Krill Hydrolysates
….. or Protein Hydrolysates?

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TABLE OF CONTENT

Shall Krill Hydrolysates Be Manufactured Instead of Krill Meals?

This is a question raised by krill operators, incumbents and insurgents.

The market has been elusive accepting krill hydrolysates as a sustainable and long-lasting supply. Beyond price and/or technical matters hindering krill hydrolysates to become part of the "*krill portfolio*", it has shown an unstable supply for the past 3 decades years.

Producers and consumers understanding is strong on krill meals and oils, less on hydrolysates, and advantage for those that master its production and sales.

THAROS' proposal starts suggesting its name to be changed to "***protein hydrolysates***", when primarily used for feed applications. This refers to any process in which the flesh is broken down by protein-digesting enzymes. Those enzymes may be present naturally in the viscera of the material being digested, or purchased from commercially available enzymes, added during the process.

Enzymes digest (process) co-products into valuable food and feed ingredients such as fish and seafood protein extracts (*FPE*s), which consist of a good-quality flavored broth, and a valuable fish oil fraction, as well as a clean bone fraction, suitable for gelatin processing.

The digestion can be stopped at different points sourcing various end products alongside. For example, the flavor of pickled or salt-cured herring comes partly from pickling or salting, but mostly from the enzymes present in the herring flesh, that soften the flesh, adding new flavors. The herring has undergone a partial protein hydrolysis.

The recommended krill digestion goes further and turns krill flesh into a liquid. Some parts of the krill are not digestible, such as the shell, and these are screened out. Proteins are composed of many amino acids chain-linked together. During the digestion process, these chains are broken so that the end product is composed of smaller chains.

Depending upon how many amino acids are left in these chains, they may be called "***dipeptides***", "***tripeptides***", or simply **"*polypeptides***". Some are single "***free***" amino acids. By changing the enzymes used and/or the time allowed for digestion, one can manipulate the amounts of free amino acids.

Which Is the Value of Breaking Down Proteins into Smaller Pieces?

There are 5 main reasons:

1. ***Hydrolysis increases aqua-feed digestibility:*** Enzyme hydrolysis mimics the process that occurs in an animal's stomach. For very young animals, who have immature digestive systems, there is a big advantage in feeds (as well as in foods as a matter of fact) that are partially pre-digested.
2. ***Smaller pieces produced by hydrolysis travel easily in water*:** Proteins this size are better recognized in the water by fish when fed, especially young fish which not always visually recognize feeds. And it stimulates the appetite, equivalent to the aroma on human food.
3. ***Hydrolysis change and intensifies flavors*:** The flavor in foods come from various sources, a relevant one is free amino acids. Intact proteins don't have much flavor, while in smaller proteins and single amino acids, flavor abounds. For example, shellfish natural sweet and distinctive flavor come from one amino acid: glycine. "*Meaty*" flavors are largely due to a different amino acid: glutamine. MSG is a salt of glutamine that adds both saltiness and meat-type flavor to foods. Depending on the degree of digestion, hydrolysis can make a food a little bit tastier (like herring) or it can make flavors so intense that only a very small amount can be added (like MSG). In pet foods, very small amounts of hydrolysates (called "***digests***") are added to make dry foods palatable. Hydrolysates are used at low levels of inclusion to flavor human foods and animal feeds. Because the use content is low, and the value is great, the price can be high.
4. ***High prices***: Depending upon the target market, the degree of digestion and how well controlled the process is, feed-grade hydrolysates prices can be anywhere from half to one fourth the price of standard-quality krill meals, with baby fish feed as an attractive market for hydrolysates. These young fish need *help* to recognize that pellets are food, since they are used to feed on live organisms, and the small molecules that hydrolysates release into the water tells them that this is food and stimulates their appetite. They benefit from the pre-digestion, as well as from the inherent nutritional benefit obtained from the raw material. And, since very small fish require very small amounts of feed, aqua farmers are willing to pay high prices for this specialized feed, a feed that offers remarkable survival and growth benefits.

5. ***The hydrolysis process can be manipulated to manufacture a variety of products:*** The duration of the process, the type of enzymes used, and the process itself (for example which temperatures are used) offer ample possibilities for product diversification, hence, amplify market portfolio, synergy, variation, extrapolation. Hydrolysis therefore empower other fraction extraction yields and quality, such as on **high quality krill oils**.

THAROS' Hydrolysate Concept and History

Starting 1994, we have run several R&D tests following THAROS' solvent-free, low-cost model applied to krill hydrolysis, on-land and at-sea. Our research focused on defining the basic conditions to properly hydrolyze Antarctic krill to obtain a feed concentrate suitable for aqua-feeds, exploring;

a) Whole vs. ground krill
b) Shelled vs. deshelled
c) Various types of exogenous enzymes
d) Individually vs. combined enzymes
e) Time and temperature conditions
f) Evaporation…..etc.

Our research started at a lab scale then on-land pilot-sized, subsequently optimized at-sea using THAROS' pilot plant running on-board factory trawlers, operating in South Antarctica waters. In one of such trials we used a custom-made evaporator to concentrate the krill hydrolysate.

The nutritional composition of the resulting feed-grade krill hydrolysate concentrate showed promising parameters:

	Protein (%)	**Moisture (%)**	**Ash (%)**	**Fat (%)**	**Astaxanthin (ppm)**	**EPA/DHA (%)**
Concentrate Krill Hydrolysate	38,3	40,0	7,7	11,5	100	24/19

Several subsequent trials were conducted on-land and onboard factory vessels using single or combined exogenous food-grade enzymes, such as *proteases, lecitases and betaglucanases* applied to freshly caught raw krill, being our main target the separation of various human-grade end-products e.g. *krill oil,* for animal and human health, and to also manufacture two other fractions:

a) ***Soluble peptide fraction***, as a palatant for feed and/or food applications.
b) ***Insoluble protein fraction,*** for the supplement/nutraceutical markets.

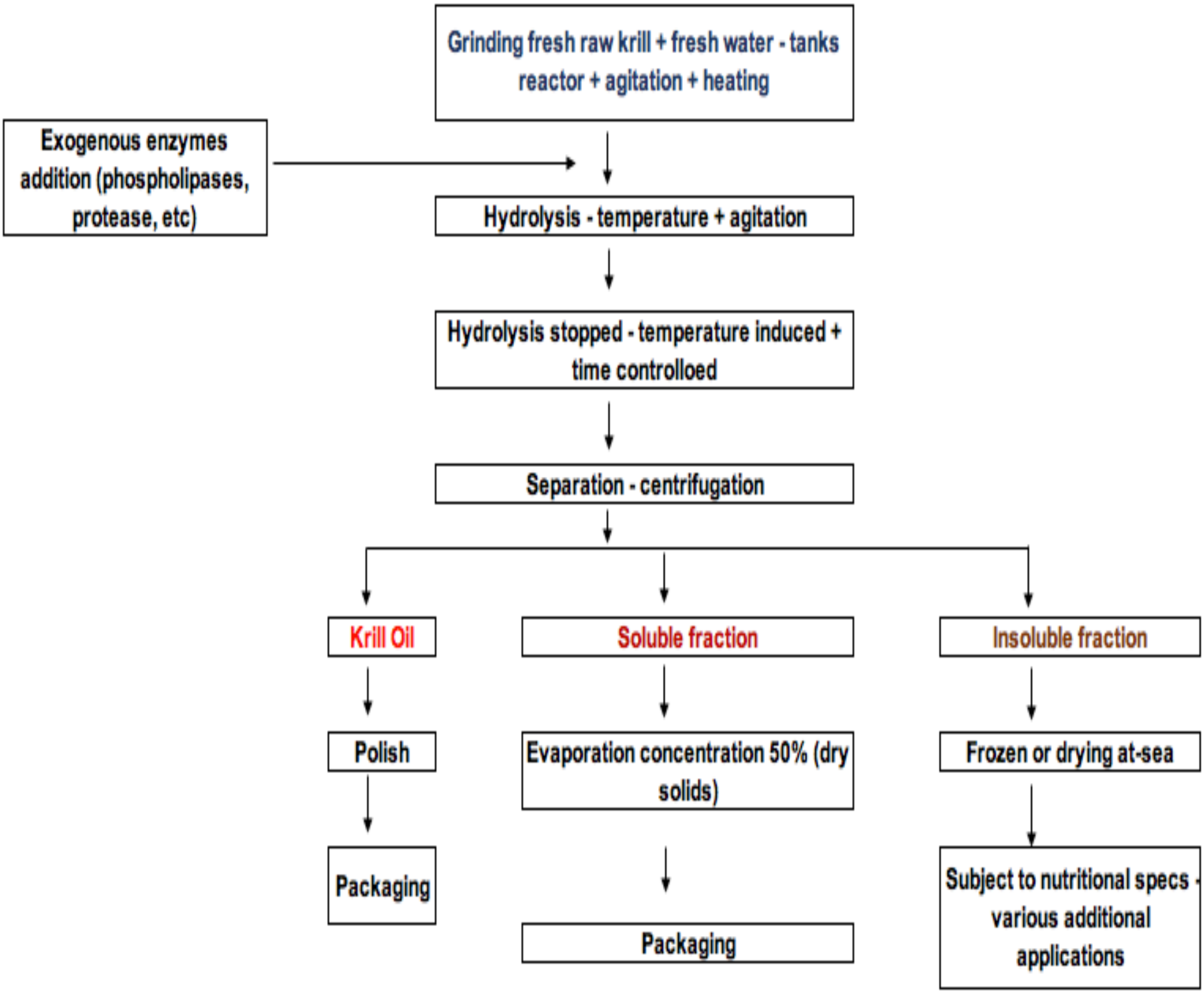
Tests' Primary Outcome:

Better results were achieved regarding krill oil extraction, with an average krill oil extraction yield circa 1.5%.

Aimed at getting the best hydrolysis outcome, our primary results were:

1. Grinding fresh whole raw krill
2. Fresh water added to grinded krill
3. Exogenous enzymes added
4. Hydrolysis temperature @ 55ºC, time @ 20 ~ 30 min
5. Enzyme inactivation @ 90ºC
6. Krill shell separation
7. Oil layer separation and other 2 fractions (soluble peptides and insoluble) using lab centrifuge @ 4 000 rpm for 15 min
8. Soluble fraction was evaporated (using rota-evaporator) to reach a soluble concentrate (moisture of about 50%) for feed or food attractancy.

Krill Hydrolysate Layout



Proximal Composition (%) and Others Analysis

	Protein[a]	Moisture	Ash[a]	Fat[a]	PL[b]	EPA/DHA[c]
Krill oil		<1			36,3	9,6/5,8
Insoluble fraction	66,0	82,8	10,0	30,8		
Soluble fraction evaporated	77,5	56,7	12,4	3,5		

a) Dry Base
b) Total Phospholipids
c) EPA Eicosapentaenoic acid + DHA Docosahexaenoic acid

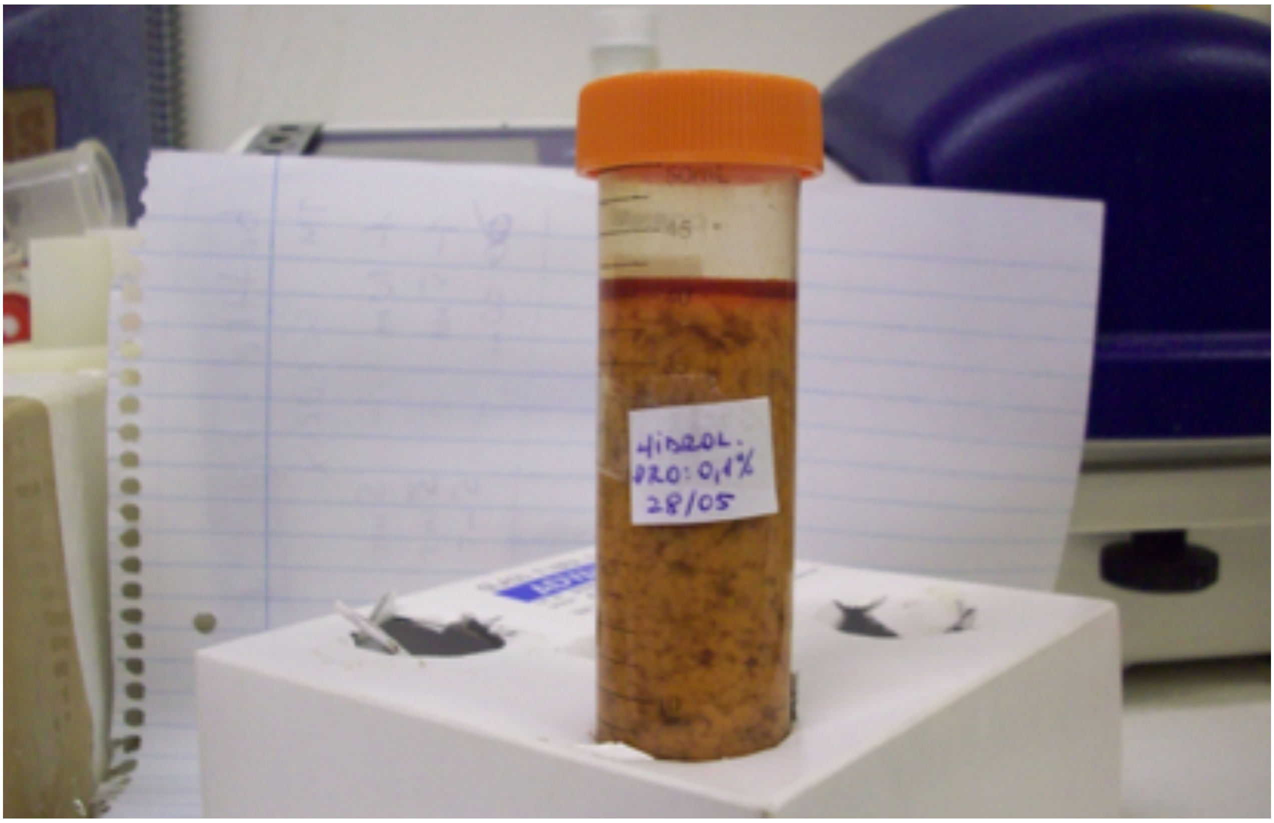
a) ***Soluble fraction*** - Shows a nice, gentle, wholesome sweet-crustacean flavor and odor.

~~b)~~ ***Insoluble fraction*** - Looks like a slurry paste, similar to what has been indicated by some Norwegian krill companies~~.~~

Some hydrolysis tests were performed onboard factory trawlers using ***minced krill,*** where the shell was separated before the hydrolysis. Nonetheless, results were not quite promising as the hydrolysates yield was lower compared to using whole grinded krill.

How a hydrolyzed raw material looks like?

Source: THAROS



Source: THAROS

Not only hydrolysates off the South Antarctic krill fishery has been available. *Euphausia pacifica*-sourced hydrolysates hit the market some years ago with very good market acceptance.

KRILL HYDROLYSATES
PRODUCT SPECIFICATION DRY WEIGHT (TYPICAL)
(*Euphausia pacifica*)

Crude Protein	60% min.
Crude Fat	18% min.
Ash	12% max.
Moisture	6% max.
Fiber	2.5% max.

AMINO ACID PROFILE (% OF DRY WEIGHT)

Threonine	2.75
Serine	2.02
Glutamic acid	7.93
Proline	2.07
Glycine	4.11
Alanine	3.42
Cysteine	1.04
Valine	3.30
Methionine	2.16
Isoleucine	2.80
Aspartic acid	6.17
Leucine	4.47
Tyrosine	2.44
Phenylalanine	2.77
Histidine	1.53
Lysine	6.37
Arginine	4.41
Tryptophan	0.78
Total protein	60.59

Fatty Acid Profile		% of total fatty acids
Myristic	14:0	3.67
Petadecanoic	15:0	0.70
Palmitic	16:0	21.50
Palmitoleic	16:1n7	7.15
Heptadecanoic	17:0	0.40
Stearic	18:0	1.37
Oleic	18:1n9	14.50
Linoleic	18:3n6	2.35
Linolenic	18:3n3	4.27
Arachidonic	20:4n6	1.14
EPA	20:5n3	22.84
DHA	22:6n3	20.01

Natural Astaxanthin pigment levels are as high as 90 - 120 ppm.

Hydrolysates Trials

Freeze-dried, spay-dried and liquid krill hydrolysates have been available throughout the years, sourcing proteins and amino acids, fatty acids, natural antioxidants and natural pigments.

	Highly Digestible Proteins with all Essential Amino Acids	Fully range of Fatty Acids (Hufas's) and Phospholipids	Strong Flavourant and Attractant Components	Astaxanthin and other Pigments	Other Nutrients and Minerals Essential	Moisture	Enzyme Activity	
Freeze -dried Krill Hydrolysate (FD-KH 1)	Yes	Yes	Yes	Yes	Yes	7 - 8%	Yes	
Spray-dried Krill Hydrolysate (SD-KH 1)	Yes	Yes	Yes	Yes	Yes	5 - 7%	No	
Liquid Krill Hydrolysate (L-KH 1)	Yes	Yes	Yes	Yes	Yes	80%	No	

This product was tested in various feeding trials; Sea bass, Sea bream, Rainbow trout flavoring response, Chinook salmon, Eel, Carp, Catfish and several other marine species. In all of them, krill hydrolysates had remarkable good results.

In the Sea bass (*Dicentrarchus labrax*) trial, it replaced 50% of fish meal in a reference larval feed without any loss of nutritional value compared to commercial larval feed (Nippai).

Feed	Mean weight (mg/fish)		Mortality (%)	
	Day 21	Day 42	Day 21	Day 42
Krill hydrolysate feed	89^{a}	516^{a}	19.0	4.0
Nippai feed	91^{a}	412^{b}	17.6	9.7

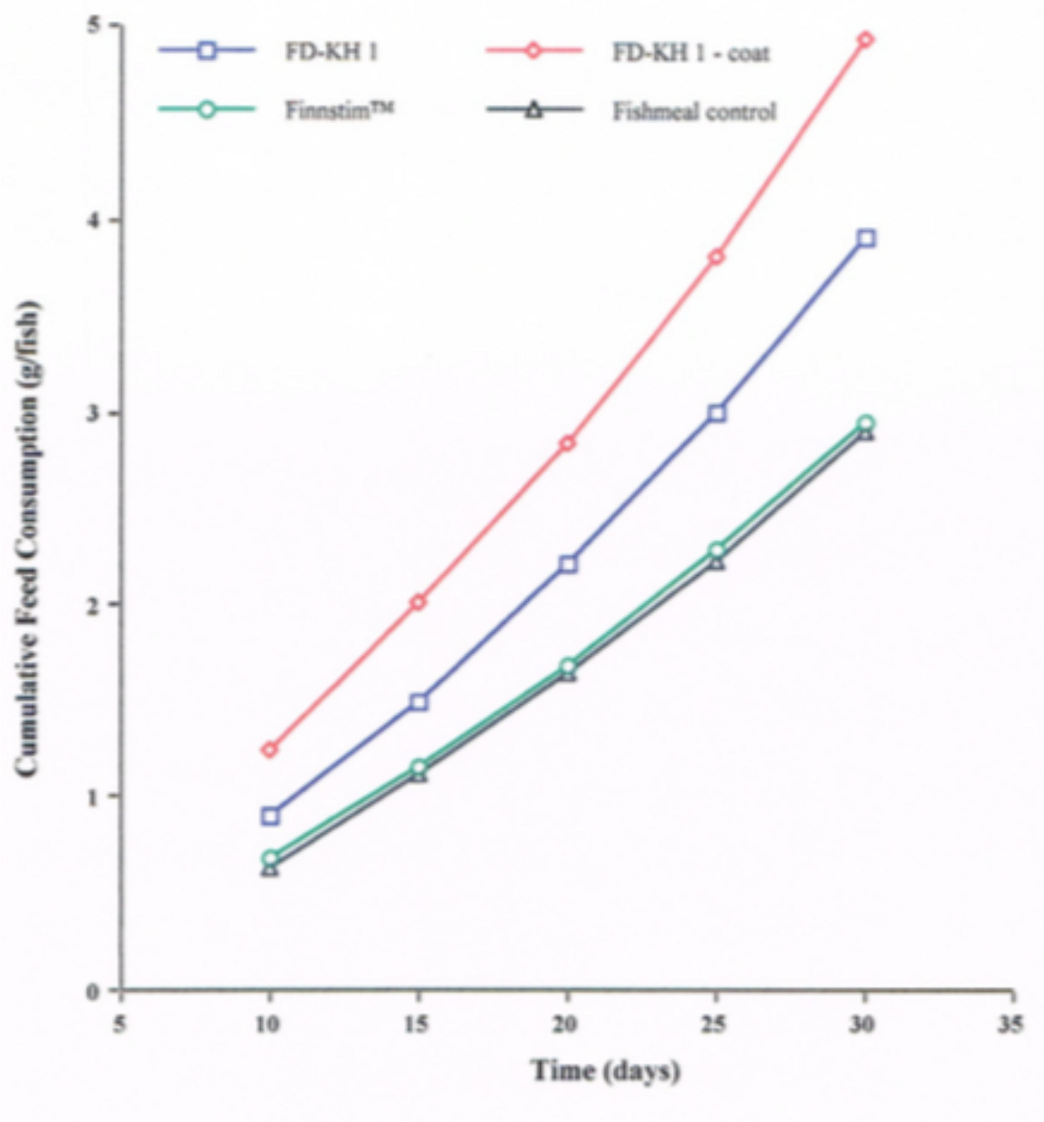
Regarding sea bream (*Sparus aurata*), a growth trial was carried out wether krill hydrolyzate can replace 50% of fish meal and improve weaning results. It resulted in an improved growth of sea bream larvae after 3 weeks, a difference that persisted throughout time.

Feed	Mean weight (mg/fish)		Mortality (%)	
	Day 21	Day 42	Day 21	Day 42
Krill hydrolysate feed	107[a]	505[a]	14.9	5.3
Nippai feed	98[b]	458[b]	15.6	5.7

In a rainbow trout (*Oncorhynchus mykiss*) flavourant response research, trials were carried out to asses the feeding stimulatory effect of krill hydrolyzates on juvenile rainbow trout in fresh water.

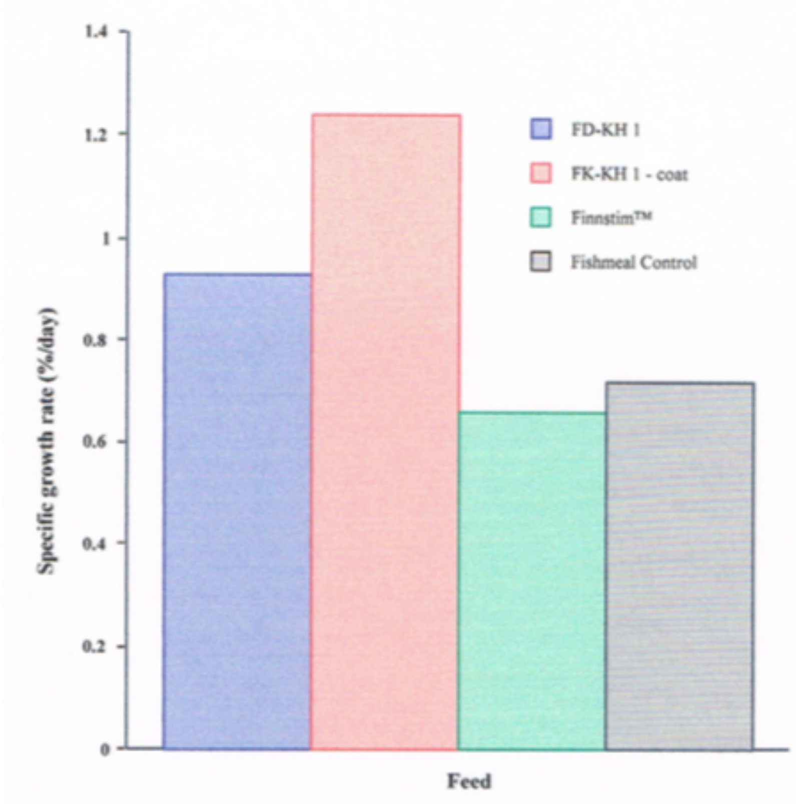
An inclusion of 2% in feeds resulted in an increased feed consumption and subsequent growth relative to fish meal or a commercial flavourant. Applying krill hydrolysate to the exterior of the pellets after pellet manufacturing, it promotes a greater feed consumption and growth than the inclusion into the pellet prior pelleting.

Cumulative feed consumption of rainbow trout fed on fishmeal based control feed, vs. control feed in which 2% of fishmeal was replaced by a commercial flavourant or freeze-dried krill hydrolysate (FD-KH).



Source: THAROS 1995

Specific growth rates of rainbow trout fed on fishmeal based control feed, vs. control feed in which 2% of fishmeal was replaced by a commercial flavourant or freeze-dried krill hydrolysate (FD-KH).



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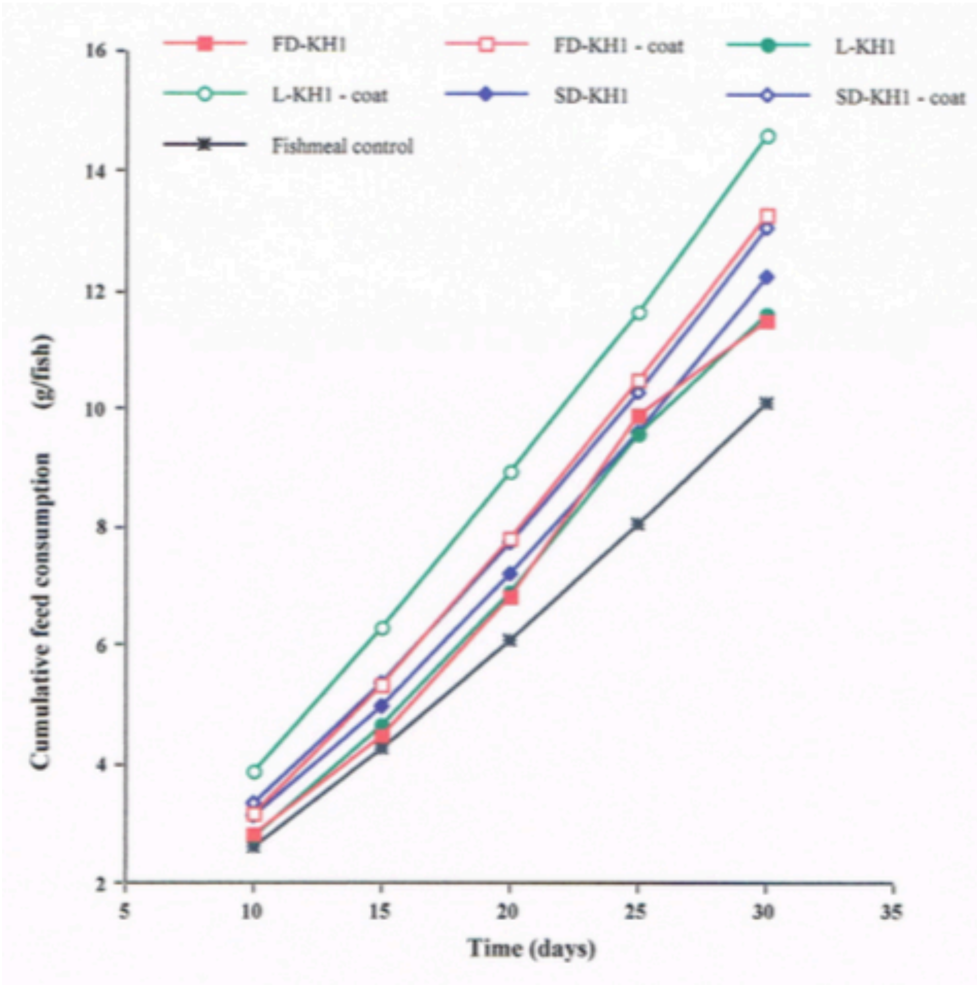
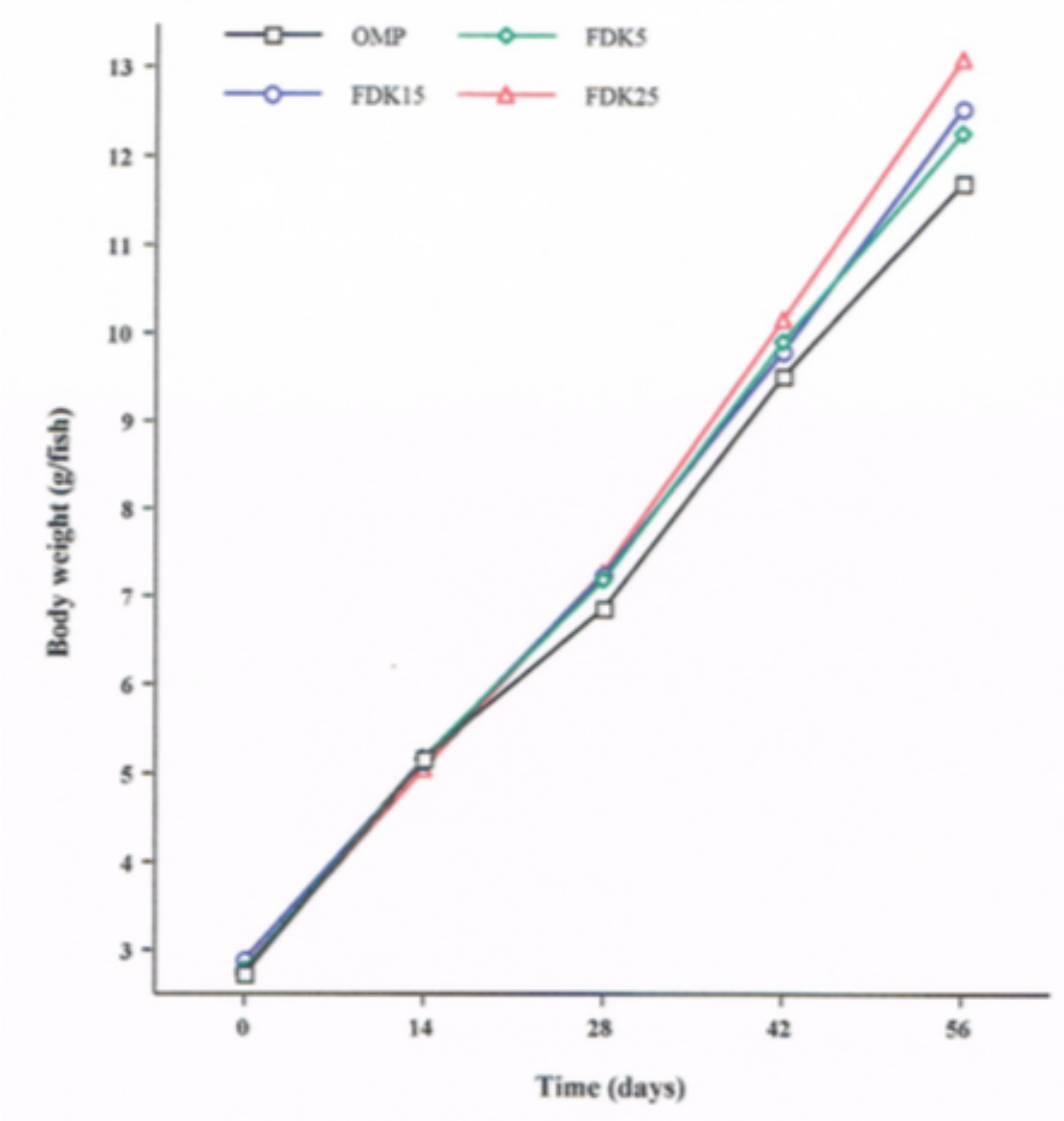


Source: THAROS 1995

Average body weight of chinook salmon (Oncorhynchus tshawytscha) reared on a common hatchery diet (OMP) and three experimental diets containing freeze-dried krill hydrolysate (FD-KH) at the level of 5, 15 and 25% (FDKH5, FDKH15 and FDKH25.)



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