GV-SOLAS
Gesellschaft für Versuchstierkunde
Society for Laboratory Animal Science

Specialist Information

from the Committee for Nutrition of Laboratory Animals

Feeding concepts and methods in laboratory animal husbandry and animal experiments

- FISH -

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Contents

Special characteristics

Compared with terrestrial animals, fish have a number of special characteristics that also have some impact on the nutrition and feeding or the husbandry of fish. Some special characteristics are listed here. The various points are discussed in more detail – if relevant to laboratory animal housing – in the corresponding chapters:

- Aquatic habitat
- Poikilothermia
- Endless growth
- High protein requirement
- NH_3/NH_4 as end products of protein catabolism
- *n3* fatty acid requirement
- Vitamin C requirement
- Requirement for pigments?

Fish are poikilothermic animals (having a variable body temperature), i.e. the body temperature of fish largely matches the temperature of the surrounding medium (water); this results in a much lower maintenance requirement than that of homoeothermic animals. As a result of their aquatic living conditions, fish also need less energy to maintain their body condition than terrestrial animals. On the other hand, these living conditions require special installations for osmoregulation, because saltwater fish are hypoosmotic to the surrounding medium, whereas freshwater fish are hyperosmotic.

The cardiovascular system of fish is comparatively simple in structure with sequential arterial and venous system. The heart consists mostly of only a single chamber and carries venous blood to the gills. Carbon dioxide is released via the gills or gill plates and the oxygen dissolved in the water is absorbed.

The air bladder, which usually consists of two parts, allows the fish to float in the water. The kidney is located close to the vertebral column, the cranial or anterior end of the kidney possessing predominantly hormonal and immunological functions.

The gastrointestinal tract of the fish is essentially comparable in its structure and function to the higher vertebrates, even if some considerable anatomical and in some cases also histological differences from terrestrial animals can be seen. For example, no villus and crypt regions are discernible in the intestinal epithelium of fish[1]. Figure 1 shows the topographic anatomy of a salmonid[2]. Differences between fish species are attributable, among other things, in their adaptation to different feeding habits: herbivorous and omnivorous fish generally have a long convoluted intestine, whereas the digestive canal of carnivorous (predatory) fish, e.g. the rainbow trout

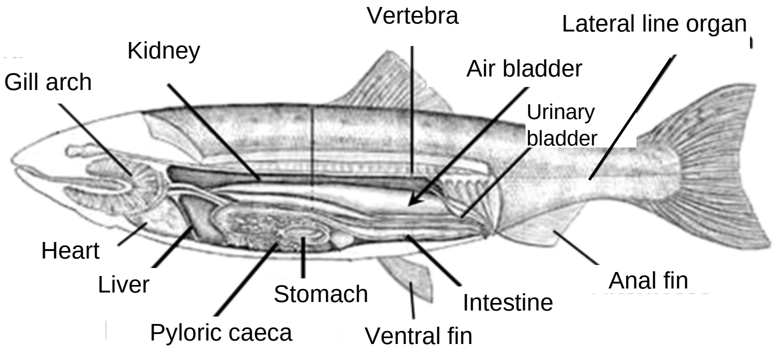

Figure 1: Topographic anatomy of a salmonid [2)]

(*Oncorhynchus mykiss*), runs more or less straight within the abdominal cavity; thus being comparatively short and nearly proportional to the body length[3,4,5]. Aside from this characteristic, carnivorous fish also possess a stomach that opens into a short intestinal segment that partly includes a number of diverticula, or so-called pyloric caeca (see Fig. 1), which increase the surface area and hence the capacity for digestion and absorption[5,6]. Omnivorous and herbivorous fish have only an elongated digestive tube, usually with a stomach-like extension (pseudo-gaster). Liver, gall bladder and pancreas – partially formed as a hepatopancreas – are also present.

Fish, especially carnivorous species, have a relatively high protein requirement due to their high basal protein metabolism and increased degradation capacity. In most species, the predominant end-products (approx. 80-90%) of protein metabolism are ammonia (NH_3) and ammonium ion (NH^+); both are primarily (>80%) eliminated via the gills by passive diffusion or active ion exchange. Ammonia is highly toxic and must therefore be bacterially oxidized in the water first to nitrite and then to nitrate. The bacterial oxidation only takes place with a high level of oxygen saturation in the water.

The approximately 30,000 species of fish can differ considerably in their appearance, the arrangement of their internal organs, and the way they reproduce. Fish that have inhabited very warm and therefore low-oxygen waters in the course of evolution have developed additional ways of utilizing atmospheric air.

Fish as laboratory animals

Numerous freshwater fish, but also some saltwater fish, may be used as laboratory animals. To date, however, the fish used are almost exclusively teleosts. Apart from a few exceptions, these are permanent aquatic inhabitants. The quality of the water is therefore of huge importance for all body functions, animal health, and hence also the reproducibility of experimental results. The following fish species are frequently found in laboratory animal facilities (see also [Roundtable discussion][7]: [REACH][8]):

- Rainbow trout — *Oncorhynchus mykiss*
- Zebrafish — *Danio rerio*
- Common carp — *Cyprinus carpio*
- Guppy (millionfish) — *Poecilia reticulata*
- Nile tilapia — *Oreochromis niloticus*
- Medaka (Japanese rice fish) — *Oryzias latipes*
- Fathead minnow (USA) — *Pimephales promelas*
- Sheepshead minnow — *Cyprinodon variegatus*
- Atlantic cod — *Gadus morhua*
- Flounder — *Paralichthys* spp./*Platichthys*
- Turbot — *Psetta maxima*

In environmental toxicology and evolutionary biology, mainly freshwater fish (see above) are used. These species are predominantly diurnally active fish which are easy to breed.

In their natural habitat, trout feed on all creatures that can be followed (carnivorous, or rather faunivorous), whereas carp, zebrafish, and guppies also feed on plants (omnivorous).

Life phases

Breeding

In the most common species kept for animal experiments - trout, carp and zebrafish - the eggs are only fertilized after they have been laid and are not cared for. Guppies belong to the live-bearing tooth carp, i.e., they are ovoviviparous. In all species, the brood may be eaten by the parents. Therefore, the fry is reared in separate containers under controlled conditions (batch of the same age and same size). This is also necessary in terms of hygiene and differing requirements with regard to the environment and food. Growth and the reproductive behaviour of fish are influenced not only by the water quality, but also by the temperature and oxygen concentration of the water. A change in water temperature and feeding can have a direct impact on the reproductive behaviour, especially in cold-water fish.

Fish keeping and Maintenance

Fish in laboratory animal facilities are usually kept in aquaria made of glass or plastic, although fiberglass and stainless steel are also suitable for larger tanks. Equipment that come into contact with the water, e.g. aquarium seals, shall be chemically inert, i.e. container materials and minerals shall not dissolve in water and, conversely, substances present in the water should not be adsorbed. Both could have a lasting impact on fish health and also on the experimental results.

The preferred temperature is about 12-18°C for cold-water fish (e.g., trout) and 20-28°C for warm-water fish (e.g., carp ≥ 20°C, zebrafish 28°C); a rapid temperature change is generally not well tolerated and should therefore be avoided in all species. The optimum oxygen concentration for trout, as cold-water fish, is set higher (at 8-11 mg O_2/L) than that for carp (6-8 mg O_2/L). The O_2 saturation increases with decreasing water temperature. Although the impact of oxygen deficiency has to be considered particularly harmful, long-term oxygen and gas supersaturation, should also be avoided, especially in young fish and eggs. The pH of the water should be around 7 (6.0-8.5) for all species.

Different fish keepings systems can be distinguished; these are open flow-through systems and semi-closed to closed recirculation systems. The function of the systems is to filter the water and maintain the biological balance in the tank. The influx of water and the flow rate has to be controlled, and adjusted to the species and fish size. The water should be free from any chlorine or copper (water pipes). In recirculation aquatic systems (RAS), mechanical cleaning with sedimentation tanks or lamella separators and sieve drum filters as well as biological cleaning (nitrification/denitrification) in biofilters are required. In addition, the water is also oxygenated and, if necessary, UV disinfected.

New aquaria should always be “started-up” without animals for about 3-4 weeks, so that the water can stabilize and a balance can be established. The addition of a bacterial culture can shorten this timespan. In tanks with animals, a partial change of water should be undertaken at regular intervals. It is essential to ensure that the existing biotope is preserved (never completely empty). Depending on the efficiency of the system, the following rule of thumb can be applied: “Partial water change every 3 weeks with 1/3 fresh, temperate water”. The stocking density should be adjusted to the requirement of the fish species. A stocking density that is either too low (hierarchical conflicts) or too high can cause stress reactions, increased aggressiveness with cannibalism, reduced growth, increased susceptibility to disease, and reduced fertility. For most fish species, hiding spaces are necessary. In the case of laboratory species, these should be designed so that an adequate observation of the animals is ensured, and dead fish can be immediately removed; they should also not release any substances (e.g. softening agents) into the water. Even zebrafish that show little territorial behaviour should be offered hiding spaces (e.g., plant-like materials), especially during mating, in order to reduce aggressive behaviour[9,10,11]. The importance of hiding spaces has been demonstrated with crucian carp (*Carassius carassius*). If no hiding materials are made available, increased serotonergic activity is triggered in the brainstem and the optic tectum as a result of olfactory stress; a lack of hiding places evidently leads to increased serotonergic activity in the brainstem even without stressors[12]. An detailed discussion about the pros and cons of enrichment, especially with regard to toxicity studies, was published by Williams et al. in 2009[13].

The day/night rhythm is ensured by artificial light (shining from above), whereby the light intensity should be increased or reduced slowly (dimming).

Nutrition

When specifying the nutrient requirement, the **minimum requirements** and **optimum requirements** are differentiated; the recommendations for the nutrient supply (**recommended allowances**) provide another information. In general, the concentration of nutrients, minerals, trace elements, and vitamins that is necessary to avoid deficiency symptoms is described as the minimum requirement. The requirement for optimum fitness (optimum requirement), e.g., realization of the full potential for growth and reproduction, is usually much higher than the minimum requirement. Recommended allowances take further factors into account, including limited availability of nutrients in natural ingredient diets or nutrient losses that can occur even when the feed is correctly processed and stored. These differences are documented again in Table 1 using vitamin C as an example.

Table 1: Differences between requirement data and recommended allowances based on the example of ascorbic acid for salmonids[14)] (per kg of dry matter)

I	Minimum requirement	20-40 mg
II	Requirement for normal collagen formation and for optimum growth	100-150 mg
III	Recommendation for intensive housing (with approx. 40-70% loss due to pelleting and drying)	200-400 mg
IV	Recommendation as above (III) and for additional effects (protection against infection, rapid wound healing)	500-1000 mg

The nutritional requirements and recommended allowances given in the following sections are limited to the most important nutrients for laboratory animal facilities.

Energy requirement

In terms of the energy requirements of a living organism, a differentiation is made between maintenance requirements (heat loss under defined conditions) and requirements for performance. Compared with homeotherms, fish (poikilotherms) have a much lower maintenance requirement because the body temperature of fish largely corresponds to the temperature of the water, and fish also have to use less energy to maintain their body position than terrestrial animals. In salmonids, the maintenance requirement reaches only 5 - 10 % of the level of mammals. This effect is particularly evident at lower temperatures and almost follows the temperature coefficient Q10[15,16,17]. Accordingly, not only body mass but also temperature has to be considered when calculating the maintenance requirement (net energy) using prediction equations. The net maintenance requirement for trout can be calculated using the following equation[18]:

$$\text{Maintenance requirement } [\text{J} \bullet \text{fish}^{-1} \bullet \text{d}^{-1}] = 22.1 \bullet e^{1.034\ T} \bullet \text{g BM}^{0.833}$$

BM = body mass [g]; T = water temperature [°C]; e = Euler's number (~2.71828)

The resulting values for the net maintenance requirement are confirmed by a similar prediction equation from a Canadian research group[17,19]. Since fish are characterized by continuous growth, energy is generally supplied to meet the requirements for maintenance and performance. However, it is not possible to provide sufficient information on the total energy requirement, as the energy requirement depends on many abiotic (see above) and biotic factors as well as the desired performance. In carnivorous fish species, fat and protein that are the primary energy sources, as complex, unprocessed carbohydrates cannot be satisfactorily utilized and would therefore result in lower growth rates and a deterioration of water quality (see below). In omnivorous and especially herbivorous species, however, complex carbohydrates play a significant part in energy supply.

Nutrient requirement

The natural diet of fish is characterized by a high concentration of ***raw protein*** (approx. 60% CP); the same applies to fish rations normally used in practice (40- 55% CP). However, not all dietary protein is used for protein accretion; protein is also used for energetic utilization, i.e. for energy production and fat synthesis. This is probably the result of the high protein supply in their natural diet, which requires less adaptability of the enzymes of protein and amino acid degradation, so that fish (similar to the cat) convert a much higher proportion of the dietary protein into energy than most other animal species. This results in a higher protein requirement. However, the protein supply (amino acid supply) should be adjusted to the actual requirements and/or energy density of the feed, especially with regard to water quality and its direct impact on animal health. It should be kept in mind, however, that the protein requirement – as in terrestrial animals – decreases with increasing age and body mass. The NRC therefore recommends protein/energy ratios between around 19 and 28 g of digestible protein per MJ of digestible energy (g DP/MJ DE;[20]) depending on the species and size of the fish. Fish meal of good quality is used as major protein source, especially for carnivorous fish; and high-quality plant protein sources (e.g., soy protein concentrate) are additionally used mainly in the diets of omnivorous fish.

There do not exist genuine requirement for ***carbohydrates***. Thus (complex) carbohydrates play a rather minor role in the nutrition of carnivorous species, but they are used for technological reasons mostly as thermally treated starch (e.g. in the extrusion process) also in the diets for these fish. However, it should be noted that the higher the starch content in the feed, the lower the digestibility of the starch, thus contributing to the eutrophication of the water. In omnivorous fish, especially cyprinids, however, carbohydrates can make a significant contribution to energy supply; the same applies particularly to herbivorous fish. Crude fibre can hardly be used for energy supply; nonetheless, crude fibre does play a certain role in the passage of chyme and excretion (e.g. of bile acids and toxins). Chitin from live feed also has a similar function to that of crude fibre.

The addition of ***fat*** serves not only to meet the energy requirements and improve the protein utilization ("protein saving effect") but primarily to supply essential fatty acids.

Table 2 informs on the recommendations for nutrient supply and nutrient concentrations in the feed of different fish species.

Table 2: Recommendations on nutrient content (% in original substance)

	Crude protein	Crude fat	Crude fibre	Carbohydrates
Salmonids	35 – 50	14 –15	0.5 – 1	max. 35
Cypriniforms	25 – 38	6 – 10	1.0 – 2	max. 65
Zebrafish	46 – 49	8 – 12	1.5 - 3	max. 35

Essential fatty acids cannot be synthesized by the organism de novo or via chain elongation, or by the introduction of double bonds; they therefore have to be supplied with the feed. Indispensable fatty acids are polyunsaturated fatty acids with at least 18 carbon atoms and two or more double bonds. Fish fat is rich in n3 series polyunsaturated fatty acids (e.g., eicosapentaenoic acid, C20:5; docosahexaenoic acid, C22:6). While only n3 fatty acids are apparently essential for salmonids, cyprinids have an additional requirement for n6 fatty acids (e.g., linoleic acid, C18:2 n6). In general, a higher requirement for essential fatty acids can be assumed for cold-water fish than for warm-water fish. Nevertheless, there should always be a balance between n3, n6 and n9 fatty acids in the feed. Table 3 shows examples of the recommended allowances for essential fatty acids for three fish species.

Based on the requirement for essential fatty acids, it can be assumed that primarily fish oils, but also small crustaceans (krill), are suitable sources to meet this requirement. Larger quantities of vegetable oils may also be used for omnivorous species. However, fats with a high melting point cannot be recommended for feeding fish in view of their relatively poor digestibility and low (absent) concentration of essential fatty acids.

Furthermore, it should be considered that a higher concentration of unsaturated fat increases the requirement for vitamin E (and antioxidants in the feed). In general, an additional concentration of 25 IU vitamin E per 10% fish oil or PUFA is recommended.

Table 3: Supply of essential fatty acids; according to NRC[20]

	% of food (original substance)		% in dietary fat (6%)	
Fatty acid type	*n3*	*n6*	*n3*	*n6*
Trout	1	—	10–20 [a)]	—
Carp	0.5–1 [a)]	1	up to15	15
Turbot	0.8 [b)]	—		

[a)] *higher value if requirements are met only with linolenic acid*
[b)] *only via n3 PUFA*

Unlike terrestrial animals, fish can meet a large proportion of their requirement for minerals and trace elements by direct absorption from the water through their gills and skin. This applies in particular to the cations calcium, sodium, magnesium, potassium, iron, copper, zinc, and selenium, whereas anionic phosphates and sulphates are absorbed better from the feed via the digestive tract[21]. The absorption of minerals from the surrounding medium makes it difficult to determine the requirement; there are therefore only a few data on the quantitative requirement. The phosphorus requirement (approx. 6 to 7 g P/kg feed) is met sufficiently with natural ingredient diets because these are usually high in phosphorus due to the (extensive) use of fish meal in the feed.

The requirement for vitamins also decreases with the body mass and age of the fish. Conversely, increasing temperatures and stress lead to higher vitamin requirements (see ascorbic acid). In commercially available complete feed, supplementation with vitamins is based on the recommended allowances, with a sufficient margin of safety e.g., for storage losses or potential leaching. Since water-soluble vitamins in particular dissolve shortly after the feed is provided, rapid feed intake is beneficial and may also be achieved by restricted feeding.

Feeding technique and feeding intensity

Fish kept as laboratory animals should be fed restrictively in order to ensure an almost complete intake of the feed particles and to reduce contamination of the water with suspended solids and excretion products. For toxicity and ecotoxicity studies, a feeding intensity of 1-2 % of body mass is recommended, so that the animals show “normal” growth with constant body fat content[8]. But in freshly hatched larvae and young fish, this feeding intensity may be much higher (up to 30% higher depending on the species).

The feed conversion ratio is about 0.8-1.4 g feed/g growth in trout and carp. Increasing the temperature increases food consumption and growth disproportionately, while lowering the temperature reduces them accordingly. The reproductive behaviour of fish may also be directly influenced by feeding and temperature.

The feed should be carefully scattered on the water surface or administered using automatic feeders. The fish should only be fed restrictively because uneaten food contaminates the water and represents a constraint on the animals; so, for almost all adult fish species one day off feed is recommended. Table 4 summarizes the feeding frequency and appropriate pellet size for trout.

Trout require floating and slowly sink feed, as they (can) feed almost exclusively while sinking and not from the bottom; whereas, other fish species, such as carp, prefer to feed from the bottom.

Table 4: Trout feeding

	Fish length	Meal	Granule size	Crude protein
Brood	2-5 cm 5-12 cm	8-12 x daily 4 x daily	0,4 - 0,8 mm 1,0 - 1,5 mm	50% 48
Fry	6 -13 cm > 13 cm	3 x daily 2 x daily	1.2 – 2.5 mm 3.5 mm	44% 40%
Broodstock		1 x daily	8.0 mm	35%

For most fish species, dry complete feed that has been adjusted to the species-specific requirements is commercially available. Although these diet allow for almost standardized feeding, it should be noted that the level of standardization and testing of the diets is not comparable to that used in GLP studies with rodents. A significantly increased incidence of neoplasia was found in zebrafish kept in recirculation systems and fed commercial feed, which was attributed to feeding and husbandry[22]. Some researchers are therefore requesting the introduction of semi-purified diets or at least feed mixtures that are tailored for use in laboratory animals and toxicological studies, e.g., with regard to a lower energy density and contaminants, etc. (see reviews:22,23,24).

Basically, only high-quality food (extruded food, flakes, tablets, sticks or floating pellets) from specialist pet shops should be used. With regard to animal health and water quality, the quantity and composition of the feed should be adapted to the actual requirements. It should further be considered that commercially available dry food is often very high in energy and nutrients.

Feeding or supplementary feeding with “natural” substrates such as zooplankton is possible – at least during experiment-free periods. However, it should be considered that this feeding regimen can lead to significant fluctuations in nutrient supply, so a standardization of the feeding procedure is not possible; slaughter waste, e.g. bovine heart or spleen, should be completely avoided, if as possible.

The use of live or fresh feed carries the risk e.g. of introducing pathogens; other disadvantages of live feed are as follows

- imbalanced nutrient concentrations, e.g. “starved” live feed (organisms stored without food) has a significantly reduced feed value,
- thiaminases (e.g. prawns, small fish)
- higher workload

Zooplankton is usually far superior to conventional complete feeds for feeding fry of most fish species in terms of nutritional physiology, food preference and food spectrum. Zebrafish in laboratory animal facilities are also reared to some extent on live feed of differing size and origin. The following live feed species/groups, including their eggs and/or larvae, are suitable and often used for fry feeding and rearing (see also 25):

Euryhaline rotifer	*Brachionus plicatilis*	approx.. 0.15 – 0.40 mm
Brine shrimp	*Artemia*	
Brine shrimp	*Artemia salina*	approx. 10 mm
Decapsulated eggs		approx. 0.2 – 0.3 mm
Nauplia		approx. 0.5 mm
Sludge worm	*Tubifex*	approx. 25 - 80 mm
Common water fleas	*Daphnia*	1 – 5 mm

If live feed is used, it should come from breeders or be bred by the facility in order to exclude any pollutants, because even the "cleaning" of live feed (watering for several days) is not able to eliminate accumulated toxins. Live feed for faunivores, such as small fish, sludge worms, and crustaceans (e.g., prawns, freshwater shrimps, water lice, etc.) should preferably be stored frozen for hygienic reasons (frozen food) and should always defrosted fresh for feeding.

Unlike with commercial fish (trout, carp), the species-specific requirements of "exotic" species, e.g., zebrafish, are not sufficiently known, and commercially available complete feed may be higher in nutrients and energy than necessary. Table 5 shows the example of a rearing and feeding regime for zebrafish in a laboratory animal facility.

Table 5: Feeding of zebrafish

Age	Meal	Type of feed
From 4th day of life	3 x daily	Paramecium [a)] ± fine powdered feed
From 10th day of life	3 x daily 1 x daily	Fine powdered food plus additional Artemia (24 h old)
From 4 weeks	2-3 x daily	Coarse powdered feed plus live Artemia
From 2 months	Feeding with	Frost food [b)] and flakes plus live Artemia
From 3 months	Exclusively	Frost food [b)] and flakes
Adults	4 x weekly 2 x weekly	Frost food [b)] and flakes Commercial dry feed

[a)] Paramecium
[b)] Artemia, Daphnia, Japanese water flea (*Moina macrocopa*)

Animals in Experiments

The fish intended for an experiment should come from the same breeding stock (same age and approximately the same length). The transfer of fish to new tanks always means considerable physical strain. The animals should therefore be kept under standardized environmental conditions with standardized feeding for at least 10-14 days.

The fish should be in good health and not have any obvious deformities. Toxic amounts of heavy metals can be dissolved, especially in soft water (e.g. copper from pipes, lead from air vents). The existing international guidelines (e.g., OECD Guidelines[26]) should be followed when selecting the fish species. Like all laboratory animals, the fish should come from known stocks (that are under veterinary supervision).

The length of the fasting period before the start of the experiment is at least 24 hours, depending on the research concerned; in ecotoxicity studies, a period of 96 hours may be necessary, which does not pose a problem for adult fish. The fish are observed after the first 2-4 hours and at least once a day. Besides mortality, visible changes such as weight loss, changes in swimming behaviour, breathing, and pigmentation, etc. should be recorded. The fish species, and in particular also the age of the fish should be considered in the design of the experiment. Young fish do not tolerate long fasting periods.

Nutrition-related diseases and disorders

(mostly from 14 and 20)

The first and most common signs on nutrition-related diseases are reduced food intake together with reduced growth and increased mortality and anaemia.

Oversupply

- Obesity or fatty liver: energy intake too high, food too rich in carbohydrates.
- Gill damage: accumulation of H2S, NH3, NH4 (extreme pH values).
- Signs of poisoning or even mass mortality: oxygen deficiency caused by rotting food. As a rule, however, only non-specific symptoms occur, such as faded colours, partial darkening (skin), apathy, insufficient fertility and changes in swimming behaviour.
- Visceral granuloma: aetiology unknown, but food-dependent.

Undersupply

- Thyroid tumour: caused by iodine deficiency, especially in trout in the Alpine foothills
- Anaemia: deficiency of essential nutrients (vitamins, trace elements, amino acids), sequelae of other nutritional diseases
- Fatty acids: growth depression, increased mortality. Trout: fatty liver, acute local myocarditis, fin erosion, specific stress reactions (after handling + sunlight exposure) with intense swimming movements and comatose states.
- Signs of vitamin deficiency (excerpt)
  - Vitamin A: Ascites, displacement of eye lens, exophthalmos, retina degeneration, depigmentation.
  - Vitamin D: Trout renal sclerosis

Vitamin E:	Anaemia, fatty liver, ascites, increased mortality, haemorrhages in the eye; exudative diathesis, muscular dystrophy, peroxidation of the fatty tissue, yellowish-brown pigment deposits
Vitamin C:	e.g. scoliosis and lordosis, lethargy, anaemia, ascites, intramuscular bleeding, trout: deformation of operculum (gill cover) and gill cartilage, delayed wound healing
Vitamin K:	Anaemia (after treatment with sulphonamide, disturbance of intestinal flora), haemorrhages, reduced haematocrit
Vitamin B1:	Balance disorder, excitation, change in swim bladder, convulsions, tremor, neuritis, paralysis
Vitamin B2:	Cataract (irreversible), blindness, haemorrhages (eye), necrosis of the gills, operculum and fin margins, dark skin coloration, uncoordinated movements
Vitamin B6:	Neural disorder - epileptiform convulsions, overexcitability, sensitivity to treatment, swimming in spirals, rapid breathing and gasping for air, sudden deaths
Pantothenic acid:	Ataxia, proliferation and adhesion of gills, abnormal or sluggish swimming movements
Folic acid:	Anaemia, macrocytic normochromic anaemia, pale gills, loss of tail fin
Biotin:	Degeneration of gill lamellae, skin lesions, “blue slime patch” disease (in the trout)

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