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The efficacy of different size of X-ray dense markers for determination of sequential feeding rates in rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792)

Gökkuşağı alabalıklarında (*Oncorhynchus mykiss* Walbaum, 1792) aralıklı olarak tüketilen yem miktarlarının tespitinde farklı büyüklüklere sahip X-ışınını yansıtan belirleyicilerin kullanılabilirliği

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Abstract

30 adult rainbow trout (*O. mykiss*) (202.1 ± 3.5 g SEM) in a fresh water recirculation system were fed the first test diet containing small radio-opaque glass beads 'ballotini' (0.6-0.9 mm) until all fish were satiated. The fish were re-fed at different time intervals (t= 0, 4, 8, 12, 24, 30 and 36 hours) with the second test diet containing large size of glass beads (1.16-1.40 mm) and X-rayed. The amount of feed consumed by fish for each time interval was calculated following the quantification of the number of different size of glass beads in the developed X-ray sheets and the appetite return of trout was modelled. A first order equation best explained the appetite revival data (Feed Intake = $106.26^{*} (1-e^{-0.079^{*}time})$). The times for 50 and 95 % of return of appetite in rainbow trout were observed as 8 and 28 hours, respectively. It was suggested that incorporation of markers of different sizes into various types of feed allows the amounts of each type of feed presented either simultaneously or as discrete meals offered within a 12 –hour-period.

Keywords: X- radiography, different size of markers, feed intake, rainbow trout

Introduction

The efficacy of X-Radiography in order to quantify individual feed intake has previously been reported (Ross & Jauncey, 1981; Talbot & Higgins, 1983; Grove, 1986; Jorgensen & Jobling, 1989; Jobling *et al.*, 1993; McCarthy *et al.*, 1993, Carter *et al.*, 1995). The use of X- radiography for the return of appetite determinations in rainbow trout was recently demonstrated by Tekinay (2001). However, during the feed intake measurements conducted by this author, only one size of X-ray dense marker (0.65-0.90 mm) was employed and the first consecutive meal eaten by fish could not be exactly quantified since it did not contain any marker. Incorporation of markers of different sizes into different types of feed permits the amounts of each type consumed to be measured when feed are presented either simultaneously or as discrete meals offered (Thorpe *et al.*, 1990; Toften *et al.*, 1995; Tekinay, 2001). This enables studies of feed preferences to be conducted using the X-Radiography. In another technique, Johnston *et al.* (1994) suggested different coloured feeds as a method to differentiate sequential feeding rate. They claimed that it would not be possible to distinguish different meals using X-Radiography. Therefore, this study was designed in order to evaluate the possibility of using two different sizes of X-ray dense marker for assessment of the first meal intake as well as the second one. Inclusion level of marker may affect the chemical compositions of diets, however, no appetite suppression was observed in fish fed the test diet containing 3.8% of ballotini (Tekinay, 1999, 2001). Another objective of the present study is also to comprehend the possible effect of a higher level of marker (9.3%) on feed acceptability of rainbow trout.

Materials and Methods

Experimental Fish and Maintenance Facilities

Rainbow trout, *Oncorhynchus mykiss* (mean weight; 202.1 ± 3.5 g SEM) were supplied from a local fish farm (Mill Leat Trout Farm, Ermington, Devon, UK) and acclimatized to aquarium conditions for 3 weeks prior to the experiment in the aquarium facility of University of Plymouth which was previously described by Tekinay (2001).

Test Diets

Formulation and chemical composition of the test diets were presented in Table 1. Chemical compositions of the diet were determined according to AOAC (1990). Test diets which had specific sizes and quantities of X-ray dense marker (3.8 or 9.3% of the diet) were prepared. The numbers of ballotini in known weights of diets were determined by X-radiography to ensure even distribution. The relationship between the weight of feed (FW) and the number of beads (N) was linear:

Diet 1: $FW= 0.0221N$, $R^2 = 0.95$, n= 20 .... (1)

Diet 2: $FW= 0.0420N$, $R^2 = 0.98$, n= 20 .... (2)

Table 1. Diet Formulation (% dry matter) and chemical composition of experimental diets.

Ingredient	*Diet 1*	*Diet 2*
LT Fish Meal[a]	47.6	45.6
Poultry Meat Meal[b]	4.9	4.7
Blood Meal[c]	2.9	2.8
Dextrin[d]	29.2	27.9
Fish Oil[e]	6.8	6.5
Vitamin/Mineral Premix[f]	2.4	2.3
Small Ballotini[g]	3.8	--
Large Ballotini[h]	---	9.3
Binder (CMC)[k]	2.4	0.9
Crude Protein (% Dry Matter)	42.2	40.4
C. Lipid (% DM)	11.1	10.6
C. Ash (% DM)	9.0	8.6
C. Carbohydrate (% DM)	29.7	28.5

a. Low Temp. Fish Meal, Norsea Mink, LT 94. Donated by Trouw Aquaculture, Wincham, Cheshire, U.K.
b. Int. Feed Number, 5-03-798, Trouw Aqua., Wincham, Cheshire, U.K.
c. Int. Feed Number, 5-00-381, " " " " "
d. Roquette Freres, Lestrem, France.
e. Atlantic Herring Oil (7-08-048), Seven Seas, Marfleet, Hull, U.K.
f. (Close Formulation). Trouw Aqua., Wincham, Cheshire, U.K.
g. Jensons Ltd. U.K. (Size 0.65-0.90 mm)
h. " " " (Size 1.16-1.40 mm)
k. Carboxymethyl Cellulose (Sigma Chemical Company, UK.)

Return of Appetite Studies and Modelling
The X-radiographic method applied for the return of appetite measurements and modelling of the experimental data were as described by Tekinay (2001). A typical X-Radiographic illustration of a rainbow trout fed two test diets containing different sizes of glass beads was demonstrated in Plate 1.

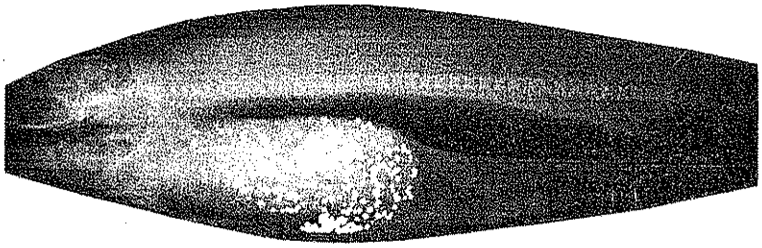

Plate 1. A picture of a trout fed the diet containing large glass beads 24 hours after the first meal containing small glass beads.

Results and Discussion

X-Radiography has successfully been utilised by Tekinay (2001) in order to monitor of feed intake in individual trout held in a 30-fish group. Since only second meal intake has been quantified individually in that study, the first meal could not be estimated. However, the present study demonstrated that first meal intake could also be quantified within a 12-hour period using different sizes of glass beads (0.65-0.90 mm and 1.16-1.40 mm). After 12 hours of feeding, the particulate marker started moving along the gut and defaecated, thus it might cause underestimations of initial feed intake.

First order and sigmoid equations were employed for the return of appetite modelling. Although both models fitted well, a first order equation resulted in a marginal better fit due to the lower residuals mean sum of squares (Figure 1). The instantaneous rate of appetite revival was supported by 89.0% relationship with time. According to the fitted model, feed intake of fish at time: 0 was chosen as 0%.

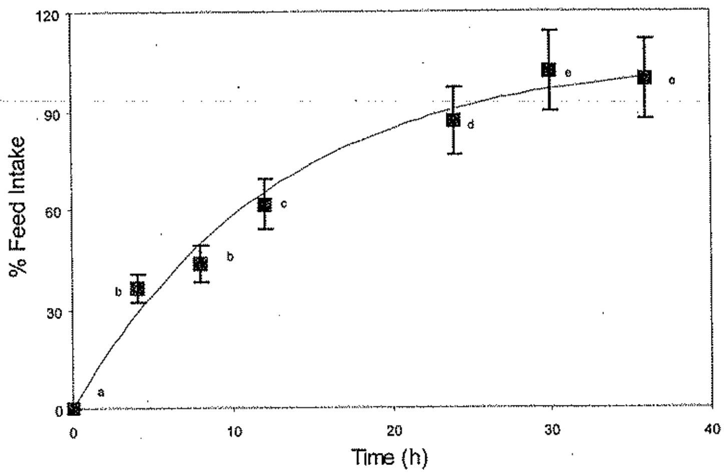

Figure 1. Rates of return of appetite rates in trout. Non-linear regression model for return of appetite (First Order), FI = 106.26* $(1\text{-}e^{-0.079*t})$, $R^2 = 0.89$ Where, 'FI' represents percentage feed intake or appetite return at time 't', n = 20. Data points in each graph allocated different letters are significantly different ($P < 0.05$). Bars denote ± 5 standard error of the mean.

A significant increase in feed intake ($P<0.05$) was evident 4 hours after the first alimentation. Return of appetite rate between the time: 4 and time: 8 and between the time: 30 and time: 36 were not observed to be significantly different ($P>0.05$). On the other hand, feed consumption of trout at times: 12 and 24 elevated significantly ($P<0.05$). The times for 50 and 95% of appetite revival in rainbow trout were predicted 8 and 28 hours, respectively.

The studies of Tekinay (1999) and (2001) did not report any alteration of palatability when the test diet consisted of 3.8% of marker. Similarly, although the second diet was diluted with 9.3 % of ballotini, no difference in feed acceptability was observed in the present study.

Johnston *et al.* (1994) used different coloured feeds as a method to differentiate sequential feeding rate. However, sacrificing of fish to recover the gastric content is both costly and prevents repeated measurements of feed intake on given individuals as critisized by Jobling *et al.* (1995). Besides, the incorporation of dyestuffs that leads to the production of feed having different colours may effect acceptability. This could cause an erroneous estimation of actual intake when the colour of the feed is changed suddenly prior to a feed intake determination (Jobling *et al.*, 1995).

Routine determination of feed intake undoubtly provides the information to study a wide range of biotic and abiotic interactions on energy partitioning of fish (Jobling *et al.*, 1995). The estimation of daily feed intake by fish of different sizes at different abiotic conditions (e.g. water temperature) provides the information required for the arrangement of feed tables and such information can be derived easily using the X-radiography. However, there might be large day-to-day variations in feed intake of fish due to handling and various stressors. Therefore the information from single meal experiments would not give reliable results, hence repeated shots should be performed for a number of fish if it is aimed to design appropriate feed tables for farmed fish species. Furthermore, adequate preliminary experiment should be carried out to validate the method against possible limitations resulting in underestimation or overestimation of feed consumption in given fish species held under defined conditions. Such problems were avoided in this investigation by conducting a preliminary experiment to consolidate the method.

One of the disadvantadges of X-radiography is that it can not be used for the continuous monitoring of feed intake. Computer operated on-demand feeding systems may be suggested to monitor each release of feed as a discrete event under conditions where feed is continuously available to the fish. Thus, these systems enable long-term automatic recording of data without disturbance to the fish (Jobling *et al.*, 1993). However, it is not possible to prove how many or which fish operated the system and consequently records do not give satisfactory information about how much feed has actually been fed by aech individual. Therefore, Jobling *et al.* (1995) suggested that the 'labelled feed' and 'on-demand' feeding techniques should be used as being complementary to each other, providing different kinds of data for the study of feeding habits of fish.

Özet

Bir kapalı devre tatlı su sisteminde, 30 tane yetişkin gökkuşağı alabalığı, (*O. mykiss*) (202.1 ± 3.5 gr) içinde küçük X-ışını yansıtan cam bilye (ballotini) (0.6-0.9 mm çapında) bulunan ilk test yemiyle doyuncaya kadar beslenmişlerdir. Yemlemeyi takiben farklı zaman aralıklarında (z= 0, 4, 8, 12, 24, 30 ve 36 saat) içinde büyük cam bilye (1.16-1.14 mm) içeren ikinci test yemiyle beslenerek röntgenleri çekilmiştir. Her zaman aralığında balıklar tarafından tüketilen yem miktarları röntgen filmlerinde belirlenen cam bilyelerin sayılması ile hesaplanmış ve alabalıkların iştaha dönüş oranları modellenmiştir. Yem tüketim verileri en uygun şekilde bir birinci mertebe (first order) eşitlik tarafından açıklanmıştır (Yem Tüketimi = 106.26* $(1-e^{-0.079*zaman})$). Bu modele göre, alabalıkların % 50 iştaha dönüş süresi 8 saat, % 95 iştaha dönüş süresi ise 28 saat olarak tahmin edilmiştir. Değişik kaliteye sahip yemlere farklı büyüklüklerde cam bilye ilave etmek suretiyle 12 saatlik bir periyot içinde, söz konusu yemlerin aynı anda veya aralıklı şekilde tüketildikleri miktarların saptanabileceği savunulmuştur.

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