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Feeding concepts and methods in laboratory animal husbandry and animal experiments

- AFRICAN CLAWED FROG -

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Contents

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1. Preliminary remarks

The housing and in particular the nutrition/feeding of amphibians generally vary widely in the way they are managed. This applies also amphibian species housed in laboratories, where conditions appear to be less standardized than they are with other laboratory animal species. This can be of relevance when it comes to the results of experiments. The aim of this booklet is to summarize existing data and provide guidance on the nutrition and feeding of African clawed frogs as laboratory animals.

Minimum requirements for the housing, feeding and transport of amphibians, including African clawed frogs, are described in the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS No. 123), Appendix A.

2. Classification of the African clawed frog

The African clawed frog belongs to the amphibians and, within this class, to the family of tongueless frogs (*Pipidae*) (according to Hilken et al. 1997, Green 2010):

Class: Amphibia

Order: Anura

Suborder: Archaeobatrachia

Superfamily: Pipoidea

Family: Pipidae

a) Subfamily Pipinae:
- *Hymenochirus* (African dwarf frog)
- *Pseudohymenochirus* (Merlin's clawed frog)
- *Pipa* (Surinam toads)

b) Subfamily Xenopodinae:
- *Silurana* (tropical clawed frogs) e.g. *Silurana tropicalis* (also *Xenopus tropicalis*) (western or tropical clawed frog)
- *Xenopus* (clawed frogs) e.g. *Xenopus laevis* (African clawed frog)

3. Peculiar characteristics of African clawed frogs

As amphibians, African clawed frogs have some peculiar characteristics that affect their nutrition, feeding and also their housing. Lakes, rivers and reservoirs in sub-Saharan African form the natural habitat of the African clawed frog, which lives entirely in water (aquatic habitat). They are poikilothermic, i.e. their body temperature adapts to the ambient temperature (water temperature) to a certain extent.

The metabolism of the African clawed frog is dependent in turn on body temperature and is therefore also subject to certain fluctuations. For example, the heart rate at 2°C is about 8 beats/min, whereas at 25°C it is about 40-60 beats/min. Since body temperature does not have to be constantly maintained at 37°C, the energy expenditure for thermoregulation tends to be minimal. So the maintenance energy requirement of African clawed frogs is comparatively low. They are able to withstand even prolonged periods of hunger or survive for a time in habitats where food is scarce. African clawed frogs can sink into a kind of hibernation (torpor, dormancy, aestivation) e.g. under unfavourable conditions, drying up of water reservoirs or temperatures <8°C. To do this, they burrow into the mud, with a tunnel to the surface for breathing, and can remain like this for 1 year or longer. African clawed frogs tolerate a wide range of temperatures, but are sensitive to cold and heat shock, so abrupt changes of water temperature should be avoided (Green 2010).

The eponymous claws of the African clawed frog are found on 3 inner toes of the webbed hindlegs (Green 2010, Weiss et al. 2014).

African clawed frogs have a slimy skin that contains a great many glands and shows no keratinization or epidermal structures such as hairs, feathers or the like (Ramelow 2009, Green 2010, Weiss et al. 2014). The skin thus has little in the way of protective structures and is very sensitive (Ferrie et al. 2014). When handling the animals, therefore, care must be taken to make sure the mucus layer is not damaged (Green 2010).

The colour / markings of the skin are adaptable to the animal's surroundings to a certain extent (Green 2010).

The skin of amphibians has diverse functions in general. It absorbs and secretes electrolytes and water (water exchange approx. 2% of bodyweight per hour), has in some cases a respiratory (exchange of CO_2, O_2) and a sensory function (e.g. lateral line), plays a role in thermoregulation and cane even serve as a source of nutrients (McWilliams 2008, Green 2010). The epidermis is shed at regular intervals and usually eaten by the frogs (Green 2010, Weiss et al. 2014).

Toxic substances can also be absorbed through the skin. Therefore the animals are dependent on clean and uncontaminated water (Green 2010).

Adult African clawed frogs possess a lung, which is why they must have the possibility of reaching the surface of the water unhindered to draw breath. Some earlier development stages possess gills (TVT 1997, Ramelow 2009, Green 2010). The African clawed frog has no ribs and no diaphragm to restrict the chest wall (Green 2010).

Adult Pipidae possess a so-called lateral line, an organ that provides a "remote sense of touch" with which they can perceive movements of the water and maintain their balance in the water. This lateral line comprises intracutaneous pores with mechanoreceptors containing cilia. The movement of the water causes a deflection of the cilia, and the information resulting from this is transmitted via the nervous system. Part of the lateral line also contains electro-receptors, so that the animals can likewise perceive electrical fields (Hilken et al. 1997, Green 2010, Weiss et al. 2014).

With an adult length about 9-14 cm (2-4 years) and a weight of up to 150 g, female African clawed frogs are much bigger than males; the males have a length of up to 9 cm and are thus about 10-30% smaller than the females.

There are visible gender-specific differences in sexually mature animals: females have trilobate cloacal papillae, while males have blackish “mating pads” on the forearms (Green 2010, Weiss et al. 2014).

4. Anatomy and physiology of the gastrointestinal tract

All adult amphibians are carnivorous (Stevens and Hume 1995, Ramelow 2009). They possess a simple gastrointestinal tract with oral cavity, pharynx, oesophagus, stomach and a small and large intestine, which are separated from each other by sphincters (Ramelow 2009, Green 2010).

The African clawed frog has no tongue, which is why it is classed as a member of the family Pipidae = “tongueless” frogs (Ramelow 2009, Weiss et al. 2014). It also lacks any real teeth but has only small sharp notches in the mouth instead (Green 2010). Live prey is not usually ground up in the mouth, but in some cases is shredded with the claws (Ramelow 2009, Green 2010).

The glandular stomach connected to the oesophagus is located on the left side of the body cavity. The production of hydrochloric acid in the stomach accelerates the death of prey that is eaten alive. The small intestine in Anurans is elongated and slightly convoluted with a small number of internal folds and villi for increasing the surface area (Ramelow 2009).

Pancreas (exocrine and endocrine) and liver (bilobate) incl. gall bladder are also present in the African clawed frog (Ramelow 2009, Green 2010). The liver serves mainly as a means of glycogen storage (Ramelow 2009).

African clawed frogs can build small fat stores in the body cavity to withstand phases of high metabolic activity and fasting (Green 2010). Lipids, the main energy source of the African clawed frog, are stored in the liver, muscle, gonads and the Corpus adiposum (fat pads) (Ramelow 2009).

African clawed frogs have a very small urinary bladder and possess a cloaca as an orifice of the urinary, intestinal and reproductive system (Green 2010). The speed of digestion depends on the ambient temperature (Ramelow 2009). Nitrogen is excreted through the skin and kidneys in the form of ammonia (Green 2010).

5. African clawed frogs as laboratory animals

It is mainly two species of clawed frog that are used as laboratory animals: the South African clawed frog (*Xenopus laevis*) or the smaller tropical clawed frog (Western clawed frog; *Xenopus tropicalis* or *Silurana tropicalis*).

The African clawed frog was previously used mainly in the form of a bioassay as a “pregnancy test”, I which the woman’s urine was injected into the female frog. If the woman was pregnant, the frog would lay eggs within 5-24 hours (effect of hCG) (Weiss et al. 2014).

Today the African clawed frog is used for, among other things, research in the fields of cell biology, development biology, neurobiology, genetics, and developmental and environmental toxicology.

6. Husbandry and breeding

6.1. Husbandry

The average life expectancy of the African clawed frog in the laboratory is 15 years (in a few cases up to 30 years). Given that the egg production in females declines after 6 years (more rarely after 12 years), the actual housing period of females is limited, as females are solely used for egg production.

The African clawed frog is highly adapted to permanent life in the water and needs water in every development stage (Green 2010, Weiss et al. 2014). Water quality is crucially important for the housing of amphibians (Ferrie et al. 2014). The intake of liquid, gases and dissolved substances occurs directly through the skin, which is why nutrition is also closely associated with water quality.

The frogs are usually housed in glass or plastic aquariums.

African clawed frogs naturally live in groups and are territorial (Green 2010).

Group housing is therefore also recommended for housing laboratory animals, but within the groups there should be no substantial differences in size (TVT 1997, Ramelow 2009).

General housing conditions (according to Hilken et al. 1997, TVT 1997, ETS No 123 2006, Green 2010, Weiss et al. 2014):

Water temperature:	17-24°C or 18-22°C; (*X. tropicalis*: 24-25°C) (>30°C lethal; *X. tropicalis* not <22°C)
Water level:	20-50 cm (adult frogs)
Space/stocking density:	Area at least 600 cm^2 per *X. laevis* frog in group housing; at least 2400 cm^2 per frog in single housing (adult frogs) according to ETS No 123: area depending on size of animals: 600 cm^2 corresponds to adult frog of 9-12 cm; for each additional frog + 150 cm^2
Background brightness:	Dark colour of tank
Lighting:	≤ 200 lux, 12-hour light/dark phases
Enrichment/cover:	Pipes (clay or plastic) for hiding (check regularly for sick or injured animals)
Change of water:	Use only stale water (approx. 48 h) for the change of water; temperature of new water only differing from water in the tank by max. 1-2°C; change at least 2-3 times a week unless there is a continuous exchange of water (individual experience shows that less change of water may also be a good idea, because it causes

	the animals less stress); always replace only part of the water with fresh water
Water treatment:	Filter purification

Requirements of water (from McWilliams 2008 and Green 2010):

pH:	6.5-8.5 – ideally 7.4-7.5
Oxygen concentration:	>7 mg/L
CO_2 concentration:	<6 mg/L
NH_4^+:	<0.5 mg/L
NH_3 (non-ionized):	<0.02 mg/L
Nitrite:	<0.1 ppm
Nitrate:	<1.5 mg/L

The ammonia concentration and pH value should be regularly checked (ETS No 123) to detect any deviations in good time and prevent negative effects on the frogs.

Chlorinated tap water can be harmful to frogs. Chlorine must therefore be removed from the water. This can be achieved by means of an activated charcoal filter, letting it stand for 24-48 h, ventilation of the water or the use of chemicals (McWilliams 2008 and Green 2010).

Purified water (distilled, reverse osmosis, deionized water) lacks salts; these must be restored to deionized water (artificial sea salt; salt content approx. 0.5 ppm or 0.5 g salt/1000 g water).

Various housing systems are used for African clawed frogs (Green 2010):

Static systems (without continuous exchange of water) are an option. These are suitable for short-term housing, quarantine or very small housing facilities. They are more work-intensive, but the equipment is not as expensive.

In flow-through systems, there is an automatic exchange of water with the flow of fresh water to wastewater (completely or partially over a defined period). These systems are relatively cheap to install and require maintenance to a certain extent. A better water quality can be achieved than in the static system, but the quality can fluctuate considerably. It is necessary to filter the water. The water consumption in the flow-through system is relatively high.

Modular/recirculating systems, in which the dirty water is fed through a filter system and then back into holding tanks, are highly effective systems for a Xenopus housing facility in which an optimum water quality can be achieved. As a rule, these systems are associated with higher costs for installation and high maintenance costs. An alarm system is also necessary to flag deficiencies in the water quality. Since the recirculating water can usually be used for several units, a more rapid spread of diseases is possible in this case.

6.2. Breeding

African clawed frogs reproduce seasonally in the wild (in spring and autumn). About 500-30,000 eggs per female are produced each season. In laboratory animal facilities, each female lays about 1000-3000 eggs. The eggs of the African clawed frog are sticky and two-toned with a dark pole, known as the animal pole, and a light pole, known as the vegetal pole, with the yolk. The tadpole (larva) develops from the egg with gills. The development from fertilized egg to juvenile frog lasts about 6-8 weeks (*X. laevis*) or 3-6 weeks (*X. tropicalis*) (Green 2010). For the metamorphosis, the African clawed frog (*X. laevis*) needs about 35-45 days at temperatures between 20 and 24°C (Hilken et al. 1997). It takes about 12 months for the frog to reach adulthood (Green 2010). These timescales can vary widely depending on housing conditions. Breeding animals are housed under the same conditions as those mentioned under section 6.1.

6.3. Transport

When transporting African clawed frogs, suitable containers must be used and the animals kept moist, for example with the aid of small, wet sponges, foam rubber pieces or similar material, otherwise they would gradually dry out (Hilken et al. 1997, Green 2010). Since African clawed frogs are aquatic creatures, they can also be transported - especially for short distances - in a plastic bag filled with water in a protective vessel (NRC 1974). Attention must also be paid to ambient temperature. African clawed frogs can be easily transported at temperatures between about 7-10°C and 30-33°C (Hilken et al. 1997, Green 2010). Outside this temperature range, appropriate means should be used to regulate the temperature in the box (ETS No. 123 2006). This can be achieved at cooler temperatures e.g. by using warmer water for the sponges or heat pads or at high temperatures e.g. by using ice or cold pads. Adult African clawed frogs do not need to be fed for short transport distances. In some cases, it is recommended that they not be fed any more directly before transport (e.g. 2-3 days before) in order to prevent regurgitation of the food during transport and spiling of the transport container (Hilken et al. 1997, NRC 1974, Green 2010). After arrival, the frogs should be placed in a clean aquarium. The water from the transport container should be mixed as far as possible with the new water and the temperature gradually adapted, if necessary, to make it easier for the animals to acclimatize to the new environment (NRC 1974).

The transport of African clawed frogs may be subject to in-house, national and/or international regulations, depending on the departure point and the intended destination of the transport or the transportation route. Staff must obtain the relevant information on these regulations beforehand.

7. Nutrition and feeding

Adult African clawed frogs are carnivorous with a preference for small live invertebrates (ETS No.123 2006). When housed in the laboratory and also in the wild they consume just about any protein-rich food which is available and which they can handle (Weiss et al. 2014). African clawed frogs are considered voracious and are also cannibalistic (large frogs eat tadpoles, larvae and their own eggs) (Green 2010). Competition in the group appears to have a positive impact on the motivation to feed (Steidle, 2016).

Tadpoles/larvae begin to feed from about the 5th day after they hatch, consuming particles suspended in the water, such as phytoplankton and zooplankton (Weiss et al. 2014).

While feeding, the adult frogs become active, swim back and forth, snap at food fragments and try to shovel them into the mouth with their forearms.

An abundant food supply can lead to excessive feeding and possibly vomiting. Following copious food intake, the animals can manage without food for weeks or months (Weiss et al. 2014).

In the wild, the African clawed frog can withstand hunger periods of 18-24 months, and yet the function of the digestive tract probably remains unaffected. Starvation for 12 months causes a weight loss of 35% in males and 45% in females. After just 6 months, fat pads disappear, glycogen in the liver, ovaries and muscles is lost, and the protein concentration is reduced in the muscles while it rises in the liver (Ramelow 2009).

Water intake takes place in the form of water and electrolyte exchange through the skin (ETS No 123 2006, Green 2010).

7.1. Energy and nutrient requirements

Energy and nutrient recommendations for African clawed frogs are only available to a limited extent (Ramelow 2009, Ferrie et al. 2014). So animal facilities are usually guided by data on species with similar physiological characteristics (poikilothermic, carnivorous), in this case especially fish such as trout or pike-perch, although they are found in completely different habitats.

The energy maintenance requirement is specified as 0.39 kcal/day for an African clawed frog weighing 70 g at a water temperature of 20°C (Green 2010).

The protein content of the feed and hence the amino acids provided have an important function for the development of the animals. In larvae of the African clawed frog, the biggest increase in body mass has been achieved with a raw protein content of 60.7%. Under optimum conditions, a more rapid growth is achieved, and the metamorphosis is shortened. For adult animals, a raw protein content of not more than 50% in the food is recommended (Ramelow 2009).

Based on the fat content of insects, a fat concentration of up 45% is recommended in some cases for carnivorous amphibians (Ramelow 2009). However, an excessive protein content can lead to limited food intake, which in turn limits the intake of other nutrients (Ferrie et al. 201).

Despite numerous studies on the lipid metabolism of mammals relatively little is known about the intermediate lipid metabolism of amphibians. Fat storage takes place mainly in the liver, muscles, gonads, tail and the fat pads (Corpus adiposum). Lipids, especially triglycerides, are the principal source of energy for African clawed frogs. When fat is metabolized, the metabolic water released is of particular importance for resting amphibians in dry areas (Ramelow 2009).

Carbohydrates play a minor role as a source of energy. A comparatively low content of <5% in the feed is recommended. Carbohydrates are stored in the form of glycogen with a certain seasonality, where maximum storage has been observed in the autumn and minimal storage in the summer (Ramelow 2009).

No reliable or precise data are available on the vitamin requirements of the African clawed frog.

As regards vitamin A, there is a demand both for carotenoids, incl. beta-carotene, and for vitamin A itself, for it is very likely that the amphibians cannot convert carotenoids into vitamin A. Therefore, vitamin A must be taken up in the food. But carotenoids are likewise important, e.g. for pigmentation of the skin and for reproduction (Clugston, Blaner 2014).

Amphibians also require vitamin D3 for calcium metabolism (McWilliams 2008). It is further known that high doses of vitamin D3 administered to amphibians can lead to osteoporosis (Ramelow 2009).

When it comes to vitamin C, the African clawed frog probably produces enough itself.

There is assumed to be a need for folic acid, because this is not synthesized by amphibians themselves. No data are available as to the amount required (Ramelow 2009).

Amphibians can absorb minerals from the water.

A sodium and potassium content in the water of >5 ppm each is important for an adequate development. Delayed development has been observed at lower concentrations, and when the concentration is very much lower (<2 ppm) it has been found that the embryos no longer develop any further and malformations have been detected (Ramelow 2009).

Calcium is not only taken up from the food in the larval stages, but also absorbed through the skin and the gills. In larvae it is stored in calcium sacs mainly as calcium carbonate, and in adult animals mainly in bones, skin and intestine (Ramelow 2009). A Ca:P ratio of 1.5:1 is generally recommended for insectivorous vertebrates (McWilliams 2008), which can serve as a guide.

Iron and iodine must be taken up with the food, the latter being considered essential for the initiation of metamorphosis (Ramelow 2009).

In their summary on the housing of amphibians, Ferrie at al. (2014) recommend the following composition of nutrients as an adequate supply for adult amphibians (after metamorphosis), these being based on the requirements specified by the National Research Council (NRC) for fish, poultry, carnivores and rats:

Table 1: Nutrient recommendations for adult amphibians (according to Ferrie at al. 2014)

Nutrient	**Unit**	**Recommendation**	**NRC for species**
Raw protein	%	44.4	Fish
Arginine	%	2.6	Fish
Glycine*	%	0.9	Poultry
Histidine	%	0.8	Fish
Isoleucine	%	1.2	Fish
Leucin	%	1.9	Fish
Lysine	%	2.6	Fish
Methionine	%	0.8	Fish
Methionine+cysteine	%	1.3	Fish
Phenylalanine	%	1.1	Fish
Threonine	%	1.3	Fish
Tryptophan	%	0.4	Fish
Valine	%	1.4	Fish
Taurine **	%	0.1	Cat
Raw fat	%	n.d.	n.d.
Calcium	%	0.6	Rat
Phosphorus	%	0.3	Rat
Sodium	%	0.2	Cat
Magnesium	%	0.04	Cat
Potassium	%	0.4	Cat
Chloride	%	0.1	Cat
Copper	ppm	12	Dog
Iodine	ppm	1	Fish
Iron	ppm	97	Poultry
Manganese	ppm	14	Fish
Selenium ***	ppm	0.3	Cat
Zinc	ppm	18	Fish
Vitamin A	IU/kg	2914	Fish
Vitamin D3 °	IU/kg	1111	Rat
Vitamin E	IU/kg	88	Fish
Vitamin K	ppm	2	Dog
Vitamin C °°	ppm	23	Fish
Biotin	ppm	1	Fish
Choline	ppm	1889	Dog
Folic acid	ppm	1	Fish
Vitamin B12	µg/kg	39	Dog
Thiamine (B1)	ppm	12	Fish
Riboflavin (B2)	ppm	8	Fish
Pyridoxine (B6)	ppm	7	Fish
Niacin	ppm	44	Cat
Pantothenate	ppm	23	Fish

Data refer to ration with 4000 kcal DE/ kg and to dry substance
n.d. = no data for recommendation
* essential for uric acid production
** limited data for essentiality of taurine
*** possible risk of toxicity
° UV-B light has a positive effect on bone mineralization; amphibians have a capacity for endogenous vitamin D3 synthesis
°° endogenous synthesis not detected for all amphibians

7.2. Feeding in the laboratory

Since larvae or tadpoles begin to take in food from about the 5th day after hatching, daily feeding should be started at this point (TVT 1997, Weiss et al. 2014). A feeding frequency of once or twice daily can help to avoid cannibalism in the group (Ramelow 2009).

The following feed components are used in practice:

Feeding of larvae/tadpoles:

- e.g. powder of dried stinging-nettle leaves + vitamins or dried yeast / algae powder 150-200 mg/L H_2O (TVT 1997, Weiss et al. 2014)
- complete feed in milled / powder form
- Fish rearing feed (Steidle, 2016)

Once metamorphosis is complete, the animals naturally prefer live food in most cases (Weiss et al. 2014).

Feeding of adult frogs (according to Hilken 1997, Ramelow 2009, Weiss et al. 2014):

- Tubifex (sludge or sewage worm)
- Drosophila larvae (standardized breeds available)
- Fish rearing feed (Steidle, 2016)
- Bovine heart or liver chopped small (can lead to heavy contamination of the water → feed immediately before change of water)
- Earthworms
- Commercial Xenopus feed or pelleted pond feed
- Often mixed rations from the above components and alternating with pelleted compound feed to avoid deficiencies
- Live mealworms, darkling beetle larvae or maggots – caution is called for here, because they are often swallowed whole and can then perforate the stomach wall

Adult African clawed frogs are fed at a frequency of once or twice a week to once every two weeks (Hilken 1997). More frequent feeding is sometimes mentioned, e.g. in connection with a more rapid attainment of sexual maturity, of up to three times a week (ETS No 123 2006, Ramelow 2009, Green 2010) or in the case of X. tropicalis daily (Steidle 2016). The frog can generally manage without food for a week or longer with no problem. Lower feeding frequency makes for better water quality but tends to adversely effect on growth and egg production (Green 2010).

It is difficult to put a figure on the exact amount of food the African clawed frog needs. It depends on age, sex, season, reproduction status and water temperature (at low temperatures the metabolic rate is lower).

Commercially available feed usually contains at least 40% raw protein and more than 6% fat with a low fibre content. A fat content of 45%, as is usually recommended, will probably not be achieved here partly also technical reasons.

A figure of 1 g feed of similar composition per adult frog can be assumed as a guide for food intake (Green 2010).

Table 2: Summary of nutrient contents in various commercial feeds for African clawed frogs:

	1	2	3	4
Raw protein %	min. 44.0	min. 40.0	min. 41.0	44.5
Raw fat %	min. 6.0	min. 10.0	min. 12.0	8.8
Raw fibre %	max. 3.0	max. 3.5	max. 4.0	1.5
Raw ash %	max. 11.0	max. 12.0	max. 11.0	8.5

The precise requirement for egg production and development / growth is not known. In the case of fish, 2-8% of bodyweight is specified for food intake, which could serve as a guide for the African clawed frog. The quantity of feed can generally be considered adequate if, after food intake, most of the feed has been eaten (after 15-20 min) and only minimal amounts are left over after 2 h (Green 2010).

Feed that slowly sinks is preferred to floating feed (Ramelow 2010). Feed that has sunk completely to the bottom of the tank is usually no longer consumed and rots, adversely affecting the water quality (Weiss et al. 2014).

Animals that are overfed or stressed or animals that are manipulated too soon after food intake may vomit (Green 2010). *X. laevis* in particular tends to need calm for feeding (Steidle 2016).

7.3. Nutritional disorders

One-sided feeding with only one component of the above-mentioned fresh feed or otherwise excess or deficiency of certain nutrients can lead to nutritional disorders (see appendix for nutrient composition of some fresh feed for amphibians). Fresh / live components can still bring certain hygienic risks with them. Some nutritional disorders known to occur in amphibians are listed below:

Overeating (obesity) is common in amphibians kept under human supervision and is a result of excessive calorie intake (e.g. very fat insect larvae) combined with minimal activity. Feeding on a lot of fat over a prolonged period can lead to corneal lipidosis (lipid keratopathy), which can ultimately result in blindness (McWilliams 2008).

Gastric overload occurs after excessive feeding and can become an acute medical emergency, because the highly distended stomach hinders breathing and circulation, and the animal may suffer a hypovolaemic shock (McWilliams 2008).

One-sided feeding on trout feed or heart can lead to wasting and death (Hilken 1997). The nutrient composition is probably imbalanced in such cases. The precise nutrients involved here have not been described in any further detail.

Insects naturally have a minimal concentration of vitamins and mineral, which is why feeding mainly or exclusively on insects can result in a deficiency of e.g. vitamins A, D3, E and B1 and also calcium, which favours the development of metabolic bone disease (Bernard et al. 1997, McWilliams 2008, Ramelow 2009, Ferrie et al. 2014).

In practice, therefore, insects are often dusted with calcium or vitamin powder, which has to be viewed critically, however, with regard to the consistency and adequacy of nutrient supply (Bernard 1997) and is rather unsuitable especially when administered in water. A more favourable method for amphibians is supplementation by means of “gut loading”, which means targeted feeding of insects e.g. with calcium to fill the digestive tract with the desired nutrients and thus make the nutrients directly available via the insect (Bernard 1997, Clugston, Blaner 2014). But in the case of the African clawed frog, it is also possible to fall back on balanced commercial complete feed.

Metabolic bone disease can be caused by calcium deficiency, an unfavourable Ca:P ratio, lack of exposure to UV light and by both a deficiency and an excess of vitamin D3. The symptoms include deficient bone mineralization, bone deformations, fractures of the long bones, abnormal posture and movement, convulsions and oedema (McWilliams 2008).

An undersupply of vitamin D3 (vitamin D deficiency) can also have a negative impact on reproduction, incl. The hatching of larvae, and cause other symptoms such as muscle weakness, reduced gut motility and constipation (McWilliams 2008).

Feeding exclusively on bovine liver leads to hypervitaminosis A in African clawed frogs, which is associated with tail resorption and chronic diarrhoea in tadpoles, general development disorders in the frogs, metabolic bone disease, weight loss, anaemia, hepatic changes and skin lesions (McWilliams 2008, Ramelow 2009, Green 2010, Clugston, Blaner 2014).

Hypovitaminosis A can also occur in amphibians if there is no direct source of vitamin A in the food, because they cannot synthesize vitamin A from the precursor beta-carotene. Signs of vitamin A deficiency are lethargy, metaplasia of the mucosal epithelia especially in the mouth, and weight loss / emaciation (McWilliams 2008, Clugston, Blaner 2014). A deficiency of the provitamin beta-carotene can lead to diseases of the eye, skin diseases and pigmentation disorders of the skin (McWilliams 2008).

If the animals are fed frozen fish, then this should always be supplemented with vitamin B1 because of the thiaminases contained in the frozen fish (McWilliams 2008, Ramelow 2009). Vitamin B (especially B1) deficiency in amphibians has been associated with various neurological and musculoskeletal disorders, such as demyelinization of the peripheral nerves, paralysis, scoliosis and so-called spindly leg syndrome (McWilliams 2008). The latter is a multifactorial development disorder that occurs in frogs in captivity and is usually in visible in the form of underdevelopment forelimbs (Camperio Ciani et al. 2018).

Iodine deficiency can prevent metamorphosis in tadpoles (McWilliams 2008).

7.4. Summary and recommendation

An imbalanced diet can lead to a variety of nutritional disorders. When it comes to live feed, there are concerns in laboratory animal facilities with regard to the introduction of parasites and pathogens (Green 2010). Ideal growth has been observed when animals are fed sludge worms, but these worms may carry microorganisms such as Aeromonas hydrophila. This is known as a cause of red-leg syndrome (bacterial dermatosepticaemia) (Hilken et al. 1997; Densmore and Green, 2007). The sludge worm is only obtainable as “wild catch” to date, which is why it is not possible to control hygiene status (Ramelow 2009). If the animals are fed on offal, there is also there is a risk of infection with chlamydia (Green 2010).

Pelleted / extruded commercial complete feed is already provided in many facilities to ensure that the feed is nutritionally and hygienically adequate and is also recommended for standardization of the housing conditions in laboratory animal facilities. Feed for African clawed frogs is available from a variety of suppliers. Care must be taken to make sure good quality feed is obtained, so that the water quality is not compromised.

Pelleted feed may be ground and the tadpoles provided with feed in powder form (Green 2010).

For the stimulation of adult frogs, fresh feed may be added, for example in the form of an alternate feeding procedure. For this purpose, It is recommended that live feed which is as standardized as possible (e.g. Drosophila larvae) or fresh feed such as bovine liver and heart chopped into small pieces be alternated with pelleted Xenopus feed (Hilken et al. 1997, Weiss et al. 2014).

In general, any change in feed should be made gradually, i.e. mixed proportionately with the accustomed feed at first (Green 2010).

8. Appendix

The tables in the appendix have been taken from the publication of Bernard et al. (1997) and included as examples illustrating the nutrient composition of insects. This list cannot be regarded as final or exhaustive but serves purely as a guide and source of information.

Table A1. Energy and nutrient concentration of invertebrates (in DS)[1)] according to Bernard et al. 1997

	DS %	**Protein** %	**Fat** %	**Ash** %	**ADF** %	**GE** kcal/g
Black worm" - tubifex-like *(Lumbriculidae)*	18.4	47.8	20.1	4.5	0.7	5.57
Non-biting midges *(Chironomus sp.)*. larvae	9.9	52.8	9.7	11.3	n.d.	n.d.
American cockroach *(Periplaneta americana)*	38.7	53.9	28.4	3.3	9.4	6.07
Corn borer (*Ostrinia nubilalis)*						
Larvae	27.3	60.4	17.2	2.9	13.1	5.69
Pupae	28.0	64.2	17.0	2.6	15.4	5.60
House cricket *(Acheta domesticus)*						
Adult	31.0	64.9	13.8	5.7	9.4	5.34
Adult. Ca-rich diet	30.3	65.2	12.6	9.8	13.2	5.40
Freshly hatched[2)]	47.4	n.d.	n.d.	n.d.	n.d.	n.d.
Earthworm – grey worm *(Allolobophora caliginosa)*	20.0	62.2	17.7	5.0	9.0	4.65
Fishflies *(Chauliodes sp.)*	26.5	63.9	19.5	5.8	10.9	5.88
Fruit fly *(Drosophila melanogaster)*						
Fly	29.6	70.1	12.6	4.5	27.0	5.12
Larva	21.2	40.3	29.4	9.8	5.9	5.57
Pupa	32.4	52.1	10.5	14.1	17.4	4.84
Housefly *(Musca domestica)*						
Larva, dry	93.7	56.8	20.0	6.8	18.0	6.07
Pupa, dry	96.4	58.3	15.8	6.8	19.9	5.70
Mealworm *(Tenebrio molitor)*						
Beetle	38.6	63.7	18.4	3.1	16.1	5.79
Larva	37.6	52.7	32.8	3.2	5.7	6.49
Pupa	39.0	54.6	30.8	3.4	5.1	6.43
Darkling beetle *(Tenebrionidae)*						
Larva	40.9	45.3	55.1	2.9	7.2	7.08
Larva. Ca-rich diet	42.2	38.9	45.4	3.5	7.7	6.79
Mosquitos *(Aedes sp.)*. larva. dried	94.0	42.2	16.1	11.8	n.d.	n.d.
Common earthworm *(Lumbricus terrestris)*	16.3	60.7	4.4	11.4	15.0	4.93
Sludge worm *(Tubifex sp.)*	11.8	46.1	15.1	6.9	n.d.	n.d.
Daphnia. dried *(Daphnia sp.)*	91.7	55.2	6.6	10.8	n.d.	n.d.
Greater wax moth *(Galleria mellonella)*						
Larva	34.1	42.4	46.4	2.7	4.8	7.06
Larva. Ca-rich diet	39.9	n.d.	n.d.	2.5	n.d.	n.d.

1) Data from: Duane E. Ullrey, Comparative Nutrition Laboratory, Michigan State University, Mary E. Allen, National Zoological Park

2) Analyses by Covance Laboratories, Inc., Madison, WI 83707; T in vacuum furnace (70°C)

DS = dry substance; ADF = Acid detergent fibre; n.b. = not determined; GE = gross energy

Table A2. Mineral and trace element concentrations of invertebrates (in DS)[1)] according to Bernard et al. 1997

Black worm" - tubifex-like *(Lumbriculidae)*	0.11	0.85	0.09	0.28	0.98	1.091	166	16	10	0.87
Non-biting midges *(Chironomus sp.).* larvae	0.38	0.90	0.12	0.62	0.35	2.940	115	22	30	0.37
American cockroach *(Periplaneta americana)*	0.20	0.50	0.08	0.27	0.87	90	57	5	14	0.36
Corn borer (*Ostrinia nubilalis)*										
Larvae	0.23	0.64	0.12	0.02	0.05	289	90	18	24	0.31
Pupae	0.22	0.67	0.13	0.02	0.05	269	98	16	20	0.20
House cricket *(Acheta domesticus)*										
Adult	0.14	0.99	0.13	0.49	1.29	58	188	31	28	0.58
Adult. Ca-rich diet	0.90	0.92	0.11	0.57	1.41	80	237	56	290.49	
Freshly hatched[2)]	0.22	1.27	0.14	0.43	1.62	200	268	33	14	n.d.
Earthworm – grey worm *(Allolobophora caliginosa)*	1.72	0.90	0.14	0.02	0.06	4.133	250	142	18	0.92
Fishflies *(Chauliodes sp.)*	0.23	1.07	0.16	0.39	1.01	216	378	6	20	1.63
Fruit fly *(Drosophila melanogaster)*										
Fly	0.10	1.05	0.08	0.42	1.06	138	171	39	18	0.07
Larva	0.59	2.30	1.89	0.09	1.28	235	176	110	16	0.49
Pupa	0.77	2.73	2.41	0.12	1.66	1.728	200	108	25	0.33
Housefly *(Musca domestica)*										
Larva, dry	0.41	1.13	0.30	0.72	1.28	658	320	167	50	1.20
Pupa, dry	0.42	1.18	0.36	0.55	1.34	574	319	302	54	1.30
Mealworm *(Tenebrio molitor)*										
Beetle	0.07	0.78	0.19	0.16	0.92	77	113	10	22	0.29
Larva	0.08	0.83	0.23	0.15	0.93	42	95	12	18	0.29
Pupa	0.11	0.77	0.22	0.14	0.91	43	100	14	19	0.31
Darkling beetle *(Tenebrionidae)*										
Larva	0.16	0.59	0.12	0.10	0.72	59	80	13	14	0.40
Larva. Ca-rich diet	0.69	0.57	0.12	0.09	0.88	58	86	24	13	0.18
Mosquitos *(Aedes sp.)*.										
Adult	0.82	1.24	0.33	n.d.	n.d.	616	1.057	70	76	n.d.
Larva, dried	0.79	1.07	0.21	0.39	0.52	3.057	281	93	57	0.57
Common earthworm *(Lumbricus terrestris)*	1.52	0.96	0.16	0.44	0.87	1.945	1.119	29	9	5.44
Sludge worm *(Tubifex sp.)*	0.19	0.73	0.09	0.46	0.79	1.702	190	30	108	2.16
Daphnia. dried *(Daphnia sp.)*	0.10	1.17	0.16	0.98	0.99	3.049	250	73	39	1.46
Greater wax moth *(Galleria mellonella)*										
Larva	0.11	0.62	0.11	0.05	0.72	22	76	3	9	0.66
Larva. Ca-rich diet	0.50	0.33	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

1) Data from: Duane E. Ullrey, Comparative Nutrition Laboratory, Michigan State University, Mary E. Allen, National Zoological Park

2) Analyses by Covance Laboratories, Inc., Madison, WI 83707; T in vacuum furnace (70°C)

DS = dry substance; ADF = Acid detergent fibre; n.b. = not determined; GE = gross energy

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