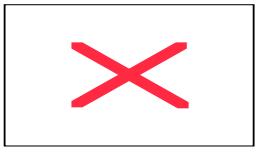
ANTARCTIC KRILL MEAL
ESSENTIAL FEED ADDITIVE IN AQUACULTURE



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Krill is a small, pelagic, shrimp-like crustacean, which is the natural food of myriad of marine species. Antarctic krill, ***Euphasia superba***,Dana is the most abundant species in the Antarctic waters (in the range of 300MM to 500Million tons as per CCAMLR commission database). Despite krill being the one of the most abundant animal creatures in the planet, its industrial usage has been hampered by technological and fishing handicaps. Today however, thanks to advances in both processing and catching techniques, it is possible to make this resource available for the aquafeed industry in the form of **Dried Krill Meal**

Krill meal has shown significant benefits for fish (salmon, red sea bream, yellow tail and shrimp, eel, others) and different species of shrimp. It is an ideal ingredient for the formulation of balanced feeds, because it preserves the main characteristics of krill. Also it is easy to handle and to incorporate during feed manufacture

Krill meal is much more than a rich source of essential aminoacids and polyunsaturated fatty acids It is also characterized as a source of (a) nitrogenous substances of powerful attractant features; (b) natural pigments; (c) micronutrients, and (d) growth enhancers, all of which provide krill meal with a high biological value and high palatability, making it the natural complement for less palatant and cost effective feed formulations.

Additionally, krill is processed at sea from whole krill on board factory vessel operating on fishing grounds in the South Atlantic waters (Antarctic Ocean within Antarctic Convergence area). The freshness of the raw material is thus assured by the immediate processing of a raw material caught in very low temperature waters of pristine purity. The product, krill meal, is therefore free of the chemical and biological pollution that affects the dried meal produced from other crustaceans. There are hardly any amount of Dioxins, PCB's and heavy metals, coincident with no human-made pollutants in the area.

Tharos has a team of engineers and technicians of first level, with several decades of krill processing knowledge and expertise who supervise production on board the factory vessel, which allow us to offer products of optimum quality and enough flexibility to rapidly respond to the needs of the customer.

Krill Meal processed under Tharos protocols offer the following benefits to the aquafeed industry:

- **Palatability**
- **Osmoregulation**
- **Minerals**
- **Phospholipids**
- **Polyunsaturated fatty Acids**
- **Chitin**
- **Essential Aminoacids**
- **Natural pigments (in the form of Astaxanthin)**

Tharos' Krill Meal as Palatant

Both fish and crustaceans live immerse in an aquatic environment, where their chemioperceptive system receives permanent stimulation by soluble compounds. These substances are usually of low molecular weight, non-volatile and most of them contain nitrogen (amino acids, nucleotides, etc.). These components stimulate the chemioperception of the animal (taste and smell), and its behavior (feeding behavior and feed intake).

Krill is natural bait for many species of fish and crustacean; for example, it is one of the main sources of food of wild salmon. Thanks to a slight processing intervention of its production process, **Tharos'** technology allows krill meals' preserve all the properties of raw krill. It is being used in the aquaculture of salmon, shrimp, sea bream and yellow tail to provide higher palatability to the commercial feeds, to mask antibiotic taste, and to provide better acceptability to less palatatant cost effective diets. **Tharos'** drying technology, unique in the krill industry, allows a sound and gentle treatment of the raw material. All of **Tharos'** krill meals are produced on human grade standard equipment (Stainless Steel human FDA grade standard).

The feature of palatability in krill meal has been found to rest in the presence of certain amino acids that stimulate the smell and taste, and in the existence of glycogenic amino acids that are appetite stimulants. Furthermore, Krill meal also features other substances of low molecular weight that are reported to have similar effects as palatants, such as TMAO (Trimethyl amine Oxide).

References:

- ***Supplemental effect of the whole body krill meal and non-muscle krill meal of Euphausia superba in fish diet Allahpichay and Shimizu (1984) Bull. Jpn. Sci Fish. 50:815-820***

Proves that the feeding behavior of several species (sea bream, Japanese eels, black sea breams y yellow tail) is stimulated when Antarctic krill meal is added to the feed.

- ***Feeding stimulation in sea bream, Pagrus major, fed diets supplemented with Antarctic krill meals Shimizu, et al (1990) Aquaculture 87:43-53***

Through the use of electrophysiological tests it proves that Krill Meal extracts stimulate the smell response of sea bream. It was also found that the non-protein portion of the meal contains smell and taste stimulants, and specific aminoacids where identified which probably responsible for this effect.

- ***Food Preference of P. vannamei. Ogle and Beaugz (1991). Gulf Research Reports 8:291-294***

It shows that *P. vannamei* has a strong preference for feed, which includes krill. The study included the use of 16 ingredients usually incorporated in commercial feed for brood stock. Of all these ingredients, krill was only outperformed by artemia.

Krill Meal and Osmoregulation

One of the most frequent uses of krill meal is in special diets, when salmonids are transferred from fresh to seawater. This is due to the osmoregulating properties of krill meal. Krill meal is rich in trimethylamine oxide (TMAO) which is a known osmoregulator.

The high salt content of the environment represents a strong physiological challenge for fish and crustaceans. They need to keep their homodynamic and a proper physiological status. This is particularly important in salmonids farmed in captivity, which are transported from fresh to seawater in a very short span.

Low molecular weight and quaternaries ammonia compounds such as trimethylamine (TMAO) are known osmoregulators.

Table N°1: Comparative levels of Trimethylamine Oxide:

	TMAO (MgN/100 sample)
Krill Meal	190
Fish Meal	<10
Crustacean Meal	20 - 50

References

Non-protein Nitrogen Compounds in Fish and Shell fish De G. Finne (Review) Advances in Sea Food Biochemistry (1992).
G. Flick pg 393

Krill Meal as a source of minerals

Krill meal has a low content of ash making it ideal for fresh water feeds. Its lower content of phosphorus compared than other marine based meals such as shrimp or brown fish meal. Its calcium/phosphorus ratio is in the vicinity of 1,5:1 that favors the absorption of both minerals, and it is significantly lower than in fish or crustacean meals (3:1 or 4:1)

Krill meal is a rich source of bio-available minerals.

Minerals are nutrients that perform essential biological functions; as enzymatic cofactors and as regulators of the immune system. The degree of bioavailability of minerals will have a significant effect on the physiological and immunological fitness of the animal. Organic minerals are more bio-available than inorganic ones since the first are chelated with aminoacids, peptides or proteins that avoid antagonism in their active transport.

Of all the minerals present in krill meal some deserve special mention Copper, which in krill meal is concentrated at a rate ten times higher than in fish meal, is found chelated with residues of lysine amino acid and has an important role in the formation of collagen and in the integrity of fin and skin. Selenium, which is also more highly concentrated in krill meal than in fishmeal, is an important player in the cellular antioxidant systems (glutathione system).

Table N°2: Comparative levels of minerals

Minerals	Krill Meal	Fish Meal
Cupper (ppm)	101	11
Zinc (ppm)	72	111
Selenium (ppm)	12	1
Calcium	1.74%	4.40%
Phosphorous	1.25%	2.60%

References:

- ***Comparative net absorption of chelated and inorganic trace minerals in channel cattfish /cta/urus punctatus diets. Paripatananont and Lovell, 1997. Journal of the World Aquaculture Society, 28:62-67***

This study shows that, in the case of catfish, the net absorption of chelated minerals (copper proteins, iron, manganese, selenium, **and** zinc) is higher than that of non-chelated forms (sulfates and selenates)

- ***The effect of dietary modification on fin quality of Erwin strain rainbow trout. Barrows and Lellis Review for U.S. fish and Wildlife Service, Bozeman fish Technology Center.***

One of the most common failures in farmed fish is the low quality of fins. Barrows and his associates have been the first in demonstrate the relevance of feed quality in skin integrity. They have shown that minerals in krill meal have a positive effect in the prevention of erosion in the fins of Trout. They suggest that the cause of this effect lies in the copper content in krill meal, since this mineral is essential in the performance of enzymes specialized in the cross linking of collagen. Distinctively, mineral pre-mixes are normally supplemented to feed where copper is added in the form of copper sulfate, copper in krill meal would be highly bio-available.

Krill meal as a source of lipids

Fatty Acids

Krill meal has a lipid content over 15%, characterized by its marine fatty acid profile rich in n-3 fatty acid, where the fatty acids EPA and DHA are over 20% of the total fatty acids.

Phospholipids: Phosphatidyl Choline.

Krill meal has a high content of phospholipids, which are as abundant as its triglicerides content. Over 25% of the phospholipids are in the form of choline esters, specifically as phosphatidyl choline. Choline has to be supplied to most of the aquafeed mainly in the esterified form that is better absorbed.

Cholesterol:

Krill meal is a rich source of cholesterol (3-8% of the fat). Cholesterol is an essential **ingredient in the diets of crustaceans.**

Table N°3: Lipids in krill meal

Lipid	**Average (% Of Lipids)**
Phosphatidyl choline	22 – 28
Phopholipids	35 – 45
Triglicerides	30 – 40
Cholesterol	3 - 8

References:

- ***Effect of polar and nonpolar lipids from Krill on quality of eggs of red Sea bream Pagrus major Watanabe, T et al (1991) Nippon Suisan Gakkaishi 57695-698.***

Previously, the authors reported the effect of frozen krill to improve the quality of sea bream eggs. Here, for frozen krill, polar and non-polar lipids were compared to vitamin E and traditional diets for brood stocks. The results clearly show the positive effect of krill and its components. It is argued that the components of krill that improve the quality of red sea bream eggs are the phosphatidyl choline and the asthaxantin since both would behave as scavengers of free radicals

- ***Effect of Broodstock diet of European Sea Bass (M Bruce Institute of Aquaculture, University of Stirling, UK). EEC Project (1996).***

Sea bass brood stocks were fed with three different diets, one of which contained krill meal. The krill meal fed groups showed consistently better growth performance and larger number of spawning batches. The egg quality of the group that received krill meal ingredient was better than the traditional diet (fish meal).

Chitin/Chitosan as immunostimulant

Chitin is natural component of several crustaceans an insect carapace. It is an energy source for several species that can degrade through special enzymes.

Disease control, prophylaxis and eradication are based on the use of vaccines and chemotherapeutical substances. But there are a number of pathologies that have no known cure. There are, for example, the case of *Piscirickettsiosis* in Chilean salmon industry and the disease that massively attack shrimp industry around the world, against which there is no known specific prevention or cure.

One of the prophylactic alternatives available is the use of agents that stimulate the immunological system of the animal. Among these agents there are the complex polysaccharides such as chitosans. Chitosan is a derivative of chitin, which is found in krill meal. Chitosan has been found to be an immunostimulant in salmon and trout when administered either orally (in the feed) or injected. Krill meal is a source of chitin. Krill meal is now being tested as a stimulant of the immune system for several species.

References:

- ***Dietary intake of immunostimulant by rainbow trout affects non-specific immunity and protection against furunculosis Siwicki AK, Anderson DP and Rumsey GL (1994) Veterinary immunology and immunopathology. 41:125- 139.***

It reports a significant increase in the specific immune system of salmon and trout. It shows a protection against furunculosis when chitosan is used in the diet.

- ***Duration of protection against Aeromona salmonicida in brook trout immunostimulated with glucan or chitosan by injection or immersion. Anderson DP and Siwicki A.K (1994) Progressive Fish-culturist, 56 (4)258-261.***

It shows that fish mortality due to *Aeromona Salmonicida* can be reduced by 50% when using injected chitosan or when it is applied in a bath by immersion.
Chitin and Chitosan sources, chemistry, biochemistry physical properties and applications G Skjak - Brack. and Paul Sanford, T Anthonsen (1988) pp 243-253, pp 269-279, pp 299-309.

Other works:

Effect of Growth Performance for P vannamei. (1997).

The addition of krill meal in experimental diets has significantly increased the growth rate of shrimp *P. vannamei. Krill meal* was added in the same proportion as fish meal was subtracted from the diet

Krill meal as a source of natural pigments (carotenoids)

Krill meal is a source of natural carotenoids. Over 95% of the pigments present in krill meal are in the form of asthaxantin, which is the only type of pigment that fixes onto the flesh of salmon when krill meal is used in the diet and the flesh color is the same that it is found in wild salmon (Hue). Pigments in krill meal are esterified giving to them more stability.

Asthaxantine is not only a pigment, but also acts as a photo protector and antioxidant. Furthermore, it has been proved that asthaxantin has an effect in higher growth rates and immunomodulation of both fish and shrimp. Finally, asthaxantin has been shown to positively influence survival rates in shrimp

References:

- ***Nutrition & Disease Shrimp: Role of Vitamins and asthaxantin Kurmaly (1996) Roche Aquaculture Center Proceedings of Feed Ingredients Asia 1995 (Review).***

Pigmentation of cultured yellowtail with krill oil Fujita *et al.* (1983). Bulletin of Japanese Soc. of Sci Fisheries 49: 1595-1600.

Based on the popular Japanese belief that krill meal is a pigment source for yellow tail, the researchers studied the effect of krill oil (1000 ppm) on the pigmentation of yellow tail. In this species the typical flesh color (green blue with a yellow line on side) is given by different pigments (tunaxantin). The study proved that yellow tail transforms the asthaxantine in krill into tunaxantine.

BIOCHEMICAL ANALYSIS OF KRILL MEAL

PROXIMATE SPECIFICATIONS

Protein % Min	58
Lipids % Max	18
Moisture % Max	10
Ash % Max	13
Carbohydrates (Chitin) Range	2-4
Antioxidant ppm Min	100
Carotenoid Pigment (As astaxantin) (ppm)	100 -200

ESSENTIAL AMINOACIDS PROFILE

AMINOACID	AVERAGE (% OF SAMPLE)
Aspartic Acid	9.5
Threoinine	3.9
Serine	3.2
Glutamic Acid	12.6
Proline	5.2
Glycine	5.8
Alanine	5.8
Cysteine	1.2
Valine	5.7
Methionine	2.5
Isoleucine	5.4
Leucine	7.3
Tyrosine	2.7
Phenylalanine	4.9
Histidine	1.4
Lysine	4.6
Arginine	4.6