De Alimenti Piscibus Compositione et Fabricatione

The Composition and Production of Fish Feeds

An Overview of Data from the Literature and the Internet

Composit et Scripsit

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Summary

Composition of fish feeds: Fish feeds like other animal feeds can be partitioned into six major compounds, (1) moisture, (2) protein, (3) fat, (4) ash, (5) crude fibre and the (6) nitrogen free extract (NFE), the so called Weende analysis. Fish feeds are mostly characterized by their protein and fat levels. The protein in the feed is primarily needed for the build-up of (muscle) tissues and the fat is a major source of energy and for accretion of fat tissue. The amount of carbohydrates in fish feeds are usually low, since fish and particularly carnivorous fish, have a low capacity to digest carbohydrates. As a consequence, the energy in the diet has to be derived from fat and fat has a higher energy density than carbohydrates. For that reason, fish feeds are more concentrated and have thus also a higher protein level (up to about 40 – 45%) and energy density than feeds for terrestrial farm animals. The digestible protein / digestible energy ratio is an important characteristic of a fish feed, and as a rule of thumb, this ratio in the fish feed should be more or less similar to the ratio of protein / energy of the growing fish itself. This way, a maximal retention of dietary protein, an expensive ingredient of (fish) feed, is achieved.

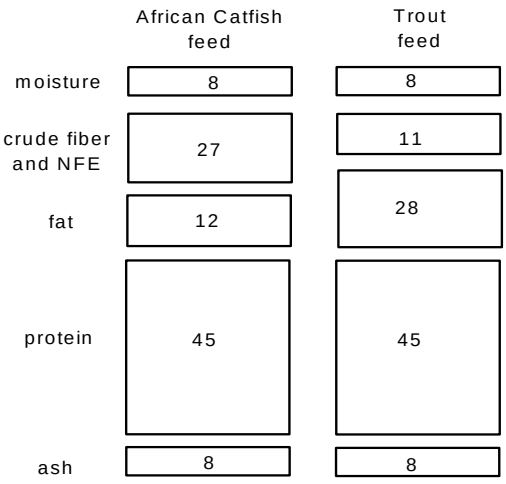


Figure: Composition in percentages of a typical African Catfish feed and a typical trout feed. The fibre content is about 1% and is in the figure included in the NFE fraction.

Ingredients of fish feeds: Fish feed are composed of various ingredients. Fish meal and fish oil are traditionally the major ingredients of fish feeds, particularly fish feeds for carnivorous fish, but nowadays, also other ingredients are used from both plant and (terrestrial) animal sources. Various by-products of e.g. the food industry can be used as ingredients such as soy, pea, and rapeseed proteins, and animal proteins and fats. These ingredients can serve as a source of protein or fat. About 50% of the protein in fish feeds can be derived from protein sources other than fish meal without compromising the growth performance.

Production of fish feeds: Fish feeds are produced by means of extrusion. The extrusion process involves high temperatures and pressures and the starch in the feed is gelatinized. This gelatinization or pressure cooking process of the starch results in a pellet with a firm structure. The extrusion technique allows the production of pellets of various sizes and shapes that are water stable and free of dust. Further, it is possible to manipulate the physical properties of the pellets and floating and sinking pellets can be manufactured. Finally, the extrusion process makes it possible to vacuum coat the pellets with large amounts of fat and feeds with a high fat content can be produced.

Contents

1. Introduction

Around the year 1860, the researchers Henneberg and Stohmann at the Agricultural Research Institute in Weende in Germany proposed to partition animal feeds into six major compounds, i.e. (1) moisture, (2) protein, (3) fat, (4) ash, (5) crude fibre and the so called (6) nitrogen free extract (NFE). The moisture, protein, ash and fibre was measured and the NFE was calculated as the difference between the total amount of the feed and these five measured compounds. This so-called Weende analysis is still being used for the analysis of (fish) feeds. We will here describe these various compounds of the Weende analysis and in addition the vitamins in fish feeds. Further, we will give an overview of the various ingredients that can be used for the production of fish feeds and a description of the extrusion process that is used to produce feed pellets suitable for fish.

2. Moisture

Fish feeds contain about 8 – 10% moisture and the ingredients that are used for the production of fish feeds contain usually 7 – 12% moisture. The moisture content should not be too high since a high moisture content can result in moulding and shorten the shelf life. The moisture content is determined by drying the feed in a dryer. The dry matter of a feed is the feed without the moisture. The composition of a fish feed can be declared as whole matter or as dry matter. The values declared on the label of a fish feed are whole matter values.

3. Proteins and amino acids

Proteins contain about 16% nitrogen and are essential for the growth of the fish; the protein in the feed drives the growth. A fish contains about 17 – 18% protein and this percentage is rather constant for the various types of fish and other animal species. The building blocks of proteins are the amino acids. An amino acid has an amino group and an acid (carboxyl) group (Figure 1) and the amino group of one amino acid is linked to a carboxyl group of another amino acid to form a protein chain.

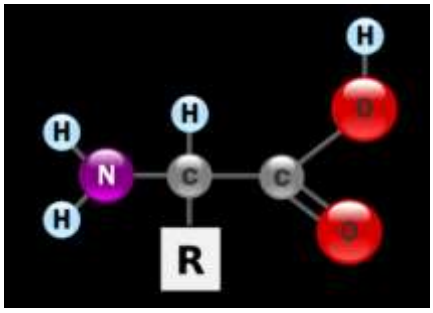


Figure 1
Structure of an amino acid. The amino group (NH_2) on the left side and the carboxyl group (COOH) on the right side. The R group is different and characteristic for the various individual amino acids.

There are a total of 20 different amino acids and 10 of these are essential (Table 1). The ten essential amino acids cannot be synthesized in the body and can only be obtained from the feed. The ten non-essential amino acids can be synthesized in the body by means of transamination. Transamination means that an amino group of an amino acid is transferred to a precursor of a non essential amino acid to form an amino acid. The semi-essential amino acids cysteine and tyrosine can only be formed from the essential amino acids methionine and phenylalanine, respectively.

Table 1

Overview of the amino acids and the composition of fish meal and the requirements according to the National Research Council (NRC,1993 and Halver and Hardy 2002).

		Requirements (% in protein)		
	Percentage in fishmeal protein	Trout	Carp	Tilapia
Essential Amino Acids				
Arginine	5.9	3.95	3.74	3.7
Histidine	2.6	1.84	1.83	1.5
Isoleucine	4.2	2.37	2.17	2.7
Leucine	7.3	3.68	2.86	3.0
Lysine	7.6	4.78	4.97	4.5
Methionine + cysteine	3.7	2.63	2.69	2.8
Phenylalanine + tyrosine	7.0	4.74	5.65	4.4
Threonine	4.2	2.11	3.40	3.3
Tryptophan	1.1	0.53	0.69	0.9
Valine	4.9	3.16	3.14	2.4
Semi-Essential Amino Acids				
Cysteine	0.9			
Tyrosine	3.1			
Non-Essential Amino Acids				
Alanine	6.3			
Asparagine and aspartate	9.3			
Glycine	6.5			
Glutamine and glutamate	13			
Proline	4.4			
Serine	4			

1. Halver, J.E. & Hardy, R.W. (2002) Fish Nutrition, Academic Press. ISBN 0-12-319652-3 (page 152 – 167 for amino acid requirement).

2. NRC, National Research Council, Nutrient Requirements of Fish (1993). National Academy Press, Washington, D.C. ISBN 0-309-04891-5 (page 63). Also on the Internet: http://www.nap.edu/books/0309048915/html/1.html See also Kaushlik, Aquaculture 199:225-241 (1995) Nutrient requirements, supply and utilization in the context of carp culture.

3 Cysteine can be derived from methionine, but also from other sources and is not really essential (semi-essential).

4. Tyrosine can only be derived from phenyl alanine and is thus more or less essential (semi-essential).

5. Arginine can be synthesized in the urea cycle, but in limited amounts. Most of the nitrogen in fish is excreted as ammonia (85%) and a minor proportion as urea (15%).

Amino acid composition of proteins

The body proteins of the fish have a specific amino acid composition and this composition is rather constant for all the various fish species (*See Appendix 1 on page 29*); the amino acid composition of the feed has no influence on the amino acid composition of the fish. The body proteins of the fish have a specific amino acid composition, and as a consequence, no body proteins can be synthesized when one or more essential amino acids are lacking in the feed. Therefore, an optimal amino acid composition of the feed is important for maximal protein accretion and thus growth of the fish (*See Appendix 2 on page 31 for the amino acid composition of various fish feed ingredients*)

Protein as source of energy

Protein in the feed is primarily used for tissue accretion and growth, but can also be used as fuel. Protein is, however, an expensive ingredient of fish feed and thus an expensive source of energy. One gram of protein has a metabolic energy density of 17.5 kJ/g or 4.2 kcal/g whereas fat has a metabolic energy density of 36.4 kJ/g of 8.7 kcal/g (1 kcal = 4.184 kJ). Further, the storage of energy in the form of protein is inefficient, 1 gram of protein is stored together with about 3 grams of water whereas fat tissue, the storage place of fat, contains predominantly fat and very little water. Moreover, the oxidation of proteins results in the formation of ammonia and thus in pollution of the water.

***Nitrogen cycle*.**

Proteins and amino acids are essential ingredients of fish feed since fish (and other animal species) are not able to synthesize amino acids from inorganic nitrogen (nitrate). Plants, however, can synthesize amino acids from inorganic nitrogen and the amino acids and proteins synthesized in the plant can be ingested by the fish. Moreover, small size fish can be eaten by larger size fish (food chain). The protein taken up by the fish can be used for the accretion of body tissues and as a source of energy. The protein used as energy is oxidized and the released nitrogen is excreted as ammonia. The ammonia is converted into nitrite and subsequently nitrate by nitrifying bacteria. This process is called the nitrogen cycle (Figure 2).

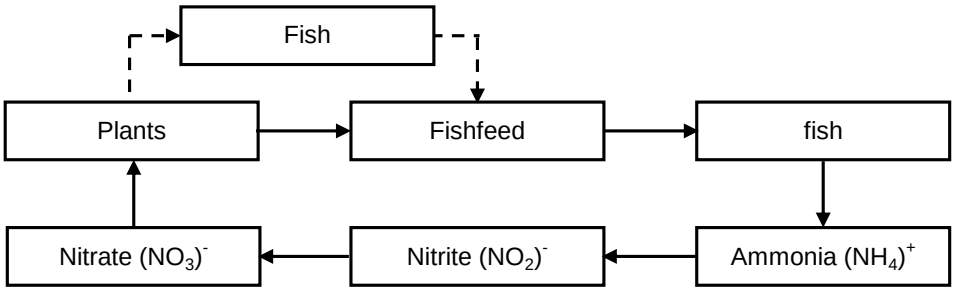


Figure 2

Nitrogen cycle. The nitrogen in the plants, fish feed and fish is organic nitrogen (amino acids and proteins) and the nitrogen in the nitrite, nitrate and ammonia is inorganic nitrogen.

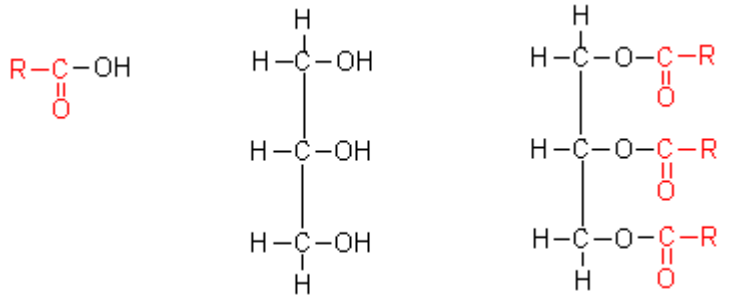
Fish feed contains both plant and fishmeal proteins (Figure 2). Fish protein (fish meal) is an excellent source of protein for fish, particularly for carnivorous fish species, which naturally also eat fish. The supply of fishmeal is, however, limited while the demand is increasing. Therefore, nowadays efforts are undertaken to replace fish meal protein (dashed line in Figure 2) by other protein sources as much as is possible without compromising the growth performance of the fish.

4. Fats and Oils

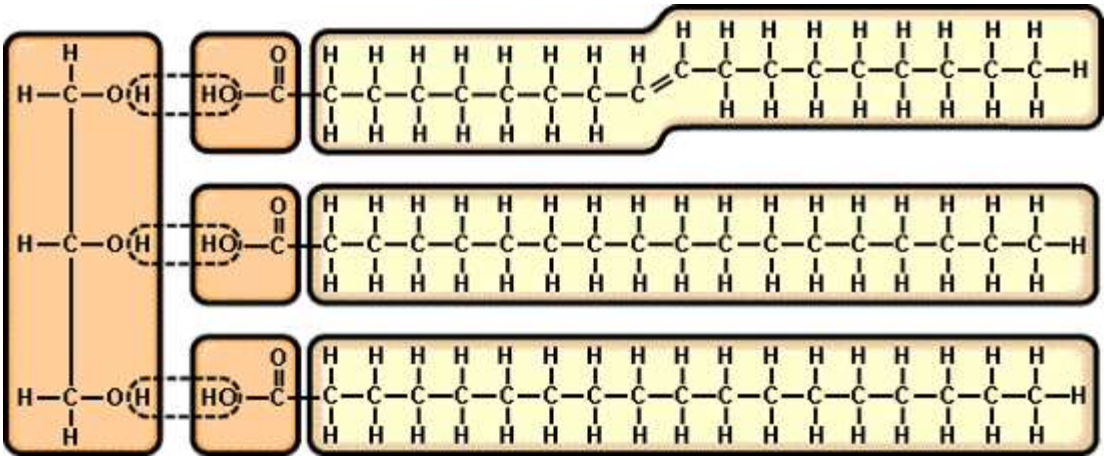
Fats are predominantly composed of triglycerides or triacylglycerols. Further, there are other non-triglyceride lipid species such as phospholipids (e.g. lecithin) and sterols (e.g. cholesterol). Triglycerides are an important source of of energy; they have a high energy density (gross energy of 39.6 kJ/g or 9.46 kcal/g, 1 kcal = 4.184 kJ) and can be stored efficiently in the fat tissue.

Structure of a triglyceride molecule.

A triglyceride molecule is composed of three fatty acids that are attached to a glycerol molecule (Figure 3). Fatty acids are long chains of carbon atoms with various lengths and can be divided into saturated and unsaturated fatty acids.



Fatty Acid Glycerol Triacylglycerol



Glycerol 3 fatty acids (1 oleic acid en 2 stearic acid molecules)

Figure 3.
Example of the structure of a triacylglycerol or triglyceride molecule

The unsaturated fatty acids can be subdivided into mono-unsaturated (one unsaturated or double bond between two carbon atoms) and poly-unsaturated fatty acids (more than one

unsaturated or double bonds between the carbon atoms). Saturated fats have a high melting point and are mostly solid at room temperature. Unsaturated fats have a lower melting point and an increasing degree of unsaturation (more double bonds) results in a further lowering of the melting point. Fish oil and vegetable oils such as soy and rapeseed oil are rich in polyunsaturated fatty acids and are liquid at room temperature, whereas coconut oil rich is in saturated fatty acids and is solid at room temperature. Olive oil contains predominantly oleic acid with a single double bond and is liquid at room temperature, but flocculates when placed in the refrigerator; fats rich in polyunsaturated fatty acids, however, remain liquid in the refrigerator. (*See Appendix 3 on page 33 for the structure of various fatty acids*).

Essential fatty acids

There are essential and non-essential fatty acids. Non-essential fatty acids can be synthesized by the fish themselves whereas the essential fatty acids have to be obtained from the feed. Essential fatty acids are characterized by the presence of more than one double bond that are positioned on specific locations in the fatty acid molecules. There are two types of essential fatty acids, the so-called n-3 and the n-6 fatty acids (or omega 3 and omega 6 fatty acids). n-3 fatty acids have the first double bond positioned after the third carbon atom from the end of the carbon chain whereas the n-6 fatty acids have the first double bond located after the 6th carbon atom. These n-3 and n-6 fatty acids are essential since the enzyme system of humans and animals can only generate a double bond after the 9th carbon atom, but not after the third and 6th carbon atom. Fish oil is rich in the n-3 fatty acids EPA (eicosapentaenoic acid, 20 carbon atoms en 5 double bonds) en DHA (docosahexaenoic acid, 22 carbon atoms en 6 double bands) (Figure 4).

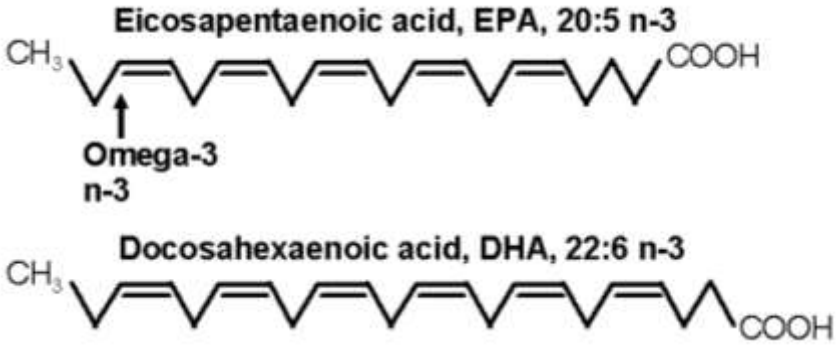


Figure 4.
Molecular structure of EPA and DHA

Vegetable fats and oils, however, do not contain EPA and DHA and fats and oils from terrestrial animals contain only very small amounts. Some vegetable oils contain the n-3 fatty acid ALA (alphalinoleic acid which has 18 carbon atoms and three double bonds). ALA is similarly as EPA and DHA an n-3 fatty acid, but has not the health promoting properties that are ascribed to EPA and DHA.

EPA and DHA are essential for both fish and humans.

The n-3 fatty acids EPA and DHA are essential for the growth and development of the fish and play an important role in the aquatic food web. These n-3 fatty acids are synthesized by phytoplankton (algae) and a further enrichment with these n-3 fatty acids takes place in zooplankton that feeds on phytoplankton. Various fish species, such as herring, feed on zooplankton and carnivorous fish species that feed on other fish have thus a high intake of these n-3 fatty acids. Moreover, carnivorous fish species have also a high requirement for n-

3 fatty acids since they have a very limited capability to synthesize these fatty acids themselves. Further, the lower the temperature of the water, the higher the level of n-3 fatty acids in the fish; a high level of these polyunsaturated fatty acids in the fat tissue of (marine) fish keeps the fat tissue soft which is very important for the fish at low water temperatures. Omnivorous and herbivorous fish species have a lower intake of n-3 fatty acids because they feed less on other (smaller size) fish that are rich in n-3 fatty acids and consume more plant materials that contains little or none of these n-3 fatty acids. Omnivorous and herbivorous fish species such as the tilapia, have also a lower requirement for EPA and DHA than carnivorous fish species, since they have a certain capability to synthesize the n-3 fatty acids EPA and DHA themselves from the n-3 fatty acid ALA that is present in various vegetable oils.

Alternative oils for fish oil

Fish feed is traditional rich in fish oil and thus rich in the n-3 fatty acids EPA and DHA. The supply of fish oil is limited and the demand is increasing; therefore, a part of the fish oil in fish feed is nowadays replaced by vegetable and oils. These vegetable oils contain, however, no EPA and DHA (See Table 9 on page 29 and Appendix 4 on page 38). Therefore, a certain amount of fish oil has to be included into fish feed to meet the requirement of the fish.

The replacement of fish oil by alternative sources of oils can also have an effect on the fatty acid composition of the fish. The fatty acid composition of the fish reflects more or less the fatty acid composition of the feed. Less EPA and DHA in the feed means also less EPA and DHA in the fish. These fatty acids are also important for humans, that is why the consumption of fish is recommended. It is thus important that the level of EPA and DHA in the fish remains high. Therefore, at the end of the grow-out period, the fish is often fed with so-called finishing diets, that are diets with exclusively fish oil. This way, alternative oils can be fed without compromising the fatty acids composition of the fish. See Appendix for the fatty acid composition of various types of oils.

Fat level in the feed and the protein/fat ratio

Important characteristics of fish feeds are the protein and fat level. Fish feeds contain considerably higher levels of protein (about 30-50%) then feeds for terrestrial animals, which contain about 15 – 20 % protein. The fat level in fish feeds can range from about 10 – 30%. The amount of carbohydrates in fish feeds are usually low, since fish and particularly carnivorous fish, have a low capacity to digest carbohydrates. As a consequence, the energy in the diet has to be derived from fat and fat has a higher energy density than carbohydrates. For that reason, fish feeds are more concentrated and have thus also a higher protein level (up to about 40 – 45%) and energy density than feeds for terrestrial farm animals.

Protein is an expensive ingredient of fish feed and in addition, the oxidation of protein results in the formation of ammonia and thus more water pollution. The digestible protein / digestible energy ratio is an important characteristic of a fish feed, and as a rule of thumb, this ratio in the feed of a growing fish should be more or less similar to the ratio of protein / energy of the fish itself. This way, a maximal retention of dietary protein, an expensive ingredient of (fish) feed, is achieved. Table 2 gives the typical protein and fat levels in the feeds for some fish species. The eel, salmon and the trout are fatty fish species and will thus require a higher fat level in the feed than fish species that are less fatty such as the tilapia and the carp. A protein / fat ratio that is tailored to a specific fish species results in a maximal utilisation of the protein for tissue accretion and a minimal utilisation of the protein as fuel; this phenomenon is called the protein sparing effect of fat.

Table 2
Typical protein and fat levels of feeds for various fish species.

Fish Species	Protein (%)	Fat (%)
Carp	40	8
Tilapia	40	9
African Catfish	45	12
Trout	43	28
Eel	46	30
Salmon	38	35

5. Ash, minerals and trace elements

The ash in the feed is the left-over after incineration of the organic materials in the feed. The ash content is determined by placing the feed for a couple of hours in a very hot oven. The ash contains the minerals and the trace elements.

Ash content in fish feeds.

Fish feeds have in usually a high ash level (about 6 – 10%) and this is due to the fish meal in the diet. Fish meal is produced from whole fish or from fish offal and contains thus also the skeleton and the bones. The skeleton and the bones are important sources of ash and minerals (e.g. calcium and phosphor) and fish feeds with a high fish meal levels have thus also a high ash level. The ash content in the feed is thus more or less an indication of the amount of fish meal in the diet.

Minerals and trace elements

Minerals and trace elements are inorganic compounds that play a role in various metabolic processes. There are 7 minerals and various trace elements (Table 3). They are important for the formation of the skeleton and for growth. Dissolved as salts, they determine the physical-chemical status of body cells and liquids and regulate the base-acid equilibrium. In addition, they can also have a function as part of enzyme systems. The requirement of the minerals, also called macro elements is expressed in grams per kg feed, whereas the requirement of the trace elements, also called micro elements, is considerably lower and is expressed in milligrams per kg feed (Table 3).

In contrast to terrestrial animals, are fish also able to absorb minerals and trace elements from the water through the gills and even through the skin. Marine fish, in contrast to fresh water fish, drink continuously and can also absorb minerals and trace elements this way.

Calcium and phosphorous

Calcium and phosphorous are the most abundant minerals in the fish and are important for the formation of the skeleton. Calcium and phosphorous in the form of hydroxyapetite ($(Ca_{10}(PO_4)_6(OH)_2$) constitutes a major part of the skeleton. The hydroxyapetite crystals in the skeleton and the bones are embedded in a matrix of collagen fibres. A trout contains about 0.43% calcium and 0.41% phosphorous and the carp about 0.61% en 0.50%, respectively. A lack of these minerals can result in reduced growth and deformations. Water is rich in calcium and the uptake of calcium from water is an important source of calcium for the fish; this way, the fish is also less dependent on calcium from the feed. The phosphorous level of water is usually low and the uptake from the feed is the major source of phosphorous for the fish.

Table 3

Mineral (7) and Trace Element Requirements for Trout and Carp[1]

	Atomic Weight	Requirements mg element/kilogram diet		
		Trout (NRC[2])	Carp (NRC[2])	General (ADCP[3])
Minerals				
Calcium (Ca)[4]	40.1	10,000	not known	5000
Phosphorus (P,available)[4]	30.9	6000	6000	7000
Magnesium (Mg)	24.3	500	500	500
Sodium (Na)	23.0	6000	not known	1000 - 3000
Potassium (K)	39.1	7000	not known	1000 - 3000
Chlorine (Cl)	35.5	9000	not known	1000 - 5000
Sulfur (S)	32.1			3000 - 5000
Trace Elements				
Iron (Fe)	55.8	60	150	50 - 100
Copper (Cu)	63.5	3	3	1 - 4
Iodine (I)	126.9	1.1	not known	100 - 300
Manganese (Mn)	54.9	13	13	20 - 50
Molybdenum (Mo)	95.9			trace
Fluorine (F)	19.0			trace
Selenium (Se)	79.0	0.3	not known	
Zinc (Zn)	65.4	30	30	30 - 100
Cobalt (Co)	58.9			5 - 10
Chromium (Cr)	52.0			trace
Nickel (Ni)	58.7			
Aluminum (Al)	26.9			
Tin (Sn)	118.7			
Vanadium (V)	50.9			

1. For more information on the minerals and the form of the minerals in fish feed: See also Hertrampf, J.W. & Piedad-Pascual, F. (2000) Handbook on Ingredients for Aquaculture Feeds. Kluwer Academic Publishers. ISBN 0-412-62760-4 (page 302-313) and Nutrient Requiremens of Warmwater Fishes and Shellfishes. (1983). National Academy Press, Washington, D.C. ISBN 0-309-03428-0 (page 90 – 92).

2. Nutrient Requirements of Fish (NRC) 1993, National Academic Press Washington D.C. ISBN 0-309-04891-5, on page 63. Also on the internet: (www.nap.edu/books/0309048915/html/1.html) (see table 7 in this internet publication). See also Kaushlik, Aquaculture 199:225-241 (1995) Nutrient requirements, supply and utilization in the context of carp culture.

3. Source: ADCP, 1983 (Aquaculture Development and Coordination Program of the FAO: Fish Feeds and Feeding in Developing Countries - An Interim Report on the ADCP Feed Development Programme ADCP/REP/83/18:97p, Chapter 4, Tables 5, 6, and 7. (http://www.fao.org/docrep/q3567e/q3567e00.htm).). Cited in: Feed and Feeding of Fish and Shrimp. Publication of the FAO by Michael B.New ((http://www.fao.org/docrep/S4314E/s4314e00.htm)

Availability of phosphor

The availability of phosphorous is dependent on the form of the phosphorous, but also on the fish species. There are various forms of phosphorous, the higher the solubility of the phosphorous in water, the higher the availability and the uptake; e.g. mono- and dicalciumphosphate are more soluble and also more available than tricalciumphosphate. Moreover, salmonids and tilapias can better utilize the various source of phosphor than carps that do not have a stomach and also no secretion of acid stomach juices; the solubility and thus the availability of various sources of phosphorous is higher in an acid environment that is a result of the acid in the stomach (*See Appendix 5 on page 39 for the availability of P from various sources in various fish species*).

Further, the phosphor in the feed can be bound to phytic acid, phytic acid can be present in ingredients derived from plants. Phytic acid is inositol with the hydroxyl groups linked to phosphate. Because of this linkage, the phosphorous is not available anymore, but the phosphate can be released by the enzyme phytase. Further, the phytic acid can also bind other elements such as calcium, zinc and magnesium and render them unavailable for the fish.

The phosphorous that is not taken up by the fish is released in the environment. It is important that the feed contains sufficient available phosphor to meet the requirements, At the same time, however, the amount of phosphorous in the feed should be as low as possible to reduce the amount of phosphorous that is released into the environment.

Other minerals and trace elements.

The other minerals have also specific metabolic functions. The minerals sodium potassium and chloride play a role in the regulation of the osmotic pressure of intra- and extracellular liquids. The mineral sulphur is part of the sulphur containing amino acids and is in the form of sulphate a component of e.g. chondriotin (in cartilage) and fibrinogen (for coagulation of blood). Zinc plays a role in various enzyme systems.

The various trace elements also play an important role in the metabolism of the fish. The trace element iron is part of the haemoglobin molecule and takes care of the transport of oxygen in the blood. Further, iron is part of the cytochrome system that has a function in the transfer of electrons that is linked to the formation of energy rich adenosinetriphosphate (ATP). Iodine is part of of the thyroid hormone thyroxin and cobalt is part of the vitamin B_{12} that plays a role in the formation of red blood cells. Other trace elements are often parts of various enzyme systems; deficiency symptoms have been shown when not sufficient amounts of these trace elements are included into the feed.

Minerals and trace elements in the feed

Many ingredients for the production of fish feed contain large quantities of minerals and trace elements. However, fish feeds are usually enriched with a mixture of minerals and trace elements to ensure that the intake of these elements is sufficient. (*See Appendix 6 on page 40 for the various forms of the minerals and trace elements in the feed and feed ingredients*)

6. Crude fibre and nitrogen free extract (NFE)

The determination of crude fibre is also a part of the Weende analysis. What is left after the determination of the moisture, protein, fat, ash and fibre is called the nitrogen free extract (NFE). Crude fibre and NFE are only present in ingredients derived from plant sources.

Composition of the carbohydrates in the feed

What is left after the determination of the moisture, protein, fat and ash in the feed are predominantly carbohydrates. Carbohydrates comprise a wide range of compounds (Figure 5). Lignin is also considered as a carbohydrate in this nomenclature, although lignin actually not a carbohydrate is but a polymer of phenol like compounds. The carbohydrates in the feed can be subdivided into crude fibre and NFE. Further, the carbohydrates can also be subdivided into non-starch polysaccharides (NSP) and starch and sugars (Figure 5). (*See also Appendix 7 on page 42 for further details*)

Composition and determination of (crude) fibre

It has always been difficult to measure the fibre and the various other carbohydrate fractions in the feed, since the fibre and the other carbohydrates consist of a wide range of

compounds. The crude fibre as originally determined by Henneberg en Stohmann around 1860 is measured by treating the feed first with acid and subsequently with alkaline. What is left after this process is considered as crude fibre and contains predominantly lignin, cellulose and hemicellulose (Figure 5). With his procedure, however, not all the cellulose, hemicellulose and lignin in the feed is measured and therefore, this method does grossly underestimate the fibre fraction of the feed.

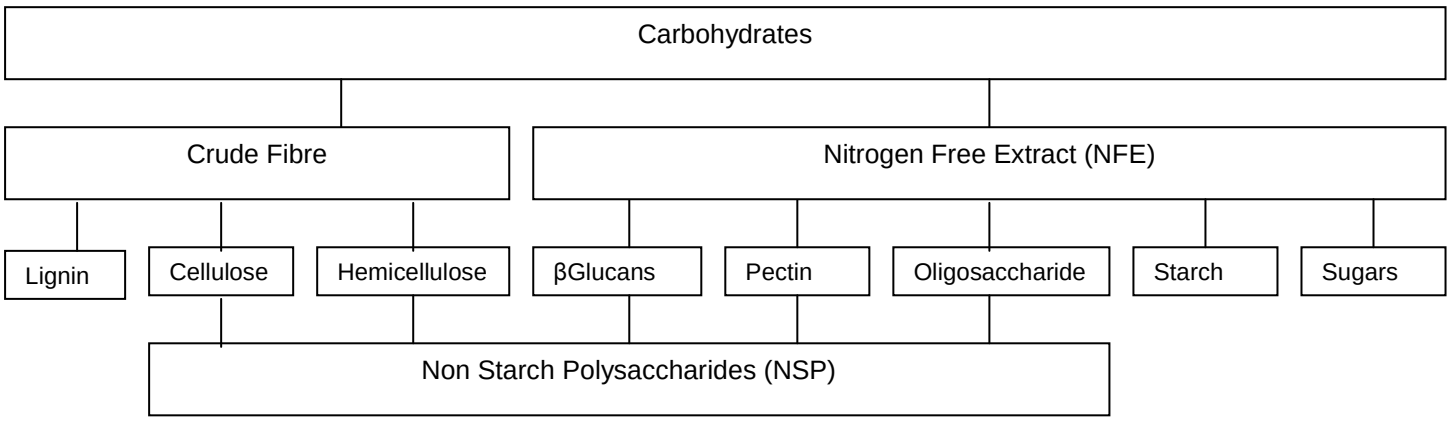


Figure 5.
The various carbohydrate fraction, the neutral detergent fraction(NDF) contains lignin, cellulose and hemicellulose; the acid detergent fraction (ADF) contains lignin and cellulose and acid detergent lignin (ADL) contains only lignin.

Later on, around 1960 -1970, the Van Soest method was introduced. This method utilizes so called “detergent solutions’, that are solutions that dissolve all the compounds such as proteins, starch and sugars in the feed except for the fibre and the ash. A correction for the ash is made by measuring the ash content in the residue. The fibre can be further fractionated by utilizing various “detergent solutions”. The use of a neutral detergent solution in combination with amylase to break down the starch results in a residue that contains the lignin, cellulose and hemicellulose (the “neutral detergent fibre” or NDF). The use of an acid detergent solution that contains sulphuric acid results in a fibre fraction that contains the lignin and the cellulose (the acid detergent fibre or ADF). The use of a detergent solution with 72% sulphuric acid results in a fibre fraction that contains only the lignin. These detergent acid methods measure all the lignin, cellulose, and hemicellulose in the feed.

Composition of the Nitrogen Free Extract

The nitrogen free extract comprises the sugars, starches, oligosaccharides, pectins and the betaglucans (Figure 5). The sugars are the monosaccharides such as glucose and fructose and the disaccharides such as sucrose or saccharose, maltose and cellobiose. These compounds are easy to digest.

Starch consists of long chains of glucose molecules. There are two types of starch, i.e. amylose, an unbranched chain of glucose molecules, and amylopectin, a branched chain of glucose molecules. Enzymes in the intestinal tract can break down the starch into small glucose molecules that are easily taken up by the intestinal cells.

Oligosaccharides are short chains composed of about 3 – 10 monosaccharides such as glucose, fructose and galactose and are water soluble and alcohol extractable. Soybeans and other pulses contain for example the oligosaccharides raffinose (3 monosaccharides), stachyose (4 monosaccharides) and verbascose (5 monosaccharides). The fish and other animals do not possess the enzymes to hydrolyze these oligosaccharides into

monosaccharides and are thus not able to digest these compounds. Oligosaccharides can, however, be fermented in the intestines and can result in the formation of gaseous compounds and in flatulence.

Pectins are longs chains composed of galacturonic acid. They can be found in sugar beet pulp and fruits and are similarly as oligosaccharides and betaglucans, indigestible. They have gelling properties and are used in the food industry because of these properties. They are hardly present in fish feed because no pectin-rich ingredients are used for the production of fish feed.

Betaglucans are branched polymers of glucose and are indigestible. They are part of the cell walls and particularly yeast products are rich in betaglucans. Betaglucans can be extracted from bakers yeast and are marketed as a supplement for both feed and food.

Carbohydrates (fibre and nitrogen free extract) in fish feed

Carnivorous fish species such as trout and salmon feed naturally on a diet that contain predominantly other fish species and is low in fibre and starch. These fish species have relatively short intestines like other carnivorous animal species (Table 4) and have a limited capacity to digest starch and sugars. As a consequence, carnivorous fish species have a preference for feed with little fibre and starch.

Table 4
Approximate length of the intestines expressed as a multiple of the body length.

Species	Length of intestines	Feeding pattern (predominantly)
Trout	0,7	carnivorous
African catfish	1,5	carnivorous
Grass carp	5	herbivorous
Milk fish	5	herbivorous
Tilapia	9	herbivorous
Humans	5	omnivorous
Cat/Dog	5	carnivorous
Pig	15	herbivorous
Cow	20	herbivorous
Sheep	30	herbivorous

Omnivorous and herbivorous fish species are in general better able to digest starch than carnivorous fish. These fish species feed naturally on a diet composed of plant materials that contain starch and fibre and have a higher activity of enzymes that can break down starch. The intestines of these fish species are usually also longer than those of carnivorous species. (Table 4).

It is important that the starch in the feed is sufficiently cooked and that the enzymes can get easily access to the starch molecules. The starch (the energy storage of the plant) in ingredients from plant origin are encapsulated into small particles that fall apart by heating and cooking. The extrusion process used for the production of fish feeds plays an important role in this process. The high temperature and pressure results in gelatinization of the starch

and makes it more easily accessible for the enzymes; raw starch is almost indigestible for the fish.

7. Vitamins

Vitamins are organic compounds other than amino acids, carbohydrates and fats and are essential for reproduction, growth and health. Vitamins are only needed in small quantities and can with a few exceptions not be synthesized by humans and animals.

Discovery of the vitamins

It was known for a long time that various diseases were caused by nutritionally deficient diets. As far back as in 1747, the Scottish marine surgeon James Lindin discovered that lemon and orange juice could cure or prevent scurvy on long sea journeys. It would take, however, almost 200 years, before the Hongarian scientist Szent-Györgyi was able to isolate and identify the “anti scurvy” factor, vitamin C. Interestingly, rats on board did not get scurvy, since rats were able to synthesize vitamin C themselves!

Similarly, it was know for a long time that rachitis could be prevented by supplementation of the diet with cod liver oil which turned many years later out to contain vitamin D. Animal experiments at the end of the 18th century showed that various diseases were the results of a deficient diet and that there were beside proteins, carbohydrates and fats also other compounds present in the diet that were essential. Many years later, in the beginning of the 20th century, these compounds were isolated and identified.

Fat soluble and water soluble vitamins

The vitamins can be divided into fat soluble (the vitamins A,D,E, and K) and water soluble vitamins. The fat soluble vitamins (ADEK) are found in fats and fatty ingredients. Fish oil and particularly cod liver oil is rich in vitamin A and D and vegetable oils are a good source of vitamin E. Vitamin K occurs in leafy ingredients such as alfalfa (Table 5).

The water soluble B vitamins occur in various ingredients from plant origin. An exception is vitamin B_{12}, that is not present plant products. Yeasts are a good source of vitamin B_{12} and are also rich in the other B vitamins (Table 5). Various animal and fish species eat plant materials (e.g. phytoplankton and algae) and as a consequence, these vitamins also end up in ingredients from animal origin. Particularly liver is rich in these vitamins since the liver is the central organ for distribution in the body. A raw material such as fish hydrolysates is also a good source of these vitamins. Fish hydrolysates are manufactured from offal of the fish industry and contains thus also the liver and other organs that are usually rich in vitamins. Fish is also a good source of B vitamins for humans, particularly of the vitamin B_{12}.

The water soluble vitamin C is predominantly present in vegetables and fruits. Insects can be a good source of vitamin C for carnivorous fish species such as trout, but also other, smaller fish species that contain vitamin C through the ingestion of plant materials with vitamin C. Most fish species are not able to synthesize vitamin C themselves and have to obtain this vitamin from their diet. An exception is the sturgeon that is able to synthesize vitamin C.

Vitamin C is very unstable and can be easily oxidized. Fish feed is manufactured by extrusion under high pressures and temperatures and as a consequence, much of the vitamin C in the feed can be lost by oxidation. Therefore, a stable form of vitamin C is used, i.e. vitamin C that is bound to phosphate. This product is marketed as “Stay C” and is

generally used as the source of vitamin C in fish feeds. Fish have an enzyme that is able to cleave off the phosphate which renders the vitamin C again available for the fish.

Table 5
Overview of the vitamins and the requirements for the trout and the carp (NRC, 1993)

		Requirement (in mg of IE per kg feed)		Source
	Scientific name	Trout	Carp	
Fat soluble vitamins				
Vitamine A	Retinol	2500 IE	4000 IE	fish oil
Vitamine D_3	Cholecalciferol	2400 IE	-	fish oil
Vitamine E	Tocopherol	50 IE	100 IE	vegetable oils
Vitamine K	Menadion	10	-	liver, vegetables, alfalfa
Water soluble vitamins				
Vitamine B_1	Thiamin	1	0,5	yeast, bran, vegetables
Vitamine B_2	Riboflavin	4	7	yeast, liver, fish hydroysates
Vitamine B_3	Niacin, nicotinic acid	20	30	yeast, liver, fish meal and hydrolysates
Vitamine B_5	Pantothenic acid	20	30	yeast, fish hydrolysates
Vitamine B_6	Pyridoxine	3	6	yeast, fish hydrolysates
Vitamine B_8	Biotin	0,15	1	yeast, liver
Vitamine B_{11} (B_9 in USA and Germany)	Folic or Pteroyglutamic acid	1	-	yeast, vegetables, afalfa
Vitamine B_{12}	Cyanocobalamin	0,01	-	yeast, liver, fish meal and hydrolysates
Vitamine C	Ascorbic acid (phosphate bound)	50	-	insects, fresh fish, fruits
Vitamine like compounds				
Choline		1000	500	fish meal, soy, wheat
Inositol		3000	440	yeast, fish meal

IU, International Units:

Vitamin A: One IU equals the activity of 0.300 µg of retinal (vitamin A) or 0.344 µg all-trans-retinyl acetate or 0.550 µg all-trans-retinyl palmitate or 0.600 µg β-carotene

Vitamin D_3: One IU equals the activity of 0.025 µg cholecalciferol and 1 µg equals 40 IU. Vitamin D_2 (cholecalciferol) is generated by UV light and *Vitamin D_2* (ergocalciferol) is derived from plants and micro organism (from the plant sterol ergosterol by UV light).

Vitamin E: One IU equals the activity of 1 mg D,L-α-tocopheryl acetates (*all-rac*-α-tocopheryl acetate)

Vitamin C: One IU equals 50 µg or 0.05 mg

Various vitamins can also be generated by the bacteria in the intestines of the fish. This capacity is higher in omnivorous and herbivorous fish since the intestines of these fish species are longer than those of carnivorous fish species (Table 4).

Requirements and biological functions of the vitamins

The requirements of the various vitamins for the trout and partially for the carp according to the National Research Council (NRC 1993) are given in Table 5 (*see Appendix 8 on page 45 for further details*). The requirements for other fish species are not completely known or have not yet been examined.

The vitamins have various functions in the metabolism of the fish. Vitamin A deficiency results in cataracts (clouding of the lens of the eye) and vitamin D plays a role in the metabolism of calcium, i.e. the uptake, the transport and the formation of the skeleton; a vitamin D deficiency can result in a deformed skeleton. Vitamin E is know as the anti sterility vitamin because a deficiency results in sterility in rats (tocopherol means literally " to carry offspring"). Deficiency of this vitamin in fish results in a low growth rate and muscle dystrophy. Further, vitamin E is a good antioxidant that can prevent the oxidation of other compounds such as polyunsaturated fatty acids in fish feed. Vitamin K is derived from the German word "coagulation" because it plays a role in the coagulation of blood; a deficiency results in a prolongation of the bleeding time. It also plays a role in bone formation.

The water soluble vitamin C is known as the "anti scurvy" vitamin and is therefore also called ascorbic acid. Vitamin C plays a role in the formation of collagen and the conversion of the amino acid proline into hydroxyproline. Hydroxyproline is an important component of collagen which forms a matrix for the calcium and phosphor (hydroxyappetite) in the skeleton. Further, vitamin C plays a role in wound healing because of its role in the formation of collagen and scar tissue.

Another important water soluble vitamin is the vitamin B_{12}. Vitamin B_{12} contains a cobalt atom and is important for the production of red blood cells. Deficiency can results in so-called pernicious anaemia.

The other vitamins also play an essential role in the metabolism of the fish, but we will not further describe their functions in this short overview. See Halver and Hardy (2002) for further details on requirements and functions of the various vitamins.

Vitamin-like compounds

There are also vitamin-like compounds beside the compounds that are called vitamins. Vitamin like compounds are essential for the metabolic processes, but some of those compounds can also be synthesized by animals and the fish themselves, although not always in sufficient amounts. Choline is a vitamin-like compound and that has lipotropic properties, i.e. it prevents the formation of a fatty liver especially when a fatty diet is consumed. Choline is a part of phospholipids (such as lecithin) and is abundantly present in fish meal and soy that is rich in lecithin. Another vitamin like compound is inositol which occurs in various forms and the myoinositol is the biologically active form. Phytic acid that is frequently found in plant products is composed of inositol where the hydroxyl groups are bound to phosphate groups. Both the inositol and the phosphate are not available anymore in this form. Moreover, phytic acid can bind various minerals and trace elements and make those unavailable. The phosphate groups can, however, be removed again by the enzyme phosphatase.

See also Appendix 8 (page 45) and 9 for more details on vitamins and vitamin like compounds.

8. Energy

Around the year 1860, the researchers Henneberg and Stohmann at the Agricultural Research Institute in Weende in Germany proposed to partition animal feeds into six major compounds, i.e. (1) moisture, (2) protein, (3) fat, (4) ash, (5) crude fibre and the so called (6) nitrogen free extract (NFE). The moisture, protein, ash and fibre were measured and the NFE was calculated as the difference between the total amount of the feed and these five measured compounds. This so-called Weende analysis is still being used for the analysis of (fish) feeds.

The fats, proteins and carbohydrates (the NFE fraction) are the major source of energy in a feed or food. The energy densities of these three compounds are different and the amount of energy in a feed or food is related to the amount of fat, carbohydrates and proteins in the feed. The energy in a feed can be expressed as gross, digestible and metabolizable energy (Figure 6).

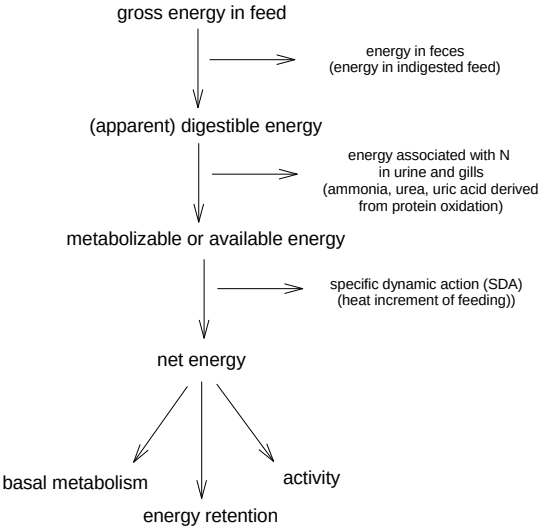


Figure 6
Gross, digestible, metabolizable, and net energy in a fish feed

Gross Energy

Gross energy is the energy that is released when nutrients, i.e. carbohydates, fats and proteins are completely oxidized, e.g. in a bomb calorimeter. The law of Hess (1838) states that the heat produced in a chemical reaction is always the same regardless of whether it proceeds directly or via a number of intermediate steps (law of constant heat summation). It means effectively that the heat of metabolizing a nutrient through a complex web of metabolic reactions in the body may be determined and duplicated by measuring the heat produced by burning the same nutrient in a bomb calorimeter. The gross energy can thus be determined by complete combustion of a feed in a so called bomb calorimeter and by measuring the amount of energy or heat that is released. This way, the amount of gross energy can be determined in a complete feed or in only fat, carbohydrates or proteins .

Digestible Energy

Digestible energy is the gross energy corrected for digestion (see Figure 6) and is the amount of gross energy in the feed that is digested and is taken up by the animal. For example when the digestibility of the energy is 95%, then the gross energy has to be multiplied by 0.95 to obtain the digestible energy. The digestibility of fat, carbohydrates and proteins is different and is dependent on various factors. Some raw materials are better digested than others and also the feeding level plays a role; a higher feed intake results usually in a lower digestion of the feed. The average digestibilities of the nutrients in fish feeds are given in Table 6.

Metabolizable Energy

The metabolizable energy is the energy in the feed that the fish can actually utilize. Metabolizable energy is the digested energy that the fish can use and is available to the fish.

The (gross) energy of the digested carbohydrates and fat are completely available for the fish. However, the (gross) energy in digested protein is not completely available to the fish. When proteins are oxidized in the body of the fish, the nitrogen in the protein has to be excreted as ammonia (85%) and ureum (15%). The fish can excrete nitrogen only in the form of these two compounds which contain a considerable amount of energy; ammonia has an energy density of 20.7 kilojoule (kJ) per gram and urea an energy density of 10.8 kJ per gram. Thus, part of the energy in the digested protein is excreted into the urine and the available (metabolizable) energy in the digested protein is thus less than the gross energy in the digested protein (see Figure 6). The energy excreted in ammonia (85%) and urea (15%) is 3.98 kJ per gram protein in the fish. Thus the metabolizable or available energy of protein is 23.65 – 3.98 = 19.67 kJ per gram protein for fish (see Appendix 11, page 52, footnote 6 (g) for the calculations).

Net Energy

The processing of the nutrients after digestion (storage, de-amination, synthesis such as synthesis of urea, uric acid etc.) requires energy and this energy is called the specific dynamic action (SDA) or the thermic effect of feed or food (TIF). The net energy is the metabolizable energy corrected for the energy of the SDA. Net energy is thus the energy that can eventually be used for the maintenance, activity and growth.

Calculation of the energy in a fish feed

The amount of energy in a fish feed can be easily calculated with the data in Table 6. The percentages of fat, protein, ash and fibre are usually declared on the label on the bag of fish feed and the percentage of moisture is usually about 8%. The percentage of carbohydrates (also called the nitrogen free extract, or NFE) is calculated as (100 - % protein - % fat - % ash - % fibre -% moisture). Table 7 gives as an example of a fish feed with 45% protein, 12% fat, 10% ash and 1.5% fibre. Thus the percentage of carbohydrates or NFE = (100 – 45% protein – 10% ash – 1.5% fibre – 8% moisture) = 23.5%.

Energy is usually expressed in joules (J) or kilojoules (kJ; 1 kJ = 1000 joules). Sometime, energy is also expressed in calories (cal) or kilocalories (kcal); 1 cal = 4.184 joule. In the metric system and in science only joules are used.

Table 6

Energy values of various dietary compounds as used in fish nutrition.

	Gross Energy in 1 gram nutrient (kJ/gram)	Metabolizable Energy in 1 gram nutrient (kJ/gram)	Digestibility (%)	Digestible energy In feed (kJ/gram nutrient)	Metabolizable energy In feed (kJ/gram nutrient)
Crude Fat	39.60	39.60	90 (90-95)	35.64	35.64
Crude Protein	23.65	19.67	95 (85-95)	22.50	18.69
NFE or Carbohydrates	17.50	17,50	70 (40-90)	12.25	12,25
Fiber and Cellulose	17.50	17,50	0	0	0

The metabolizable energy in 1 gram of fat or carbohydrate is similar to the gross energy in 1 gram of fat or carbohydrate. However, the metabolizable energy in 1 gram of protein is the gross energy (23.65 kJ) minus the energy that is excreted into the urine in the form of ammonia (85%) and urea (15%) (a total of 3.98 kJ, see Appendix 11, footnote 6 (g), thus 23.65 – 3.98 = 19.67 kJ). The values for gross energy and for the metabolizable energy in 1 gram nutrient can be used for all fish species. However, the values for the digestibilities (and thus the values for the digestible and metabolizable energy in the feed) may vary and is dependent on the type of the diet and the fish species.

Fish metabolize and oxydize predominantly fat and proteins and the average energy equivalent of oxygen (Eeq O_2) for fat (13.72 kJ per gram oxygen) and for protein (13.79 per gram oxygen in ammoniatelic fish) (see

Appendix 11, page 52) is about 13.75 kJ per gram oxygen. Thus, the energy expenditure or heat production of the fish in kJ can be calculated by multiplying the oxygen uptake (grams) of a fish by 13.75.

Table 7
Example of the calculation of the energy density of a fish feed (a typical trout feed).

Nutrient	% in diet	Gross Energy in 1 gram nutrient (kJ/g)	Metabolizable Energy in 1 gram nutrient (kJ/g)	Gross Energy in 1 gram feed (kJ/g)	Digestibility (%)	Digestible Energy in 1 gram feed	Metabolizable Energy in 1 gram feed
Protein	45	23,65	19.67	10,64	95	10,11	8,40
Fat	28	39,6	39,60	11,088	90	9,98	9,98
Ash	9						
Moisture	5						
Fiber	1	17,5	0	0,175	0		
NFE or carbohydrate	12	17,5	17,50	2,1	60	1,26	1,26
Total	100			24,01		21,35	19.64

Fat, protein, fibre, ash, and moisture are measured with the so called Weende Analysis. The carbohydrate fraction (also called the nitrogen free extract or NFE) is calculated as 1000 – (protein – fat – ash – fibre – moisture).

*DP/DE (digestible protein/digestible energy) = (450*0.95) / 21.35 = 20.02 mg/kJ.*

9. Ingredients for fish feed

Fish meal and fish oil are traditionally the major ingredients of fish feeds, particularly fish feeds for carnivorous fish. Nowadays, also other ingredients are used from both plant and (terrestrial) animal sources. This way, the fish feed production is less dependent on fish meal and fish oil; the demand for these ingredients is increasing while the availability is decreasing.

Natural diet of the fish

Various fish species and in particular marine fish and some fresh water species like the trout, the pike and the pike perch are carnivorous. Fish contains predominantly water (about 65-80%), protein (about 17%), fat (about 2 – 15%) and ash (about 2.5%). The natural diet of carnivorous fish contains thus predominantly protein and fat and no fibre and almost no carbohydrates, only the small amount present in the liver of the captured fish as glycogen. The activity of starch cleaving enzymes in the intestines of carnivorous fish is therefore low and the ideal diet for carnivorous fish would thus be fish. For that reason, the traditional fish feeds were mainly composed of fish meal and fish oil.

There are also various fish species that are omnivorous or herbivorous. These fish species consume plant materials and have a higher capacity to handle and to digest starch in their feed than carnivorous species. Their intestines are also longer than those of carnivorous fish as we have seen earlier (Table 4).

Protein and fat levels in fish feeds

Important characteristics of fish feeds are the protein and fat level. Fish feeds have usually a high protein level (about 40%) and a fat level that ranges from about 10 – 30% dependent on the fish species. Because of this high protein levels of fish feeds, the ingredients for fish feeds also need to have high levels of protein and fat.

Ingredients as a source for protein

An important source of protein for fish feeds is fish meal. Fish that is not suitable for human consumption, trash fish, and offal of the fish industry are processed to fish meal. Fish

meal contains predominantly protein and further ash and fat (Table 8). Fish meal is produced from the whole fish and fish offal and contains also all the bones of the fish. Therefore, the ash content of fish meal is usually high.

Table 8
Various ingredients that can be used as a protein source for fish feed

	Average Composition			
	Protein (%)	Fat (%)	Ash (%)	Moisture(%)
Animal				
Fish meal LT[1]	72	12	13	10
Standard fish meal	66	9	16	9
Bloodmeal	94	0	1	5
Haemoglobin powder[2]	96	1	1	2
Feathermeal	82	7	2	8
Plant				
Horse or faba beans	30	1	3	12
Soybeans	36	19	5	11
Soybean meal	49	1	7	13
Soy protein concentrate	56	3	7	8
Rapeseed	20	41	4	7
Rapeseed meal	34	2	7	12
Sunflower cake	26	2	6	12
Corn	9	4	1	14
Corngluten	60	6	2	8
Peas	21	1	3	13
Pea protein concentrate	73	4	5	8
Wheat (for expansion)[3]	11	1	2	15
Wheat middlings	15	3	5	14
Wheatgluten	79	7	1	7
Rice Protein Concentrate	65	2	4	12

1. *Fish meal LT is fish meal that is dried at a low temperature (Low Temperature), i.e. lower than 70 centigrade and with steam.*
2. *Haemoglobin powder consists of red blood cells that are separated from the blood plasma and that are spray dried.*
3. *Wheat is used as a source of starch for the expansion of the pellets (see chapter on extrusion).*

There are various quality fish meals. Standard fish meal is dried at a temperature of 95 – 100 centigrade with flame driers, but fish meal LT (Low Temperature) is dried at lower temperatures (lower than 70 centigrade and with steam); this fish meal LT is therefore considered as the best quality fish meal since little protein degradation had taken place. In addition, LT fish meal has a higher protein level than other qualitities fish meal. Fish meal LT is more expensive than standard fish meal and is predominantly used for the production of high quality fish feeds such as for larval feeds.

The demand for fish meal is increasing while the availability is decreasing. Therefore, other protein sources are nowadays also used in the fish feed industry. It is, however, important that these alternative ingredients have a high protein content. Fish feeds have a

high protein level and this high level can only be achieved with ingredients that also have a high protein level. The ingredients can be both from animal and vegetable origin. Table 8 gives an overview of various ingredients that can be used in fish feeds as a source of protein and sometimes at the same time as a possible source of fat.

There are various criteria that determine whether a raw material is suitable as a raw material for fish feed, such as the amino acid composition, the digestibility, the availability the cost price and the possible presence of so called antinutritional factors (ANFs), that can be found in many plant products. It is important that amino acid composition of the protein source matches the requirements of the fish. The amino acid composition of fish meal is often used as a reference since fish is the natural protein source of various fish species, particularly of carnivorous fish species (*see Appendix 2 on page 31*). It is also possible to enrich the fish feed with single amino acids when the level of a particular amino acid (too) low is. For instance, the sulphur containing amino acid methionine is added to soy protein and lysine to wheat gluten.

Protein sources of plant origin may contain so called antinutritional factors (ANFs). ANFs are compounds that can have a negative effect on the digestibility of the feed and the growth of the fish. Examples of ANFs are protease inhibitors, allergens, phytoestrogens, saponins, phytic acid and flatulence factors such as oligosaccharides. These ANFs can be inactivated by heating, by extraction (oligosaccharides) or by enzymatic hydrolysis (phytic acid).

Tabel 9
Various ingredients that can be used as a fat source for fish feed

	Average fatty acid composition (%)					
Ingredient	Palmitic acid C-16:0	Oleic acid C18:1 (n-9)	Linoleic acid C18:3 (n-6)	Linolenic acid C-18 (n-3)	EPA C-20:5 (n-3)	DHA C-22:6 (n-3)
Fish oil	18	15	2	2	10	13
Soy oil	10	23	51	7	-	-
Raapseed oil	4	56	20	9	-	-
Linseed oil	5	20	13	53	-	-
Palm oil	38	37	10	1	-	-
Coconut oil	8	6	2	-	-	-
Poultry fat	23	37	20	-	-	-
Tallow	25	36	3	-	-	-
Lard	24	14	10	-	-	-

Source: USDA Food Tables on the Internet (http://www.nal.usda.gov/fnic/foodcomp/search/).
See Appendix 3 on page 33 for the structure of the fatty acids and Appendix 4 on page 38 for more details on the fatty acid composition of various oils.

Ingredients as a source for fat

Fish oil was traditionally the only source of oil that was used for the production of fish feed. The fish oil is produced from fish species that are not suitable for human consumption and from offal of the fish industry. The availability of fish oil is limited and therefore, also other sources of fat are used now in the fish feed industry (Table 9). Fish oil is, however, an important ingredient for fish feeds, particularly for carnivorous fish species. Fish oil contains

the fatty acids EPA and DHA that are essential for these fish species. The requirement for these essential fatty acids is less for fresh water fish species since these species have a higher capability to synthesize these fatty acids themselves.

Special ingredients and additives

Fish feeds are often enriched with feed supplements and other additives. Pigments such as astaxanthin can be added to trout and salmon feeds to give the fillets a pink colour; wild trout and salmon eat for example shrimps that also contains astaxanthin. Ornamental fish feeds are usually supplemented with algae (spirulina) and red pepper extracts that contain pigments, so called carotenoids or xanthophylls that colour the skin of the fish (*see Appendix 10 on page 49 for the various carotenoids*). Further, also supplements such as betaglucans can be added that may have an effect on the health status of the fish.

Formulation of fish feed.

The most important ingredients for a fish feed are (1) a source of protein, (2) a source of fat, (3) a mineral and vitamin mixture and (4) a source for starch (wheat) that serves as a binder for the production of the pellets (see chapter on extrusion). Further, ingredients can be added (5) that have a particular function such as pigmentation of the fish, or other feed supplements.

Fish feeds are formulated with a computer program, a so called optimalization program. This program has all the available ingredients and the composition of all the nutrients in the ingredients. The various criteria for a particular fish feed can be entered into the program such as the protein and fat level, the amino acid composition, fatty acid composition, vitamins and minerals and the maximum or minimum amount of a particular ingredient. Subsequently, the program generates a composition that meets all the required criteria and gives a complete overview of all the nutrient composition of the feed. The feed is subsequently produced in the factory with this recipe.

10. Production of fish feeds by extrusion

Feeds for pets and production animals such as poultry and pigs can be easily produced in the form of meal, slurry or pelletized particles. However, fish live in water and the way the feed is administered to the fish is very important. The feed has to be stable in water and free of dust in order to prevent spillage and pollution of the water. Further, the size of the pellet has to match the size of the fish since the fish swallows the feed as an intact particle

At first, fish feed was produced in the pelletized form. Pelletized feed is still being used for manufacturing feed for most of the production animals such as poultry, pigs, and cows. Pelletized feed is rather stable and the cost for producing these pellets is low. A disadvantage of the pelletizing process is that there are limited possibilities to influence the physical properties of the pellets, such as density and the shape of the pellet. Further, it is difficult to make pellets with a high fat content; the pellets become soft and the oil easily leaks out of the pellet. Moreover, the starch in the pellets produced by the pelletizing process is only partly cooked or gelatinized and fish can hardly digest raw, uncooked starch.

Extrusion process

Around 1970 – 1980, the extrusion process was introduced to produce fish feed. The extrusion technique has already been used for a long time and originates from the rubber and plastic industry. As far back as in 1797, Joseph Bramah constructed a hand driven plunger press to manufacture lead pipes. Later on, these same types of machines were used to produce soap, pastas and building materials. The screw extruder was developed at the same time when the electricity was introduced, since the cable industry needed a continuous process to mantle electricity cables with rubber. The first concepts of double screw extruders

originate from 1873 and the first double screw extruder for plastics was already built in Italy before the second world war.

Extruders are in principle pumps, but special pumps that can also transport, melt, cook, heat and mix. An extruder is designed to transport under high pressure and temperature a continuous flow of melted or cooked material to a die.

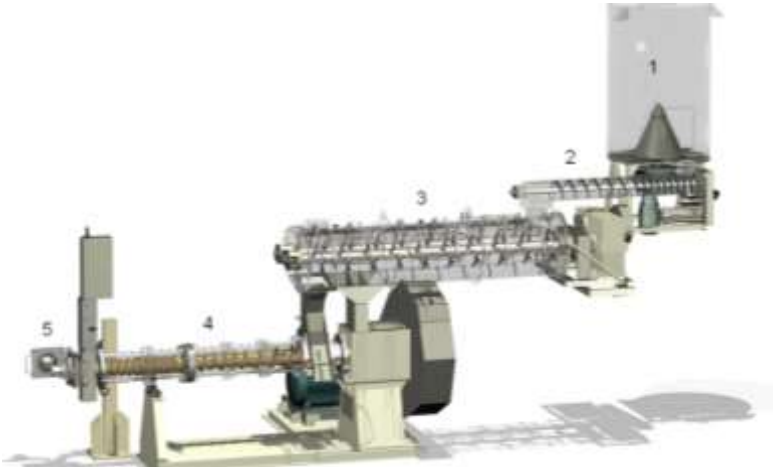


Figure 7

Overview of the extrusion process of fish feed. 1. Storage of the mixed and ground ingredients. 2. System for transport of the ingredients to the conditioner. 3. Conditioner where hot water, steam and other liquid ingredients can be added. 4. Barrel of the extruder with the Archimedes screw. (see also figure 8) 5. Die and rotating knives for cutting off the pellets (see also figure 9).

Extrusion process of fish feed

An extruder is a kind of a pressure cooker; the Archimedes screw in the extruder builds up a high pressure which results in an increase of the boiling point of the water and high temperatures are generated by conversion of mechanical energy into heat (Figure 7). The high temperatures in the extruder result in a fast cooking or gelatinization of the starch which makes the starch digestible for the fish. At the same time, the cooked starch acts as a binder to be able to produce a pellet with a firm structure that is water stable and dust free. Further, the extrusion technique allows to manipulate the physical properties of the product, such as shape and density. Finally large amounts of fat can be coated onto the pellets because of their light structure. This all together, makes the extrusion technique very useful for the production of fish feed.

Figure 8

Barrel of the extruder with an Archimedes screw (see also figure 7)

Grinding and mixing

The extrusion process involves various steps. The various ingredients of the fish feed are first mixed and ground with a hammer mill. A hammer mill has a set of hammers that turns around and crushes the particles. A sieve is constructed around the hammer mill and only the small particles can pass the sieve that has a particular mesh size. The ingredients should be ground properly particularly for the production of feeds for small larvae.

Conditioner

The ground mixture of ingredients is transferred to a conditioner, a kind of cooking device where steam and water (up to 30%) is added to the mixture and where the ingredients are thoroughly mixed. This conditioner step involves heating, moisturizing, and mixing of the ingredients, thus a pre-treatment or conditioning of the ingredients. The temperature in the conditioner can go up to 100 ^{o}C (boiling point of water) and the temperature is dependent on the type of feed that is produced or the type of ingredients that are used.

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Extruder

Subsequently, the precooked or conditioned feed mixture is transferred to the extruder. The Archimedes screw will knead and force the wet dough through the extruder and the wet mass of feed is eventually pressed through the die at the end of the extruder (Figure 9). The shape and the size of the holes in the die will determine the shape and the size of the pellets. The feed leaves the die as spaghetti like strings that are cut off by rotating knives; the rotation speed of the knives determines the length of the pellets.

The Archimedes screw induces a high pressure in the barrel and high temperatures are achieved by the conversion of the mechanical energy into heat. The high pressure in the barrel will also result in an increase of the boiling point of the water in the extruder; an increase of the pressure by 1 bar or 1 atmosphere will increase the boiling point of water by 26 ^{o}C. This means that the water in the extruder can be heated to temperatures higher than 100 ^{o}C which will accelerate the cooking process of the feed. Thus, the extruder is comparable to a high pressure cooker, where also temperatures higher than 100 ^{o}C are achieved by increasing the boiling point due to the high pressure; because of these higher temperatures, the food in the pressure cooker will be cooked faster. The residence time of the feed in the extruder is only a few minutes, and therefore, the cooking process has to take place in only a short period. The feed contains starch that has to be fully cooked or gelatinized, since fish and particularly carnivorous fish have difficulties to digest raw starch

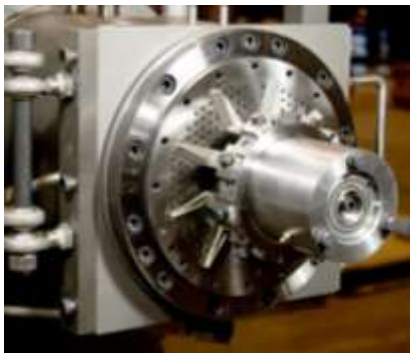

Figure 9
Die with rotating knives for cutting off the pellets (see also figure 7).

Further, the gelatinizing process is also important for the structure of the fish feed pellet. When the pellets leave the die of the extruder, the pressure is lowered and the water in the pellet will suddenly start to boil and evaporate. As a consequence, the pellet will

expand and a light and porous pellet is obtained. The gelatinized starch in the feed will act as a binder and give the pellet a firm structure that is water stable and dust free. The degree of expansion is related to various factors such as the temperature and pressure in the extruder, and the amount and type of starch. The degree of expansion also determines the physical properties of the pellet, a high degree of expansion results in a light pellet that will float, whereas a low degree of expansion will result in a dense pellet that will easily sink in the water. Some fish species such as African catfish, tilapia and carp require a floating pellet, whereas other fish species such as salmon, trout and cod require a slow sinking pellet. Thus, the feeding habit of a fish species determines the physical properties of a feed.

Drying

The pellets that leave the extruder are very wet and can contain more than 30% moisture. A high moisture content can easily result in the formation of moulds and reduce the shelf life of a feed. Moreover, the pellets are very soft. Therefore, the pellets have to be dried. This process takes place in a drier; the wet pellets are dried with hot air on a belt of a mesh wire.

Vacuum coating

The amount of oil that can be extruded into the pellets is very limited. A high fat content of the wet mass of feed results in a smooth consistency which makes it difficult to built up a high pressure in the extruder. Moreover, extruding a fatty feed mass results in little expansion when the pellet leaves the extruder. Therefore, most of the oil is coated on the pellet with a vacuum coater. The dried pellets are transferred in batches into a vacuum coater, a kind of a large drum. Subsequently, the coater is placed under vacuum and the oil is sprayed onto the pellets. The vacuum is released again and the oil is pressed into the pellets. This way, large amounts of oil can be coated onto the pellets and feed with a fat level (up to 40%) can be produced while only 5% of fat can be coated onto pelletized feed. Particularly feeds for salmonids require a high fat level. Finally, the pellets are cooled with air from outside.

Fish feeds are thus nowadays exclusively produced by means of extrusion. Extrusie is a technique that makes it possible to produce feed pellets of various sizes and shapes. The extrusion process results in pellets that have a firm structure and are water stable and free of dust. Further, it is possible to manipulate the physical properties of the pellets and floating and sinking pellets can be manufactured. The feed is exposed to high temperatures and pressure during the extrusion process which kills off bacteria and moulds. Finally, the extrusion process makes it possible to coat the pellets with large amount of fat and feeds with a high fat content can be produced.

Figure 10
Fish feed pellets manufactured by extrusion

11. Literature

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Nutrient Requirements of Warmwater Fishes and Shellfishes. (1983). National Academy Press, Washington, D.C. ISBN 0-309-03428-0 (out of press)''

Nutrient Requirements of Fish (1993). National Academy Press, Washington, D.C. ISBN 0-309-04891-5. This book can also be read at the internet (www.nap.edu/books/0309048915/html/1.html)

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(Volume 1: http://www.fao.org/docrep/field/003/AB470E/AB470E00.htm
(Volume 2: http://www.fao.org/docrep/field/003/AB468E/AB468E00.htm
(Volume 3: http://www.fao.org/docrep/field/003/AB467E/AB467E00.htm)

Websites.

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FAO Fisheries and Agricultural Department. http://www.fao.org/fishery/en

FAO Stat. Website of the FAO with statistical data etc. . http://faostat.fao.org/site/291/default.aspx

Feed and Feeding of Fish and Shrimp. Publication of the FAO by Michael B. New: (http://www.fao.org/docrep/S4314E/s4314e00.htm)

Nutrition of Fish and Crustaceans: A Laboratory Manual: (http://www.fao.org/docrep/field/003/AB479E/AB479E00.htm) An overview of laboratory methods for feed analysis.

FAO website: Food Composition Tables for International Use (1955): http://www.fao.org/docrep/x5557e/x5557e00.htm#Contents

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DTU Food Tables on the Internet (Danish Technical University): http://www.foodcomp.dk/v7/fcdb_default.asp

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http://www.fao.org/docrep/006/y5022e/y5022e03.htm#bm3.1

Appendix 1

Whole Body Amino Acid Composition of various Fish Species (% of total protein)

Product	Fishmeal[1]	Whole Trout[2]	Whole Salmon[2]	Channel catish[3]	Carp (Cyprinus carpio L)[4]	Sturgeon[5]	Goldfish[6]	Golden Shiner[6]	Fathead Minnow[6]	Silver Perch (Bidyanus bidyanus) Ngamsnae[7]
Source Composition Data	CVB	Halver[9] (2002)	Halver[9] (2002)	Halver[9] (2002)	Schwarz (1988)	Ng (1994)	Gatlin (1987)	Gatlin (1987)	Gatlin (1987)	(1999)
Essential Amino acids										
Arginine[1]	5,9	6,41	6,61	6,67	6,02	7,62	6,81	6,41	6,37	5,82
Histidine	2,6	2,96	3,02	2,17	2,82	3,10	2,61	2,62	2,53	2,19
Isoleucine	4,2	4,34	4,41	4,29	3,73	4,81	3,99	4,11	4,19	3,95
Leucine	7,3	7,59	7,72	7,40	6,87	7,25	7,49	7,62	7,78	7,75
Lysine	7,6	8,49	9,28	8,51	6,55	8,06	8,59	8,85	8,88	7,88
Methionine	*2,8*	*2,88*	*1,83*	*2,92*	*2,11*	*3,09*	*3,08*	*3,21*	*3,01*	*3,25*
Cysteine[9]	*0,9*	*0,80*	*0,95*	*0,86*	*0,68*	*0,56*	*1,02*	*0,92*	*0,73*	*0,86*
Methionen+Cysteine	3,1	3,68	2,78	3,74	2,79	3,65	4,10	4,13	3,74	4,11
Phenylalanine	*3,9*	*4,38*	*4,36*	*4,14*	*3,78*	*5,03*	*4,10*	*4,47*	*4,55*	*4,76*
Tyrosine[9]	*3,1*	*3,38*	*3,50*	*3,28*	*2,28*	*3,74*	*3,11*	*3,12*	*3,09*	*2,96*
Phenylal.+Tyrosine	7,0	7,76	7,86	7,42	6,06	8,77	7,21	7,59	7,64	7,72
Threonine	4,2	4,76	4,95	4,,41	3,95	5,26	4,66	4,97	5,15	5,95
Tryptophane	1,1	0,93	0,93	0,78	0,81	0,74	0,94	0,93	1,11	n.d.
Valine	4,9	5,09	5,09	5,15	4,26	5,24	4,49	4,42	4,36	4,92
Sub Total[10]	47,9	52,0	52,7	46,1	43,9	54,5	50,9	51,7	51,8	50,3
Semi-Essential Amino Acids										
Cysteine	0,9	0,80	0,95	0,86	0,68	0,56	1,02	0,92	0,73	0,86
Tyrosine	3,1	3,38	3,50	3,28	2,28	3,74	3,11	3,12	3,09	2,96
Non Essential Amino Acids										
Alanine	6,3	6,57	6,52	6,31	6,34	6,09	7,09	6,77	6,56	8,95
Asparagine	9,3	9,94	9,92	9,74	9,21	8,12	9,13	9,50	9,25	7,47
Glycine	6,5	7,76	7,41	8,14	7,94	7,54	8,57	7,87	7,53	10,25
Glutamine	13,0	14,22	14,32	14,39	13,23	13,83	14,46	14,42	14,39	10,17
Proline	4,4	4,89	4,64	6,02	5,44	5,07	5,01	4,81	4,80	5,48
Serine	4,0	4,66	4,61	4,89	3,97	4,86	4,84	4,97	5,74	7,38
Sub Total	43,5	48,04	47,42	49,49	46,13	45,51	49,10	48,34	48,27	49,70
Grand Total	91,4	100,1	100,1	95,6	90,0	100,0	100,0	100,0	100,0	100,0

1. CVB, Veevoeder tabel of the CVB (Centraal VeevoederBureau, Runderweg 2, Postbus 2176, 8203 AD Lelystad, The Netherlands.

2. Wilson RP & Cowey CB (1985) Amino acid composition of whole body tissue of rainbow trout and atlantic salmon. Aquaculture 48: 373-376. (PDF) Summarized in : Halver, J.E. & Hardy, R.W. (2002) Fish Nutrition. Academic Press, London ISBN 0-12-319652-3 on page 171.

3. Wilson RP & Poe WE (1985) Relationship of whole and egg essential amino acid patters to amino acid requirement patterns in channel catfish , Ictalurus punctatus. Comp. Biochem. Physiol. 80B: 385-385. (PDF) Summarized in : Halver, J.E. & Hardy, R.W. (2002) Fish Nutrition. Academic Press, London ISBN 0-12-319652-3 on page 171.

4. Schwarz, F.J. & Kirchgessner, M. (1988) Amino acid composition of carp (Cyprinus carpio L.) with varying protein and energy supplies. Aquaculture 72: 307-317. The average body weight of the carps were 1014 grams.

5. Ng, W.K. & Hung, S.S.O. (1994) Amino acid composition of whole body, egg and selected tissues of white sturgeon (Aciper transmontanus). Aquaculture 126: 329-339. The average weight of the sturgeons were 535 grams.

6. Gatlin, D.M. (1987) Whole body amino acid composition and comparative aspects of amino acid nutrition of the goldfish, golden shiner and fathead minnow. Aquaculture 60: 223-229.

7. Ngamsnae, P. Desilva, S.S. & Gunasekera, R.M. (1999) Arginine and phenylalanine requirement of juvernile silver perch Bidyanus bidyanus and validation of the use of body acid composition for estimating individual amino acid requirements. Aquaculture Nutrition 5: 173-`180.

8. Further, Mai et al. (1980) described the amino acid composition of 6 fresh water fish species (Mai et al. Protein and amino acid composition of selected fresh water fish, J. Agric. Food Chemistry 28: 884 – 885)

9. Other studies describing the whole body amino acid composition of various fish species:

(a) Ramseyer, L.J. & Garling, D.L.J.R. (1994) Amino acid composition of the ovaries, muscle and whole body of yellow perch. Prog. Fish-Cult. 56: 175-291 .
(b) Mohanty, S.N., & Kaushlik, S.J. (1991) Whole body amino acid composition of Indian carps and its significance. Aquat. Living Resour. 4: 61-64.
(c) Ostrowski, A.C. & Divakaran S. (1989) The amino acid and fatty acid composition of selected tissues of the dolphin fish (Coryphaena hippurus) and their nutritional implications. Aquaculture 80: 285-299.
(d) Njaa, L.R. & Utne, F. (1982) A comparison of the amino acid composition of fifteeen species of whole fish. Fisk. Skr. Ernaehrung 2: 25-33 (cited in Nutr. Abstr. Rev. Ser B (1984), 54: 3091.
(e) Dabrowska, H & Wojno, T (1977) Studies on the utilization of rainbow trout of feed mixtures containing soybean meal and an addition of amino acids. Aquaculture 10: 297-310
(g) Kim, J-D & Lall, S.P. (2000) Amino acid composition of whole body tissue of atlantic halibut, yellow tail flounder and japanes flounder. Aquaculture 187: 367-373
(h) Oellermann, L/K.% Hecht, T. (2000) Comparison of the fillet yield, protein content and amino acid profile of Claria Gariepinus and the claris garipienus x heterobranchus longifilis hybrid. Aquaculture Research 31: 553-556. (Amino acid composition of the fillet)
(i) Clement, S. & Lovell, R.T. (1994) Comparison of processing yield and nutrient composition of tilapa and channel catfish. Aqauculture 119: 299-310 Composition of fillet.
(j) Haaland, H., Arnesen, E. & Njaa, L.R. (1990) Amino acid composition of whole mackerel (scomber scrombrus) stored anaerobically at 20 ^{o}C and 2 ^{o}C. Int J Food Sci Technol 25: 82-87
(k) Kaushik, S.J. (1998) Whole body amino acid composition of european seabass, guilthead seabream and turbot with an estimation of their IAA requirement profiles. Aquatic Living Resources 11: 355-358.

10. This subtotal does include the cysteine and methione.

Appendix 2

Amino acid composition of various fish feed ingredients that are used as a protein source

Product Manufacturer	Potato protein AVEBE	Porcine Plasma Powder Sonac	Hemo- globin Powder Sonac	Feather meal	Rice Protein	Rice Protein Sopropeche	Casein	Sweet Lupins	Corn Gluten	Wheat Gluten	Wheat	Soy 50	Egg (dried)	Pea Conc.	Whey Protein Conc.	Fishmeal	Require-ments[11] (Trout)
% Protein	80	70	90	82.4		66	84.0	36.0	60	76.3	11.1	48.9	47.4	78.0	78	69.9	
Source Composition	Protastar[5]	Sonac[7]	Sonac[7]	CVB[6]	CVB[6]	Manuf.	CVB[6]	CVB[6]	CVB[6]	CVB[6]	CVB[6]	CVB[6]	USDA[9]	Manuf.[8]	Volac[10]	CVB[6]	NRC 1993
Essential AA																	
Arginine[1]	5,5	5,4	3,9	6,9	7,8	9,4	3,6	10,8	3,2	3,6	4,7	7,4	6,0	8,5	2,3	5,9	4,0
Histidine	2,4	3,4	7,3	1,0	2,7	2,3	3,1	2,5	2,1	2,1	2,3	2,7	2,4	2,5	2,0	2,6	1,8
Isoleucine	5,9	3,1	0,8	4,8	3,7	4,2	5,2	4,1	4,1	3,7	3,4	4,6	5,5	5,1	5,9	4,2	2,4
Leucine	10,8	8,9	13,2	8,3	7,3	8,6	9,7	7,0	16,6	7,0	6,6	7,7	8,5	8,5	10,3	7,3	3,7
Lysine	8,0	8,1	8,9	2,5	4,2	3,1	8,0	4,8	1,7	1,7	2,8	6,2	7,2	7,5	8,9	7,6	4,8
Methionine	*2,4*	*0,5*	*0,9*	*0,7*	*2,1*	*2,9*	*3,0*	*0,7*	*2,4*	*1,6*	*1,6*	*1,4*	*3,1*	*1,2*	*2,1*	*2,8*	
Cysteine[2]	*1,6*	*3,3*	*0,8*	*5,0*	*2,2*	*2,2*	*0,4*	*0,8*	*1,8*	*2,2*	*2,2*	*1,5*	*2,3*	*1,2*	*2,3*	*0,9*	
Meth+Cysteine	4,0	3,8	1,7	5,7	4,3	5,1	3,4	1,5	4,2	3,8	3,8	2,9	5,4	2,4	4,4	3,7	2,6
Phenylalanine	*6,9*	*5,4*	*7,1*	*4,9*	*4,7*	*5,4*	*5,2*	*3,9*	*6,3*	*5,3*	*4,5*	*5,1*	*5,3*	*5,5*	*3,1*	*3,9*	
Tyrosine[3]	*5,8*	*5,1*	*2,4*	*3,1*	*3,4*	*5,2*	*5,6*	*4,0*	*5,2*	*3,4*	*2,8*	*3,7*	*4,1*	*3,2*	*2,3*	*3,1*	
Phenylal.+Tyrosine	12,7	10,5	9,5	8,0	8,1	10,6	10,8	7,9	11,5	8,7	7,3	8,8	9,4	8,7	5,4	7,0	4,7
Threonine	6,0	5,4	3,7	4,7	3,7	3,8	4,3	3,5	3,4	2,5	2,9	3,9	4,8	3,9	7,0	4,2	2,1
Tryptophane	1,3	1,5	1,4	0,7	1,1	1,1	1,3	0,9	0,5	0,9	1,2	1,3	1,2	1,1	1,3	1,1	0,5
Valine	7,1	6,1	8,3	7,3	5,5	5,7	6,7	3,9	4,7	4,0	4,3	4,8	6,1	5,3	5,5	4,9	3,2
Sub Total[4]	63,7	56,2	58,7	49,9	48,4	53,9	56,1	46,9	52,0	38,0	39,3	50,3	56,5	53,5	53,0	48,5	29,8
Semi Essential AA																	
Cysteine	1,6	3,3	0,8	5,0	2,2	2,2	0,4	0,8	1,8	2,2	2,2	1,5	2,3	1,2	2,3	0,9	
Tyrosine	5,8	5,1	2,4	3,1	4,7	5,2	5,6	4,0	5,2	3,4	2,8	3,7	4,1	3,2	2,3	3,1	
Non Essential AA																	
Alanine	5,1	5,1	8,2	4,7	5,9	5,7	3,2	3,4	8,9	2,7	3,7	4,4	5,7	4,2	4,8	6,3	
Asparagine	12,9	8,7	11,0	7,0	9,0	9,0	7,3	10,1	6,3	3,4	5,3	11,6	10,0	12,0	10,7	9,3	
Glycine	5,3	3,2	4,6	7,7	5,1	4,8	2,0	4,1	2,7	3,4	4,0	4,3	3,4	4,0	1,7	6,5	
Glutamine	12,2	15,6	9,3	10,9	14,6	19,3	22,0	20,9	21,6	34,3	28,3	18,1	13,1	17,6	17,5	13,0	
Proline	5,2	5,8	3,4	9,6	4,5	3,0	11,2	4,1	9,4	12,6	9,7	5,1	4,0	4,2	7,4	4,4	
Serine	5,7	5,3	4,5	10,7	3,4	4,9	5,7	4,9	5,3	4,8	4,6	5,2	7,4	4,8	4,9	4,0	
Sub Total	46,4	43,7	41,0	50,6	42,5	46,7	51,4	47,5	54,2	61,2	55,6	48,7	43,6	46,8	47,0	43,5	
Grand Total	110,1	99,9	99,7	100,5	90,9	100,6	107,5	94,4	106,2	99,2	94,9	99,0	100,1	100,3	100,0	92,0	

1. Arginine can be synthesized in the urea cycle, but in limited amounts. Most of the nitrogen in fish is excreted as ammonia and a minor proportion as urea.
2. Cysteine can be derived from methionine, but also from other sources and is not really essential.
3. Tyrosine can only be derived from phenyl alanine and is thus more or less essential.

4. This subtotal does include the cysteine and the tyrosine.

5. Protastar, data from AVEBE b.a., Feed Department, P.O Box 15, 9640 AA Veendam, The Netherlands

6. CVB, Veevoeder tabel of the CVB (centraal Veevoederbureau, Runderweg 2, Postbus 2176, 8203 AD Lelystad, The Netherlands.

7. Sonac b.v., Kanaaldijk Noord 20 -21, P.O. Box 9, 5690 AA Son, The Netherlands. Internet: www.sonac.biz

8. Roquette Freres, 62080 Lestrem Cedex France.

9. USDA Food Tables on the Internet (http://www.nal.usda.gov/fnic/foodcomp/search/).

10. Volac International Ltd, Volac House, Orwell, Royston, Hertfordshire SG8 5QX. Produced by DV Nutrition, Buitenvaart 4023, 7905 TC Hoogeveen, The Netherlands.

11. NRC, National Research Coucil (1993).

Appendix 3

Structure of Fatty Acids

Notation: e.g.18:2n-6 (or 18:2ω-6, a so-called omega 6 fatty acid) indicates that the fatty acid has 18 carbon atoms and 2 double bonds and that the first double bond is located at the sixth carbon atom from the end of the carbon chain (thus not from the carboxyl group). However, when the systematic name is used, then the position of the double bonds starts from the carboxyl group (see below). Thus, linoleic acid is18:2n-6 (or 18:2ω-6) and the systematic name is 9,12-octadecadienoic acid ("octadeca" means 18 (18 carbon atoms) and "dien" means that it is a "diene" and that there are two double bonds (not conjugated in this case).

Iso and ante-iso-methyl branched fatty acids: Isomethyl branched fatty acids have the branch point on the penultimate carbon (one from the end), while ante-iso methyl branched fatty acids have the branch point on the ante penultima carbon atom (see also the webstie: www.lipidlibrary.co.uk) for further information on structure, compositions etc.)

18 17 16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1
COOH - CH_2 - CH_2 -CH_2 - CH_2 - CH_2 - CH_2 - CH_2 - CH_2 - CH - CH_2 - CH_2 - CH - CH_2 - CH_2 - CH_2 - CH_2 - CH_3
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

octadecanoic acid
(stearic acid)

18:1n-9 or 18:1ω-9

18 17 16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1
COOH - CH_2 - CH_2 -CH_2 - CH_2 - CH_2 - CH_2 - CH_2 - CH = CH - CH_2 - CH2 - CH2 - CH_2 - CH_2 - CH_2 - CH_2 - CH_3
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

9 - octadecenoic acid
(oleic acid)

Table 13

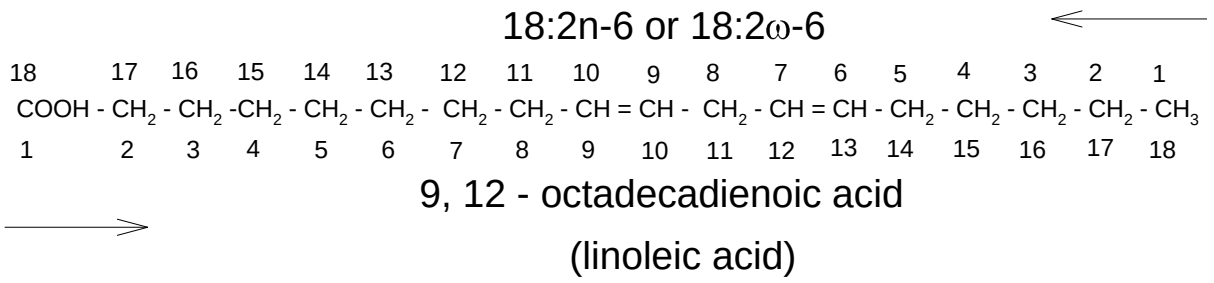
Some Selected Fatty Acids in Fats and Oils.

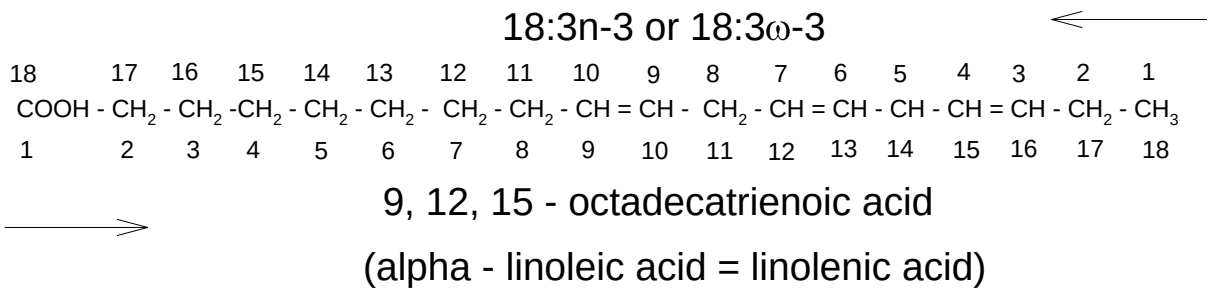
Symbol Saturated Fatty Acids	Systematic Name	Common Name	Typical Fat Source
1:0	Methanoic	Formic Acid	
2:0	Ethanoic	Acetic Acid	
3:0	Propanoic	Propionic Acid	
4:0	Butanoic acid	Butyric	Butterfat
5:0	Pentanoic acid	Valeric acid	
5:0	3-methylbutanoic acid	Isovaleric acid	
6:0	Hexanoic	Caproic	Butterfat
7:0	Heptanoic acid	Enanthic acid	
8:0	Octanoic	Caprylic	Coconut Oil
9:0	Nonanoic acid	Pelargonic acid	
10:0	Decanoic	Capric	Coconut Oil
11:0	Undecanoic		
12:0	Dodecanoic	Lauric	Coconut Oil

Symbol Saturated Fatty Acids	Systematic Name	Common Name	Typical Fat Source
13:0	Tridecanoic	Tridecylic acid	
14:0	Tetradecanoic	Myristic	Coconut Oil, Butterfat
15:0	Pentadecanoic	Pentadecylic acid	
16:0	Hexadecanoic	Palmitic	Most Fats and Oils
17:0	Heptadecanoic	Margaric acid	
18:0	Octadecanoic	Stearic	Most Fats and Oils
20:0	Eicosanoic	Arachidic	Lard, Peanut Oil
20:0	3,7,11,15-tetramethylhexadecanoic acid	Phytanic acid	
22:0	Docosanoic	Behenic	Peanut oil
24:0	Tetracosanoic	Lignoceric	
26:0	Hexacosanoic acid	Cerotic acid	
28:0	Octacosanoic	Montanic acid	
Mono Unsaturated Fatty Acids			
Monos (n-1, n-5, n-5, n-6)			
10:1n-1	cis-9-decenoic	Caproleic	Butterfat
14:1n-5	cis-9-tetradecenoic	Myristoleic	Butterfat
10:1n-6	cis-4-decenoic	Obtusilic	
Monos (n-7)			
12:1n-7	cis-5-dodecenoic	Lauroleic	Butterfat
16:1n-7	cis 9-hexadecenoic (cis)	Palmitoleic	Fish oils
16:1n-7	Trans-9-hexadecenoic (trans)	Palmitelaidic	HVO
18:1n-7	trans-11-octadecenoic	Vaccenic	Butterfat, Beef fat
18:1n-7	cis-11-octadecenoic	cis-vaccenic acid	
Monos (n-8)			
12:1n-8	cis-4-dodecenoic	Linderic	
Monos (n-9)			
14:1n-9	cis-5-tetradecenoic	Physeteric	
18:1n-9	cis -9-octadecenoic	Oleic	Most fats and oils
18:1n-9	trans-9-octadecenoic	Elaidic	Butterfat, Beef fat, HVO
20:1n-9	11-eicosenoic	Gondoic	Rapeseed oil
22:1n-9	cis-13-docosenoic	Erucic	
22:1n-9	Trans-13-docosenoic	Brassidic acid	
Monos (n-10)			
14:1n-10	cis-4-tetradecenoic	tsuzuic	
24:1n-10	cis-15-tetracosaenoic	Nervonic	
Monos (n-11)			
20:1n-11	cis-9-eicosenoic	Gadoleic	**Fish oils**
22:1n-11	cis-11-docosenoic	Cetoleic	
Monos (n-12)			
18:1n-12	cis-6-octadecenoic	Petroselinic	
Poly Unsaturated Fatty Acids			
Polys (n-3)			
18:3n-3	trans-9,trans-12,trans-15-octadecatrienoic	Linolenelaidic	
18:3n-3	cis-9,trans-11,trans-13-octadecatrienoic	α-Eleostearic	Tung oil
18:3n-3	trans-9,trans-11,trans-13-octadecatrienoic	β-Eleostearic	
18:3n-3	trans-9,cis-11,trans-13-	Cis-eleostearic acid	

Symbol Saturated Fatty Acids	Systematic Name	Common Name	Typical Fat Source
	octadecatrienoic		
18:3n-3	9,12,15-octadecatrienoic (cis)	α-linoleic (linolenic)	Soybean, Canola, Walnut, Flaxseed oils
18:4n-3	6,9,12,15-octadecatetraenoic	Stearidonic acid	
20:3n-3	11,14,17-eicosatrienoic acid		
20:4n-3	8,11,14,17-eicosatetraenoic		
20:5n-3	5,8,11,14,17-eicosapentaenoic	**EPA**, Timnodonic	**Fish oils**
22:3n-3	13,16,19-docosatrienoic acid		
22:5n-3	7,10,13,16,19-docosapentaenoic		
22:6n-3	4,7,10,13,16,19-docosahexaenoic	**DHA**, Cervonic	**Fish oils**
Polys (n-6)			
18:2n-6	9,12-octadecadienoic (cis, cis)	Linoleic	Corn, Safflower, Soybean
18:2n-6	trans-9, cis-12- octadecadienoic		HVO
18:2n-6	trans-9, trans-12-octadecadienoic	Linolelaidic	HVO
18:3n-6	6,9,12-octadecatrienoic	γ-linoleic	Primrose and Borage oil
20:2n-6	11,14-eicosadienoic		
20:3n-6	8,1,14-eicosatrienoic	Dihomogammalinoleic	
20:4n-6	5,8,11,14-eicosatetraenoic (all-trans?)	Arachidonic	Lard
22:2n-6	13,16-docosatetraenoic acid		
22:4n-6	7,10,13,16-docosatetraenoic	Adrenic	
22:5n-6	4,7,10,13,16-docosapentaenoic		
Polys (n-9)			
20:3n-9	5,8,11-eicosatrienoic	Mead	EFA-deficient animals

HVO, hydrogenated vegetable oils.





18:3n-6 or 18:3ω-6 ←

18 17 16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1
COOH - CH_2 - CH_2 -CH_2 - CH_2 - CH = CH - CH_2 - CH = CH - CH_2 - CH = CH - CH_2 - CH_2 - CH_2 - CH_2 - CH_3
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

→ 6, 9, 12 - octadecatrienoic acid
(gamma linoleic acid)

18 17 16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1
COOH - CH_2 - CH_2 -CH_2 - CH_2 - CH_2 - CH_2 - CH_2 - CH = CH - CH = CH - CH_2 - CH_2 - CH_2 - CH_2 - CH_2 - CH_3
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

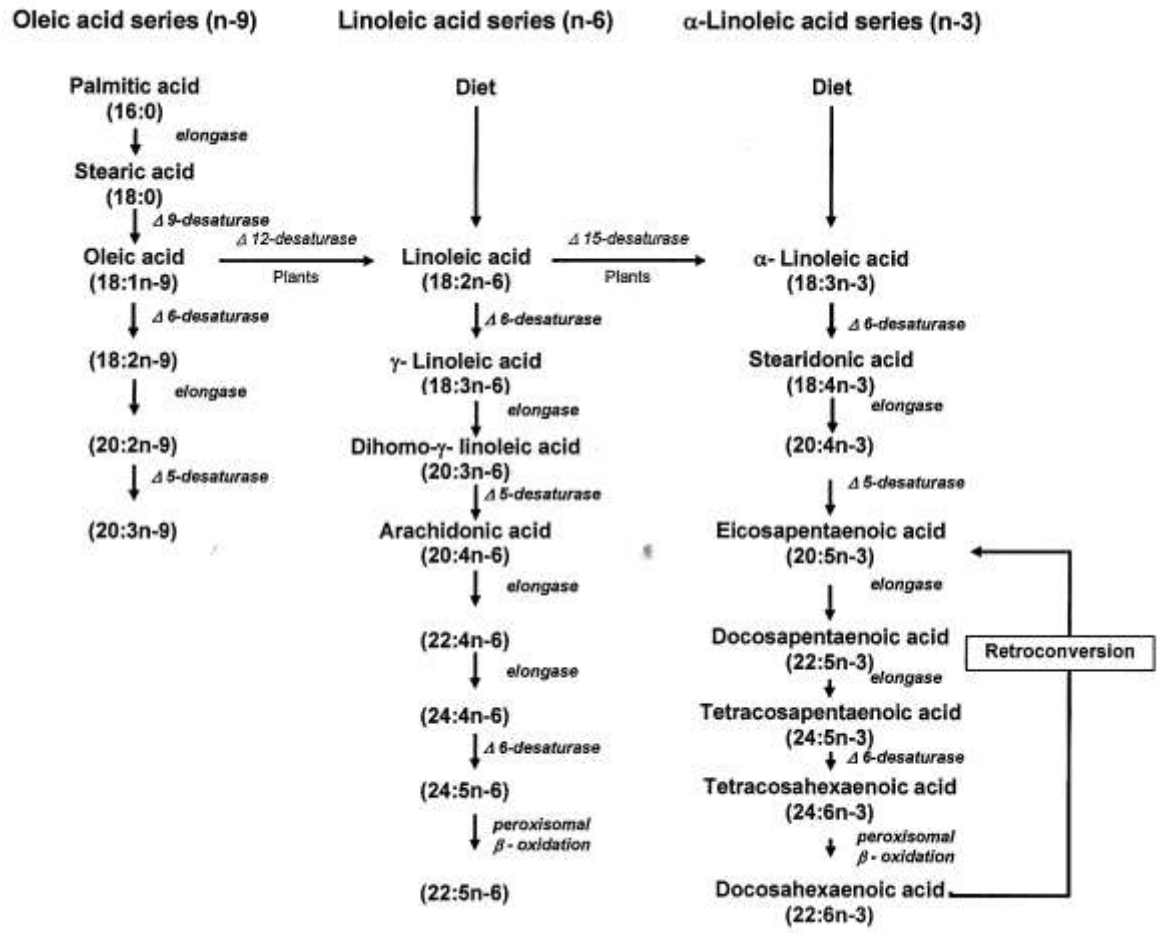
→ 9 cis, 11 trans - octadecadienoic acid
(conjugated linoleic acid)

18 17 16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1
COOH - CH_2 - CH_2 -CH_2 - CH_2 - CH_2 - CH_2 - CH_2 - CH_2 - CH = CH - CH = CH - CH_2 - CH_2 - CH_2 - CH_2 - CH_3
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

→ 10 trans, 12 cis - octadecadienoic acid
(conjugated linoleic acid)

Chain of desaturation and elongation of fatty acids of the n-9, n-6 and n-3 series

Oleic acid can be desaturated (delta 12 desaturase, only present in plants and not in mammels and fish) to linoleic acid and linoleic acid can be desaturated to linolenic acid (delta 15 desaturase, only present in plants, not in mammals and fish). Mammals and fish have the delta 9, 6, and 5 desaturases and the elongases needed to elongate the fatty acids at the carboxyl group end. The desaturation and elongation of linoleic and linolenic acid to HUFAs is not very effective in mammals and fish (*Opsahl-Ferstad, Plant Science 165: 349-357 (2003)*).



From: *Opsahl-Ferstad, Plant Science 165: 349-357 (2003).*

Appendix 4

Fatty acid composition of various oils that are used as an oil source in fish feeds

	Common Name	Cod Liver Oil	Salmon Oil	Sardine Oil	Herring Oil	Menhaden Oil	Olive Oil	Sunflower Oil	High Oleic Sunflower Oil	Linseed Oil	Soybean Oil	Rapeseed Oil	Palm Oil
C-12:0	Lauric acid			0,103	0,157								
C-14:0	Myristic acid	3,6	3,3	6,5	7	8					0,1		1,0
C-15:0	Pentadecanoic acid								0,8				
C-16:0	Palmitic acid	10,6	9,8	17	12	15	11,29	5,9	4	5,3	10,3	4	43,5
C-16:1 (n-7)	Palmitoleic acid	8,3	5	8	10	10	1				0,2	0,2	0,3
C-17:0	Margaric acid						0,022						
C-18:0	Stearic acid	3	4	4	0,818	4	2	4,5	4	4,1	3,8	1,8	4,3
C-18:1 (n-9)	Oleic acid (cis)	21	17	15	12	15	71	19,5	83	20,2	22,8	56,1	36,6
C-18:2 (n-6)	Linoleic acid (cis, cis)	0,935	2	2	1	2	10	65,7	4	12,7	51	20,3	9,1
C-18:3 (n-3)	Linolenic acid (cis, cis, cis)	0,935	1	1	0,763	1	0,761		0,192	53,3	6,8	9,3	0,2
C-18:4 (n-3)	Stearidonic acid	0,935	3	3	2	3							
C-20:0	Arachidic acid						0,414					0,7	
C-20:1 (n-11)	Gadoleic acid	10	4	6	14	1	0,311		0,964		0,2	1,7	0,1
C-20:5 (n-3)	Eicosapentaenoic acid (EPA)	7	13	10	6	13							
C-22:0	Behenic acid						0,129		1			0,4	
C-22:1 (n-11)	Cetoleic acid	7	3	6	21	0,352							
C-22:5 (n-3)		0,935	3	2	0,619	5							
C-22:6 (n-3)	Docosahexaenoic acid (DHA)	11	18	11	4	9							
Total		85	86	90	91	87	97	96	97	96	95	95	95
Saturated		17,0	17,4	27,2	19,9	26,9	13,8	10,4	9,8	9,4	14,2	6,9	48,8
Mono-unsaturated		46,7	29,0	33,8	55,8	26,7	72,8	19,5	83,6	20,2	23,2	58,0	37,0
Poly-unsaturated		21,6	39,6	29,1	15,3	33,0	10,5	65,7	3,8	66,0	57,8	29,6	9,3
EPA + DHA		17,9	31,3	20,8	10,5	21,7	0,0	0,0	0,0	0,0	0,0	0,0	0,0

Data from the USDA Food Tables on the Internet: (http://www.nal.usda.gov/fnic/foodcomp/search/).

Appendix 5

Reported Dietary Phophorus Availability (%) in Carp and Trout

Dietary Source	Chemical Formula	Common Carp	Rainbow trout
Sodium PO_4, monobasic	NaH_2PO_4 H_2O	94	98
Potassium PO_4, monobasic	KH_2PO_4	94	98
Calcium PO_4, monobasic	$Ca(H_2PO_4)_2$ H_2O	94	98
Calcium PO_4, dibasic	$Ca(HPO_4)$ $2H_2O$	46	71
Calcium PO_4, tribasic	$Ca_3(PO_4)_2$	13	64
White Fishmeal		0 - 18	66
Brown Fishmeal		24	74
Casein		97	90
Brewers Yeast		93	91
Wheat Germ		57	58

The availibilities of P from various ingredients is given in:

(1) Nutrient Requirements of Warmwater Fishes and Shellfishes. (1983). National Academy Press, Washington, D.C. ISBN 0-309-03428-0, page 26.

(2) Nutritional Fish Pathology Morphological signs of nutrient deficiency and toxicity in farmed fish by Albert G.J. Tacon (http://www.fao.org/DOCREP/003/T0700E/T0700E00.HTM).

(3) Feed and Feeding of Fish and Shrimp. Publication of the FAO by Michael B. New: (http://www.fao.org/docrep/S4314E/s4314e00.htm).

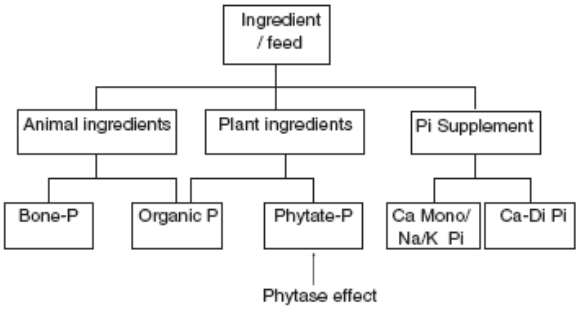
(4) Kaushlik, Nutrient requirements, supply and utilization in the context of carp culture, Aquaculture 129: 225-241 (1994).

The availability of P from plant products is low (between 30 – 50%, See NRC 1993 page 18.

Monobasic acid: *"is having only one replaceable hydrogen atom, or having only one hydrogen atom to donate to a base in an acid base reaction".* Monobasic means that one H atom of the phosphoric acid (H_3PO_4) is replaced for the formation of a salt, dibasic that two H atoms are replaced and tribasic that 3 H atoms are replaced. Monobasic calciumphosphate = Monocalciumphosphate = calciumdihydrogen-phosphate = $Ca(H_2PO_4)_2$.and dicalciumphosphate = calcium-mono-hydrogen-phosphate = $Ca(HPO_4)$. (Ortho) phosphoric acid is H_3PO_4. When a salt of of P-acid is formed by the donation of one hydrogen atom by the P-acid, then is it a mono basic salt; this salt is also called a calcium-di-hydrogen-phosphate because the formed salt still contains two hydrogen atoms. When a salt is formed by the donation of two hydrogen atoms by the P-acid, then it is a dibasic salt and this salt is also called a calcium-mono-hydrogen-phosphate, because this salt molecule contains only one hydrogen atom. The same is true for tricalcium phosphate.

Monocalcium phosphate is more soluble than di- and tricalcium phosphate and therefore better absorbed. P in bones (and thus also in fish meal) is present in the form of hydroxy apetite ($Ca_{10}(PO_4)_6(OH)_2$) which is the most stable form of P, but the least soluble form of P. Hydroxy apetite ($Ca_{10}(PO_4)_6(OH)_2$) is a tribasic form of phosphate and only soluble in acid. The carp (similarly as the shrimp and the abalone) lack a stomach where acids are produced and tribasic phosphate(in e.g. fish meal) is therefore poorly absorbed in the carp and shrimp.

Further, also P-acid can be used as a source of P. P-acid is very soluble in water, but a disadvantage may be that P-acid also will leach out very easily.



Classification scheme for P compounds in ingredients and feeds

Appendix 6

The various chemical forms of minerals and trace elements in feed[1]

Element	Chemical Form
Minerals	
Calcium[2] (Ca, M = 40.1)	
Mono-calcium phosphate free of H_2O	$Ca(H_2PO_4)_2$
Mono-calcium phosphate monohydrate	$Ca(H_2PO_4)_2\ H_2O$
Di-calcium phosphate free of H_2O	$Ca(HPO)_4$
Di-calcium phosphate didihydrate	$Ca(HPO)_4\ 2H_2O$
Tri-calcium phosphate	$Ca_3(PO_4)_2$
Calcium carbonate	$CaCO_3$
Calcium carbonate dihydrate	$CaSO_4\ 2H_2O$
Calcium sulfate dihydrate	$CaSO_4\ 2H_2O$
Phosphorus (P, M = 30.9)	
Mono-calcium phosphate free of H_2O	$Ca(H_2PO_4)_2$
Mono-calcium phosphate monohydrate	$Ca(H_2PO_4)_2\ H_2O$
Di-calcium phosphate free of H_2O	$Ca(HPO)_4$
Di-calcium phosphatedihydrate	$Ca(HPO)_4\ 2H_2O$
Tri-calcium phosphate	$Ca_3(PO_4)_2$
Di-magnesium phosphatetrihydrate	$MgHPO_4\ 3H_2O$
Mono-sodium phosphatedihydrate	$NaH_2PO_4\ 2H_2O$
Di-sodium phosphatedecahydrate	$Na_2HPO_4\ 12H_2O$
Di-sodium phosphatedihydrate	$Na_2HPO_4\ 2H_2O$
Di-sodium phosphate	Na_2HPO_4
Magnesium (Mg, M = 24.3))	
Magnesium oxide	MgO
Magnesium chloride hexahydrate	$MgCl_2\ 6H_2O$
Magnesium fumarate	$Mg(CH\text{-}COO)_2$
Magnesium sulfate heptahydrate	$MgSO_4\ 7H_2O$
Magnesium sulfate	$MgSO_4$
Magnesium carbonate	$MgCO_3\ Mg(OH)_2$
Magnesium acetate	$Mg(CH_3COO)_2$
Sodium (Na, M = 23)	
Sodium chloride	NaCl
Sodium bicarbonate	$NaHCO_3$
Sodium propionate	$Na(COO\text{-}CH_3)$
Mono-sodium phosphate monohydrate	$Ca(H_2PO_4)_2\ H_2O$
Sodium selenite pentahydrate	$Na_2SeO_3\ 5H_2O$
Sodium sulphate decahydrate	$Na_2SO_4\ 10\ H_2O$
Sodium tripoly phosphate	$Na_5P_3O_{10}$
Potassium (K, M - = 39.1))	
Potassium iodide	KI
Potassium bicarbonate	$KHCO_3$

Element	Chemical Form
Chlorine (Cl, M = 35.5)	
Sodium chloride	NaCl
Sulfur (S, M = 32.1))	
Sulfates	$(SO_4)^{2-}$
Trace Elements	
Iron (Fe), M = 55.8)	
Ferrous sulphate heptahydrate	$FeSO_4\ 7H_2O$
Ferrous (II) carbonate	$FeCO_3$
Ferrous oxide (black color)	FeO
Copper (Cu, M = 63.5))	
Copper sulfate pentahydrate	$CuSO_4.5H_2O$
Copper (II) carbonate	$CuCO_3\ Cu(OH)_2$
Copper (II) oxide	CuO
Copper (II) hydroxide	$Cu(OH)_2$
Iodine (I, M = 126.9))	
Potassium iodide	KI
Potassium iodate	KIO_3
Calcium Iodate	$Ca(IO_3)2\ H_2O)$
Manganese (Mn, M = 54.9)	
Manganese sulfate monohydrate	$MnSO_4.H_2O$
Manganese oxide	MnO
Zinc (Zn, M = 65.4)	
Zinc sulfate dihydrate	$ZnSO_4.2H_2O$
Zinc carbonate	$ZnCO_3$
Zinc oxide	ZnO
Cobalt (Co, M = 58.9),	
Cobalt carbonate	$CoCO_3$
Cobalt sulfate monohydrate	$CoSO\ H_2O$
Selenium (Se, M = 79.0), sodium selenite	$Na_2SeO_3.5H_2O$
Fluorine (F, M = 19 0)	NaF
Chromium (Cr, M = 52.0)	$CrCl_3.6H_2O$
Nickel (Ni, M = 58.7)	$NiSO_4.6H_2O$
Tin (Sn, M = 118.7)	$SnCl_2.2H_2O$
Vanadium (V, M = 50.9)	NH_4VO_3
Molybdenum (Mo, M = 95.9)	
Aluminum (Al, M = 26.9)	

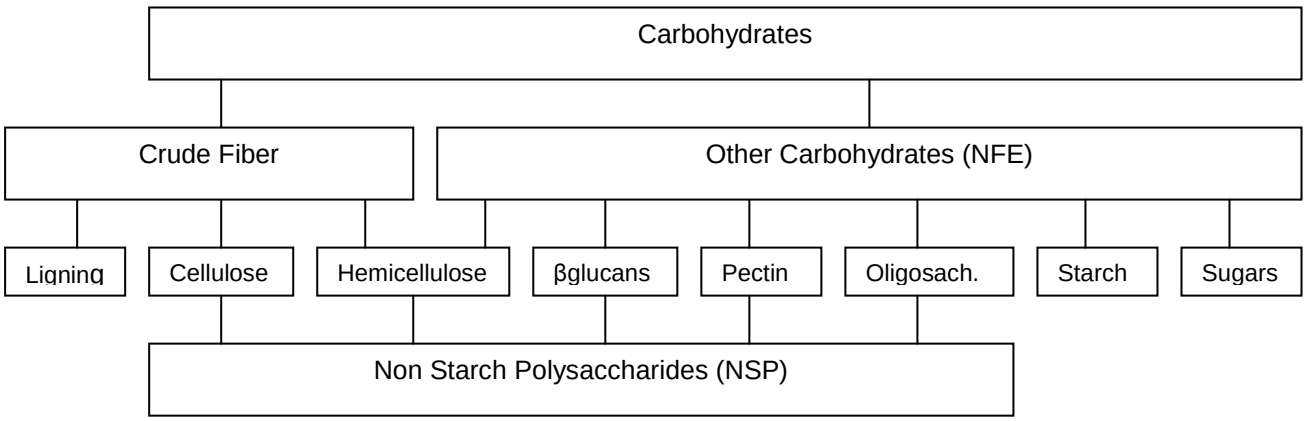
1. For more detailed information on the form of the minerals in fish feed: See also Hertrampf, J.W. & Piedad-Pascual, F. (2000) Handbook on Ingredients for Aquaculture Feeds. Kluwer Academic Publishers. ISBN 0-412-62760-4 (page 302-313) and Nutrient Requiremens of Warmwater Fishes and Shellfishes. (1983). National Academy Press, Washington, D.C. ISBN 0-309-03428-0 (page 90 – 92)

2. Calcium oxide and calcium hydroxide are insoluble calcium sources and should not be used in animal diets. Mono-di-calcium phosphate is a mixture of mono-calcium phosphate and di-calcium phosphate.

Appendix 7

Fiber and Carbohydrates in fish feeds

With the Weende analysis, the protein, fat, ash, fiber and moisture is measured. The Nitrogen Free Extract (NFE) is calculated as 100 – (moisture + protein + fat + fiber + ash). The carbohydrates comprise the crude fiber and the other carbohydrates, and the NFE comprise the total carbohydrates – other carbohydrates (See diagram below)



Note that part of the hemicellulose is included in the crude fiber fraction and part is included in the NFE fraction.

There are various extraction fractions of the fiber:

1. Extraction (mild) with neutral detergent gives as residue:

   NDF (Neutral Detergent Fiber) and comprises lignin and cellulose and hemicellulose.

2. Subsequent extraction (strong) with 0.5 M sulfuric acid (extraction of hemicellulose) gives as residue:

   ADF (Acid Detergen Fiber) and comprises lignin and cellulose

3. Subsequent extraction with 72% sulfuric acid gives and gives as residue:

   ADL (Acid Detergent Lignin) and comprises the lignin

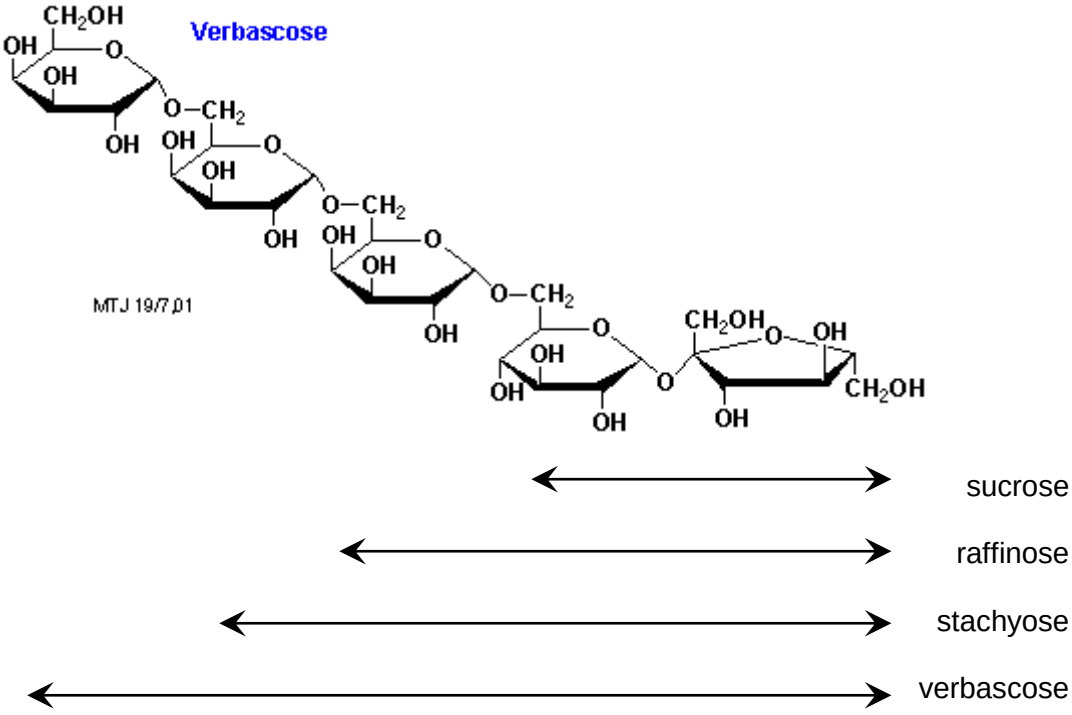
The other organic materials (OOM) is calculated as 100 - protein – fat – ash – moisture – sugars – starch, and includes the crude fiber. This fraction can be considered as the undigestible fraction in the diet for fish.

The carbohydrates can be divided into storage polysaccharides (starch) and structure polysaccharides (for the structure of cell walls).

1. *Sugars:* Monosaccharides (e.g. glucose, arabinose, fructose etc) and disaccharides (e,g, sucrose, maltose, cellobiose) (digestible)

2. *Starch* (polysaccharides): amylose and amylopectine (digestible). Amylose is unbranched chain of glucose molecules with an α-1,4 linkage and amylo pectine is amylose with α-1,6 branches of glucose. Hydrolysis of amylose and amylopectine results in glucose molecules.

3. *Oligosaccharides:* (soluble and alcohol extractable):
   1. Digestible glucose oligosaccharides (GOS), like sucrose
   2. Indigestible oligosaccharides (e.g. α-galacto-oligosaccharides) e.g. verbascose (penta-saccharide of three galactose, a fructose and a glucose (sucrose) molecule), stachyose (tetra-saccharide of two galactose, a fructose and a glucose (fructose + glucose = sucrose) molecules, less sweet than sugar), raffinose (tri-saccharide of galactose, fructose and glucose (sucrose)) and inuline (fructan).



4. *Pectine:* α -1,4 polygalacturonic acid.

5. *β-glucans:* polysaccharide consisting of a branched chain of glucose molecules (= glucan) which are connected by β-1,3 and β-1,6 linkages (immuno stimulant) or β-1,3 and β-1,4 linkages.

6. *Cellulose:* Linear molecule and is a polysaccharide of β-(1,4) glucose

7. *Hemicellulose:* Less defined branched polysaccharides other than pectines, cellulose, and β-glucans. Hemicellulose can contains various poly saccharides and are named after the predominant sugar they contain, e.g. glucans, mannans, galactans (hexosans) or xylans and arabinans (pentosans). Hemicellulose is part of the cell wals, but are in general considered as storage polysaccharides.

8. *Lignine:* Lignine is strictly taken not a polysaccharide. It is a polymer of phenolic compounds, like cinnamic acid, ferulic acid, p-coumarylalcohol, coniferol, sinapylalcohol, coumaric acid.

In animals, glycogen is storage polysaccharides (in liver), and chitine (a polymer of 2-acetamido-2-desoxy-D-glucose connected with β(1-4) bonds) is a structure polysaccharide in insects and shrimps.

Appendix 8

Vitamin Requirements for Trout and Carp.

Common Name	Scientific name	Requirements[1] (mg or IU /kilogram diet)				Source
		Trout		Carp		
		Halver et al.[2] (2002)	NRC[3] (1993)	Halver et al.[2] (2002)	NRC[3] (1993)	
Fat Soluble Vitamins						
Vitamin A	Retinyl acetate/palmitate	2000 – 2500 IU	2500 IU	1000 – 2000 IU	4000 IU	fish oil
Vitamin D_3	Cholecalciferol	2400 IU	2400 IU	not known	not known	fish oil
Vitamin E	*all-rac*-α-tocopheryl acetate	30 IU	50 IU	80 – 100 IU	100 IU	vegetable oils
Vitamin K_3	Menadione	10	required	required	not known	liver, vegetables, alfalfa
Water Soluble Vitamins						
Vitamin B_1	Thiamin Hydrochloride	10 – 12	1	2 – 3	0.5	yeast, bran, vegetables
Vitamin B_2	Riboflavin	20 – 30	4	7 – 10	7	yeast, liver, fish hydroysates
Vitamin B_3	Niacin or Nicotinic acid (Niacinamide = nicotinamide = nicotinic acid amide)	120 – 150	10	30 – 50	28	yeast, liver, fish meal and hydrolysates
Vitamin B_5	Ca-pantothenate, Pantothenic acid	40 – 50	20	30 – 40	30	yeast, fish hydrolysates
Vitamin B_6	Pyridoxine hydrochloride	10 – 15	3	5 – 10	6	yeast, fish hydrolysates
Vitamin B_8	Biotine (vit H, of Haut or Huid)	1 – 1.2	0.15	1 – 1.5	1	yeast, liver
Vitamin B_{11} (B9 in USA and Germany)	Folic Acid (Folacin) or Pteroglutamic acid	6 – 10	1	not known	not known	yeast, vegetables, afalfa
Vitamin B_{12}	Cyanocobalamin	required	0.01	not known	not known	yeast, liver, fish meal and hydrolysates
Vitamin C	Ascorbic acid (phosphate bound Stay-C or Aquastab)	100 – 150	50	30 – 50	required	insects, fresh fish, fruits
Other vitamin like compounds[4]						
Choline Chloride[5]			1000		500	fish meal, soy, wheat
Betaine						
Inositol[6]	Meso- or Myo-inositol (acitve isomer)		3000		440	yeast, fish meal
Para-amino-benzoic acid (PABA)						
Lipoic acid						

1. IU, International Units:

Vitamin A: One IU equals the activity of 0.300 µg of retinal (vitamin A) or 0.344 µg all-trans-retinyl acetate or 0.550 µg all-trans-retinyl palmitate or 0.600 µg β-carotene (β-carotene can be converted into vitamin A).

Vitamin D_3: One IU equals the activity of 0.025 µg cholecalciferol and 1 µg equals 40 IU. Vitamin D_2 (cholecalciferol) is generated by UV light and *Vitamin D_2* (ergocalciferol) is derived from plants and micro organism (from the plant sterol ergosterol by UV light).

Vitamin E: One IU equals the activity of 1 mg D,L-α-tocopheryl acetates (*all-rac*-α-tocopheryl acetate)

Vitamin C: One IE equals 50 µg or 0.05 mg

2. From: Halver, J.E. & Hardy, R.W. (2002) Fish Nutrition. Academic Press, London ISBN 0-12-319652-3 on page 70.

3. NRC (1993), National Research Council National, Nutrient Requirements of Fish, Academic Press Washington D.C. ISBN 0-309-04891-5, on page 63.Also on the internet: (www.nap.edu/books/0309048915/html/1.html)

4. *Vitamin like compounds* have vitamin like activity but are not all essential and some can be synthesized in the body. Choline can be made from serine and methionine. Choline, methionine, and serine are methyl donors. Other vitamin like compounds are ubiquinone, lipoic acid, taurine (synthesized from cysteine) and carnitine that also can be synthesized in the body. Carnitine is part of the enzyme carnitine palmitoyl transferase. Fish (trout) seem to have a high requirement for p-Aminobenzoic acid (PABA), another vitaminlike compound.

5. *Choline chloride:* Choline chloride can be converted into betaine (oxidized choline), but not the other way around. Betaine (not choline) functions as a methyl donor for the conversion of homocysteine into methionine. About 50% of the choline chloride requirement of trout could possibly be met by betaine in the trout (NRC 1993). Choline is a part of lecithine or phosphatidylcholine, a phospholipid that is part of the cell membranes. For an overview of choline, see: Zeisel (1994), Choline and human nutrition Ann Rev Nutr vol 14, page 269.

6. *Inositol.* There are seven optically active and two optically inactive isomers. One of the optically active forms, meso- or myo (myo means muscle) has the biologically activity. Phytic acid is inositol with the phosphate groups linked to hydroxygroups. The P in phytic acid is not available, but the enzyme phytase can cleave of the phosphate groups from the phytic acid molecule and make the P available.

7. *Further literature on requirements*:

Tacon, A.G.J. in: Standard methods for the nutrition and feeding of farmed fish and shrimp. Volume 1 (the essential nutrients),volume 2 (Nutrient sources and composition), and volume 3 (Feeding methods). Argent laboratories Press, Redmond, Washington, 98502 USA, page 86 (1990). Also available on the Internet as a FAO (Food and Agricultural Organization) publication:
(Volume 1: http://www.fao.org/docrep/field/003/AB470E/AB470E00.htm
(Volume 2: http://www.fao.org/docrep/field/003/AB468E/AB468E00.htm
(Volume 3: http://www.fao.org/docrep/field/003/AB467E/AB467E00.htm)

DeSilva, S.S. & Anderson, T.A. (1995) Fish Nutrition in Aquaculture. Chapman and Hall. ISBN 0-412-55030.

Appendix 9

Conversion of Choline (chloride) into Betaine and methionine into homocysteine

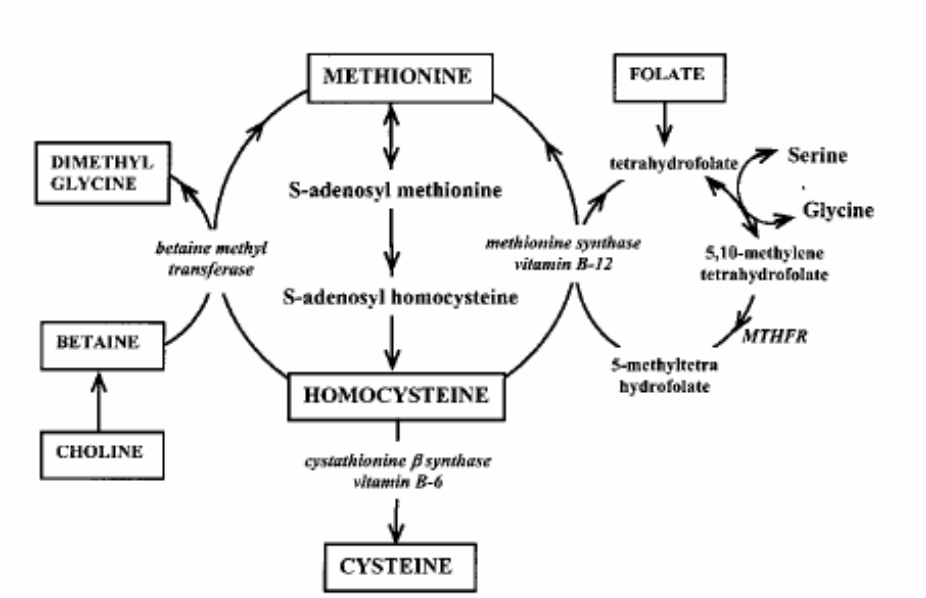
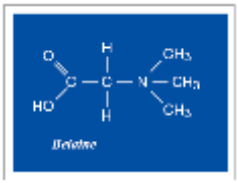


FIGURE 1 Schematic representation of homocysteine metabolism. MTHFR, methylenetetrahydrofolatereductase.

Figure from: Steenge et al. J. Nutr. 133: 1291-1295 (2003)



Betaine: name is derived from the Beta Vulgaris (sugar beet).

Functions of Betaine:
(= trimethyl glycine, and choline can be converted into betaine).

1. **Osmolyte:** Protects from stress when moving from low to high salinity. Thus important for marine fish like salmon (actively uptake by liver mitochondria).

2. **Methyl donor:** for conversion of homocysteine into methione (trimethy glycine (=betaine) > dimethyl glycine).
   Thus spares:
   1. Choline chloride (e.g. for P-lipid synthesis)
   2. Methionine (homocysteine -> methione), homocysteine is a breakdown product of methione.

Functions of Choline:

1. *Per se* for the synthesis of neurotransmittor acetyl choline
2. *Per se* for the synthesis of phosphatidyl choline (lecithin) and other complex choline containing P-lipids.
3. As a source of methyl groups via betaine for the synthesis of various methylated metabolites (like methione).

Choline is lipotropic, it prevents fatty livers, P-lipids (e.g. lecithin which contains choline) is needed for the synthesis of lipoproteins that transport the lipids from the liver to the peripheral tissues (See Hertramph, Handbook of ingredients for Aquaculture feeds, on page 387). Choline can be synthesized in the liver (e.g. from serine) provided that sufficient methyl donors are available, but synthesis can be insufficient in fast growing animals.

Lecithin (phosphatidyl choline):
Is a good source of choline and is important for fish larvae and the requirement is 1 – 3% in the larval diet (see Couteau et al, Review of the dietary effects of phospholipids in fish and crustacean larviculture, Aquaculture 155: 149-164 (1997).

Choline

The choline requirement for fish is 1 gram per kg of diet. The raw materials used in fish diets are abundant in choline and supply enough choline to meet this requirements:

Fish meal	-	3056 – 5507	mg / kg
Soy dehulled	-	2731	mg / kg
Soy seed heated	-	2307	mg / kg
Blood meals	-	780 – 850	mg / kg
Wheat	-	1000 – 1250	mg / kg

From: Nutrient Requirement of Swine at the Internet: http://darwin.nap.edu/books/0309059933/html, page 130-131.

Appendix 10

Carotenoids

Carotenoids can be subdivided into:

1. **Carotenes** (oxygen-free carotenoids) such as lycopenes (in tomatoes) and α, β and γ carotenes (in carrots). Carotenes have a yellowish color but *do not color the fish*.

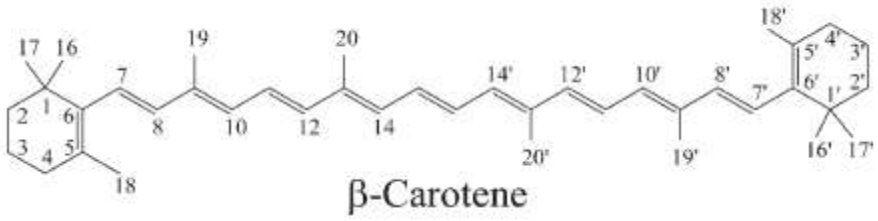
.

2. **Xanthophylls** or oxy-carotenoids (oxygen-containing carotenoids, xanthophylls means yellow leaves). These compounds have in contrast to carotenes one or more oxygen atoms. These compounds can color the fish, either the fillet or the skin and color egg yolks.

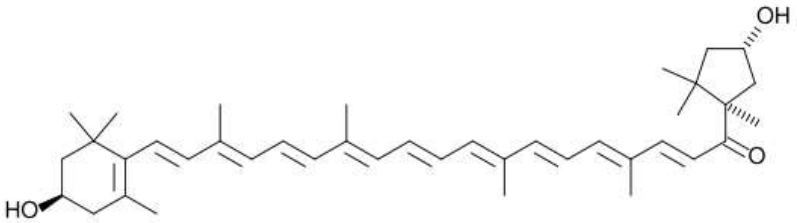
Examples of xanthophylls are:

- Astaxanthine (in shrimps and trout, used to color trout and salmon fillet, Carophyll pink))
- Canthaxanthine (in cantharelle mushrooms, used to color eggs, Carophyll red)
- Capsanthine (in red pepper, the capsicum)
- Zeaxanthine (in Zea Mais, red mais, spirulina, alfalfa, grass meal)
- Luteine (luteus means yellow, in mais, spirulina, alfalfa, grass meal)
- Apo ester (used to color eggs, Carophyll yellow, Food Orange 7 E 160f, the ethyl ester of β – apo – 8'- carotenic acid). It is only found in small quantities in some plants, but is predominantly commercially produced from Beta – apo – 8 –carotenal, E 160e Food Orange 6, that is found in spinach and citrus fruits.

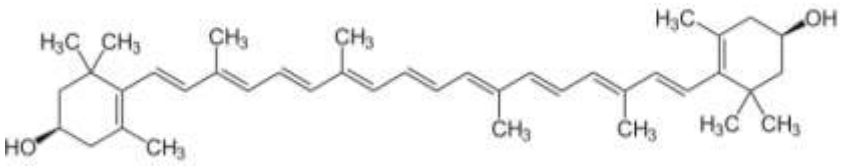
Structures of various carotenoids



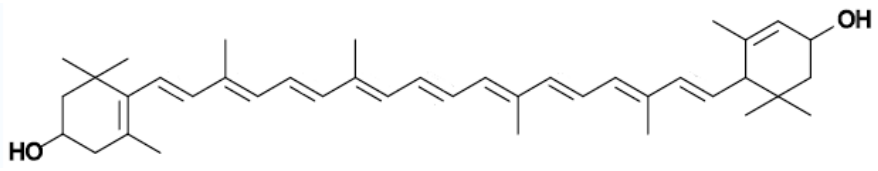
Beta carotene



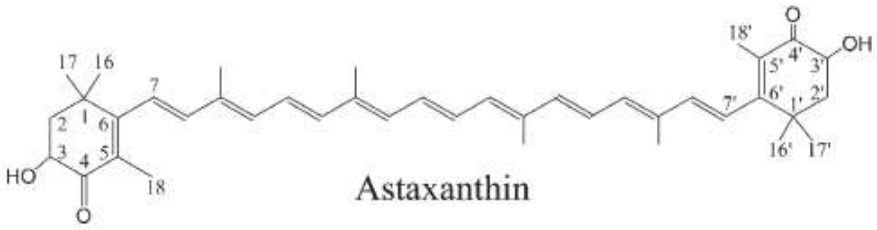
Capsanthine
(in red peppers or paprika)



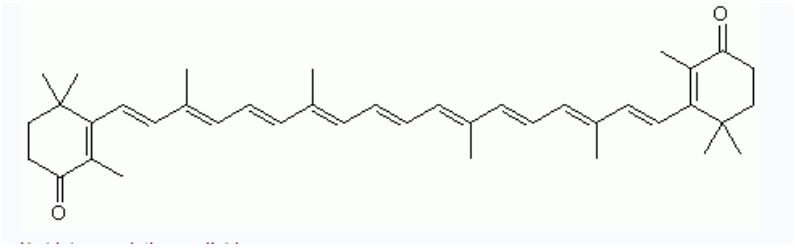
Zeaxanthin (orange color)
(in spirulina and mais)



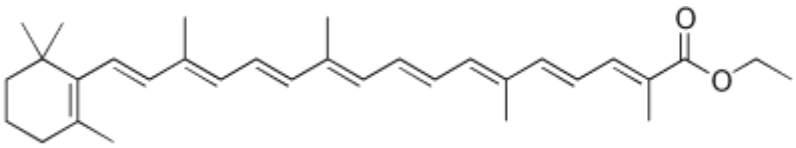
Lutein (orange – red color) in Marigold flowers
(Flora Glo by Kemin or Xangold by Cognis)



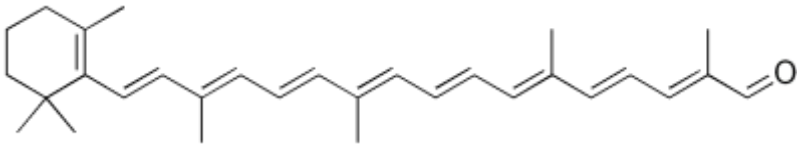
To color trout and salmon (Carophyll pink of DSM or Lucanthin pink by BASF)



Canthaxanthin
To color egg yolk (Carophyll red of DSM)



Ethyl ester of β – apo – 8'- carotenic acid (Apo Ester)
Food Orange 7
To color egg yolks (Carophyll yellow of DSM)



β – apo – 8'- carotenal
Food Orange 6
(used to produce Apo Ester)

Many of the xanthophylls have an E-number and are used as a food coloring agent.

- E 160e Beta – apo – 8 carotenal
- E 160f Ethyl ester of β – apo – 8'- carotenic acid made from Beta – apo – 8 carotenal (can be prepared synthetically from beta – apo – 8 – carotenal, DSM)
- E 161a Flavoxanthin
- E 161b Lutein
- E 161c Cryptoxanthin
- E 161d Rubixanthin
- E 161e Violaxanthin
- E 161f Rodoxanthin
- E 161g Canthaxanthin (can be prepared synthetically, DSM)
- E 161h Zeaxanthin
- E 161i Citranaxanthin (can be prepared syntheticaly)
- E 161j Astaxanthin (can be prepared synthetically, DSM

Appendix 11

The values for energy generated in the body, Respiratory Quotient (RQ) and the Energy Equivalents $EeqO_2$ and $EeqCO_2$ for carbohydrate, fat, protein and alcohol according to data from Elia and Livesey (1992).

		Energy Generated in the Body[1]		H_2O generated		O_2 consumed			$C0_2$ generated			RQ	$EeqO_2$[4]		$EeqCO_2$[4]		Atwater Digest.Coeffic	Metabol Energy
	MW	(kJ/mol)	(kJ/g)	(mol/mol)	(g/g)	(mol/mol)	(g/g)	(L/g)	(mol/mol)	(g/g)	(L/g)	(CO_2/O_2)	(kJ/g)	(kJ/L)	(kJ/g)	(kJ/L)	(%)	(kJ/g)
Protein (combustion)[2]	2260,0	53448	23,65	79,50	0,63	125,2	1,77	1,24	100,0	1,95	0,99	0,799	13,35	19,06	12,14	23,85	92	21,76
Protein (in body)[3]	2260,0	45376	20,08	50,60	0,40	104,0	1,47	1,03	86,6	1,69	0,86	0,833	13,64	19,47	11,91	23,38	92	18,47
Fat (dioleylpalmitate)[3]	859,4	34022	39,59	51,00	1,07	77,5	2,89	2,02	55,0	2,82	1,43	0,710	13,72	19,59	14,06	27,61	95	37,61
Carbohydrate (glucan)[3]	162,1	2840	17,52	5,00	0,56	6,0	1,18	0,83	6,0	1,63	0,83	1,000	14,79	21,12	10,76	21,12	97	16,99
Sacharose ($C_{12}H_{22}O_{11}$)	342,3	5641	16,48	11,00	0,58	12,0	1,12	0,79	12,0	1,54	0,79	1,000	14,69	20,98	10,68	20,98	97	15,99
Glucose ($C_6H_{12}O_6$)	180,2	2803	15,56	6,00	0,60	6,0	1,07	0,75	6,0	1,47	0,75	1,000	14,60	20,85	10,62	20,85	97	15,09
Alcohol (C_2H_6O)	46,1	1367	29,67	3,00	1,17	3,0	2,08	1,46	2,0	1,91	0,97	0,667	14,24	20,34	15,53	30,50	97	28,78
Kleibers standard protein Data from Elia and Livesey[5]																		
Protein to mixture [3]	2260,0	45376	20,08	50,60	0,40	104,0	1,47	1,03	86,6	1,69	0,86	0,833	13,64	19,47	11,91	23,38	92	18,47
Protein to urea	2260,0	45950	20,33	52,80	0,42	105,3	1,49	1,04	87,0	1,69	0,86	0,826	13,64	19,47	12,00	23,57	92	18,71
Protein to uric acid	2260,0	41880	18,53	65,00	0,52	95,5	1,35	0,95	67,5	1,31	0,67	0,707	13,71	19,57	14,10	27,69	92	17,05
Protein to ammonia	2260,0	46450	20,55	13,80	0,11	105,3	1,49	1,04	100,0	1,95	0,99	0,950	13,79	19,69	10,55	20,73	92	18,91
Protein to creatinine	2260,0	33960	15,03	48,47	0,39	79,3	1,12	0,79	65,3	1,27	0,65	0,824	13,38	19,11	11,81	23,20	92	13,82
Protein to allantoin	2260,0	43254	19,14	59,30	0,47	98,8	1,40	0,98	74,0	1,44	0,73	0,749	13,68	19,54	13,28	26,09	92	17,61
Kleibers standard protein Calculated[6]																		
Protein to mixture[3]	2260,0	44415	19,68	50,60	0,40	104,0	1,47	1,03	86,6	1,69	0,86	0,833	13,35	19,06	11,65	22,89	92	18,08
Protein to urea	2260,0	45037	19,93	52,80	0,42	105,3	1,49	1,04	87,0	1,69	0,86	0,826	13,37	19,09	11,76	23,10	92	18,33
Protein to uric acid	2260,0	40962	18,12	65,00	0,52	95,5	1,35	0,95	67,5	1,31	0,67	0,707	13,40	19,14	13,79	27,08	92	16,67
Protein to ammonia	2260,0	44270	19,59	13,80	0,11	105,3	1,49	1,04	100,0	1,95	0,99	0,950	13,14	18,76	10,06	19,76	92	18,02
Protein to creatinine	2260,0	33193	14,69	48,47	0,39	79,3	1,12	0,79	65,3	1,27	0,65	0,824	13,08	18,68	11,54	22,67	92	13,51
Protein to allantoin	2260,0			59,30		98,8			74,0									

Data are from:

M. Elia and G. Livesey (1992) Energy expenditure and fuel selection in biological systems: the theory and practice of calculations based on indirect calorimetry and tracer methods, World Review of Nutrition and Dietetics, volume 70, page 68-131 (see pages 71 and 78 for the equations of the oxidations of the carbohydrtaes, fats and proteins).

1.The energy generated is the energy generated in the body. For the protein, a correction is made for the energy excreted in the urine in the form of form of urea, ammonia, uric acid, creatine, creatinine, and allantoin. The protein in this Table refers to the Kleiber's standard protein ($C_{100}\,H_{159}\,N_{26}\,O_{32}\,S_{0.7}$ (MW = 2260, contains 16.1% N). The energy generated from the carbohydrates and the fat and alcohol in the body is identical to the energy generated in a bomb calorimeter.

2. Complete combustion of the Kleiber's protein in a bomb calorimeter. The heat of complete combustion of protein in the bomb calorimeter is 23.65 kJ/g (gross energy). The equation of the complete combustion is: $C_{100}\,H_{159}\,N_{26}\,O_{32}\,S_{0.7} + 124.8\,O_2 = 100\,CO_2 + 78.8\,H_2O + 13\,N_2 = 0.7\,H_2SO_4 + 53448$ kJ.

3. The Kleibers standard protein is metabolized to urea, creatinine and ammonia in the nitrogen mass ratio of 90:5:5 (See Elia and Livesey 1992, page 71):

$C_{100}H_{159}N_{26}O_{32}S_{0.7}$ + 104 O_2 (= 22.414 x 104 = 2331.06 liters) = 86.6 CO_2 (= 22.414 x 86.6 = 1941.05 liters) + 50.6 H_2O + 11.7 N_2H_4CO (urea) + 1.3 NH_4OH (ammonia) + 0.43 $N_3C_4H_7O$ (creatinine) + 0.7 H_2SO_4
For the heat of combustion released from the oxidation of fat and carbohydrates, see Elia and Livesey 1992, page 71 and for the oxidation of saccharose and glucose and alcohol (ethanol): K. Blaxter 1989, page 296.(*K. Blaxter (1989)* Energy metabolism in animals and man, Cambridge University press).

4. Eeq, energy equivalent. All values for the volumes of O_2 and CO_2 are at 1 bar and a temperature of 0 ^oC (273.15 ^oK).
1 mg O_2 = 0.700 ml O_2 and 1 ml O_2 = 1.428 mg O_2. Further 1 mg CO_2 = 0.509 ml CO_2 and 1 ml CO_2 ° 1,963 mg CO_2

Data on energy equivalents of oxygen consumption for protein, fat and carbohydrates have also been given in earlier literature , see: J.M. Elliot and W. Davison (1975) Energy equivalents of oxygen consumption in animal energetics. Oecologia (Berlin) Volume 19, pages 195-201.

5. Data are from Elia and Livesey 1992 (page 71 and 78).

6.These data are calculated as following: The N in the protein can be excreted in the form of ammonia, urea, creatinine, creatin, or allantoin. These compounds contain a considerable amount of energy (See Appendix Table 4 and 5).

(a). <u>*Excretion of the nitrogen in the form of urea*</u>: the energy density of urea (in solution) is 647 kJ per mol (647 / 60.056 = 10.77 kJ per gram). The oxidation of 1 mol of Kleiber's protein results in the formation of 13 mol urea (Elia and Livesey 1992, page 78). This amount of urea contains thus 13 x 647 = 8411 kJ of energy, which is excreted in the urine. The gross energy of protein is 23.65 x 2260 = 53448 kJ. Thus 53448 – 8411 = 45037 kJ is left. Thus, the available energy of the protein is then 45037 / 2260 = 19.93 kJ per gram protein.
Oxidation of Kleiber's protein (Kleiber's protein contains 16.1% protein): $C_{100}H_{159}N_{26}O_{32}S_{0.7}$ + 105.3 O_2 = 87 CO_2 + 52.8 H_2O + 13 N_2H_4CO (urea) + 0.7 H_2SO_4

The complete combustion of Kleiber's protein is
(1) $C_{100}H_{159}N_{26}O_{32}S_{0.7}$ + 124.8 O_2 = 100 CO_2 + 78.8 H_2O + 13 N_2 + 0.7 H_2SO_4 + 53448 kJ and (complete combustion of protein)
(2) 13 N_2H_4CO (urea) +19.5 O_2 = 13 CO_2 + 26 H_2O + 13 N_2 + 13 * 647 kJ (= 8411 kJ) (complete combustion of urea)
Substract (2) from (1): (compare McLean and Tobin 1987, page 33, and Blaxter 1989, page 12, law of Hess, law of constant heat summation).
$C_{100}H_{159}N_{26}O_{32}S_{0.7}$ + 105.3 O_2 = 87 CO_2 + 52.8 H_2O + 13 N_2H_4CO (urea) + 0.7 H_2SO_4 + 45037 kJ or 45037 / 2260 = 19.93.

We can also assume that protein in general contains 16% nitrogen (The Kleiber's protein contains16.1% protein). Thus the oxidation of 1 gram of protein results in the generation of 0.16 gram nitrogen. Urea contains 46.6% nitrogen, thus the oxidation of 1 gram of protein results in the formation of 0.16 / 0.46 = 0.34 grams of urea. The energy density of 1 gram of urea is 10.77 kJ, thus the energy of 0.34 grams of urea is 0.34 x 10.77 = 3.66 kJ and the available energy in 1 gram protein is then 23.65 – 3.66 = 19.99 kJ.

(b) <u>*Excretion of the nitrogen in the form of uric acid*</u>: the energy density of uric acid is 1921 kJ per mol (1921 / 168.112 = 11.42 kJ per gram). The oxidation of 1 mol of Kleiber's protein results in the formation of 6.5 mol uric acid (Elia and Livesey 1992, page 78). This amount of uric acid contains thus 6.5 x 1921 = 12487 kJ of energy, which is excreted in the urine. The gross energy of protein is 23.65 x 2260 = 53448 kJ. Thus 53448 – 12487 = 40961 kJ is left. Thus, the available energy of the protein is then 40961 / 2260 = 18.12 kJ per gram protein.
Oxidation of Kleiber's protein (Kleiber's protein contains 16.1% protein: $C_{100}H_{159}N_{26}O_{32}S_{0.7}$ + 95.5 O_2 = 67.5 CO_2 + 65 H_2O + 6.5 $C_5H_4O_3N_4$ (uric acid) + 0.7 H_2SO_4

We can also assume that protein in general contains 16% nitrogen. Thus the oxidation of 1 gram of protein results in the generation of 0.16 gram nitrogen. Uric contains 33.3% nitrogen, thus the oxidation of 1 gram of protein results in the formation of 0.16 / 0.33 = 0.48 grams of urea. The energy density of 1 gram of uric is 11.40 kJ, thus the energy of 0.48 grams of uric acid is 0.48 x 11.40 = 5.47 kJ and the available energy in 1 gram protein is then 23.65 – 5.47 = 18.18 kJ.

(c) *Excretion of the nitrogen in the form of ammonia*: the energy density of ammonia (in solution) is 353 kJ per mol (353 / 17.031 = 20.73 kJ per gram). The oxidation of 1 mol of Kleiber's protein results in the formation of 26 mol ammonia (Elia and Livesey 1992, page 78). This amount of ammonia contains thus 26 x 353 = 9178 kJ of energy, which is excreted in the urine. The gross energy of protein is 23.65 x 2260 = 53448 kJ. Thus 53448 – 9178 = 44270 kJ is left. Thus, the available energy of the protein is then 44270 / 2260 = 19.59 kJ per gram protein.
Oxidation of Kleiber's protein (Kleiber's protein contains 16.1% protein): $C_{100}H_{159}N_{26}O_{32}S_{0.7} + 105.3\ O_2 = 100\ CO_2 + 13.8\ H_2O + 26\ NH_4OH$ (ammonia) $+ 0.7\ H_2SO_4$

We can also assume that protein in general contains 16% nitrogen. Thus the oxidation of 1 gram of protein results in the generation of 0.16 gram nitrogen. ammonia contains 82.2% nitrogen, thus the oxidation of 1 gram of protein results in the formation of 0.16 / 0.822 = 0.195 grams of ammonia. The energy density of 1 gram of ammonia is 20.73 kJ, thus the energy of 0.13 grams of ammonia is 0.195 x 20.73 = 4.04 kJ and the available energy in 1 gram protein is then 23.65 – 4.04 = 19.61 kJ.

(d) *Excretion of the nitrogen in the form of creatinine*: the energy density of creatinine is 2337 kJ per mol (2337 / 113.120 = 20.66 kJ per gram). The oxidation of 1 mol of Kleiber's protein results in the formation of 8.667 mol creatinine (Elia and Livesey 1992, page 78). This amount of creatinine contains thus 8.667 x 2337 = 20255 kJ of energy, which is excreted in the urine. The gross energy of protein is 23.65 x 2260 = 53448 kJ. Thus 53448 – 20255 = 33193 kJ is left. Thus, the available energy of the protein is then 33193 / 2260 = 14.69 kJ per gram protein.
Oxidation of Kleiber's protein (Kleiber's protein contains 16.1% protein): $C_{100}H_{159}N_{26}O_{32}S_{0.7} + 79.3\ O_2 = 65.332\ CO_2 + 48.466\ H_2O + 8.667\ N_3C_4H_7O$ (creatinine) $+ 0.7\ H_2SO_4$

We can also assume that protein in general contains 16% nitrogen. Thus the oxidation of 1 gram of protein results in the generation of 0.16 gram nitrogen. creatinine contains 37.147% nitrogen, thus the oxidation of 1 gram of protein results in the formation of 0.16 / 0.371 = 0.43 grams of creatinine. The energy density of 1 gram of creatinine is 20.66 kJ, thus the energy of 0.43 grams of creatinine is 0.43 x 20.66 = 8.88 kJ and the available energy in 1 gram protein is then 23.65 – 8.88 = 14.77 kJ.

(e) *Excretion of the nitrogen in the form of creatine*: the energy density of creatine is 2324 kJ per mol (2324 / 115.136 = 20.18 kJ per gram). The oxidation of 1 mol of Kleiber's protein results in the formation of 8.667 mol creatine (Elia and Livesey 1992, own calculation). This amount of creatine contains thus 8.667 x 2324 = 20142 kJ of energy, which is excreted in the urine. The gross energy of protein is 23.65 x 2260 = 53448 kJ. Thus 53448 – 20142 = 33306 kJ is left. Thus, the available energy of the protein is then 33306 / 2260 = 14.74 kJ per gram protein.
Oxidation of Kleiber's protein (Kleiber's protein contains 16.1% protein): $C_{100}H_{159}N_{26}O_{32}S_{0.7} + 79.288\ O_2 = 65.332\ CO_2 + 39.779\ H_2O + 8.667\ N_3C_4H_9O_2$ (creatine) $+ 0.7\ H_2SO_4$

We can also assume that protein in general contains 16% nitrogen. Thus the oxidation of 1 gram of protein results in the generation of 0.16 gram nitrogen. Creatine contains 36.497% nitrogen, thus the oxidation of 1 gram of protein results in the formation of 0.16 / 0.365 = 0.44 grams of creatine. The energy density of 1 gram of creatine is 20.18 kJ, thus the energy of 0.44 grams of creatine is 0.44 x 20.18 = 8.88 kJ and the available energy in 1 gram protein is then 23.65 – 8.88 = 14.77 kJ.

(f) *Excretion of nitrogen in the form of a mixture of urea (90%), creatinine (5%) and ammonia (5%).* We can assume that protein in general contains 16% nitrogen. Thus the oxidation of 1 gram of protein results in the generation of 0.16 gram nitrogen. Urea contains 46.6% N and 10.77 kJ per gram urea, creatinine contains 37.1%N and 20.66 kJ per gram creatinine and ammonia contains 82.2% N and 20.73 kJ per gram ammonia. Thus the loss of energy is ((0.16 x 0.90 / 0.466) x 10.77) + ((0.16 x 0.05) / 0.371) x 20.66) + ((0.16 x 0.05 / 0.822) x 20.73) = 3.975 kJ per gram protein. Thus the available energy of 1 gram of protein is 23.65 – 3.975 = 19.68 kJ per gram protein.

(g) *Excretion of nitrogen in the form of a mixture of ammonia (85%) and urea (15%) as in fish.* We can assume that protein in general contains 16% nitrogen. Thus the oxidation of 1 gram of protein results in the generation of 0.16 gram nitrogen. Ammonia contains 82.2% N and 20.73 kJ per gram ammonia and urea contains 46.6% N and 10.77 kJ per gram urea, Thus the loss of energy is ((0.16 x 0.85 / 0.822) x 20.73) + ((0.16 x 0.15 / 0.466) x 10.77) = 3.98 kJ per gram protein. Thus the available energy of 1 gram of protein is 23.65 – 3.98 = 19.67 kJ per gram protein in fish.