac. Nutr. 4, 1-11.

El-Mowafi, A.F.A., Maage, A., 1998. Magnesium requirement of Atlantic salmon (*Salmo salar* L) parr in seawater-treated fresh water. Aquac. Nutr. 4, 31-38.

El-Mowafi, A.F.A., Maage, A., Lorentzen, M., Hassanein, E.I., Julshamn, K., 1997. Tissue indicators of element status in Atlantic salmon (*Salmo salar*) post-smolts: Effect of fasting. Aquac. Nutr. 3, 73-80.

Fjelldal, P.G., Nordgarden, U., Hansen, T., 2007. The mineral content affects vertebral morphology in underyearling smolt of Atlantic salmon (*Salmo salar* L.). Aquaculture 270, 231-239.

Hardy, R.W., Shearer, K.D., 1985. Effect of dietary calcium phosphate and zinc supplementation on whole body zinc concentration of rainbow trout (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci. 42, 181-184.

Lall, S.P., Lewis-McCrea, L.M., 2007. Role of nutrients in skeletal metabolism and pathology – An overview. Aquaculture 267, 3-19.
Lall, S.P., 2002. The minerals. In: Halver, J.E., Hardy, R.W. (Eds.), Fish Nutrition, 3rd ed. Academic Press, London, pp. 259-308.
Maage, A., Lorentzen, M., Bjørnevik, M., Julshamn, K., 1991. Zinc requirements of Atlantic salmon (*Salmo salar*) fry during start feeding. In: Kaushik, S.J., Luquet, P. (Eds.), Fish Nutrition in Practice. INRA Editions, Paris, No 61, 873-882.
Maage, A., Julshamn, K., Berge, G.E., 2001. Zinc gluconate and zinc sulphate as dietary zinc sources for Atlantic salmon. Aquac. Nutr. 7, 183-187.
National Research Council (NRC), 1993. Nutrient Requirements of Fish. National Academy Press, Washington DC.
Ogino, C., Takashima, F., Chiou, J.Y., 1978. Requirement of rainbow trout for dietary magnesium. Bull. Japan. Soc. Sci. Fish. 44, 1105-1108.
Rodehutscord, M., 1996. Response of rainbow trout (*Oncorhynchus mykiss*) growing from 50 to 200 g to supplements of dibasic sodium phosphate in a semipurified diet. J. Nutr. 126, 324-331.
Rydland, R., 1998. Fordøyingsgrad av formineral med vekt på fosfor (Digestibility of feed minerals with emphasize on phosphorus). Hovedoppgåve, Norges Landbrukshøgskole. 53 pp. In Norwegian.
Satoh, S., Yamamoto, H., Takeuchi, T., Watanabe, T., 1983. Effects on growth and mineral composition of rainbow trout of deletion of trace elements and magnesium from fish meal diet. Bull. Japan. Soc. Sci. Fish.49, 425-429.
Shearer, K.D., 1984. Changes in elemental composition of hatchery-reared rainbow trout, *Salmo gairdneri*, associated with growth and reproduction. Can. J. Fish Aquat. Sci. 41, 1592-1600.
Shearer, K.D., 1995. The use of factorial modeling to determine the dietary requirements for essential elements in fishes. Aquaculture, 133, 57-72.
Shearer, K.D., Åsgård, T., 1992. The effect of water-borne magnesium on the dietary magnesium requirement of the rainbow trout (*Oncorhynchus mykiss*). Fish Physiol. Biochem. 9, 387-392.
Shearer, K.D., Åsgård, T., Andorsdottir, G., Aas, G.H., 1994. Whole body elemental and proximate composition of Atlantic salmon (*Salmo salar*) during the life cycle. J. Fish Biol. 44, 785-797.
Storebakken, T., Shearer, K.D., Roem, A., 1998. Availability of protein, phosphorus and other elements in fish meal, soy-protein concentrate and phytase-treated soy-protein-concentrate-based diets to Atlantic salmon, *Salmo salar*. Aquaculture 161, 365-379.
Sugiura, S.H., Kelsey, K., Ferraris, R.P., 2007. Molecular and conventional responses of large rainbow trout to dietary phosphorus restriction. J. Comp. Physiol. B. 177, 461-472.
Thodesen, J., Storebakkken, T., Shearer, K.D., Rye, M., Bjerkeng, B., Gjerde, B., 2001a. Genetic variation in mineral absorption of large Atlantic salmon (*Salmo salar*) reared in seawater. Aquaculture 194, 263-271.
Thodesen, J., Gjerde, B., Grisdale-Helland, B., Storebakken, T., 2001b. Genetic variation in feed intake, growth and feed utilization in Atlantic salmon (*Salmo salar*). Aquaculture 194, 273-281.
Tvenning, L., 1998. Ny veksttabell for settefisk. I Dybden, nr 1, p 87.
Vielma, J., Lall, S.P., 1998. Control of phosphorus homeostasis of Atlantic salmon (*Salmo salar*) in fresh water. Fish Physiol. Biochem. 19, 83-93.

7. Carbohydrates

Barbara Grisdale-Helland

Carbohydrates are not specifically required in the diet of fish, but a growth-promoting effect is found when, with an adequate dietary protein level, gelatinized starches are included in the diet at low levels (reviewed by Hemre et al., 2002). Starches are added to the diet mainly for binding and expansion during extrusion. But, increasing dietary starch levels reduce both starch and fat digestibility in salmon (reviewed by Storebakken, 2002). Similarly, feed utilization is reduced with increasing dietary starch levels in diets for Atlantic cod and Atlantic halibut (Hemre et al., 1993; Helland and Grisdale-Helland, 1998). The protein-sparing effect of carbohydrates varies with species, temperature, light regime, season, and the type and amount of carbohydrate included in the diet (reviewed by Hemre et al., 2002). Simple sugars in gelatinized forms are most effective for protein sparing (Hemre et al., 2002). Oligosaccharides in soybean meal may be indigestible and reduce the uptake of fat and minerals in salmon (Refstie et al., 1998; Refstie et al., 2005). In addition, dietary soybean non-starch polysaccharides may be responsible for reducing the digestibility of fat and protein in salmon (Refstie et al., 1999). According to Bæverfjord and Krogdahl (1996), enteritis in salmon caused by exposure to soybean meal is reversible.

As reviewed by Hemre et al. (2002), high glycogen deposition as a result of feeding diets with high starch levels may reduce liver function in rainbow trout. No effect of high carbohydrate diets on non-specific immunity has been found in trout, whereas serum haemolytic activity was negatively associated with dietary carbohydrate level in salmon. The effect of dietary starch level on mortality when fish are challenged with pathogens seems to vary with type of pathogen, environment and fish size.

7.1. References

Bæverfjord, G., Krogdahl, Å., 1996. Development and regression of soybean meal induced enteritis in Atlantic salmon distal intestine. A comparison with the intestines of fasted fish. J. Fish Dis. 19, 375-387.

Helland, S.J., Grisdale-Helland, 1998. Growth, feed utilization and body composition of juvenile Atlantic halibut (Hippoglossus hippoglossus) fed diets differing in the ratio between the macronutrients. Aquaculture 166, 49-56.

Hemre, G.-I., Lie, Ø., Sundby, A., 1993. Dietary carbohydrate utilisation in cod (Gadus morhua): metabolic responses to feeding and fasting. Fish Physiol. Biochem. 10, 455-463.

Hemre, G.-I., Mommsen, T.P., Krogdahl, Å., 2002. Carbohydrates in fish nutrition: effects on growth, glucose metabolism and hepatic enzymes. Aquac. Nutr. 8, 175-194.

Refstie, S., Sahlstrom, S., Brathen, E., Baeverfjord, G., Krogedal, P., 2005. Lactic acid fermentation eliminates indigestible carbohydrates and antinutritional factors in soybean meal for Atlantic salmon (*Salmo salar*). Aquaculture 246, 331-345.

Refstie, S., Storebakken, T., Roem, A.J., 1998. Feed consumption and conversion in Atlantic salmon (*Salmo salar*) fed diets with fish meal, extracted soybean meal or soybean meal with reduced content of oligosaccharides, trypsin inhibitors, lectins and soy antigens. Aquaculture 162, 301-312.

Refstie, S., Svihus, B., Shearer, K.D., Storebakken, T., 1999. Nutrient digestibility in Atlantic salmon and broiler chickens related to viscosity and non-starch polysaccharide content in different soybean products. Anim. Feed Sci. Tech. 79, 331-345.

Storebakken, T., 2002. Atlantic salmon, *Salmo salar*. In: C.D. Webster, C. Lim (eds.), Nutrient Requirements and Feeding of Finfish for Aquaculture. CAB International, Oxon, UK, pp. 79-102.

8. Carotenoids

Trine Ytrestøyl and Bjørn Bjerkeng

8.1. Introduction

The pink flesh colour unique to salmonid fishes is caused by deposition of carotenoids such as astaxanthin (3,3´-dihydroxy-β,β-carotene-4,4´-dione) and canthaxanthin (β,β-carotene-4,4´-dione) in the muscle where the carotenoids are associated with the muscle protein fraction (Henmi et al., 1987, 1989). Salmonid fishes are able to deposit appreciable amounts of carotenoids in their muscle tissues. Carotenoids are synthesised *de novo* by plants, algae, certain types of bacteria and fungi. In plants, the main function of carotenoids is as light-harvesting accessory pigments and as protectants against photooxidation and quenchers of singlet oxygen. No animals, including fishes, are able to biosynthesise carotenoids and thus depend on a dietary supply. In animals, carotenoids are important in species-specific colouration of integuments and plumage and thus contribute to camouflage and communication (Brush 1981; Shahidi et al., 1998; Schiedt 1998). Colourful ornaments are often displayed by males and play an important role in reproduction through sexual selection. One of their most important physiological functions in animals is their action as vitamin A precursors. Almost all animals are able to enzymatically convert certain carotenoids into vitamin A. Canthaxanthin and astaxanthin serve as vitamin A precursors in salmonid fishes (Schiedt et al., 1985; Al-Khalifa and Simpson, 1988; Guillou et al., 1989; White et al., 2003). Carotenoids have also been shown to have beneficial effects on health and fecundity probably related to their effects as antioxidants, quenchers of free radicals such as singlet oxygen and as immunostimulants (Palace et al., 1999; Stahl and Sies, 2003; Amar et al., 2004; Fraser and Bramley, 2004; Ahmadi et al., 2006).

8.2. Reproduction and growth

Carotenoids accumulate in the reproductive organs of a wide range of organisms and their role in reproduction has been debated for more than 50 years (Goodwin, 1950; Craik, 1985; Palace and Werner, 2006). The carotenoids have been suggested to act as fertilisation hormones, to enhance growth, maturation and fecundity, as well as to reduce mortality during embryonic development and increase the ability to tolerate harsh environmental conditions such as high temperature, light and low oxygen (reviewed by Tacon, 1981; Craik, 1985; Choubert, 1986). The experimental evidence for this is conflicting and knowledge on this topic has been hampered by the lack of controlled fish experiments in which carotenoid

supply was the only variable. Some studies have found carotenoids to positively affect egg production and offspring growth or survival, whereas several others have failed to detect such effects. This could be due to the choice of parameters. Fertilization rate has often been used as an indicator of egg or embryo quality, but this may not always be the case. In the two-spotted goby (*Gobiusculus flavenscens*), females fed diets supplemented with 135 mg astaxanthin per kg developed a stronger nuptial coloration, were more likely to spawn and also produced larvae that had a higher phototactic response compared to females fed diets without astaxanthin (Svensson et al., unpublished results). Phototaxis is crucial for survival of the larvae. However, commonly reported parameters for reproductive success, such as fertilization and hatching rates, were unaffected. In aquaculture, optimal broodstock diet formulation is essential for producing offspring with good quality. During sexual maturation, the carotenoids in the muscle of salmonid fishes are redistributed to the ovaries and accumulate in the eggs. The carotenoid concentration in the eggs usually reflects the concentration in the broodstock diet (Christiansen and Torrissen, 1997; Ahmadi et al., 2006). In female rainbow trout, dietary supplementation of astaxanthin to broodstock diets increased the fertilization rate, percentage of eyed and hatched eggs, and reduced mortality of eyed eggs. In males, astaxanthin supplementation had a positive effect on fertilization rate (Ahmadi et al., 2006). There seemed to be a critical dietary level between 0.07 and 12.5 mg astaxanthin per kg. Above 12.5 mg/kg there were no significant differences between the dietary astaxanthin concentrations. Thus, the astaxanthin concentration in broodstock diets for rainbow trout should be kept above this level. The critical concentrations in the rainbow trout eggs were somewhere between 2 and 10 mg/kg (Ahmadi et al., 2006). A critical level of 1-3 mg/kg in the eggs was suggested by Craik (1985) after reviewing many of the early studies on rainbow trout. In contrast, Tveranger (1986) fed diets containing astaxanthin concentrations between 1.4 and 4.2 mg/kg to rainbow trout broodstock and could not find any differences between the treatments on fertilisation rate, survival of eggs and alevins, and growth of fry during the start-feeding period. This is in accordance with Choubert et al. (1998) who fed diets supplemented with canthaxanthin to rainbow trout broodstock before spawning without observing any effect on the frequency of maturing females and the subsequent growth of fry. However, the carotenoid concentration of the control diet was not given in the study of Choubert and coworkers, and it could be above the critical level due to the contribution from the dietary ingredients. Deufel (1965) found an increase in both fertilization rate and the percentage fertile females after canthaxanthin supplementation (40 mg/kg) in the broodstock diet.

Astaxanthin supplementation to a broodstock diet (98.7 mg/kg vs. control level of 0.4 mg/kg) did not have a significant effect on the percentage of fertilised eggs, egg viability and embryo quality in Atlantic salmon (Christiansen and Torrissen, 1997). Torrissen (1984) did not find any effect of the carotenoid content of the eggs on survival of eggs and alevins in Atlantic salmon, but fry fed diets supplemented with 30 mg astaxanthin or canthaxanthin per kg grew faster than fry fed unsupplemented diets in the early start feeding period. Dietary supplementation of astaxanthin to semi-purified casein/gelatine based diets has later been shown to improve growth performance in first feeding fry, juveniles and parr of Atlantic salmon (Christiansen et al., 1994, 1995a,b; Christiansen and Torrissen, 1996). However, these studies were done without replication of some of the dietary treatments. Christensen et al. (1994, 1995a) found that astaxanthin had a positive effect on growth and survival in Atlantic salmon fry apart from its function as a source of vitamin A, but the authors questioned the bioavailability of the vitamin A source used in the study (a mixture of retinol palmitate and retinol acetate). A concentration of 5 mg/kg was recommended as a minimum in start feeding diets to secure maximum growth and survival (Christensen et al., 1995a). Torrissen and Christiansen (1995) suggested that astaxanthin and canthaxanthin should be considered as a vitamin for fish and supplemented in the diet at a level of minimum of 10 mg/kg dry diet. However, Atlantic salmon smolts fed diets supplemented with high levels of astaxanthin (48 mg/kg) had a reduced cataract frequency compared to smolts fed lower dietary astaxanthin levels (11 mg/kg) (Waagbø et al., 2003). Thus, dietary astaxanthin levels above 10 mg/kg might be necessary to secure optimal health in Atlantic salmon.

In his pioneering study, Steven (1949) believed that all the egg carotenoids of brown trout (*Salmo trutta*) were transferred to the embryo and fry where it was found predominantly as esters in the integument, but later studies with wild Atlantic salmon showed that about 30% of the egg astaxanthin disappeared during development to fry (Craik and Harvey, 1986). Irreversible transformation into vitamin A and retinoic acids and subsequent secretion via catabolic pathways may be responsible for at least part of the observed loss. In rainbow trout, the loss of egg carotenoids is about 60% when the fish reaches the start feeding stage (Bazyar Lakeh et al., unpublished results). Since these fishes are not eating at this stage the carotenoids must have been transformed to colourless metabolites. Astaxanthin is probably not an important vitamin A source at the egg stage, whereas it may serve as a precursor at the fry stage when a functional liver has been developed (Ørnsrud et al., 2004). The contribution of astaxanthin to the retinoid pool in eggs and fry of salmonid fishes remains to be determined. Effects of intact carotenoids or metabolites other than vitamin A should also be

considered. At present there is no knowledge on the biochemical basis of the effects of carotenoids during embryogenesis and later stages in salmonid fishes. However, a gene from zebrafish (*Danio rerio*) encoding the enzyme responsible for vitamin A formation, β,β-carotene-15,15´-oxygenase (*bcox*), has been cloned (Lampert et al., 2003). Targeted gene knockdown caused severe malformations of eyes, craniofacial skeleton and pectoral fins during embryonic development. Furthermore, retinoic acid formation depending on local *de novo* formation of retinal from provitamin A via *bcox* appeared to be essential. This suggests a crucial role of carotenoids in the early development of fish. The substrate requirements and putative roles of such enzymes in formation of retinoids in developing embryos of salmonid fishes and other farmed species require investigation.

8.3. Flesh quality

In the seawater grow-out phase, the need for carotenoids is not defined by health and growth parameters but by the need to obtain a sufficient colouring of the flesh. Flesh colour is an important quality criterion for Atlantic salmon, and a mean astaxanthin level of 6-7 mg/kg in the muscle is considered sufficient to obtain an acceptable flesh colour. To achieve this target it is common practice to fortify diets with 20-75 mg carotenoids per kg during the entire seawater phase. The dietary dose may be changed during the grow-out phase and in general, small fish are fed higher doses of pigment than larger fish. In Norway, the main carotenoid source is synthetically manufactured astaxanthin. Canthaxanthin is also used by some feed producers, but the restrictions on the use of canthaxanthin posed by the EU (maximum 25 mg per kg feed) have reduced the importance of this carotenoid for the salmon industry. The muscle retention of carotenoids decreases with increasing dietary dose in a curvilinear fashion. For both Atlantic salmon and rainbow trout, the gain in flesh pigmentation is only minor when applying diet concentrations higher than 50-60 mg/kg (Bjerkeng et al., 1990; Torrissen et al., 1995; Forsberg and Guttormsen, 2006a). Apart from dietary concentration, the amount of carotenoid that is deposited in the flesh is also affected by several other factors such as diet composition, fish age, size, physiological status and genetics (Torrissen et al., 1989; Storebakken and No, 1992). In pre-smolts, the carotenoids are deposited mainly in the skin and the deposition in the muscle is limited (Storebakken et al., 1987; Bjerkeng et al., 1992). In post-smolts the carotenoid concentration in the muscle increases with size in a curvilinear fashion when the dietary concentration is kept constant throughout the seawater phase (Storebakken et al., 1987; Bjerkeng et al., 1992; Torrissen et al., 1995; Forsberg and Guttormsen, 2006a). The deposition of carotenoids ceases when the fish become sexually

mature, and instead there is a mobilisation of carotenoids from the muscle to the skin and reproductive organs (Bjerkeng et al., 1992; Schiedt, 1998). A pigmentation model for farmed Atlantic salmon based on published experimental data was recently published (Forsberg and Guttormsen, 2006a). This model may be used to predict the concentration of astaxanthin in the fillet as a function of fish weight and dietary astaxanthin concentration in the seawater phase. The model has been included in a mathematical program designed to predict the dietary astaxanthin concentration that gives an adequately pigmented fish at the minimum cost (Forsberg and Guttormsen, 2006b). The model is built on data from 6 different Norwegian pigmentation trials performed in the period 1993-2002. The model simulations show three general trends: 1) Smaller fish should be fed higher pigment concentrations than larger fish (fish of 0-2 kg are fed 20-40 mg more per kg diet then fish of 4-6 kg). 2) The optimal diet concentration is dependent on the flesh target concentration and increases by 10-30 mg/kg if the Salmofan score is increased from 27 to 28. This would increase the pigment cost by around 50%. 3) The optimal pigmentation strategy depends on the desired harvest size and thus how fast the targeted Salmofan score of 27 should be reached. The model of Forsberg and Guttormsen (2006a) assumes that fish weight and dietary astaxanthin concentration are the only variables that determine the astaxanthin concentration in the flesh. Specific dose-response relationships are in principle only valid under the experimental conditions under which they were obtained with respect to pigment dose and dietary composition, growth rate, temperature, genetic origin and physiological state of the fish material. Fish in small experimental units do not always grow as fast as fish under commercial production regimes and as the authors point out, the model should be used with caution and there is a need to validate the model on fast growing and well-performing Atlantic salmon.

How effective the dietary carotenoids are used for muscle pigmentation is defined as the bioavailability of the carotenoid (Jackson, 1997). A major determinant of nutrient bioavailability is the proportion absorbed from the gastrointestinal tract, often referred to as digestibility. The proximal intestine is the major site of absorption (Choubert et al., 1987; Al-Khalifa and Simpson, 1988; Torrissen et al., 1990; White et al., 2002). Digestion involves several steps from breakdown of the food matrix, solubilization of carotenoids into mixed bile salt micelles, movement across the unstirred water layer adjacent to the microvilli, uptake by the enterocyte and incorporation into chylomicrons (Furr and Clark, 1997; Tyssandier et al., 2001, 2002). Biotic or abiotic factors interfering with any of these steps may potentially have an effect on carotenoid bioavailability. The apparent digestibility of carotenoids in salmonid

fishes is quite low compared to that of essential nutrients, typically between 40 and 60% (Bjerkeng et al., 1997a; Bjerkeng and Berge, 2000; Ytrestøyl et al., 2005, 2006). The digestibility of carotenoids is negatively correlated with the dietary pigment concentration (Choubert and Storebakken, 1989; Bjerkeng et al., 1990; Torrissen et al., 1990, 1995) and recently, water temperature and feed intake were found to affect carotenoid digestibility. Reducing the ration level by 60%, from 0.44 to 0.17% of biomass, led to a 1.5 fold increase in astaxanthin digestibility (Ytrestøyl et al., 2006) whereas increasing the temperature from 8 to 12 °C improved the digestibility by 10% (Ytrestøyl et al., 2005). Thus, carotenoid digestibility and retention may vary with season, explaining the spring drop often observed in muscle pigmentation (Mørkøre and Rørvik, 2001).

The composition of the diet may be important for the utilisation of carotenoids. A high fat content in the diet has a positive effect on absorption and retention of carotenoids (Choubert et al., 1991; Torrissen et al., 1990; Bjerkeng et al., 1997b, 1999; Clark et al., 2000; Hamre et al., 2004). The quality of the dietary fat may also be important. A high content of polyunsaturated fatty acids has a positive effect on carotenoid bioavailability (Waagbø et al., 1993; Bjerkeng et al., 1999, 2000; Regost et al., 2004; Rørå et al., 2005), whereas a high content of water-soluble dietary fibre has a negative impact on digestibility of carotenoids (Riedl et al., 1999). Antinutritional factors present in many vegetable feedstuffs have also been found to have negative effects on nutrient and carotenoid digestibility (Storebakken et al., 2000; Refstie et al., 2005). Furthermore, in the gilthead sea bream (*Sparus aurata*), the effect of ration level on nutrient digestibility was dependent on the composition of the diet. Ration level had no effect on nutrient digestibility when the diet was based on capelin meal but there was a negative effect of a higher ration level when the diets were made from brown fish meal and trash fish meal (Fernández et al., 1998). The bioavailability of carotenoids should therefore, be considered when formulating diets for rapidly growing salmon; possible negative effects of certain feed ingredients on carotenoid digestibility may not become evident until growth rates are approaching the upper limit. A basic understanding of how temperature, growth rate and diet composition interact to affect solubilization and uptake of carotenoids from the gut is vital to develop a pigment strategy for salmonid fishes that ensures a flesh carotenoid concentration above the market demand.

8.4. Concluding remarks

It is documented that carotenoids have beneficial effects on health and fecundity of fish and should be included in the diet to ensure maximum growth and welfare of the animal.

Based on the present knowledge, a diet concentration of at least 10 mg/kg seems adequate in most cases. However, the dietary recommendations for Atlantic salmon fry and juveniles are based mainly on studies done more than ten years ago and where the fish had been fed synthetic casein/gelatine based diets which may have resulted in lower growth rates compared to commercial diets. The digestibility of carotenoids is rather low and is negatively affected by feed intake. In fast growing fish, particularly juveniles, the bioavailability may be very low and the dietary concentration should perhaps be increased. The use of vegetable feed ingredients is becoming more popular and this can also interfere with utilisation of carotenoids in the diet. Currently, there is limited information on how the astaxanthin concentration in broodstock diets for Atlantic salmon affects fecundity and growth of fry and fingerlings. In rainbow trout, recent results suggest that the dietary concentration should be at least 12.5 mg/kg. In the seawater grow-out phase, the need for carotenoids is not defined by health and growth parameters but by the need to obtain a sufficient colouring of the flesh. There is currently work in progress on how to optimize the dietary pigment concentration in Atlantic salmon, whereas there is a lack of studies aimed to develop an optimal pigmentation strategy for rainbow trout.

8.5. References

Ahmadi, M.R., Bazyar, A.A., Safi, S., Ytrestøyl, T., Bjerkeng, B. 2006. Effects of dietary astaxanthin supplementation on reproductive characteristics of rainbow trout (*Oncorhynchus mykiss*). J. Appl. Ichtyol. 22, 388-394.

Al-Khalifa, A.R., Simpson, K.L. 1988. Metabolism of astaxanthin in the rainbow trout (*Salmo gairdneri*). Comp. Biochem. Physiol. 91B, 563-568.

Amar, E.C., Kiron, V., Satoh, S., Watanabe, T. 2004. Enhancement of innate immunity in rainbow trout (*Oncorhynchys mykiss* Walbaum) associated with dietary intake of carotenoids from natural products. Fish Shellfish Immunol. 16, 527-537.

Bjerkeng, B., Berge, G.M. 2000. Apparent digestibility coefficients and accumulation of astaxanthin *E/Z* isomers in Atlantic salmon (*Salmo salar* L.) and Atlantic halibut (*Hippoglossus hippoglossus* L.). Comp Biochem Physiol 127B, 423-432

Bjerkeng, B., Storebakken, T., Liaaen-Jensen, S. 1990. Response to carotenoids by rainbow trout in the sea: resorption and metabolism of dietary astaxanthin and canthaxanthin. Aquaculture 91, 153-162.

Bjerkeng, B., Storebakken, T., Liaaen-Jensen, S. 1992. Pigmentation of rainbow trout from start feeding to sexual maturation. Aquaculture 108, 333-346.

Bjerkeng, B., Følling, M., Lagocki, S., Storebakken, T., Olli, J.J., Alsted, A. 1997a. Bioavailability of all-*E*-astaxanthin and *Z*-isomers in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 157, 63-82.

Bjerkeng, B., Refstie, S., Fjalestad, K. T., Storebakken, T., Rødbotten, M., Roem, A.J. 1997b. Quality parameters of the flesh of Atlantic salmon (*Salmo salar*) as affected by dietary fat content and full-fat soybean meal as a partial substitute for fish meal in the diet. Aquaculture 157, 297-309.

Bjerkeng, B., Hatlen, B., Wathne, E. 1999. Deposition of astaxanthin in fillets of Atlantic salmon (*Salmo salar*) fed diets with herring, capelin, sandeel, or Peruvian high PUFA oils. Aquaculture 180, 307-319.

Bjerkeng, B., Hatlen, B., Wathne, E. 2000. Corrigendum to "Deposition of astaxanthin in fillets of Atlantic salmon (*Salmo salar*) fed diets with herring, capelin, sandeel, or Peruvian high PUFA oils" [Aquaculture 180, 307-319]. Aquaculture 189, 389-390.

Brush, A.H., 1981. Carotenoids in wild and captive birds. In: Bauernfeind, J.C., (Ed.), Carotenoids as Colorants and Vitamin A Precursors. Technological and Nutritional Applications. Academic Press, Inc., New York, USA, pp. 539-563.

Choubert, G. 1986. Carotenoid pigments and their role in the reproduction of fish. Bull. Fr. Pêche Piscic. 300, 25-32.
Choubert, G., Guillou, A., Fauconneau, B. 1987. Absorption and fate of labelled canthaxanthin 15,15′-$^{3}H_2$ in rainbow trout (*Salmo gairdneri* Rich.). Comp. Biochem. Physiol. 87A, 717-720.
Choubert, G., Storebakken, T. 1989. Dose response to astaxanthin and canthaxanthin pigmentation of rainbow trout fed various dietary carotenoid concentrations. Aquaculture 81, 69-77.
Choubert, G., de la Noüe, J., Blanc, J-M. 1991. Apparent digestibility of canthaxanthin in rainbow trout: effect of dietary fat level, antibiotics and number of pyloric caeca. Aquaculture 99, 323-329.
Choubert, G., Blanc, J.-M., Poisson, H. 1998. Effects of dietary keto-carotenoids (canthaxanthin and astaxanthin) on the reproductive performance of female rainbow trout *Oncorhynchus mykiss* (Walbaum). Aquac. Nutr. 4, 249-254.
Clark, R.M., Yao, L., She, L., Furr, H.C. 2000. A comparison of lycopene and astaxanthin absorption from corn oil and olive oil emulsions. Lipids 35, 803-806.
Christiansen, R., and Torrissen, O.J. 1996. Growth and survival of Atlantic salmon, *Salmo salar* L., fed different dietary levels of astaxanthin. Juveniles. Aquac. Nutr. 2, 55-62.
Christiansen R., Torrissen, O.J. 1997. Effects of dietary astaxanthin supplementation on fertilization and egg survival in Atlantic salmon (*Salmo salar* L.). Aquaculture 153, 51-62.
Christiansen R., Lie, Ø., Torrissen, O.J. 1994. Effect of astaxanthin and vitamin A on growth and survival during the first feeding of Atlantic salmon, *Salmo salar* L. Aquac. Fish Man. 25, 903-914.
Christiansen R., Lie, Ø., Torrissen, O.J. 1995a. Growth and survival of Atlantic salmon, *Salmo salar* L., fed different dietary levels of astaxanthin. First feeding fry. Aquac. Nutr. 1, 189-198.
Christiansen, R., Glette, J., Lie, Ø., Torrissen, O.J., Waagbø, R. 1995b. Antioxidant status and immunity in Atlantic salmon, *Salmo salar* L., fed semi-purified diets with and without astaxanthin supplementation. J. Fish Diseases 18, 317-328.
Craik, J.C.A. 1985. Egg quality and egg pigment content in salmonid fishes. Aquaculture 47, 61-88.
Craik, J.C.A., Harvey, S.M. 1986. The carotenoids of eggs of wild and farmed Atlantic salmon and their changes during development to the start of feeding. J. Fish Biol. 29, 549-565.
Deufel, J. 1965. Pigmentieringsversuche mit canthaxanthinbei regenbogenforellen. Arch. Fischereiwiss. 16, 125-132.
Fernández, F., Miquel, A.G., Guinea, J., Martínez, R. 1998. Digestion and digestibility in gilthead sea bream (*Sparus aurata*): the effect of diet composition and ration size. Aquaculture 166, 67-84.
Forsberg, O.I., Guttormsen, A. G. 2006a. A pigmentation model for farmed Atlantic salmon: Nonlinear regression analysis of published experimental data. Aquaculture 253, 415-420.
Forsberg, O.I., Guttormsen, A. G. 2006b. Modelling optimal dietary pigmentation strategies in farmed Atlantic salmon: Application of mixed-integer non-linear mathematical programming techniques. Aquaculture 261, 118-124.
Fraser, P.D., Bramley, P.M. 2004. The biosynthesis and nutritional uses of carotenoids. Prog. Lipid Res. 43, 228-265.
Furr, H. C., Clark, R.M. 1997. Intestinal absorption and tissue distribution of carotenoids. Nutr. Biochem. 8, 364-377.
Goodwin,T.W. 1950. Carotenoids and reproduction. Biol. Rev. Cambr. 25, 391-413.
Guillou, A., Choubert, G., Storebakken, T., de la Noüe, J., Kaushik, S. 1989. Bioconversion pathway of astaxanthin into retinol$_2$ in mature rainbow trout (*Salmo gairdneri* Rich.). Comp. Biochem. Physiol. 94B, 481-485.
Hamre, K., Christiansen, R., Waagbø, R., Maage, A., Torstensen, B.E., Lygren, B., Lie, Ø., Wathne, E., Albrektsen, S. 2004. Antioxidant vitamins, minerals and lipid levels in diets for Atlantic salmon (*Salmo salar*, L.): effects on growth performance and fillet quality. Aquac. Nutr. 10, 113-123.
Henmi, H., Iwata, T., Hata, M., Hata, M. 1987. Studies on the carotenoids in the muscle of salmons I. Intracellular distribution of carotenoids in the muscle. Tohoku J. Agric. Res. 37, 101-111.
Henmi, H., Hata, M., Hata, M., 1989. Astaxanthin and/or canthaxanthin-actomyosin in salmon muscle. Nippon Suisan Gakkaishi 55, 1583-1589.
Jackson, M.J. 1997. The assessment of bioavailability of micronutrients: Introduction. Eur. J. Clin. Nutr. 51, suppl. 1, 1-2.
Lampert, J.M., Holzschuh, J., Hessel, S., Driever, W., Vogt, K., von Lintig, J. 2003. Provitamin A conversion to retinal via the β,β-carotene-15,15′-oxygenase (*bcox*) is essential for pattern formation and differentitation during zebrafish embryogenesis. Development 130, 2173-2186.
Mørkøre, T., Rørvik, K.-A. 2001. Seasonal variations in growth, feed utilisation and product quality of farmed Atlantic salmon (*Salmo salar*) transferred to seawater as 0+ smolts or 1+ smolts. Aquaculture 199, 145-157.

Palace, V.P., Werner, J. 2006. Vitamins A and E in the maternal diet influence egg quality and early life satage development in fish: a review. Sci. Mar., 70S2, 41-57.
Palace, V.P., Khaper, N., Qin, Q., Singal, P.K. 1999. Antioxidant potentials of vitamin A and carotenoids and their relevance to heart disease. Free Radic. Biol. Med. 26, 746-761.
Refstie, S., Sahlström, S., Bråthen, E., Baeverfjord, G., Krogedahl, P. 2005. Lactic acid fermentation eliminates indigestible carbohydrates and antinutritional factors in soybean meal for Atlantic salmon (*Salmo salar*). Aquaculture 246, 331-345.
Regost, C., Jakobsen, J.V., Rørå, A.M.B. 2004. Flesh quality of raw and smoked fillets of Atlantic salmon as influenced by dietary oil sources and frozen storage. Food Res. Int. 37, 259-271.
Riedl, J., Linseisen, J., Hoffman, J., Wolfram, G. 1999. Some dietary fibers reduce the absorption of carotenoids in women. J. Nutr. 129, 2170-2176.
Rørå, A.M.B., Ruyter, B., Skorve, J., Berge, R.K., Slinning, K-E. 2005. Influence of high content of dietary soybean oil quality of large fresh, smoked and frozen Atlantic salmon (*Salmo salar*). Aquac. Int. 13, 217-231.
Schiedt, K. 1998. Absorption and metabolism of carotenoids in birds, fish and crustaceans. In: (Eds.) Carotenoids. Vol. 3 Biosynthesis, Birkhäuser, Basel, pp. 285-358.
Schiedt, K., Leuenberger, F.J., Vecchi, M., Glinz, E. 1985. Absorption, retention, and metabolic transformations of carotenoids in rainbow trout, salmon and chicken. Pure Appl. Chem. 57, 685-692.
Shahidi, F., Metusalach, Brown, J.A. 1998. Carotenoid pigments in seafoods and aquaculture. Crit. Rev. Food Sci. 38, 1-67.
Stahl, W., Sies, H., 2003. Antioxidant activity of carotenoids. Mol. Asp. Med. 24, 345-351.
Steven, D.M. J. 1949. Studies on animal carotenoids. II. Carotenoids in the reproductive cycle of brown trout. J. Exp. Biol. 26, 295-303.
Storebakken, T., Foss, P., Schiedt, K., Austreng, E., Liaen-Jensen, S., Manz, U. 1987. Carotenoids in diets for salmonids IV. Pigmentation of Atlantic salmon with astaxanthin, astaxanthin dipalmitate and canthaxanthin. Aquaculture 65, 279-292.
Storebakken, T., No, H.K. 1992. Pigmentation of rainbow trout. Aquaculture 100, 209-229.
Storebakken, T., Refstie, S., Ruyter, B. 2000. Soy products as fat and protein sources in fish feeds for intensive aquaculture. In: Drackley, J.K. (Ed)., Soy in Animal Nutrition. Federation of Animal Science Societies, pp. 127-170.
Tacon, A.G.J. 1981. Speculative review of possible carotenoid functions in fish. Prog. Fish. Cult. 43, 205-208.
Torrissen, O.J. 1984. Pigmentation of salmonids. Effect of carotenoids in eggs and start-feeding diet on survival and growth rate. Aquaculture 43, 185-193.
Torrissen, O.J., Christiansen, R. 1995. Requirements for carotenoids in fish diets. J. Appl. Ichthyol. 11, 225-230.
Torrissen, O.J., Hardy, R.W., Shearer, K.D. 1989. Pigmentation of salmonids-carotenoid deposition and metabolism. CRC Crit. Rev. Aquat. Sci. 1, 209-225
Torrissen, O.J., Hardy, R.W., Shearer, K.D., Scott, T.M., Stone, F.E. 1990. Effects of dietary canthaxanthin and lipid level on apparent digestibility coefficients for canthaxanthin in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 88, 351-362.
Torrissen, O.J., Christiansen, R., Struksnæs, G., Estermann. R. 1995. Astaxanthin deposition in the flesh of Atlantic salmon, *Salmo salar* L., in relation to dietary astaxanthin concentration and feeding period. Aquac. Nutr. 1, 77-84.
Tveranger, B. 1986. Effect of pigment content in broodstock diet on subsequent fertilization rate, survival and growth rate of of rainbow trout (*Salmo gairdneri*) offspring. Aquaculture 53, 85-93.
Tyssandier, V., Lyan, B., Borel, P. 2001. Main factors governing the transfer of carotenoids from emulsion lipid droplets to micelles. Biochim. Biophys. Acta 1533, 285-292.
Tyssandier, V., Choubert, G., Grolier, P., Borel, P., 2002. Carotenoids, mostly the xanthophylls, exchange between plasma lipoproteins. Int. J. Nutr. Res. 72, 300-308.
Waagbø, R., Sandnes, K., Torrissen, O.J., Sandvin, A., Lie., Ø. 1993. Chemical and sensory evaluation of fillets from Atlantic salmon (*Salmo salar*) fed three levels of N-3 polyunsaturated fatty acids at two levels of vitamin E. Food Chem. 46, 361-366.
Waagbø, R., Hamre, K., Bjerkås, E., Wathne, E., Lie, Ø., Torstensen, B. 2003. Cataract formation in Atlantis salmon, *Salmo salar* L., smolt relative to dietary pro- and antioxidants and lipid level. J. Fish Diseases 26, 213.229.
White, D.A., Page, G.I., Swaile, J., Moody, A.J., Davies, S.J. 2002. Effect of esterification on the absorption of astaxanthin in rainbow trout, *Oncorhynchus mykiss* (Walbaum). Aquac. Res. 33, 343-350.
White, D.A., Ørnsrud, R., Davies, S.J. 2003. Determination of carotenoid and vitamin A concentrations in everted salmonid intestine following exposure to solutions of carotenoid in vitro. Comp. Biochem. Physiol. 136, 683-692.

Ørnsrud, R., Wargelius, A., Sæle, Ø., Pittman K., Waagbø, R. J. 2004. Influence of egg vitamin A status and egg incubation temperature on subsequent development of the early vertebral column in Atlantic salmon fry. J. Fish Biol. 64, 399- 417.

Ytrestøyl, T., Struksnæs, G., Koppe, W., Bjerkeng, B. 2005. Effects of temperature and feed intake on astaxanthin digestibility and metabolism in Atlantic salmon, *Salmo salar*. Comp. Biochem. Physiol. 142B, 445-455.

Ytrestøyl, T., Struksnæs, G., Rørvik, K.-A., Koppe, W., Bjerkeng, B. 2006. Astaxanthin digestibility as affected by ration levels for Atlantic salmon, *Salmo salar*. Aquaculture 261, 215-224.