Influence of dietary composition on growth and energy reserves in tench (Tinca tinca)


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Summary

The effects of different protein, lipid and carbohydrate diets on growth and energy storage in tench, Tinca tinca L., were studied. Over a 2-month period fish were fed four different diets: control, protein-enriched, carbohydrate-enriched and lipid-enriched. The best growth rates were obtained with the control and protein-enriched diets; the carbohydrate diet produced the worst results (lowest specific growth rate, weight gain, nutritional index and hepatosomatic index). These results suggest that it is not advisable to reduce dietary fish protein below 35% and that it is not possible to obtain a protein-sparing effect of either lipids or carbohydrates, at least in our experimental conditions. The high-protein diet resulted in the storage of energy excess as muscle proteins and hepatic glycogen. Tench fed the high-carbohydrate diet stored carbohydrates as muscle glycogen and reduced plasma triglycerides. Finally, both liver and muscle lipid content were in positive correlation to dietary lipid.

Introduction

Tench (Tinca tinca L.) is a cyprinid species of particular interest in European pond fish farming (Pérez-Regadera 1997). However, manufactured feed development is restricted by the lack of knowledge on the feeding and nutrition requirements of this species; thus, a main objective in this study was to optimise the feed composition and feeding strategies of this species.

An important prerequisite for optimum nutrition and growth is an appropriate supply of dietary macronutrients. This optimal nutrient composition varies among fish species. Generally, fish appear to require 30–50% dietary protein to achieve maximum growth rates (Wilson and Halver 1986; Cowey 1992). Lipids also play an important role as dietary energy sources for both carnivorous and herbivorous fish (Takeuchi et al. 1978). In relation to carbohydrates (CH), herbivorous and omnivorous fish accept more than 25% CH in their diet, while carnivorous fish show an optimum below 20% (Wilson 1994).

Feed represents one of the major costs in fish farming, proteins being the most expensive component. A cost reduction can be obtained by replacing part of the protein energy with energy from lipids or carbohydrates. The protein-sparing effect of lipids has been established in fish (Takeuchi et al. 1978; De Silva et al. 1991; Bjornson et al. 1992; García-Gallego et al. 1993; Grisdale-Helland and Helland 1997). CH are a cheaper source of energy than proteins and lipids, but carnivorous fish have limitations in their absorption and metabolism of CH; an excessive intake can result in pathological conditions (Lall 1988; Roberts and Bullock 1989). Nevertheless, recent studies have indicated that high levels of CH are well tolerated by Salmo salar L. (Hemre et al. 1994), and a protein-sparing effect of CH in Oreochromis niloticus L. (Anderson et al. 1984) and Anguilla anguilla L. (Hidalgo et al. 1993; Sanz et al. 1993) has been shown.

It is generally accepted that the main energy reserves in fish are stored in the liver and muscle (Lie et al. 1988; Berge and Storebakken 1991; Hemre et al. 1992). CH are stored as glycogen in these tissues in all vertebrates so far studied. Moreover, fish store their surplus energy as lipids and proteins in muscle and the liver. However, the influence of diet composition on such energy storage in the tench is unknown. Taking this into account, the objective of the present study was to evaluate the effects in the tench of diets with different protein, lipid and carbohydrate contents on their growth rate and energy storage.

Materials and Methods

Fish and diets

Experiments were carried out on tench, T. tinca (L.) (5.26±0.24 g body weight) provided by the Ipescon fish farm (Salamanca, Spain). The tench were kept at the laboratory in glass aquaria (50–100 L), with walls painted black to reduce the stress during the acclimatization period. The fish were maintained in flowing and aerated tap water under 12 L:12 D photoperiod and 20 ± 2°C water temperature. All fish were fed Sera Biogran (Heinsberg, Germany) pellets at a daily ratio of 1% body weight (bw).

Experimental procedure

Following a 30-day period of adaptation to laboratory conditions, the tench were divided into four groups (n = 7–9/group) and fed with the respective experimental diet for 2 months, at a daily ratio of 2% bw. Four experimental diets were formulated (Table 1) varying in protein, lipid and carbohydrate content: control diet (C), and diets enriched with proteins (P), carbohydrates (CH) or lipids (L), respectively. All experiments were carried out in duplicate. In order to obtain individual data, each tench was marked with a visible implant (fluorescent elastomer tags, Northwest Marine Technology, ICN, Washington, USA) using a special biocompatible elastomer material containing fluorescent colouring which provided internal marks that were externally visible. The best place to locate and identify the tags was tested in a previous experiment whereby the tench were marked with yellow and red elastomers on different body locations: top of the head (n = 60), the base of both dorsal and pectoral fins, and between the caudal fin rays (n = 60). Retention of tags was monitored weekly for 6 weeks.

Fish were individually weighed once each week throughout the 2-month experimental period. Daily rations were adjusted...
Table 1
General composition of the experimental diets (g/100 g dry diet)

<table>
<thead>
<tr>
<th>Diet</th>
<th>C</th>
<th>P</th>
<th>CH</th>
<th>L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish meal</td>
<td>35.00</td>
<td>50.00</td>
<td>17.50</td>
<td>24.25</td>
</tr>
<tr>
<td>Casein</td>
<td>9.33</td>
<td>13.33</td>
<td>4.67</td>
<td>6.45</td>
</tr>
<tr>
<td>Fish oil</td>
<td>1.95</td>
<td>-</td>
<td>4.22</td>
<td>8.35</td>
</tr>
<tr>
<td>Sunflower oil</td>
<td>3.50</td>
<td>3.50</td>
<td>8.50</td>
<td>8.50</td>
</tr>
<tr>
<td>Dextrine</td>
<td>35.00</td>
<td>17.90</td>
<td>55.00</td>
<td>24.30</td>
</tr>
<tr>
<td>Betaine</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Vitamin mixture</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Mineral mixture</td>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Cellulose</td>
<td>5.22</td>
<td>5.27</td>
<td>5.11</td>
<td>18.15</td>
</tr>
<tr>
<td>Carboxymethylcellulose</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Total protein (%)†</td>
<td>35.00</td>
<td>50.00</td>
<td>17.50</td>
<td>24.23</td>
</tr>
<tr>
<td>Total fat (%)†</td>
<td>10.00</td>
<td>10.00</td>
<td>10.00</td>
<td>20.00</td>
</tr>
<tr>
<td>Total carbohydrate (%)†</td>
<td>35.00</td>
<td>17.90</td>
<td>55.00</td>
<td>24.23</td>
</tr>
<tr>
<td>Energy (MJ/100 g)*</td>
<td>1.68</td>
<td>1.68</td>
<td>1.68</td>
<td>1.68</td>
</tr>
</tbody>
</table>

C: control diet; P: protein-enriched diet; CH: carbohydrate-enriched diet; L: lipid-enriched diet.

* 1 g protein: 19.6 KJ; 1 g fat: 39.5 KJ; 1 g carbohydrate: 17.2 KJ.
†% according to formula and raw material composition.

Weekly on the basis of bw. At the end of the 2-month growth trial the fish were decapitated after 24 h food deprivation. Blood samples were collected using heparinized capillary tubes and centrifuged (6000 r.p.m. for 3 min) to obtain plasma samples. The livers and muscle were immediately removed and frozen on solid CO2. All plasma and tissue samples were stored at –80°C until biochemical analyses were performed.

Calculations
The weight increase (body weight gain) and specific growth rate (SGR) were determined. The SGR was calculated as a percentage of bw per day according to the formula: $\text{SGR} = \left( \frac{\ln W_f - \ln W_i}{t} \right) \times 100$, where $W_f$ is the weight of the fish at the end of experiment, $W_i$ is the weight at the start of the experiment, and $t$ is the duration of the growth period in days. Two biometric indices were also calculated: the nutritional index, $\text{NI} = \text{final body weight} \times 100 / [\text{length (cm)}]^2$, and the hepatosomatic index, $\text{HSI} = \text{liver weight} \times 100 / \text{body weight}$.

Biochemical analysis
Plasma glucose levels were determined by the glucose-oxidase method using a commercial kit (Peridochrom Glucose, Boehringer Mannheim, Barcelona, Spain). Plasma levels of triglycerides (TG) were quantified by the GOP-PAP method (Dubois et al. 1956) after extraction with ethanol and previous digestion with KOH (Cifonelli et al. 1956; Montgomery 1957). Hepatic and muscle glycogen were measured by spectrophotometry. Protein content in liver and muscle homogenates was determined by applying the method of Lowry et al. (1951) using bovine serum albumin as a standard. Total lipids from liver and muscle samples were extracted with chloroform/methanol (Folch et al. 1957) and determined by turbidimetry.

Statistics
A one-way ANOVA was performed to ascertain statistical differences between duplicate tanks exposed to the same treatment (diet). When no statistical differences were found, we considered both tanks per diet as two homogeneous populations, and the individual data were used to calculate the mean and SEM of the different groups. A Student’s $t$-test was performed to examine statistical differences between the control fish and the fish fed the protein-, carbohydrate- and lipid-enriched diets. A probability level of $P < 0.05$ was considered statistically significant.

Results
Retention of fin elastomer tags was 95% after 1 week and 83.3% after 6 weeks (Fig. 1). At the end of the experiment there was an 88.9% loss of tags placed in the head region, indicating that the elastomer fin tag locations are more reliable and probably better detectable, and therefore a useful marking system for T. tinca, at least for 6 weeks. Fish that lost their markings were retagged each week.

Weight gain, SGR and both NI and HSI obtained at the end of the 2-month experiment are shown in Table 2. Fish fed the carbohydrate-enriched diet showed significantly reduced weight gains, SGR, NI and HSI. No significant differences in these indices were observed when dietary proteins were increased. Weight gain and SGR were reduced ($P < 0.05$), compared to the control diet, when dietary lipids were increased.

Plasma glucose levels did not vary among tench fed different diet compositions (Fig. 2a). However, the carbohydrate-enriched diet significantly ($P < 0.001$) decreased plasma triglycerides levels. There were no significant differences in these values in the other diets (Fig. 2b).

The hepatic contents of glycogen, proteins and lipids after 2 months of feeding the different diets are shown in Fig. 3. Fish fed the high-protein diet contained more hepatic glycogen than did the other groups (Fig. 3a). The different diets caused no significant changes in liver proteins (Fig. 3b). An increase in liver lipid content was observed in the tench fed the lipid-enriched diet (Fig. 3c).

Figure 4 summarizes the results for muscle content of glycogen, proteins and lipids. Glycogen increased in the fish fed the protein-, lipid- and carbohydrate-enriched diets compared to the control diet; this increase was statistically significant for the
CH and L diets. The muscle protein content significantly (P < 0.05) increased in both the protein- and lipid-enriched diets. Finally, an increase in muscle lipids was found in the tench fed the lipid-enriched diet.

Discussion
In our study, the best growth rate was obtained in tench fed the control and protein-enriched diets; the carbohydrate diet produced the lowest SGR, body weight gain, NI and HSI. The negative results obtained with the CH-enriched diet might indicate that the tench did not tolerate large amounts of CH. In fact, the natural diet for tench contains little CH and it could be expected that this species would not be able to digest or metabolize large amounts thereof. This hypothesis is in accordance with results obtained for other carnivorous teleosts, which tolerate approximately 10% dietary starch, but they have significant problems in efficiently metabolizing higher starch amounts (Hemre et al. 1992, 1993). Similarly, when the dietary CH content exceeded 22%, feed utilization was reduced in S. salar (Hemre et al. 1995). The present tench growth results suggest that, in this species, reduction of dietary fish protein below 35% is not recommendable. On the other hand, an increase in dietary protein from 35 to 50% did not result in further improvements in tench growth. These results are in agreement with previous data for halibut (Hippoglossus hippoglossus L.), by Aksnes et al. (1996).

Table 2
Influence of diet composition on weight gain, SGR and biometrical indices in Tinca tinca (L.)

<table>
<thead>
<tr>
<th>Diets</th>
<th>C</th>
<th>High P</th>
<th>High CH</th>
<th>High L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final body weight gain (%)</td>
<td>12.97 ± 2.65</td>
<td>13.33 ± 2.28</td>
<td>-14.11 ± 1.56</td>
<td>1.64 ± 2.44†</td>
</tr>
<tr>
<td>Standard growth rate (% day⁻¹)</td>
<td>0.16 ± 0.05</td>
<td>0.19 ± 0.03</td>
<td>-0.23 ± 0.03†</td>
<td>0.02 ± 0.04*</td>
</tr>
<tr>
<td>Initial length (cm)</td>
<td>6.98 ± 0.22</td>
<td>6.42 ± 0.16</td>
<td>6.47 ± 0.16</td>
<td>6.59 ± 0.34</td>
</tr>
<tr>
<td>Final length (cm)</td>
<td>7.10 ± 0.22</td>
<td>6.58 ± 0.16</td>
<td>6.51 ± 0.16</td>
<td>6.76 ± 0.33</td>
</tr>
<tr>
<td>Nutritional index (g cm⁻³)</td>
<td>18.64 ± 0.99</td>
<td>18.60 ± 0.21</td>
<td>15.26 ± 0.34†</td>
<td>16.57 ± 0.62</td>
</tr>
<tr>
<td>Hepatosomatic index (%)</td>
<td>1.98 ± 0.18</td>
<td>1.67 ± 0.07</td>
<td>1.48 ± 0.09†</td>
<td>1.63 ± 0.15</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SEM. *P < 0.05; †P < 0.01; ‡P < 0.005 compared to control group.
C, control (n = 15); P, protein (n = 18); CH, carbohydrate (n = 19); L, lipid (n = 7).
The storage of energy as muscle protein in tench fed an enriched protein diet is in accord with the maintenance of high-protein deposition, one of the roles of the muscle. Thus, it was shown that protein-synthesis rates in the muscle of cod (Gadus morhua L.) fed with different protein level diets increased when the metabolizable energy in the form of protein was increased from 10 to 48% (Lied and Braaten 1984). Peragon et al. (1994) reported that an enhancement of dietary protein caused a significant increase in both protein-accumulation and protein-synthesis rates in the muscle of the rainbow trout.

Despite a poor utilization of dietary CH, fish require glucose as the main fuel for certain tissues, such as brain and active gonads. Gluconeogenesis from amino acids is an important metabolic pathway in salmonids (Walton and Cowey 1982), representing a certain waste of dietary protein. This could explain the increase in hepatic glycogen observed in tench fed with a high-protein diet in the present study, probably due to the activation of gluconeogenesis from amino acids, as suggested by Hemre et al. (1993) in G. morhua.

The tench fed with the high-carbohydrate diet stored CH as muscle glycogen, without correlation between dietary CH and hepatic glycogen. Similarly, no correlation between dietary starch and liver glycogen was found in Atlantic salmon, S. salar (Hemre et al. 1996). In this species the liver was not the main target organ for plasma glucose, and muscle could reserve more glucose than the liver, as reported by García-Riera and

![Fig. 3. Hepatic content of (a) glycogen, (b) proteins, and (c) lipids in tench fed diets of different composition: C, control diet (n = 8); P, protein-enriched diet (n = 8); CH, carbohydrate-enriched diet (n = 8) and L, lipid-enriched diet (n = 7). Data refer to samples taken at the end of the experiment and are expressed as mean ± SEM. **P < 0.01, ***P < 0.001 compared to control diet.](image1)

![Fig. 4. Muscle content of (a) glycogen, (b) proteins, and (c) lipids in tench fed diets of different composition: C, control diet (n = 8); P, protein-enriched diet (n = 8); CH, carbohydrate-enriched diet (n = 8) and L, lipid-enriched diet (n = 7). Data refer to samples taken at the end of the experiment and are expressed as mean ± SEM. *P < 0.05 compared to control diet.](image2)
Hemre (1996). On the other hand, it was reported extensively that feeding (Walton and Cowey 1982) or dietary CH (Hemre et al. 1996) increased plasma glucose levels. However, it was found that the plasma glucose response after feeding or a glucose load showed an initial increase but returned to basal levels within 24 h (García-Riera and Hemre 1996). Thus, the lack of hyperglycemia in tench fed a high CH diet could be explained by the previous 24 fasting carried out to obtain plasma samples.

Earlier studies have shown that glucose stimulates triglyceride hydrolysis in fish, probably mediated by glucagon (Harmon and Sheridan 1992). A similar mechanism could be functioning in tench since there was a significant drop of plasma triglyceride levels when they were fed a high CH diet. Accordingly, recent studies on glucose tolerance in turbot, Scophthalmus maximus L., demonstrated that a glucose load decreased plasma TG levels (García-Riera and Hemre 1996).

The increase in lipid storage found in the liver and muscle of the tench fed with high dietary lipid is in accord with previous reports (García-Gallego et al. 1993; Aksnes et al. 1996; Koskela et al. 1998). The interdependence between dietary lipids levels and storage of surplus energy as lipid depots has been corroborated in different species and tissues.

Given the present results, two principal conclusions can be drawn. On the one hand, because of the negative results in the tench, at least under our experimental conditions, reduction of dietary fish protein below 35% is not recommended. On the other hand, neither lipid nor CH had a pronounced protein-sparing effect. Finally, dietary composition plays an important role in the regulation of protein, carbohydrate and lipid reserves in tench, as is the case with other fish.

Acknowledgements

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References


Bjornson, B.; Sigurthorsson, G.; Hemre, G.-I.; Lie, O., 1992: Haematological values and storage of surplus energy as lipid depots has been corroborated in different species and tissues. The increase in lipid storage found in the liver and muscle of the tench fed with high dietary lipid is in accord with previous reports (García-Gallego et al. 1993; Aksnes et al. 1996; Koskela et al. 1998). The interdependence between dietary lipids levels and storage of surplus energy as lipid depots has been corroborated in different species and tissues.

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