

# Health effects of functional feeds for farmed salmonids

The project and its report, sponsored by the Norwegian Seafood Research Fund (FHF), aims to summarise and scrutinise the scientific data underlying the current use of specific diets (functional feeds) to support fish health management and disease control in salmonid aquaculture. In addition to a scientific overview of published scientific articles, the report discusses experimental designs and statistical methods used in live fish studies, and provides recommendations on trial setups and statistical methods to document clinical health effects of fish feeds.

#### **Review of published results**

There is a striking abundance of review papers and overview publications (more than 70) covering the health effects of diets, in particular dealing with probiotics (24 papers), followed by various immunostimulants including beta-glucans (21 papers).

Among 152 published articles providing original results from studies of *in vivo* effects derived from nutritional interventions, the majority report indirect outcome measures of health, predominantly immune parameters such non-specific immune responses, immune cell activity, antibody levels, or expression of various immune genes. The number of reports showing beneficial <u>clinical</u> effects, e.g. from cell wall polysaccharides (including beta-glucans) and nucleotides is, however, much lower. Supplementation of feed with probiotics (live bacteria or yeasts) and with vitamins or carotenoids (pigments) has yielded moderate clinical benefits in freshwater reared rainbow trout, but not in marine rearing of salmonids that is predominant in

Norwegian aquaculture. There is a striking paucity of reports from trials with Atlantic salmon, the dominant farmed species among the salmonid fishes.

A summary assessment of the results from the published trials given in tabular format:

Overall evaluation of published evidence for beneficial effects of various feed ingredients for salmonids from controlled trials and field studies

Substance	Summary evaluation
Cell wall polysaccharides (beta-	Beneficial clinical effects on bacterial, viral and ectoparasite
glucans, mannan	infections shown in controlled trials with rainbow trout. Limited
oligosaccharides)	effects on sea lice settlement shown in one Atlantic salmon field
	trial.
Other immunostimulants	Some support for beneficial clinical effects in controlled clinical
	trials in rainbow trout. No reports of clinical effects from the field.
Lipids, bile salts, organic acid	Some support for improved survival or reduced gut pathology from
salts, and others	field trials in Atlantic salmon and rainbow trout. No reports from
	controlled clinical trials.
Nucleotides	Beneficial clinical effects on bacterial, viral and ectoparasitic
	infections shown in controlled trials with Atlantic salmon and
	rainbow trout. No reports of clinical effects from field trials.
Prebiotics	No support for beneficial clinical effects neither from controlled
	clinical trials nor from field trials.
Probiotics	Lactic acid bacteria, Pseudomonas/Aeromonas and Micrococci:
	beneficial clinical effects on bacterial, viral and ectoparasitic
	infections shown in some controlled trials with rainbow trout.
	Yeasts: some support for increased survival from field trials.
Vitamins and carotenoids	Some support for beneficial clinical effects in controlled clinical
	trials in rainbow trout. No support from field trials.

Compared to the relatively widespread use of so-called "health diets" in the farming industry, there are surprisingly few field studies that can confirm the findings done under controlled conditions. The report argues strongly that the direct health benefits that can be obtained by functional feed formulae must be verified in large scale field trials. Quantification and publication of such data are necessary in order to enable autonomous and independent benefit-cost assessments by the aquaculture industry. The number of papers giving results obtained with proprietary ingredients or feed recipes is also rather limited:

Overview of published salmonid trials containing data that show beneficial clinical effects after use of commercially protected feed or ingredient brands (reference to literature list numbers in brackets).

Proprietary brand	Articles reporting beneficial clinical effects
Macrogard (B-1.3/1.6 glucan)	Raa et al. 1992 [103]
	Refstie et al. 2010 [102]
	Sealey et al. 2008 [104]
ProVale (beta-glucan)	Guselle et al. 2010 [96]
Unidentified ingredient cocktail	Xueqin et al. 2012 [204]
Unidentified glucan	Lauridsen and Buchmann 2010 [98]
Ascogen/Optimun (nucleotide mixture)	Burrells et al. 2001 [134]
	Burrells et al. 2001 [135]
	Leonardi et al. 2003 [136]
	Tahmasebi-Koyani et al. 2011 [138]
Rovimix Stay-C (Vitamin C)	Wahli T et al. 1986 [235]
	Verlhaq et al. 1996 [234]
	Wahli, T et al. 1998 [237]
	Verlhaq et al. 1998 [110
	Wahli et al. 2003 [236]
Carophyll (pigment)	Amar et al. 2012 [218]

#### Assessment and recommendations regarding study design

There are significant methodological challenges when studying infectious diseases in groups of animals or fish, and in many of the papers, trial design and statistical methods pay little tribute to how factors other the feed itself (in particular transmission of infection from fish to fish) might affect the outcome parameters.

The report gives several examples of trial setups that are recommended in order to avoid pitfalls in design and statistical analysis of both controlled and field studies. Examples of comparing up to three feed types in the field, that easily can be repeated in parallel sites are provided. One of these examples is visualized in Figure 1.

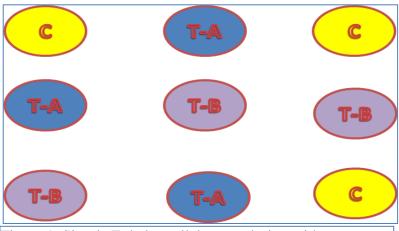


Figure 1. Simple Enkel parallel gorup design with two test feeds (T-A and T-B) and one control feed (C).

Field trials are of particular importance in order to confirm that the benefits observed in smallerscale and well-controlled trials can be reproduced in everyday industrial situations. Above all are trials in several sites needed in order to realistically quantify the magnitude of achievable benefits. In such studies should "commonplace" statistical techniques be abandoned and so-called multilevel regression techniques should be used, allowing the analysis to adjust for environmental and sitespecific factors, and thereby reducing the probability of misinterpreting the results.

In grand summary, the dominant weakness of the current scientific literature on health feeds is the paucity of data showing clear clinical effect, and which magnitude of improvements can be reached in today's industrial salmon farming conditions. Reports on indirect benefits (immunostimulatory and other physiological effects) are more abundant. Field trials with an improved and robust design are needed to significantly demonstrate moderate, but economically important biological effects, and also to prevent from being led astray by single experimental outcomes. The authors and the Norwegian Seafood Industries Research Fund hope that the report will contribute a good background for own judgment by the farming industry's technical specialists and decision makers.

### **Report title:**

Clinical studies on the effects of functional feeds for cultured salmonids; a critical assessment of health indicators, results, experimental designs and statistical methods.

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## **Full project title:**

"Studier av helseeffekter av fôr til oppdrettsfisk. En kritisk gjennomgang av helseindikatorer, forsøksdesign og statistiske metoder."

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