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The use of X-Radiography for the return of appetite measurements in rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792)

Gökkuşağı alabalıklarının (*Oncorhynchus mykiss* Walbaum, 1792) iştaha dönüş sürelerinin tespitinde X-Radiyografi tekniğinin kullanılabilirliği

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Abstract

Quantification of return of appetite in fish is considerably important with respect to the estimation of optimum feeding protocole. Therefore, 30 adult female rainbow trout (*O. mykiss*) (205.6 ± 2.2 g) in a fresh water recirculation system were fed the control diet containing 42.6% of digestible protein and 19.7 MJ kg^{-1} of digestible energy until all fish were satiated. The fish were re-fed at different time intervals (t= 0, 4, 8, 12, 24 and 48 hours) with the test diet containing radio-opaque glass beads 'ballotini' (0.6-0.9 mm) and X-rayed. The amount of feed consumed by fish for each time interval was calculated following the quantification of the number of glass beads in the developed X-ray sheets and the appetite revival of trout was modelled. According to the results, the appetite revival data was best explained by a sigmoid model since the model gave a higher correlation coefficient with a lower residual mean square. The times for 50 and 95 % of return of appetite in rainbow trout were observed as 17 and 43 hours, respectively. It was suggested that the X-radiography technique for the sequential feed intake measurements could be used to understand feeding behaviour of cultured fish species in order to obtain maximum growth performance and feed efficiency.

Keywords: Rainbow trout, return of appetite, X- radiography

Introduction

X-radiography has been used in a wide area in order to obtain scientific information on feeding behaviour, digestion physiology and mechanism of
fish (Grove, 1986; McCarthy *et al.*, 1993; Charter *et al.*, 1995; Sims *et al.*, 1996). Especially, it has significant applications in studies in which quantitative information is required about the feed intake of individual fish. This technique can be employed for the quantitative determination of gastrointestinal content of fish under different environmental conditions (Jobling *et al.*, 1995). However, there are different opinions on this technique for the determination of stomach evacuation. For instance, Dos Santos and Jobling (1988), Jørgensen and Jobling (1989), Jobling *et al.* (1993) and Tekinay (1999b) reported that the information derived from X-ray technique was not reliable since the radio opaque glass beads did not move with the digesta and retained in the cardiac stomach of the fish, collectively. On the other hand, the same method has been used widely for the determination of feed in the stomach. Therefore, this study was conducted to evaluate the feed intake of rainbow trout individually at different time intervals and to model the return of appetite pattern by using X-radiography tecnique.

Materials and Methods

Experimental Fish and Maintenance Facilities
Rainbow trout, *Oncorhynchus mykiss* (mean weight; 205.6 ± 2.2 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 University of Plymouth. Throughout the study, the fish were maintained in 400 l, fiberglass tanks within a closed, fresh water recirculation system with a parallel flow of 6.8 l through the tanks per minute at a temperature of 15 ± 0.2 ° C. Approximately 20% of the system water was changed weekly to ensure that the physico-chemical conditions were at the optimum level. Photoperiod was set as 12 h light/12 h dark (8:00 am: 8:00 pm) using fluorescent discharge lamps (480 lux). During the acclimation period, the trout did not exhibit any unusual behaviour and continued to feed normally on the test diet.

Test Diet

Formulation and chemical composition of the test diet was as used by Tekinay (1999b) and presented in Table 1. Chemical compositions of the diet were determined according to AOAC (1990). The test diet which had a specific size and quantity of X-ray dense marker (3.8% of the diet) were prepared. The numbers of ballotini in known weights of diet were determined by X-radiography to ensure even distribution as outlined by McCarthy *et al.* (1993). The relationship between the weight of feed (FW) and the number of beads (N) was linear:

$FW = 0.0255N$, $R^2 = 0.950$, $n = 20$ .... (1)

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

Ingredient	*Control Diet*	*Test Diet*
LT Fish Meal[a]	52.6	51.3
Poultry Meat Meal[b]	12.0	11.7
Blood Meal[c]	3.0	2.9
Extruded Wheat Meal[d]	15.3	14.9
Fish Oil[e]	10.8	10.6
Vitamin/Mineral Premix[f]	2.0	2.0
∝-cellulose[g]	1.9	1.9
Cr_2O_3[g]	0.4	
Ballotini	-	3.8
Binder[g, h]	2.0	0.9
C. Protein (% DM)	48.7	47.6
C. Lipid (% DM)	20.5	20.0
C. Ash (% DM)	10.4	10.1
C. Carbohydrate (% DM)	13.2	12.9

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. Int. Feed Number, 4-05-205, " " " " "
e. Atlantic Herring Oil (7-08-048), Seven Seas, Marfleet, Hull, U.K.
f. (Close Formulation). Trouw Aqua., Wincham, Cheshire, U.K.
g. Sigma Chemical Company, Poole, Dorset, U.K.
h. Carboxymethyl Cellulose

Return of Appetite Determinations

A protocol was designed to allow each diet to be assayed at set time intervals (t = 0, 4, 8, 12, 24, 48 h) so that no fish was X-rayed more than once in a 120 h period. Following a 72 h starvation period, the fish were fed diets without an X-ray dense marker and then fed a second meal with the X-ray opaque beads (0.6-0.9 mm) after a set time interval until all fish reached satiation. The level of re-alimentation at the specified time interval was equal to the extent of appetite return (Elliott, 1975). Subsequently, all fish were weighed and X-rayed using a portable Phillips Practix X-ray unit with light beam diaphragm attachment following anaesthetisation by ethyl p-amino benzoate (Benzocaine, Sigma; 1 g dissolved in 100 ml of ethanol, this added to fresh water at a concentration of 5 ml l^{-1}). The technique employed was that of Tekinay (1999a). The recovered group of fish were then maintained on the same diet for 2 days and deprived of food for three days before beginning further appetite revival measurements. During the X-radiographic studies, no fish vomited or died. The X-radiographic pictures (Plate 1) of rainbow trout were viewed on a light table (PLH Scientific Ltd, UK) and glass beads were counted. Weight of feed consumed by each fish was calculated using the above formulae and expressed in weight specific terms. Feed intake of fish for each set time interval was expressed as a percentage of the mean feed intake of fish at time = 0.

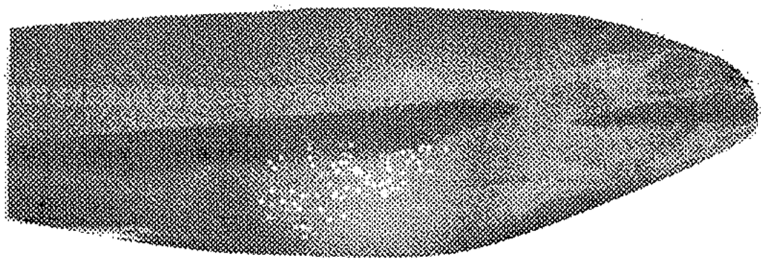

Plate 1. A typical X-Radiographic picture of a trout fed the diet containing glass beads 12 hours after the first meal.

Modelling of Return of Appetite

The appetite return determination was performed using either a sigmoid or a first-order relationship as used by Tekinay (1999a).

$$FI = 1/(a+b*e^{-k*t}) \quad \text{.......(2)}$$

$$FI = a*(1-e^{-k*t}) \quad \text{.......(3)}$$

where, 'FI' represents the return of appetite or feed intake at time 't'. 'a', 'b' and 'k' are fitted parameters, with 'a' being the Y-intercept (asymptote to appetite return at t = 0) and 'k' the rate constant of appetite return. 'a' and 'b' are the asymptotes to appetite return and 'k' is the rate constant of appetite revival at the given time 't' for the regressions. Each model was fitted to the appetite return data using least square regression (Marquardt, 1963). The goodness of fit of the two models was compared by noting the magnitude of the residual mean square (RMS) produced by each model and by comparison of the resultant r^2 values (Zar, 1996).

Results

In this study, a sigmoid relationship (Fig. 1) was preferred for the return of appetite modelling since the model gave a higher correlation coefficient with a lower residual mean square compared to first order model. The linear relationship would not apply because fish continued to eat after reaching 100% of appetite. The instantaneous rate of appetite revival was supported by 76.0% relationship with time. According to the model, feed intake of fish at time: 0 was chosen as 0%. Return of appetite rates at times: 4, 8 and 12 were not observed to be significantly different ($P>0.05$).

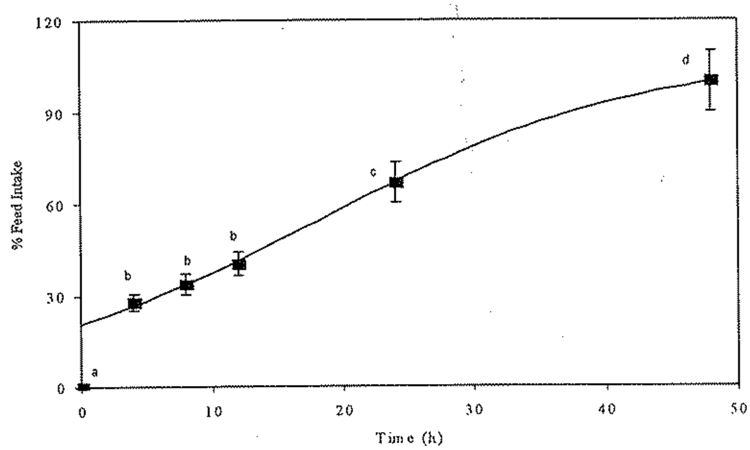

Fig.1. Rates of return of appetite rates in trout. Regression equation for return of appetite, $FI = 1/\ 0.0092 + 0.039^{*}\ e^{-0.08^{*}t}$, $R^2 = 0.76$, where FI represents percentage feed intake or appetite return at time t, n = 30. Data points allocated different letters are significantly different from each other ($p< 0.05$).

On the other hand, feed intakes of trout at times: 24 and 48 were significantly higher compared to those of times 4, 8, and 12 ($P<0.05$). Considerable difference was also observed in return of appetite rates of fish between the times 24 and 48 ($P<0.05$). The times for 50 and 95% of return of appetite in rainbow trout were estimated as 17 and 43 hours, respectively.

Discussion

Nutritional studies are basically carried out in order to determine the effects of the amount or the quality of the diet on growth parameters. Feeding tanks of fish and measuring the growth rates of separately fed groups of fish with different feeds is the conventional method to achieve feeding experiments. Another technique is to quantify the food intake of the individual fish and to model from the data an individual fish's food consumption-growth rate relationship (Carter *et al.*, 1995). In some species of fish which can be held individually, e.g. cod, *Gadus morhua*, there is not a problem in measuring food intake and growth rate relationship (McCarthy *et al.*, 1993). However, in the group feeding of experimental fish, a major problem has been to advance a reliable method to make repeated measurements of feed intake individually. Results of the present investigation demonstrated that X-radiography method for the determination of return of appetite in rainbow trout relating to feed intake physiology have relevance to aquafeed formulation and fish nutrition.

There may be large variations in feed intake by individual fish as indicated by Jobling *et al.* (1993), however, this problem was avoided in this study by using 30 fish and X-raying the same group of fish no sooner than 120 h.

X-radiography is also a valuable method in the study of social relationships within groups of fish (Jobling *et al.*, 1995). Besides, same technique has been used by Arnesen *et al.* (1993) (cited in Jobling *et al.*, 1993) to study changes in feed intake of Arctic charr (*Salvelinus alpinus* Lin. 1758) following sudden transfer from fresh water to water with salinities ranging from 10 to 35 ppt. Jobling *et al.* (1993) also described a method to investigate the effects of different levels of exercise on feed-growth relationships. In addition to such studies and return of appetite measurements, the use of X-Radiography technique could be widened in order to quantify sequential meals which have a likely role in consecutive appetite regulation. Also choice feeding practices can be established towards better understanding of the response of fish to diets differing in quality (Jobling *et al.*, 1995) Such a basis for allowing feed selection is quite commonly employed for terrestrial domestic animal production (Grove *et al.*, 1978; Bromley, 1994; Tekinay, 1999a).

In conclusion, X-radiography is a non-invasive technique and permits repeated sampling of individual and groups of fish. It is also likely to have application in studies to determine the effects of different biotic and abiotic factors on feed-growth relationships and energy partitioning (Jobling *et al.*, 1995). As a result of an integration of physiological / biochemical information and practical considerations, fish nutritionists would be able to regulate feed intake of fish to obtain maximum growth rate whilst reducing the feed waste under production condition.

Özet

Balıklarda iştaha dönüş süresinin saptanması, optimum yemleme protokolünün belirlenmesi bakımından oldukça önemlidir. Bu amaçla 30 tane yetişkin dişi gökkuşağı alabalığı, (*O. mykiss*) (205.6 ± 2.2 gr) kapalı devre tatlı su sisteminde % 42.6 sindirilebilir protein ve 19.7 MJ kg^{-1} sindirilebilir enerji içeren yemle doyuncaya kadar yemlenmiş, yemlemeyi takiben farklı zaman aralıklarında (z= 0, 4, 8, 12, 24 ve 48 saat) tekrar içinde X-ışını yansıtan cam madde (ballotini) (0.6-0.9 mm çapında) bulunan test yemiyle beslenerek röntgenleri çekilmiş ve yedikleri yem miktarları belirlenmiştir. Farklı zaman aralıklarında balıkların tükettikleri yemler dikkate alınarak gökkuşağı alabalığının iştaha dönüş süresi modellendirilmiştir. Sonuçlara göre, iştaha dönüş verileri en yüksek korelasyon katsayısı ve en düşük kareler ortalaması dikkate alınarak en uygun şekilde bir sigmoid model tarafından açıklanmıştır. Söz konusu özelliklere sahip yem ile beslenen gökkuşağı alabalıklarında % 50 iştaha dönme süresi 17 saat, % 95 iştaha dönme süresi ise 43 saat olarak tahmin edilmiştir. Röntgen tekniğinin, yetiştiriciliği yapılan balık türlerinde maksimum büyüme performans ve yem değerlendirme oranlarını sağlamak için gereksinim duyulan beslenme davranışlarını anlamada kullanılabileceği savunulmuştur.

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