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Digestibility measurements in juvenile tench [*Tinca tinca* (L.)] by using a continuous filtration device for fish faeces

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Summary

Effective pond production of tench [*Tinca tinca* (L.)] requires the use of formulated diets. However, insufficient information is available regarding the nutrition of this species. Therefore, for the first time, the digestibility of macronutrients by tench was investigated using a sieving method of faeces collection and chromic oxide. A pelleted experimental diet rich in untreated wheat starch revealed the following mean apparent digestibility coefficients (ADCs): dry matter 77.2%, crude protein 89.4%, crude fat 94.3%, ash 30.5%, nitrogen-free extracts 78.2%, organic matter 83.3% and energy 85.1%. Generally, these ADCs indicate that the ability of tench to digest the macronutrients from pelleted feed correlates with reports of other fish species. However, the tench is more able to digest untreated wheat starch than are carnivorous and coldwater fish species. Compared with carp (*Cyprinus carpio* L.), the digestibility of untreated wheat starch is less in tench. The faeces sieving method as described in this paper is a useful procedure for further digestibility trials with tench. However, compared with the approach presented here, several adaptations are recommended, e.g. long adaptation times to the rearing facility, high stocking densities, usage of more waterproof food pellets and the addition of formalin to the collection pans.

Introduction

The tench [*Tinca tinca* (L.)] is an Eurosiberian, warm-water, cyprinid fish species that has been cultured for centuries as a so-called secondary fish in polyculture with carp (*Cyprinus carpio* L.) in ponds in many European countries and the former USSR (Steffens, 1995). On the European market tench is bought as a food, for stocking open waters (especially for recreational fishing) and as an ornamental fish (Steffens, 1995). When the yield of carp in ponds increases, the yield of tench in polyculture decreases (Müller, 1961). This is mainly due to a strong competition for food between tench and carp (Sukop and Adámek, 1995), with the latter dominating (Füllner and Pfeifer, 1998). Carp yields have decreased over the past years in Europe due to, *inter alia*, reduced demand (Steffens, 1999). Thus, there is a potential for an increase in tench production. However, Füllner and Pfeifer (1998) clearly showed that the effective culture of tench is not possible unless farmers begin to regard this fish species independently as a primary cultured pond fish and not merely as a secondary species. As the tench is likely to become more significant for pond culture (Arlinghaus and Rennert, 2000), increased research, particularly in tench nutrition, is needed, and farmers are encouraged to test new pond management methods.

Tench are known to be a very shy and delicate fish that frequently tend to hide. Under controlled culture conditions, e.g. in recirculating systems, tench often stop feeding after exogenous disturbances (Fleig and Gottschalk, 2001). Hence, growth rate of tench in recirculating systems is very low (e.g. Wolnicki and Myszkowski, 1998), to a great extent explained by their sensitivity to stress. These circumstances may also be the reason for the lack of nutritional investigations dealing with tench (e.g. Billard and Flajšhans, 1995) because nutrition research requires controlled conditions in recirculating systems and handling of the fish.

This is the first published investigation on tench digestibility and its ability to digest macronutrients of a pellet feed. It is important to determine nutrient digestibility in dry diets in order to formulate diets that maximize the growth of cultured fish with appropriate amounts of nutrients and also limit the produced wastes. In the present study, special emphasis was placed on carbohydrate digestibility because the digestion and utilization thereof is species-specific and evidence in the literature is controversial (Hepher, 1988; Steffens, 1989; Wilson, 1994), especially concerning tench (Steffens, 1995; Wirth et al., 1997; Füllner and Pfeifer, 1998). Thus far, no dietary requirement for carbohydrates has been demonstrated in fish (Wilson, 1994). However, carbohydrates are the least expensive form of dietary energy and supplementary grain feeding is popular in European pond aquaculture. Füllner and Pfeifer (1998) concluded from pond experiments with supplemental wheat feed that the digestibility and utilization of carbohydrates by tench is too low to allow for an effective aquaculture. In order to verify the assumption of Füllner and Pfeifer (1998), one particular aim of the present study was to assess tench ability to digest carbohydrates with untreated starch as a main carbohydrate source. According to Wirth et al. (1997), tench are able to utilize untreated wheat starch and transform the absorbed glucose into body fat. Therefore, in the present digestibility trial, it was hypothesized that tench ability to digest raw starch is substantial and thus the apparent digestibility coefficient of nitrogen-free extracts (NFE) (mainly carbohydrates) amounts to at least 50%. Another aim of the investigation was to determine methodological possibilities and constraints in digestibility trials using tench.

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Materials and methods

Fish and culture conditions

The experiment was carried out at the Institute of Freshwater Ecology and Inland Fisheries Berlin (IGB). Experimental fish were hybrids of a wild population from Lake Döllnsee (approximately 80 km north of Berlin) and a pond strain from Königswartha in Saxony, Germany. Both strains were held in ponds at the IGB since 1996. In 1997, spawners were caught in the ponds, brought indoors into the rearing facility of IGB, hormonally treated with carp pituitary gland extracts and stripped. The incubation of eggs took place in gauze frames. During the first 100 days after hatching 0-group tench were fed *ad libitum* with *Artemia* nauplii, zooplankton, macrozoobenthon and a trout starter feed. Thereafter, the juvenile tench were fed solely with a commercial trout feed (Trouvit) at 2% of wet body weight per day. The crude protein and crude fat content (% of dry matter) in this feed amounted to 45 and 20%, respectively. After 100 days, when the body weight was more then 1 g, the juvenile tench were stocked into circular tanks (500 L) with a water volume of 285 L in a recirculating system with warm-water conditions. These tank-reared, two-summer-old, juvenile tench were used for the digestibility trials which began in November 1998.

Fish were held indoors in two semi-recirculating systems. Each system consisted of three cylindro-conical tanks of dark synthetic material strengthened by fibreglass. The volume of each tank was 60 L. Via a 21 mm internal diameter spigot at the bottom of each tank, water was delivered through a plastic tube to the faeces filtering screen with a falling height of 10 mm, at a constant down-flow of 4 L min^{-1}. Small sieves were fitted to each water outlet inside the tanks in order to prevent the tench from becoming stuck in the outlet. Fresh water was added to the system at a rate of 0.12 L min^{-1} to compensate for splashing and evaporation losses. Water temperature was maintained with the help of an immersion heater. During the experimental period, the water quality parameters (n = 15 determinants for each parameter) ranged from 21.3 to 22.7°C (temperature), 766–799 μS cm^{-1} (conductivity), 5.92–7.48 mg L^{-1} (dissolved oxygen), 7.76–8.05 (pH), < 100 mg L^{-1} (nitrate), < 0.1 mg L^{-1} (nitrite) and < 0.01 mg L^{-1} (ammonia). Experimental fish were subjected to a 12 h light/dark photoperiod. During the 12 h light period, light intensities were very low because of the opaque tank, the low light intensities entering the rearing facility from outside during the winter months, and the avoidance of using artificial light in the direct surroundings of the fish tanks. Such low light conditions should have enhanced feeding, as the tench is known to show a higher feeding activity in the dark (Perrow et al., 1996; Martín et al., 1998).

Two weeks prior to the start of the digestibility trial, a total of 60 tench (30 per semi-recirculating system) were stocked in order to acclimatize them to the rearing facility and the experimental diet. Ten fish per each of the six tanks were held in stocking densities between 4.3 and 6.0 kg m^{-3}. Thus, digestibility was measured in six replicates which corresponds to three replicates per semi-recirculating system. The fish were anaesthetized (MS-222, dosage 0.2 ml L^{-1}) and weighed 3 days prior to the start and at the end of the faeces collection period. Before weighing, fish were dehumidified with a paper towel. At the start of the experiment, mean weight ± SD was 36.0 ± 8.4 g; mean total length ± SD was 14.1 ± 0.9 cm.

Diet formulation and preparation

An experimental diet rich in wheat starch was compounded (Table 1). Chromic oxide was added as a non-digestible marker to allow calculation of digestibility (indirect method) as seems suitable in starch-based diets (Ng and Wilson, 1997). Chromic oxide was first mixed with a small amount of wheat meal and then mixed with the feedstuffs to allow for a homogenous distribution of the marker substance.

Pelleting of the feed was carried out by passing the mixture through a mincer with 1.0-mm-diameter holes. The wet pellets were air-dried for 1.5 weeks. At the beginning of the faeces collection period one sample was freeze-dried for analysis. Pellets were stored in a refrigerator (4°C) to minimize loss of nutrients sensitive to degradation. The experimental diet composition is given in Table 2.

Feeding and faecal collection

An automatic continuous collector for fish faeces (Choubert et al., 1982) was attached to each tank. In the collector, effluent water flowed from the fish tanks through successive rotating metallic screens (650 μm mesh), thus separating (filtering or sieving) the faeces and dropping them into a collection pan. The faeces were ejected into the pans within 6–15 s after being excreted by the fish, thereby minimizing leaching. Faeces were collected for about 5 weeks from 30 November 1998 until 8 January 1999.

During this period, the fish were fed with 2% of their wet body weight in late afternoon and thus in the dark (winter months). The daily ration was divided into three portions and feeding was carried out by hand without showing oneself or ones shadow on the tank surface. By adding water to the collection pans, uneaten pellets were reapplied and poured into the tanks again using a clear glass cup. The water–pellet mixture was poured slowly so that no swirls were created in the water column, thus minimizing exogenous disturbances during feeding; this procedure was repeated until most pellets were ingested. The next portion was then given.

Feeding lasted in total from 3 to 4 h. One hour thereafter, feed residue in the collection pans was cleared by hand and the pans were reset at an inclined angle for water to drop off the faeces after being filtered (Yeshak, 2000). The following day, the filtered faeces were collected and stored at −20°C. At the end of the digestibility trial, the frozen faeces were freeze-dried, ground and refrigerated at 4°C.

Analytical procedures and calculations

Samples of feed (n = 1) and faeces (n = 6) were chemically analysed in triplicate (Table 2) using the following procedures: dry matter and moisture freeze-dried at −54°C until reaching a constant weight; crude protein (Nitrogen × 6.25) according to the Kjeldahl distillation method (Kjeltec System Tecator); crude fat by petroleum ether extraction in a Soxhlet apparatus (Soxtec System HT Tecator); ash by incineration for 4 h at 750°C; chromic oxide by a spectrophotometer (Petry and Rapp, 1970); NFE by subtracting the percentage values of moisture, crude protein, crude fat and ash from 100%; organic matter by subtracting percentage value of ash from dry matter; gross energy in an oxygen bomb calorimeter (Framo-MK 200). Apparent digestibility coefficients (ADCs) of dry matter, crude protein, crude fat, ash, NFE, organic matter and energy were estimated indirectly as follows (e.g. Castell and Tiews, 1980):

Table 1
Composition of the experimental diet in % of wet weight

Ingredient	%	Company supply
Wheat flour (1 : 1 mixture of white and wholemeal)	44.2	Aurora, Weinheim, Germany
Low temperature fish meal	23	M + M Baits, Vörden, Germany
1 : 1 mixture of fish oil and sunflower oil	6	Kraftfutterwerk Beeskow e.G., Brandenburg, Germany
Mixture of vitamins and minerals[a]	3	Spezialfutter Neuruppin GmbH & Co. KG., VM BM 36/22 EX, Germany
Chromic oxide (Cr_2O_3)	0.8	Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany
Water (H_2O)	23	Tap water, Berlin, Germany

[a]Vitamins and minerals supplied per 1 kg of dry feed: Vitamin A 120 000 IE, Vitamin D3 20 000 IE, Vitamin E 400 mg, Vitamin K3 300 mg, Vitamin B1 230 mg, Vitamin B2 100 mg, Vitamin B6 110 mg, Vitamin B12 320 mg, Nicotinic acid 923.08 mg, Biotin 5000 mg, Folic acid 70 mg, Pantothenic acid 670 mg, Inositol 10 000 mg, Vitamin C 363.6 mg, Antioxidants BHT 1650 mg, Calcium 0.772%.

Table 2
Chemical composition (mean ± SD, % of dry matter) and gross energy (kJ g^{-1}) of the experimental diet at the start of the experiment (30.11.1998) and the faecal samples for the duration of the experiment (30.11.1998–08.01.1999)

Macronutrients and gross energy	Experimental diet (30.11.98)	Faecal sample (30.11.98–08.01.99)
Dry matter	94.96	–
Moisture (% wet weight)	5.04	–
Crude protein	27.87	12.92 ± 0.50
Crude fat	13.75	3.46 ± 0.07
Ash	11.56	35.27 ± 0.01
Cr_2O_3	1.02	4.46 ± 0.02
NFE	44.82	43.90 ± 0.47
Organic matter	88.45	64.74 ± 0.01
Gross energy (kJ g^{-1})	20.45	13.40 ± 0.35

Table 3
Apparent digestibility coefficients (ADCs, mean ± SD, n = 6) of the macronutrients and digestible energy of the tench experimental diet

Macronutrients and energy	ADCs
Dry matter	77.2 ± 0.4
Crude protein	89.4 ± 1.2
Crude fat	94.3 ± 0.2
Ash	30.5 ± 0.5
NFE	78.2 ± 0.7
Organic matter	83.3 ± 0.3
Energy	85.1 ± 0.6

$$\mathrm{ADC}(\%) = 100 - 100 \times \frac{\%\ \mathrm{Cr_2O_3feed}}{\%\ \mathrm{Cr_2O_3faeces}} \times \frac{\%\ \text{nutrient faeces}}{\%\ \text{nutrient feed}}$$

In order to calculate the ADC of dry matter, only the percentage of the indicator (chromic oxide) in feed and faeces was taken into consideration. Digestible (gross) energy was estimated by their respective values (kJ g^{-1}) in both feed and faeces instead of nutrient % in feed and faeces (see formula above, Table 2).

Differences in mean ADCs between the two semi-recirculating systems were tested by Student's *t*-test at the 95% level of confidence.

Results

Regarding mean ADCs, there were no significant differences ($P > 0.05$) between the two semi-recirculating system tanks. Therefore, the six tanks were considered as replicates and mean ADCs were calculated (Table 3). In general, high ADCs were determined for the different macronutrients. The mean ADC of NFE was estimated by nearly 78.2% in tench. Thus, the digestibility of untreated wheat starch as a main NFE source was substantial. The digestible energy ranged around 17.4 kJ g^{-1} of dry matter of the diet.

Although exogenous disturbances were kept to a minimum during the experiment, the tench showed a rather poor intake of feed and growth performance. Some fish did not grow at all or lost some weight. This precluded (a) calculation of growth parameters such as specific growth rate and feed conversation ratio and (b) meaningfulness of the chemical analysis of body tissue composition. One tench died during the experiment, thus the mortality rate was 1.6%.

Discussion

Factors affecting the digestibility of food by fish are complex and depend on the type of food, composition of the feed (e.g. the content of crude fibre), size of the particles, feed amount eaten, enzyme activity, and the period of time the food is exposed to the enzymes. Further factors affecting digestibility include species of fish, age and size of fish, physiological condition, and environmental parameters such as water temperature (Hepher, 1988). Furthermore, a daily variation of digestibility has been demonstrated (De Silva and Perera, 1983). However, the method by which digestibility is calculated is the decisive factor that determines accuracy in digestibility measurements (e.g. Vens-Cappell, 1985; Spyridakis et al., 1989; Storebakken et al., 1998; Austreng et al., 2000). Due to the filtering procedure and reusing of uneaten feed, leakage of nutrients was the major source of error for the ADCs in the present study (Table 3) leading to an overestimation of apparent digestibility (e.g. Vens-Cappell, 1985; Storebakken et al., 1998). Therefore, only literature results obtained by faeces sedimentation methods (e.g. Kirchgessner et al., 1986) are comparable with the ADCs of tench.

Normally, fish are able to digest and utilize dietary proteins, particularly fish proteins, and dietary fats efficiently

(Wee, 1992). In the experimental diet, a high digestible low temperature (LT) fish meal (NRC, 1993) and high digestible oils (Wee, 1992) were used as the main crude protein and crude fat sources. Thus, a high digestibility could be expected. The ADCs of protein and fat in tench (Table 3) are in the upper range of the apparent digestibility values reported by NRC (1993) and other literature summaries. This demonstrates that the tench is able to digest fish proteins and also (mainly unsaturated) fatty acids well. However, the high level of carbohydrates (NFE) in the diet may have lowered the ADC of crude protein in the present study (Steffens, 1989).

It is known that digestibility of carbohydrates is significantly affected by the source of the carbohydrates (Wee, 1992). The experimental diet used was rich in wheat starch and low in crude fibre. The relatively small quantity of the latter means that its negative effect on NFE-digestibility (Steffens, 1989) was negligible. The ADC of the NFE-fraction in tench was well above 50% (Table 3), which confirmed the main working hypothesis that tench ability to digest raw starch is substantial (see introduction). This ADC of NFE in tench is a relatively high value for the digestion of raw wheat starch as the main NFE-source when compared with carnivorous or coldwater fish species such as salmonids (Hepher, 1988; Steffens, 1989; Wilson, 1994). However, the digestibility of raw wheat starch by tench is low compared with carp, another cyprinid fish species. For carp, ADCs for raw starch usually range between 85 and 90% (Kirchgessner et al., 1986; Schwarz, 1998). This general ranking of the tench also correlates with the enzymatic activities. Tench have a total amylase activity only 18% of that in carp (Hidalgo et al., 1999), which contributes to the lower ability of tench to hydrolyse starch. In addition to the competition for food (e.g. Sukop and Adámek, 1995), these differences in digestibility of carbohydrates help to explain the reduced tench growth in polyculture with carp in ponds when supplemental grain feeding is applied (Füllner and Pfeifer, 1998). Another explanation could be that tench have only recently been domesticated whereas carp has been selected for efficient carbohydrate (starch) utilization for centuries (Kamler et al., 1995).

However, digestibility does not necessarily equate with utilization (Wee, 1992). Ability of the fish to utilize the absorbed nutrient should also be taken into account. The general inabilities of fish to utilize high amounts of carbohydrates (Wilson, 1994) may also contribute to the low yields in tench monoculture in ponds under supplemental grain feeding (Füllner and Pfeifer, 1998). However, it can be assumed that the true starch digestibility from cereals is lower than the ADC of NFE revealed in the present study. This is because grains contain much higher starch and fibre levels than the compounded diet used in this experiment. Moreover, pelleting can be considered a gently hydrothermical treatment which increases the ADC of NFE (Wilson, 1994). Further investigations on digestibility and growth are recommended to evaluate tench utilization of untreated starch.

The potential influence of stress on the digestion (Hepher, 1988) of tench in this trial was negligible because of the high ADCs of organic matter and energy obtained (Table 3, compare values reported by Kirchgessner et al., 1986; Schwarz, 1998). ADC of organic matter and ADC of energy summarize the ADCs of the single macronutrients and facilitate the evaluation of the whole diet. In contrast to these digestibility coefficients, the ADCs of ash and dry matter cannot be regarded as meaningful measures of macronutrient uptake (Storebakken et al., 1998). The reason is that elements from the water may be excreted into the gut, affecting the estimates for absorption of ash and dry matter (Steffens, 1989; Storebakken et al., 1998).

Recommendations for further digestibility measurements using tench

Chromic oxide is the most widely used external marker in feed digestibility studies of aquatic species (De Silva and Anderson, 1995). Recent studies have raised doubts about its suitability in salmonids and percids (e.g. Shiau and Liang, 1995; Austreng et al., 2000). However, there are only few concerns about the use of chromic oxide in cyprinid fish species (Hepher, 1988; Hertz et al., 1989; De Silva et al., 1997). Hence, and because the indirect method and the use of chromic oxide with an inclusion level of 0.5–1% is somehow the only standard in digestibility measurements worldwide (NRC, 1993), chromic oxide is recommended for further digestibility measurements in tench. Standardization of methods at the family or species level is essential in order to make literature comparable (Hepher, 1988; De Silva and Anderson, 1995).

With regard to tench, any faecal collection method that involves handling and disturbing the experimental fish is unsuitable because of their aforementioned sensitiveness (Fleig and Gottschalk, 2001). Therefore stripping (e.g. Vens-Cappell, 1985), metabolic chambers (Smith, 1971) or suction (Spyridakis et al., 1989) are not suitable methods unless large fish groups are available to compensate for reduced feed intake. Three other methods can be considered for tench: dissection out of the gut (e.g. Austreng, 1978), sedimentation ('guelph system' and adaptations after Cho and Slinger, 1979), and automatic faecal collection or sieving (Choubert et al., 1982; Vens-Cappell, 1985). If researchers are planning to use the faecal collection method used in the present investigation, the following adaptations are recommended: (a) experimental tench should have a body height > 2.5 cm, a size large enough to prevent them getting stuck in the water outlet and thus sieves on the outlets would be unnecessary; (b) experimental tench should be acclimatized to the rearing facility and the experimental diet for a period of several weeks or months; tench should not be handled long before or during the experimental period; (c) the feed intake of tench could probably be increased if the stocking densities were above 6 kg m^{-3} (compare Füllner and Pfeifer, 1998); (d) uneaten feed should not be used again; under conditions of low feed intake, no growth trials should be carried out; it seems crucial to use more waterproof food pellets to minimize crumbling of feed and faeces which tends to accelerate leaching (Vens-Cappell, 1985; De la Noüe and Choubert, 1986; Spyridakis et al., 1989); (e) filtered faeces should be frozen automatically in the collection pans (Choubert et al., 1982), or formalin should be added to the pans to prevent bacterial proliferation in the faeces (Spyridakis et al., 1989).

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