ARRAINA

Advanced Research Initiatives for Nutrition & Aquaculture

FEED INGREDIENTS IN AQUACULTURE

TECHNICAL BOOKLET

A DATABASE OF AQUACULTURE FEED INGREDIENTS

Technical content prepared by SPAROS Lda. with contributions from other ARRAINA project partners.

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A database of aquaculture feed ingredients covering the nutrient composition, functional properties and their effects on feed processing conditions and physical quality of extruded pellets

FEED INGREDIENTS IN AQUACULTURE

INTRODUCTION

About This Booklet

This booklet is part of a series of three technical booklets on the nutrition and feed of fish farmed in Europe produced under the framework of the EU FP7 project **ARRAINA (Advanced Research Initiatives for Nutrition & Aquaculture)**. The aim is to make these booklets widely available to both targeted stakeholders and society in general, in order to raise awareness on the science-based knowledge supporting the development of high-quality, safe and environmentally sustainable aquaculture feeds.

This booklet is specifically aimed at **aquaculture feed producers**, but also targets other industrial segments (e.g. fish farmers, feed additives companies, retailers) and individuals interested in gaining further knowledge on the raw materials that are currently used in the feeds of farmed fish. It is composed of a database of key ingredients used in aquaculture feeds and focuses on their nutrient composition, functional properties and their effects on feed processing conditions and physical quality of extruded pellets.

The inspiration behind the development of this booklet was to provide a user friendly resource for aquaculture feed producers which would allow them to understand at a glance the different properties of specific feed ingredients for aquaculture. The information provided in this booklet could serve as common ground for more clear and transparent communication across the aquaculture production chain (farmer, feed producer/retail) and address some unfounded fears and barriers.

Two further technical booklets are planned to be produced within the timeframe of the project. The second of the series will focus on the nutrient requirements of Atlantic salmon, rainbow trout, carp, sea bass and sea bream. The third and final technical booklet will showcase a new integrative tool to predict the effects of alternative aquaculture feeds on fish farmed with known levels of contaminants in relation to food safety by testing the flesh.

About Aquaculture Feeds in Europe

Today's fish feeds are produced from a large variety of ingredients. These have different nutritional and physical properties. During feed production, the physical and functional characteristics of ingredients can cause considerable variation in the quality of finished feeds. Sustainable fish feeds, manufactured based on solid scientific knowledge and reliable raw materials, can contribute to ensure aquaculture is a very efficient, environmentally sustainable, and fish welfare friendly industry that produces highly nutritious quality food for humans.

The production of high-quality aquaculture feeds starts with the selection of high quality ingredients, and therefore it is important to understand what "quality" implies in aquaculture feed manufacturing. A quality ingredient can be one that

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contributes to the nutritional value of the feed (such as its profile in terms of amino acids, fatty acids, vitamins and minerals, its digestibility and palatability which impact feed efficiency), or one that improves the physical integrity of the feed (such as pellet hardness, durability, binding, starch gelatinisation, density, oil absorption capacity and ultimately the pellet water stability).

While information on the nutritional value of a given ingredient is available [1, 2, 3, 4, 5], the contribution of individual ingredients to the physical properties of fish pellets is less well known. This is particularly true for plant ingredients. One of the aims of the **ARRAINA** project is to evaluate the contribution of plant raw materials to the physical properties of produced pellets, in particular when plant-protein rich formulations are used.

About the ARRAINA Project

Traditionally, fish feeds for cultured fish are based on fishmeal and fish oil derived from capture fisheries. However, there is increasing pressure on this raw material due to growing demands from a variety of users including the expanding aquaculture sector and the human health market (e.g. fish oil food supplements). Hence the sustainability and competiveness of aquaculture may depend on the replacement of fishmeal and fish oil with alternative ingredients such as plant-based feeds. **ARRAINA** has been responding to this need by measuring the long-term effects these changes in diet will have on the full life cycle of fish for which presently little is known.

By developing applied tools and solutions of technological interest to the European fish feed industry, in collaboration with SMEs, **ARRAINA** will further strengthen the links between the scientific community and the EU feed industry and will contribute to increasing the productivity and performance of the aquaculture sector leading to competitive advantage of the whole sector at a global level.

For general information on the project, the project factsheet can be downloaded from the **ARRAINA** website, **www.arraina.eu** as a PDF. For more information please contact the **ARRAINA** Project Coordinator, Sadasivam Kaushik (kaushik@st-pee.inra.fr) or the Project Manager, Heloise Simonson (Heloise.Simonson@paris.inra.fr).

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[1] NRC (2011) Nutrient Requirements of Fish and Shrimp. National Research Council, The National Academies Press, Washington, D.C.

[2] Feedipedia: an open access information system on animal feed resources (http://www.feedipedia.org/).

[3] Tacon, A.G.J.; Metian, M.; Hasan, M.R. (2009). Feed ingredients and fertilizers for farmed aquatic animals: sources and composition. FAO Fisheries and Aquaculture Technical Paper. No.540. Rome, FAO. 209p.

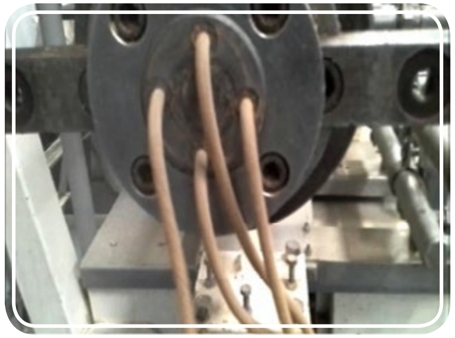
[4] Hertrampf, J. W. ; Piedad-Pascual, F., 2000. Handbook on ingredients for aquaculture feeds. Kluwer Academic Publishers, 624 pp.

[5] Sauvant D., Perez J.M., Tran G., 2004. Tables of composition and nutritive value of feed materials : Pigs, poultry, cattle, sheep, goats, rabbits, horses, fish., INRA Editions Versailles, 304p.

MANAGING THE PHYSICAL QUALITY OF EXTRUDED FISH PELLETS

Extrusion cooking is now the preferred technology to manufacture high-quality fish feeds. Extrusion processing is a baro-thermo-mechanical treatment by which moistened, expansible, starch and/or proteinaceous materials are plasticised and cooked in a tubular structure by a combination of moisture, pressure, temperature and mechanical shear. Feed pellets are shaped when the dough is forced through a die opening at the extruder outlet.

Various reactions take place during extrusion cooking: starch gelatinisation, protein denaturation, hydration, expansion, and thermal destruction of microorganisms and other anti-nutritional compounds.



The complexity of the extrusion process, and limitations in basic knowledge of protein properties and protein/starch-interaction, make it a demanding task to obtain consistent product quality based on different types of raw materials. Physical quality of extruded pellets is affected by several variables, among which formulation and extruder parameters are recognised as having great influence (Figure 1).

Extrusion technology is an efficient feed manufacturing process and plays an essential role in ensuring competitiveness of the European aquaculture industry. Advantages of extruded aquaculture feeds include enhanced feed efficiency, a better control of pellet density and water stability, all aspects that contribute towards a reduction of feed wastes and a minimisation of the environmental impact of farming systems.

Figure 1: Affects of formulation and extrusion parameters on physical quality of extruded pellets

Raw Materials
Chemical composition
Functional properties
- Protein
- Starch
- Fat
Particle size
Bulk Density

Process Variables
Conditioning
- Steam
- Residence time
Mash feeding rate
Torque speed
Screw configuration
Drying

Functional Changes
Starch swelling and gelatinisation
Protein denaturation

↓

Physical Quality
Durability & Hardness
Post-extrusion oil absorption capacity
Buoyancy control

PELLET QUALITY ASSESSMENT CRITERIA

Bulk Density: is defined as the pellet weight per unit of volume. Bulk density can change depending on how the material is handled (e.g. particle size). The bulk density of pellets can be used as an indicator of floatability/sinking speed in the water column in order to comply with the feeding behaviour of a given species or farming system.

Starch Gelatinisation: changes in the crystalline structure of native starch granules, which in the presence of heat and moisture results in irreversible modifications of swelling and viscoelastic properties. The high degree of starch gelatinisation (>90%) that takes place during extrusion cooking has been associated to enhanced pellet durability and nutrient digestibility.

Pellet Durability: is the amount of fines (fraction of feed pellets smaller than the specified minimum size) produced from pellets subjected to mechanical or pneumatic agitation. Low durability is associated with high levels of fines and results in feed wastage and sub-optimal feeding.

Pellet Hardness: is the maximum force needed to break a pellet. High hardness is beneficial to avoid the generation of fines during bulk transport and in pneumatic feed delivery systems.

Pellet Expansion Ratio: is the relative differential between the measured diameter of the pellets and the extruder die diameter. The expansion ratio is controlled by a combination of the amount of energy supplied to the mass via the extruder in the form of heat from steam and friction, and the viscoelastic properties of the mash. Control of the pellet expansion ratio allows the modulation of the post-extrusion oil absorption capacity and the fine tuning of the sinking speed in the water column.

Pellet Water Stability: changes on the physical integrity of pellets and nutrient leaching after water immersion.

MARINE PROTEIN SOURCES

Fishmeal has always played an important role in aquaculture feeds due to an ideal amino acid supply and high palatability for most aquatic species. Fishmeal is generally manufactured from wild-caught, small marine fish (e.g. anchovy, horse mackerel, menhaden, capelin and sandeel), usually deemed not suitable for direct human consumption. Trimmings and processed by-products (e.g. from fish filleting and cannery operations) are also a growing fraction of the raw material used for fishmeal production.

There was a general concern that aquaculture growth would contribute to the over-exploitation of certain types of wild-fisheries. However, time-series data show that there has been no upward trend in the catch of fish for feed. Major developments in fish feed formulations and the adoption of stringent measures to ensure that the feed-grade fisheries respect various sustainability criteria, allows aquaculture to contribute significantly to global seafood supply, without compromising marine fishery resources. Fishmeal and other high quality marine-derived protein sources (such as squid meal and krill meal) are becoming less of a bulk feedstuff and more of a strategic ingredient used at production stages (e.g. larvae, young juveniles and broodstock feeds) where their unique nutritional properties can give the best results and where price is less critical.

Fishmeal and other processed fishery by-product protein sources are an excellent source of highly digestible protein, long chain omega-3 fatty acids (eicosapentaenoic acid (EPA) and docosaheaxenoic (DHA)) and essential vitamins and minerals. Fishmeal quality and specifications are highly dependent on the raw material used and the processing methods involved.

Table 1. Overview of Nutritional Composition of Marine Protein Sources

As fed basis	Unit	Fishmeal LT 70	Fishmeal 60	Fish protein concentrate	Squid meal	Krill meal
Moisture	%	7 - 10	6 - 9	3 - 5	8 - 10	7 - 9
Crude Protein	%	69 - 72	57 - 64	80 - 85	78 - 80	58 - 62
Lipid	%	10 - 12	7 - 9	6 - 12	2 - 3	16 - 20
Ash	%	9 - 12	15 - 25	4 - 8	3 - 4	9 - 11
Gross Energy	MJ/kg	20.0 - 20.5	18.8 - 19.3	21.0 - 21.5	21.0 - 21.4	22.3 - 22.8
Essential AA						
Arginine	%	3.7	3.7	7.8	7.9	3.8
Histidine	%	1.4	1.4	1.8	2.0	1.4
Isoleucine	%	2.5	2.5	3.4	4.1	2.8
Leucine	%	4.5	4.3	5.8	6.9	4.7
Lysine	%	4.7	4.5	7.0	8.2	4.9
Threonine	%	2.5	2.5	3.5	4.0	2.7
Tryptophan	%	0.7	0.6	0.8	0.9	0.5
Valine	%	2.7	2.9	4.1	4.2	3.4
Methionine	%	1.8	1.6	2.7	2.7	1.6
Cysteine	%	0.4	0.5	0.4	0.4	0.7
Phenylalanine	%	2.4	2.3	3.9	3.7	2.5
Tyrosine	%	1.9	1.9	2.8	2.3	2.7
Non Essential AA						
Alanine	%	3.9	3.8	5.4	4.6	3.5
Aspartic acid	%	5.6	5.5	6.5	8.1	5.7
Glutamic acid	%	8.1	7.6	10.0	12.6	7.6
Glycine	%	3.8	3.8	9.4	4.4	2.9
Proline	%	2.4	2.5	5.2	3.6	2.4
Serine	%	2.6	2.3	3.8	4.0	2.7
Other valuable nutrients						
n-3 HUFAs (EPA+DHA)	%	2.0	1.8	0.6	1.7	3.0
Total phospholipids	%	2.6	2.5	0.8	1.4	8.1
Taurine*	%	0.3	0.4	0.5	0.1	0.5 - 0.7
Astaxanthin	mg/kg	-	-	-	-	90

Values presented were compiled from various literature sources[1-5] and completed with internal analytical data from SPAROS. Values are indicative only and do not exempt users from specific analysis of their ingredients.
*Taurine or 2-aminoethanesulfonic acid is not an amino acid, as it lacks a carboxyl group. The extent of its nutritional relevancy in fish is still under debate, but several studies demonstrate its importance while replacing fishmeal by plant protein sources.

Table 2. Functional and Technological Properties of Marine Protein Sources on Extruded Fish Feeds

	Fishmeal LT 70	Fishmeal 60	Fish protein concentrate	Squid meal	Krill meal
Functional role					
Feed Palatability	++	++	+++	+++	+++
n-3 LC PUFA & Phospholipids supply	+	+	+	-	++
Oxidative potential	-	-	-	-	-
Impact on physical pellet properties					
Bulk density (kg/m³)	480-570	510-600	402	640	325
Pellet Binding	+	-	++	-	-
Starch gelatinisation	+	-	++	-	-
Pellet durability	+	-	++	-	-

Minor detrimental or no effect (-)/Minor beneficial effect (+)/Moderate beneficial effect (++)/Major beneficial effect (+++). Effects of each ingredient on the physical pellet properties of complete feeds are highly dependent on incorporation levels and remaining formulation.

Findings:

- High quality fishmeal (LT-type) and fish protein concentrate have positive effects on binding, starch gelatinisation and pellet durability. When they are replaced by lower quality fishmeal these effects seem to disappear. The various quality grades of fishmeal are produced by heat coagulation combined with mechanical and thermal dewatering process. Changes in the raw material type (i.e. fish species), freshness and drying conditions affect their functional properties (e.g. viscosity, water holding capacity) and consequently the binding properties among fishmeals.
- Pre-processed proteins with enhanced levels of small peptides and amino acids (e.g. protein concentrates or hydrolisates) may have an additive plasticising effect together with moisture and may also lead to higher starch gelatinisation[6].
- At high inclusion levels, the relatively high fat content of krill meal tends to lubricate the feed mash inside the extruder and makes it difficult to impart mechanical energy into the products. Moreover, fat molecules may coat the starch granules and do not allow an effective hydration and cooking of native starch.
- Detrimental changes in pellet binding properties due to an increased use of lower quality fishmeal may be compensated by the inclusion of raw materials from plant origin, or processed hemo-derivatives, which have good binding properties.

[6] Draganovic, V., Van der Goot, A.J., Boom, R., Jonkers, J., 2011. Assessment of the effects of fish meal, wheat gluten, soy protein concentrate and feed moisture on extruder system parameters and the technical quality of fish feed. Anim. Feed Sci. Technol., 165, 238–250.

PROCESSED ANIMAL PROTEIN SOURCES

Processed Animal Proteins (PAPs) are ingredients derived only from by-products of animals which are fit for human consumption (category 3 under the European conditions), while being fully traceable and quality assured. They are manufactured in specialised facilities and their use is thoroughly controlled within the entire feed chain, to ensure that the ban on intra-species recycling is respected.

PAPs provide a safe protein-rich alternative to fishmeal and oilseeds. PAPs are rich in most essential amino acids, with the exception of methionine. However, not all PAPs are the same and great care must be taken to assess the nutritional value of the various products on a digestible basis. Poultry meal and feather meal hydrolysate have an essential amino acid profile comparable to that of fishmeal, but generally show lower levels of methionine, lysine, histidine and tryptophan. Pressurised cooking conditions used in the manufacture of feather meal products can have drastic effects on the digestibility of protein and amino acids, requiring a careful assessment. Blood meal, with high levels of lysine and histidine, is a suitable complementary protein to use with plant-derived protein sources. However, the isoleucine content in blood meal is very low and care should be taken to avoid a sub-optimal amino acid supply. Moreover, the drying method used during the manufacture of blood meal is relevant because there is an inverse relationship between the amount of heat applied and protein digestibility. Particularly, lysine content and lysine availability decrease when the amount of heat increases. Poultry meal and feather meal hydrolysate can be valuable sources of phosphorus in fish feeds. Blood meal is extremely rich in iron and its use at high incorporation levels requires attention regarding the pro-oxidative status of the feeds.

PAPs are available in considerable quantities in the EU market and their use can reduce the dependency on third country supplies and counterbalance the -70% protein deficit that currently characterises the EU animal feed sector.

Table 3. Overview of Nutritional Composition of Processed Animal Protein Sources

As fed basis	Unit	Poultry meal 65	Feather meal hydrolysate	Porcine blood meal
Moisture	%	6 - 8	6 - 8	3 - 5
Crude Protein	%	66	79 - 85	88 - 92
Lipid	%	10 - 14	10 - 12	1 - 2
Ash	%	10 - 15	2 - 5	2
Gross Energy	MJ/kg	21.8 - 22.5	21.0 - 21.6	22.0 - 22.8
Essential AA				
Arginine	%	3.4	4.9	3.5
Histidine	%	0.9	0.6	5.1
Isoleucine	%	2.0	3.6	0.9
Leucine	%	3.6	5.8	10.0
Lysine	%	2.3	1.5	7.2
Threonine	%	2.0	3.3	3.9
Tryptophan	%	0.4	0.4	1.2
Valine	%	2.8	5.2	7.0
Methionine	%	0.7	0.5	1.0
Cysteine	%	1.3	3.1	0.9
Phenylalanine	%	2.0	3.4	5.7
Tyrosine	%	1.3	1.8	2.5
Non Essential AA				
Alanine	%	2.8	3.3	6.5
Aspartic acid	%	3.5	4.9	8.9
Glutamic acid	%	5.6	7.7	7.9
Glycine	%	4.5	5.3	3.7
Proline	%	4.1	6.8	3.3
Serine	%	3.2	8.3	4.1
Other valuable nutrients				
Total Phospholipids	%	1.5	0.0	0.5
Total Cholesterol	%	0.2	0.1	0.4
Taurine*	%	0.3	0.01	0.2

Values presented were compiled from various literature sources[1-5] and completed with internal analytical data from SPAROS.
Values are indicative only and do not exempt users from specific analysis of their ingredients.
*Taurine or 2-aminoethanesulfonic acid is not an amino acid, as it lacks a carboxyl group. The extent of its nutritional relevancy in fish is still under debate.

Table 4. Functional and Technological Properties of Processed Animal Protein Sources on Extruded Fish Feeds

	Poultry meal 65	Feather meal hydrolysate	Porcine blood meal
Functional role			
Feed Palatability	+	+	-
n-3 LC PUFA & Phospholipids supply	-	-	-
Oxidative potential	-	-	++
Impact on physical pellet properties			
Bulk density (kg/m^3)	570	480	610
Pellet Binding	+	-	++
Starch gelatinisation	-	-	-
Pellet durability	-	-	++

Minor detrimental or no effect (-)/Minor beneficial effect (+)/Moderate beneficial effect (++)/Major beneficial effect (+++).
Effects of each ingredient on the physical pellet properties of complete feeds are highly dependent on incorporation levels and remaining formulation.

Findings:

- At below 10% incorporation levels, blood meal has positive effects on the binding properties and durability of extruded fish pellets. Extra care should be taken in balancing inclusion levels of blood-origin ingredients and antioxidants, as these PAPs significantly increase the oxidative potential of the feed due to their high iron contents.
- Little is known about the effects of PAPs on starch gelatinisation. But extrusion tests performed in the framework of **ARRAINA** showed that 10 and 15% incorporation of feather meal hydrolsate had little effect on starch gelatinisation, hardness, durability and expansion ratio of extruded feeds for common carp and gilthead seabream. However, the highest inclusion level of feather meal (15%) tended to increase the bulk density of the feeds and affected the pellet sinking speed.

PLANT PROTEIN SOURCES

Oilseed meals, cereal proteins and grain legumes are now important protein sources in aquaculture feeds. In comparison to animal derived proteins, plant proteins show a lower biological value for fish (essential amino acid imbalance, impaired phosphorus availability, presence of anti-nutritional factors, higher carbohydrate fraction). Most plant proteins are deficient in the sulfur-containing amino acids (methionine and cysteine) and approximately two thirds of the total phosphorus in plants is in the form of phytate, which is poorly digested by fish. However, solid knowledge gained on the nutritional requirements of fish, and technological advances in plant ingredients processing, have allowed the progressive use of plant protein sources in fish feeds without detrimental effects on fish performance, welfare and flesh quality. The **ARRAINA** project is currently assessing the long-term effects that these dietary changes will have on the full life cycle of fish for which presently little is known.

Table 5. Overview of Nutritional Composition of Vegetable Protein Sources

As fed basis	Unit	Wheat gluten	Corn gluten meal	Pea protein concentrate	Soy protein concentrate	Soybean meal 48	Rapeseed meal	Sunflower meal
Moisture	%	3 - 5	8 - 10	4 - 6	4 - 6	7 - 9	7 - 9	6 - 8
Crude Protein	%	79 - 82	60 - 62	76 - 78	60 - 63	45 - 47	34 - 36	26 - 29
Lipid	%	4 - 6	2 - 4	1 - 2	< 0.5	2 - 4	2 - 4	2 - 3
Crude fibre	%	< 0.5	1 - 2	1 - 2	3 - 4	5 - 7	10 - 13	24 - 26
Starch	%	6 - 8	13 - 16	8 - 10	< 1	4 - 6	1 - 2	1 - 2
Ash	%	0.8	1.9	4.5	6.5	6.24	6.93	6.32
Gross Energy	MJ/kg	22.3	20.8	21.6	18.6	17.3	17.2	17.3
Essential AA								
Arginine	%	3.4	1.6	7.8	5.7	3.0	1.8	2.1
Histidine	%	2.2	1.1	2.6	2.4	1.0	0.8	0.6
Isoleucine	%	2.8	2.2	3.0	2.6	1.8	1.2	1.1
Leucine	%	5.7	8.7	6.9	5.5	3.0	2.0	1.6
Lysine	%	1.8	0.9	6.2	4.4	2.4	1.7	0.9
Threonine	%	2.4	1.8	3.3	3.1	1.6	1.3	0.9
Tryptophan	%	1.1	0.3	0.5	0.8	0.5	0.4	0.3
Valine	%	3.3	2.4	4.3	3.2	1.9	1.5	1.3
Methionine	%	1.8	1.3	1.4	1.6	0.6	0.6	0.6
Cysteine	%	1.8	1.0	1.0	1.1	0.6	0.7	0.4
Phenylalanine	%	4.4	3.3	4.8	4.0	2.0	1.2	1.1
Tyrosine	%	3.5	2.6	3.9	3.4	1.4	0.9	0.6
Non essential AA								
Alanine	%	2.4	4.6	3.7	3.2	1.8	1.3	1.1
Aspartic acid	%	2.8	3.2	9.7	7.9	4.5	2.1	2.3
Glutamic acid	%	27.9	10.9	13.9	12.7	7.1	5.0	4.9
Glycine	%	2.8	1.4	3.3	2.9	1.7	1.5	1.4
Proline	%	9.6	4.7	3.7	3.8	2.0	1.8	1.1
Serine	%	4.4	2.7	4.6	4.1	2.0	1.3	1.1

Values presented were compiled from various literature sources[1-5] and completed with internal analytical data from SPAROS.
Values are indicative only and do not exempt users from specific analysis of their ingredients.

As part of their natural defence mechanisms, plants may produce several metabolites, often referred to as anti-nutritional factors (ANFs). Their presence in the plant derived ingredients can reduce nutrient utilisation and/or feed intake by the fish. Some of these ANFs are easily eliminated during feed extrusion, while others are resistant to thermal processing conditions (Figure 2). The **ARRAINA** project is currently assessing the biological effects of some relevant ANFs (e.g. non-starch polysaccharides) on fish digestion and exploring several strategies to mitigate their detrimental effects.

Figure2: Extrusion of elevated temperatures (120°-130°) is an effective way to eliminate several types of ANFs

Heat Labile ANFs

- Protease inhibitors
- Haemogglutinins
- Anti-vitamins

Heat Stable ANFs

- Phytate
- Saponins
- Non-starch polysaccharides
- Antigenic proteins
- Estrogens
- Phenolic compounds
- Glucosinolates

Table 6. Functional and Technological Properties of Vegetable Protein Sources on Extruded Fish Feeds

	Wheat gluten	Corn gluten meal	Pea protein concentrate	Soy protein concentrate	Soybean meal 48	Rapeseed meal	Sunflower meal
Functional role							
Feed Palatability	-	-	-	-	- -	- -	- -
n-3 LC PUFA & Phospholipids supply	-	-	-	-	-	-	-
Oxidative Potential	+	+	+	+	+	+	+
Impact on physical pellet properties							
Bulk Density (kg/m³)	520	645 - 660	420	630 - 670	640 - 670	625 - 650	470 - 500
Pellet Binding	+++	+	++	+	+	+	+
Starch Gelatinisation	-	-	-	+	+	-	-
Pellet Durability	+++	+	++	+	+	+	+

Minor detrimental or no effect (-)/Minor beneficial effect (+)/Moderate beneficial effect (++)/Major beneficial effect (+++).
Effects of each ingredient on the physical pellet properties of complete feeds are highly dependent on incorporation levels and remaining formulation

Findings:

- Within certain limits, vegetable protein sources have no detrimental effects on feed palatability. However, at elevated incorporation levels, vegetable ingredients with low protein and high fibre contents can affect feed intake.
- Most vegetable protein sources tested, but particularly wheat gluten and pea protein concentrate, have a beneficial effect on the binding properties, expansion and durability of extruded pellets. Such effects on the pellet structuring capacity are probably associated to the adhesiveness of globular proteins in plant meals[5, 7, 8].
- However, the presence of variable levels of non-starch polysaccharides (NSP) in plant ingredients may result in less expansion, which is an important criterion for controlling the sinking velocity of the pellets and the post-extrusion application of fats.
- When attempting to reduce the fishmeal levels in fish feed, the effect of both typical high-protein meals (e.g. protein concentrates derived from wheat and corn glutens, soy, peas) and combined protein-starch sources (e.g. soybean, rapeseed, sunflower meals) on the physical pellet properties should be considered. This gains particular relevance given that the viscoelastic properties of the feed dough often relate also to starch gelatinisation and the protein/starch-interaction.
- Knowledge generated in the framework of the **ARRAINA** project confirms that within the "commonly used" incorporation levels and with a careful adjustment of extrusion conditions, the use of plant ingredients results in fish pellets with high physical quality, without detrimental effects on the environmental loads associated to feed losses.

[7] Sørensen, M., Stjepanovic, N., Romarheim, O.H., Krekling, T., Storebakken, T., 2009. Soybean meal improves the physical quality of extruded fish feed. Anim. Feed Sci. Technol., 149, 149–161.

[8] Sørensen, M., 2012. A review of the effects of ingredient composition and processing conditions on the physical qualities of extruded high-energy fish feed as measured by prevailing methods. Aquaculture Nutrition, 18: 233–248.

CEREALS, PULSE AND LEGUME STARCH SOURCES

Even though they contain some protein, cereals, pulses and legumes are incorporated in aquaculture feeds mostly as a source of starch, an energy yielding substrate. Moreover, starch plays an important role as a pellet binding agent. Depending on the starch content and the manufacturing conditions, starch gelatinisation is a key element for pellet expansion, allowing not only the addition of high levels of fat, but also to control the floatability of fish pellets.

Table 7. Overview of Nutritional Composition of Cereals and Legume Starch Sources

As fed basis	Unit	Whole wheat	Wheat bran	Corn meal	Whole peas
Moisture	%	9 - 12	10 - 12	9 - 12	8 - 12
Crude Protein	%	11	15.1	8.1	20.7
Lipid	%	1.5	3.4	3.7	1
Crude fibre	%	2.3	9	2.2	5.2
Starch	%	69	20.2	63.3	44.4
Ash	%	1.6	4.9	1.2	3
Gross Energy	MJ/kg	15.8	16.4	16.1	15.8
Essential AA					
Arginine	%	0.4	0.9	0.3	1.5
Histidine	%	0.2	0.4	0.2	0.4
Isoleucine	%	0.3	0.4	0.2	0.8
Leucine	%	0.6	0.8	0.8	1.3
Lysine	%	0.3	0.5	0.2	1.3
Threonine	%	0.3	0.4	0.3	0.7
Tryptophan	%	0.1	0.2	0.0	0.2
Valine	%	0.4	0.6	0.3	0.9
Methionine	%	0.2	0.2	0.1	0.2
Cysteine	%	0.2	0.3	0.2	0.3
Phenylalanine	%	0.4	0.5	0.3	0.8
Tyrosine	%	0.3	0.4	0.3	0.6
Non essential AA					
Alanine	%	0.3	0.6	0.5	0.8
Aspartic acid	%	0.5	0.9	0.5	2.1
Glutamic acid	%	2.7	2.5	1.3	3.0
Glycine	%	0.4	0.7	0.3	0.8
Proline	%	0.9	0.8	0.6	0.8
Serine	%	0.4	0.5	0.3	0.8

Values presented were compiled from various literature sources[1-5] and completed with internal analytical data from SPAROS.
Values are indicative only and do not exempt users from specific analysis of their ingredients.

Aquaculture feeds can also use concentrated starches (86-95%) from various botanical origins like wheat, corn, peas, cassava and potato. Starch is a mixture of two polymers of glucose, amylose and amylopectin. Depending on the botanical origin, the ratio between amylose and amylopectin can vary. Amylose presents a tight linear helical structure making the α-glycosidic bonds less available to enzymatic cleavage, whereas amylopectin is highly branched and, thus, more susceptible to enzymatic degradation. Therefore, the amylose/amylopectin ratio of the starch granule may affect its nutritional availability. Although not widely used at industrial level, through selective plant breeding there are now starch products (waxy corn starch) that contain virtually 100% amylopectin.

Table 8. Amylose/Amylopectin Ratio in Various Starches

Botanical source	Amylose, %	Amylopectin, %
Wheat	26	74
Corn	28	72
Pea	35	65
Cassava	15	85
Potato	20	80
Waxy corn	1	99

Table 9. Functional and Technological Properties of Cereals and Legume Starch Sources on Extruded Fish Feeds

	Whole wheat	Wheat bran	Corn meal	Whole peas
Functional role				
Feed Palatability	-	-	-	-
n-3 LC PUFA & Phospholipids supply	-	-	-	-
Oxidative Potential	-	-	-	-
Impact on physical pellet properties				
Bulk Density (kg/m^3)	600 - 680	170 - 250	600 - 640	750 - 800
Pellet Binding	+++	+	++	++
Starch Gelatinisation	++	+	++	+
Pellet Durability	+++	+	+++	+++

Minor detrimental or no effect (-)/Minor beneficial effect (+)/Moderate beneficial effect (++)/Major beneficial effect (+++).
Effects of each ingredient on the physical pellet properties of complete feeds are highly dependent on incorporation levels and remaining formulation.

Findings:

- High-starch ingredients are of prime importance in extruded fish feeds. Starch, upon gelatinisation by thermal processing during extrusion, is not only a digestible source of energy, but also a key element on the physical structure of the feed pellets due to its viscoelastic properties.
- **ARRAINA** results confirmed that legume starch from peas is harder to gelatinise than cereal-derived starches (wheat, corn). However, it also showed that processing conditions (steam pressure in the pre-conditioner, residence time, moisture level, extrusion temperature and screw configuration) can all affect starch gelatinisation rate, which implies careful adjustments of manufacturing conditions.

OILS

The primary lipid source for aquaculture feeds has traditionally been supplied by marine oils produced as a by-product of fishmeal production. However, their limited supply instigated the need to find and use other sustainable oil sources. Salmon oil, a by-product from farmed salmon industry, tuna oil, krill oil and copepod oil are also available but their use in aquaculture feeds is still limited by cost constraints. Krill oil and copepod oil are rich sources of phospholipids and can be used in fish larvae and broodstock stages. Most successful alternatives to fish oil are plant oils due to their global availability and favourable price.

The fatty acid composition of fish fillets is dependent on the fatty acid profile of the feed that they consume. Feeding marine oils results in the accumulation of long-chain polyunsaturated fatty acids (LC-PUFAs), EPA and DHA, which have been unequivocally associated with a protective role against a number of human diseases. The inclusion of alternative lipid sources in fish feed causes a reduction in EPA and DHA contents, because plant- and animal-based oils do not contain EPA or DHA. Some plant oils (soybean, rapeseed and linseed) contain polyunsaturated fatty acids of the omega-3 series (C18:3 n-3, alpha-linolenic acid). However, this should not be confused with LC-PUFAs in terms of the health beneficial effects to humans. Fish are the main source of LC-PUFAs in human health. The **ARRAINA** project is currently assessing the long-term effects (full cycle) of replacing fish oil by blends of vegetable oils on the performance and health status of fish, without compromising the production of fish of high nutritional value, quality and safety.

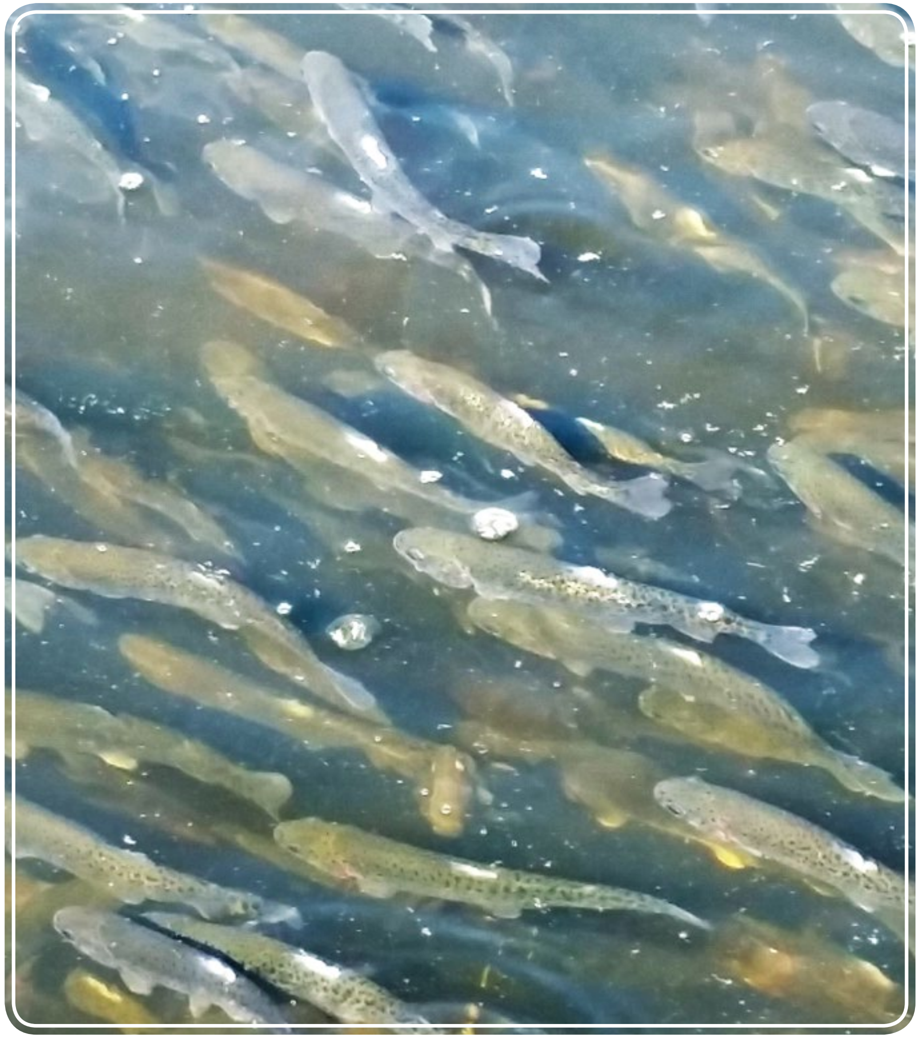


Table 10. Summarised Fatty Acid Composition of Oils

Fatty acid (% of oil)	Herring Fish oil Northern Hemisphere	Anchovy Fish oil Southern Hemisphere	Salmon oil	Tuna oil	Krill oil	Copepod oil	Soybean oil	Rapeseed oil	Linseed oil	Palm oil	Poultry fat
C14:0	8.3	7.2	4.7	4.6	13.6	7.3	0.1	0.1	0.1	0.9	0.9
C16:0	14.8	17.6	12.6	21.8	24.2	20.6	11.4	4.4	6.1	43.6	21.6
C18:0	1.8	4.2	2.4	5.5	1.8	2.1	3.4	1.7	5.5	4.3	6
C20:0	0.2	0.4	0.3	0.4	0.1	0.1	0.3	0.5	0.2	0.2	0.15
C16:1 n-7	7.7	10.5	5.7	6	6.3	6.5	0.1	0.3	0.1	0.1	5.7
C18:1 n-7	2	2.2	2.4	2.8	6.6	4.5	1.5	3	0.7	0.2	1.2
C18:1 n-9	8.8	9.7	13.1	17.1	12.8	11.6	22.9	59.2	20.6	36.9	37.3
C20:1 n-11	11	1.6	5.7	1.5	0.7	8.9	0.5	1.6	0	0.1	0.1
C22:1 n-11	15.6	1.3	5.7	0.3	0.1	7.1	0	0	0	0	0
C18:2 n-6	2.7	2.8	2.2	2.6	3.2	1.9	52.9	19	16.3	9.2	19.5
C18:3 n-6	0.1	1.8	0.1	0.2	0.2	0.1	0.3	0.4	0.2	0	0
C20:4 n-6	0.1	0.1	0.6	0.1	0.1	0.1	0	0	0	0	0
C18:3 n-3	1.5	1.1	0.9	0.9	1.6	1.1	5.1	8.6	49.7	0.2	1
C18:4 n-3	4.3	1.7	1.5	1.0	5.4	1.9	0.1	0	0	0	1.1
C20:5 n-3 (EPA)	**8.7**	**13.4**	**7.9**	**7.2**	**14.2**	**7.8**	**0**	**0**	**0**	**0**	**0**
C22:5 n-3	0.7	1.6	3.3	1.3	0.3	0.5	0	0	0	0	0
C22:6 n-3 (DHA)	**5.7**	**11.4**	**10.1**	**16.0**	**4.7**	**11.5**	**0**	**0**	**0**	**0**	**0**

Values presented were compiled from various literature sources[1-5] and completed with internal analytical data from SPAROS.
Values are indicative only and do not exempt users from specific analysis of their ingredients.

MICRONUTRIENTS

Vitamins and minerals are nutrients required in trace amounts and are essential for normal fish growth, reproduction and general health status. Fish cannot synthesise vitamins. Fish are able to absorb some minerals from the aquatic environment, although not in sufficient amounts to fulfil their daily requirements. Therefore, vitamins and minerals must be supplied through feed. Knowledge on the micronutrient requirements of fish is still fragmented. When dietary protein and lipid sources are changed from fish-based to plant-based ingredients, there is an associated change in a range of dietary nutrients, especially micronutrients. Large variations are found in vitamin and mineral contents when we compare fishmeal with plant-protein sources. The **ARRAINA** project has a strong focus on identifying minerals, vitamins, amino acids, lipid components (e.g. cholesterol), and other fishmeal and fish oil specific nutrients, that need to be added to feeds with high levels of plant products to avoid reduced performance and health status in farmed fish.

Vitamin Content of Select Feed Ingredients

	Biotin	Choline	Folic acid	Niacin	Panthothenic acid	Pyridoxine	Riboflavin	Thiamin	Vitamin B12	Vitamin E
As fed basis	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Fishmeal LT 70	0.5	5200	0.3	85	17.3	4.8	9.7	0.4	430	22.1
Fishmeal 60	0.2	4400	0.2	100	15.0	4.6	7.1	0.1	352	5.0
Fish protein concentrate	0.2	3200	0.3	20	30.0	0.2	8.8	0.1	140	-
Squid meal		650	-	194	-	1.3	8.0	0.1	-	-
Krill meal	-	9000	-	-	1.6	0.0	0.1	0.1	-	2.6
Poultry meal 65	0.3	1896	0.7	178	46.6	6.4	9.1	5.7	0	2.9
Feather meal hydrolysate	0.1	6000	0.5	47	11.0	4.4	10.5	0.2	301	2.2
Porcine blood meal	0.3	800	0.2	22	3.2	4.5	2.9	0.3	0	20.0
Wheat gluten	-	-	-	-	-	-	-	-	-	-
Corn gluten meal	0.2	352	0.3	60	3.5	6.9	2.0	0.3	-	23.4
Pea protein concentrate	-	-	-	-	-	-	-	-	-	-
Soy protein concentrate	0.3	2609	0.6	28	16.3	6.0	2.9	6.0	-	2.4
Soybean meal 48	0.3	2731	1.4	22	15.0	6.4	3.1	3.2	0	2.3
Rapeseed meal	1.0	6700	0.8	160	9.5	7.2	5.8	5.2	0	13.4
Sunflower meal	0.9	3700	2.3	242	40.6	13.7	3.5	3.1	-	11.1
Whole wheat	0.1	920	0.4	54	9.4	3.1	1.5	4.7	-	12.0
Wheat bran	0.4	1232	1.8	2	28.0	8.5	3.6	8.4	-	14.3
Corn meal	0.1	504	0.3	23	5.1	4.7	1.1	3.7	-	20.9
Whole peas	0.2	532	0.2	33	21.6	1.0	1.9	4.6	-	0.2

Values presented were compiled from various literature sources[1-5] and completed with internal analytical data from SPAROS.
Values are indicative only and can vary significantly with raw material source/botanical origin and processing conditions.
Values do not exempt users from specific analysis of their own ingredients.

Mineral Content of Selected Feed Ingredients

	Calcium	Phosphorus	Sodium	Potassium	Magnesium	Copper	Iron	Manganese	Selenium	Zinc
Unit	%	%	%	%	%	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Fishmeal LT 70	2.2	2.1	1.0	1.1	0.3	5	114	8	1.6	125
Fishmeal 60	5.5	4.1	1.1	0.8	0.2	6	320	14	1.2	100
Fish protein concentrate	0.7	0.4	0.5	0.5	0.1	4	1208	81	1.9	125
Squid meal	0.1	1.4	0.1	0.3	0.0	19	7	0	0.5	15
Krill meal	1.8	1.2	1.5	0.1	0.7	78	395	6	12.0	60
Poultry meal 65	0.3	1.9	0.6	0.5	0.1	12	220	16		60
Feather meal hydrolysate	1.4	1.2	0.1	0.1	0.1	9	575	15	0.8	130
Porcine blood meal	0.1	0.2	0.4	0.4	0.0	6	2050	1		23
Wheat gluten	0.1	0.3	0.0	0.1	0.0	2	52	0	0.4	9
Corn gluten meal	0.0	0.4	0.1	0.1	0.1	12	100	8	0.8	34
Pea protein concentrate	0.1	0.8	0.0	2.0	0.2	15	85	25	0.1	59
Soy protein concentrate	0.3	0.7	0.0	2.1	0.3	23	140	31	0.1	52
Soybean meal 48	0.3	0.6	0.0	2.1	0.3	16	304	40	0.3	47
Rapeseed meal	0.8	1.1	0.0	1.2	0.5	9	131	58	1.1	71
Sunflower meal	0.4	1.0	0.0	1.5	0.5	28	241	34	0.5	85
Whole wheat	0.1	0.3	0.0	0.4	0.1	5	68	35		27
Wheat bran	0.1	1.0	0.0	1.2	0.4	12	137	98		77
Corn meal	0.0	0.3	0.0	0.3	0.1	2	32	4		18
Whole peas	0.1	0.4	0.0	1.0	0.2	7	93	9		32

Values presented were compiled from various literature sources[1-5] and completed with internal analytical data from SPAROS.
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Values do not exempt users from specific analysis of their own ingredients.

Approximately two-thirds of the total phosphorus in plant-based ingredients is in the form of phytate. Fish, as other monogastric animals, are not able to digest phytate. Therefore the phytate phosphorus fraction present in the diet is eliminated in the excreta and represents a source of environmental pollution. Furthermore, phytate acid has strong chelating properties and can make up complexes with minerals, starch and protein, thereby reducing their bioavailability. To avoid phosphorus deficiency, aquaculture feeds with high levels of plant-based ingredients are commonly supplemented with inorganic feed phosphates. Another option which is under evaluation in the **ARRAINA** project is the supplementation of plant-rich feeds with phytase, an enzyme capable of releasing phytate-bound phosphorus, making it available to fish.

CONCLUDING REMARKS

Formulating a nutritionally balanced feed for aquaculture fish requires an in depth knowledge on the nutrient composition of the vast array of ingredients available. However, the efficacy of the feeds for farmed fish relies also on a series of technical criteria (e.g. pellet durability, starch gelatinisation, expansion rate) which define the pellet water stability and its adequacy to the species feeding behaviour. When changing aquaculture feeds from fish-derived ingredients to plant- or animal-based ingredients, even slight changes on formulations can have profound effects on the physical properties of extruded feeds.

The complexity of the extrusion process, and limitations in basic knowledge on protein properties and protein/starch-interaction, make it a demanding task to obtain consistent product quality based on different types of raw materials. Physical quality of extruded pellets is affected by several variables, among which ingredient formulation and extrusion parameters are recognised as having great influence. Data generated in the framework of the **ARRAINA** project confirm that within the “commonly used” incorporation levels and with a careful adjustment of extrusion conditions, the use of plant ingredients originates fish feeds with high physical quality and without detrimental effects on the environmental load of aquafeeds.

Credits:

This technical booklet was prepared under the EU seventh Framework Programme by the **ARRAINA** project No. 288925: Advanced Research Initiatives for Nutrition & Aquaculture. The views expressed in this work are the sole responsibility of the **ARRAINA** team involved and do not necessary reflect the views of the European Commission. The technical content was prepared by SPAROS Lda with contributions by NIFES, Wageningen University, and INRA. AquaTT is responsible for the production of the booklet.

Disclaimer

Composition and technological properties of feed ingredients commonly used in aquaculture feeds may have significant variations both due to origin, season and processing. Therefore, the values provided in this booklet should be used only as guidelines. For practical formulation of aquaculture feeds, SPAROS and the **ARRAINA** project recommend that each raw material batch used is analysed.

Tables in digital format:

If you wish to access the tables presented in this booklet in spreadsheet format, please go to:

www.sparos.pt/index.php/en/resources/booklet_alternative_ingredients

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