Influence of Dietary Energy Level on Stomach Emptying and Appetite Revival Rates in Rainbow Trout, *Oncorhynchus mykiss*

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Abstract: D1, D2 and D3 diets containing 15.5, 12.5 and 9.0 MJ kg^{-1} digestible energy concentrations were offered to rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792), and the rates of gastric evacuation and feed intake were determined. Gastric evacuation determinations were achieved by slaughtering eight fish every 6 h until no residue was found in the cardiac stomach. Return of appetite experiments were conducted by re-feeding groups of trout every 6 h following the first feeding. The data provided from each process was modelled by regression analysis and compared statistically.

Square root equations best explained the gastric evacuation data, whereas first-order models were used for the description of return of appetite. The gastric emptying slope of D1 was found to be different (P < 0.05) to the other two treatments, whilst no significance (P > 0.05) was apparent between the slopes of D2 and D3. Similarly, the return of appetite slope of D1 was significantly different (P < 0.05) to the slopes of D2 and D3, although no noticeable difference (P > 0.05) was evident between the return of appetite slopes of D2 and D3. A close relationship between appetite revival and gastric evacuation rates in rainbow trout was established.

Key Words: Feed intake, low energy diets, gastric evacuation, return of appetite, rainbow trout, *Oncorhynchus mykiss*

Yem Enerjisi Düzeyinin Gökkuşağı Alabalıklarının (*Oncorhynchus mykiss*) Mide Boşaltım ve İştaha Dönüş Oranları Üzerine Etkileri

Özet: Sırasıyla 15,5, 12,5 ve 9,0 MJ kg^{-1} sindirilebilir enerji içeren D1, D2 ve D3 yemleri ile beslenen Gökkuşağı alabalıklarında (*Oncorhynchus mykiss* Walbaum, 1792), mide boşaltım ve yem alma isteğine dönüş oranları hesaplanmıştır. Mide boşalım süresi, yemlemenin bitimini izleyen (t = 0 saat) midenin tamamen boşalıncaya kadar her 6 saatlik zaman aralığında tespit edilmiştir. Bu amaçla 8 balık öldürülerek mide içerikleri analiz edilmiştir. Yem alma isteğine dönüş süresi de, gruplar halinde bulunan alabalıkların son yemlemeyi izleyen her 6 saatte tekrar yemlenmesiyle ölçülmüştür. Her iki denemenin verileri, regresyon analizi yardımıyla modellendirilerek istatistiki olarak karşılaştırılmıştır.

Her üç yemle beslenen alabalıkların mide boşaltım oranlarını en iyi şekilde üç karakök eşitliği açıklamıştır. D2 ve D3 grubu balıkların midelerini D1 grubuna göre daha hızlı (P < 0,05) boşalttığı saptanmış olup, D2 ve D3 ile yemlenen balıkların mide boşaltım oranlarında önemli bir farklılık gözlenmemiştir (P > 0,05). İştaha dönüş verileri de üç adet birinci aşama eşitlik ile tanımlanmış; yine D2 ve D3'le yemlenen balıkların iştaha dönüş oranlarının D1 ile yemlenenlerden farklı (P < 0,05) oldukları saptanmıştır. D2 ve D3 ile yemlenen balıkların iştaha dönüş oranlarında ise önemli bir farklılık saptanmamıştır (P > 0,05). Diğer taraftan bütün grupların mide boşaltım ve iştaha dönüş oranları arasında çok yakın bir ilişki belirlenmiştir.

Anahtar Sözcükler: Yem tüketimi, düşük enerjili yemler, mide boşaltım oranı, yem alma isteğine dönüş, Gökkuşağı alabalığı, *Oncorhynchus mykiss*

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Introduction

Gastric evacuation rates in mammals are strongly influenced by the energy and nutritive density of their diet (1,2). In fish, however, meal volume and dietary composition (e.g. protein, lipid, energy levels) are considered to be the most important factors affecting rates of both gastric emptying and appetite revival (3).

The influence of dietary protein and energy concentration on these parameters has recently been studied by Tekinay and Güner (4), who observed similar (P > 0.05) rates of gastric evacuation in rainbow trout (*Oncorhynchus mykiss*) fed diets containing different levels of digestible energy (18.8, 20.3 and 21.3 MJ kg^{-1}). They also reported no significant difference (P > 0.05) in the rates of appetite revival in fish fed these diets. In addition, Tekinay and Davies (5) assessed the effects of dietary carbohydrate level as a dietary filler on stomach evacuation and return of appetite in trout fed test diets including different levels of carbohydrate and digestible energy (16.1, 17.3 and 20.2 MJ kg^{-1}). A higher (P < 0.05) relative instantaneous rate of gastric evacuation occurred in trout after consumption of diets with 16.1 and 17.3 MJ kg^{-1} digestible energy compared to those fed a diet with 20.2 MJ kg^{-1} digestible energy. This was explained by the general view that rainbow trout increase their feed intake in order to obtain sufficient nutrient, energy and consequently maximum growth potential when the energy concentration of a diet is diluted (5-10). This is achieved by increasing the stomach emptying rate. Similar results were reported in goldfish, *Carassius carassius* (11), turbot, *Scapthalmus maximus* (12) and plaice, *Pleuronectes platessa* (13). Despite a number of studies, it has not clearly been demonstrated at what dilution rates of dietary energy levels trout empty their stomachs, significantly (P < 0.05).

This investigation was therefore carried out by formulating three diets containing 9.0, 12.5 and 15.5 MJ kg^{-1} DE (digestible energy) levels, and feeding trout in order to evaluate the dilution effect of DE on gastric evacuation and appetite revival under laboratory conditions.

Materials and Methods

Seventy-two rainbow trout, (*Oncorhynchus mykiss*), (mean weight 195.6 ± 12.6 g SEM) for the return of appetite analysis and 180 trout (186.2 ± 15.1 g SEM) for the subsequent gastric evacuation determinations

were supplied from a local fish farm (Mill Leat Trout Farm, Ermington, Devon, UK) and placed into duplicate 400 L, fibreglass tanks within a closed, fresh water recirculation system. There was a parallel flow through the tanks of 6.8 L per minute and temperature was maintained at 15 ± 0.2 °C. Approximately 20% of the water was changed weekly. The photoperiod was set to 12 h light, 12 h dark (08^{00}-20^{00}). The light phase illumination was at the water surface, at 480 lux.

The formulation and chemical composition of the experimental diets are presented in Table 1. The manufacturing technique of the experimental diets was explained by Tekinay (14).

Table 1. Dietary formulation and chemical composition of the test diets.

Ingredients	Diet 1	Diet 2	Diet 3
LT Fish Meal[1]	40.0	35.0	28.5
Blood Meal[2]	3.0	3.0	3.0
Poultry Meat Meal[3]	8.3	8.3	8.3
Extruded Wheat Meal[4]	15.7	4.7	-
Fish Oil[5]	11.2	10.4	9.0
Vitamin/Mineral Premix[6]	2.5	2.5	2.5
α- Cellulose[7]	18.1	35.0	47.5
Binder[7] (CMC*)	1.0	1.0	1.0
Nutrient Analysis[8]			
Crude Protein	40.3	34.6	29.2
Crude Lipid	16.7	15.4	13.0
Crude Ash	8.5	7.2	6.5
NFE[9]	34.5	42.8	51.3
Digestible Protein (DP)(%)	35.2	29.0	23.6
Digestible Energy (DE)(MJ kg^{-1})	15.5	12.5	9.0
DP/DE Ratio (g DP MJ^{-1} DE)	22.8	23.3	26.3

1. Low temperature fish meal, Norsea Mink, LT 94. Trouw Aquaculture, Wincham, Cheshire, UK.
2. Int. Feed Number, 5-00-381, Trouw Aquaculture, Wincham, Cheshire, UK.
3. Int. Feed Number, 5-03-798, Trouw Aquaculture, Wincham, Cheshire, UK.
4. Int. Feed Number, 4-05-205, Trouw Aquaculture, Wincham, Cheshire, UK.
5. Int. Feed Number, 7-01-994, Boost Oil, Cod liver oil, Seven Seas, Hull, UK.
6. (Closed Formulation). Trouw Aquaculture, Wincham, Cheshire, UK.
7. Sigma Chemical Company, Poole, Dorset, UK.
* Carboxy methyl cellulose
8. Dry matter
9. Nitrogen Free Extract

Return of Appetite Determinations

Return of appetite determinations were performed by re-feeding fish as separate groups. Following a 72 h starvation period, fish were fed their respective diets for about 45 min until all fish reached apparent satiation (15,16). This was determined by monitoring the bottom of the tanks where one or two feed pellets remained. After removing and weighing the residual feed, the amount of feed consumed was recorded. Fish were again fed their respective diets, to apparent satiation 4 h after first feeding. The level of re-feeding at the specified time interval was equal to the extent of appetite return. The uneaten feeds were collected and weighed and subtracted from the amount of the subsequent feed consumed. Then all groups were starved for 72 h and the same procedure was repeated for subsequent time periods of 6 h, 12 h, 24 h, 30 h and 36 h. Appetite return determinations were performed four times for each time interval. During the course of the experiment, the total biomass of fish was weighed during the second day of starvation, without anaesthetic, in order to perform weight specific calculations.

Gastric Evacuation Analysis

After completion of the return of appetite measurements, the fish used for the return of appetite experiments and those provided for the gastric evacuation investigation were pooled. Fifty-six fish were placed in each of the three tanks and were fed for one week on their respective diets prior to post-mortem analysis of the stomach contents.

The sampling procedure was that same as detailed previously (14). In summary, eight fish from each six treatments were sacrificed following the feeding of all groups with their respective diets. After weighing sampled fish, paper plugs were placed in the buccal cavity of the trout following individual weighing and measuring to prevent the regurgitation of digesta. Digesta from each fish were carefully recovered and analysed as explained by Tekinay (14).

Statistical Analysis

Return of appetite and gastric evacuation data were subjected to analysis of variance (ANOVA) and Duncan's multiple range test (P < 0.05) (15) using the statistical software package Statgraphics (Manugistics Incorporated, Rockville, MD, USA) following the arcsin transformation. Regression analyses were applied to the gastric evacuation and return of appetite data and the following equations were fitted where necessary:

$$S_t = S_0 - k*t \text{ (Linear)} \quad \text{......................} \quad (1)$$
$$S_t = (S_0 - k*t)^2 \text{ (Square root)} \quad \text{.......................} \quad (2)$$
$$RA = 1/(a+b*e^{-k*t}) \text{ (Sigmoid)} \quad \text{.......................} \quad (3)$$
$$RA = a(1-e^{-kt}) \text{ (First Order)} \quad \text{............................} (4)$$

where 'S_0' is the meal amount consumed at time = 0, 'S_t' represents the gastric content at the given time 't' and 'k' is the instantaneous rate of stomach evacuation for the first three regressions. In addition, 'a' and 'b' are the asymptotes of appetite return and 'k' is the rate constant of appetite revival at the given time 't' for the last two regressions. The fitted curves for gastric evacuation and return of appetite measurements were statistically compared by multiple regression analysis in order to test whether there was any significant difference (P < 0.05) between the slopes.

Results

The gastric evacuation and return of appetite models for D1, D2 and D3 are displayed in Figures 1-3, respectively. The amount of meal ingested is presented in each figure as a percentage of the average satiation amount. The gastric evacuation curve of the population of fish following a satiation meal at the same temperature (15 $^{\circ}$C) is presented on the same graph.

Square root equations best explained the stomach emptying data according to the minimum residual mean sum of squares (RMS) and the highest R^2. Following the comparisons of fitted models by multiple regression analysis (Table 2), the gastric evacuation rates of D1 and D2, and D1 and D3 were observed to be significantly different (P < 0.05). However, no significant difference (P > 0.05) was evident between the emptying rate of D2 and D3. Three first-order equations were used for the description of return of appetite data (Figures 1-3). Similar to comparisons of gastric evacuation data, the return of appetite slopes of D1 and D2, and D1 and D3 were significantly different (P < 0.05) (Table 2), whilst the return of appetite rates of D2 and D3 displayed a similar pattern (P > 0.05).

There was always a significant increase in the feed intake (P < 0.05) of all groups at each time interval up to time 36 h. Percentages of feed intake of all groups at
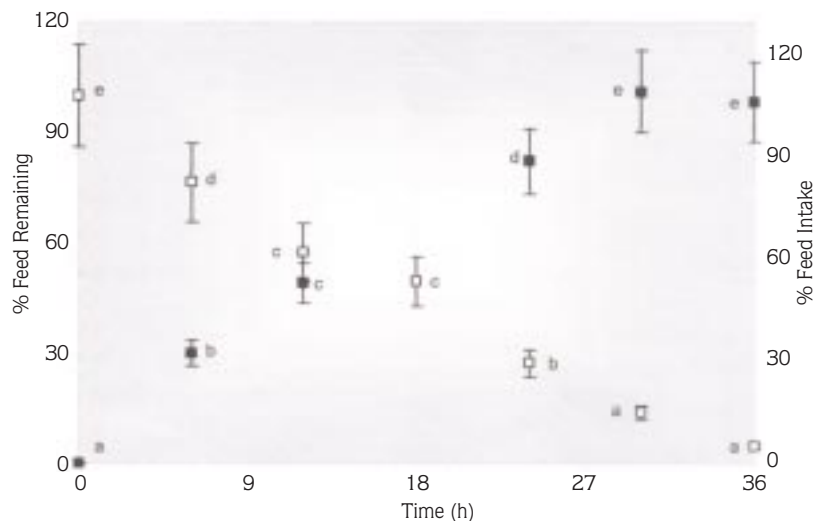
Figure 1. Percentages of stomach evacuation (□) and return of appetite (■) in trout fed D1. The stomach evacuation rate was described by a square root model $S_t = (10.03 - 0.2*t)^2$, $R^2 = 0.91$, Where, 'St' denotes percentage stomach content at time 't', n = 56. Non-linear regression model for return of appetite (First-Order); FI = $144.2*(1-e^{-0.041*t})$, $R^2 = 0.98$, where, 'FI' represents percentage feed intake or appetite return at time 't', n = 24. Data points in each graph allocated different letters are significantly different from each other (P < 0.05). Bars denote ± 5 standard error of the mean.
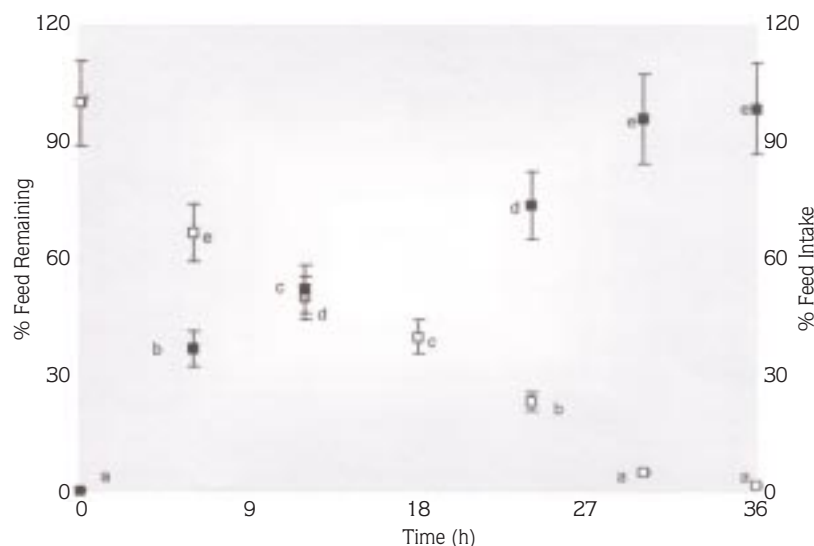

Figure 2. Percentages of stomach evacuation (□) and return of appetite (■) in trout fed D2. Stomach evacuation rate was described by a square root model; $S_t = (9.85-0.22*t)^2$, $R^2 = 0.94$, where 'St' denotes percentage stomach content at time 't', n = 56. Non-linear regression model for return of appetite (first-order); FI = $116.5* (1-e^{-0.05*t})$, $R^2 = 0.97$, where 'FI' represents percentage feed intake or appetite return at time 't', n = 24.

times 30 h and 36 h were not significantly different (P > 0.05). The time required for 95% appetite revival was estimated as 26.2, 28.8 and 27.5 h for D1, D2 and D3 treatments, respectively (Table 3), according to the fitted first order equations.

A significant evacuation (P < 0.05) was observed every 6 h until the sampling time of 30 h in fish fed D2. However, the trout fed D1 and D3 cleared their stomachs significantly up to 12 h (P < 0.05), and a similar instantaneous emptying rate (P>0.05) was evident
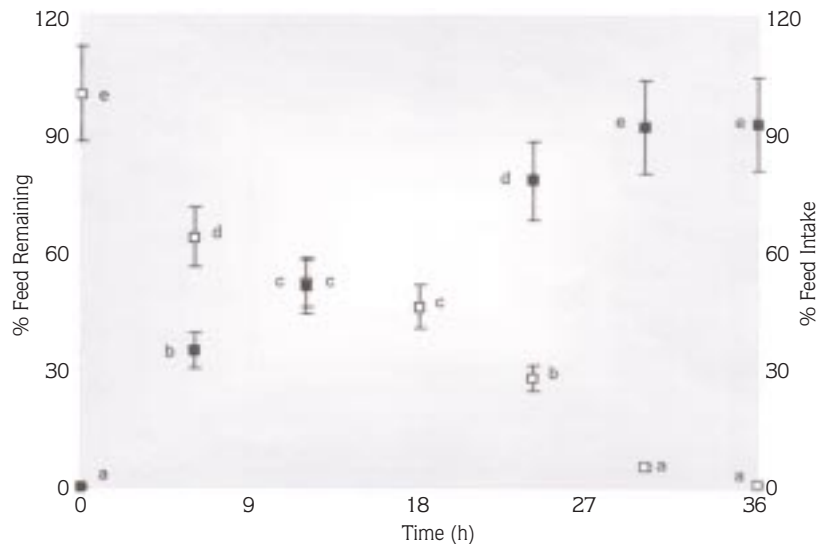
Figure 3. Percentages of stomach evacuation (□) and return of appetite (■) in trout fed D3. Stomach evacuation rate was described by a square root model $S_t = (9.8-0.21*t)^2$, $R^2 = 0.88$, where 'St' denotes percentage stomach content at time 't', n = 56. Non-linear regression model for return of appetite (first-order); $FI = 107.16* (1-e^{-0.058* t})$, $R^2 = 0.98$ where 'FI' represents percentage feed intake or appetite return at time 't', n = 24.

Table 2. Statistical summary of comparison of the fitted gastric evacuation and return of appetite slopes.

Treatments	Gastric Evacuation[1] ANCOVA[3] d.f. (3:108)		Return of Appetite[2] ANCOVA d.f. (3:46)	
	f	P	f	P
D1 & D2	5.4	< 0.05	4.8	< 0.05
D1 & D3	4.3	< 0.05	4.2	< 0.05
D2 & D3	0.0	> 0.05	0.0	> 0.05

[1] The fitted square root model $St = (S_0-k*t)^2$, where 'St' is the percentage meal remain in the cardiac stomach at time 't'.
[2] The fitted first-order model $FI = a*(1- e^{-k* t})$, where 'FI' is the percentage of feed consumed.
[3] Significant differences at the 95% confidence level (P< 0.05) in shape of slopes determined by multiple regression analysis.

Table 3. Predicted gastric evacuation and return of appetite times.

	Calculated times (h) for gastric evacuation (%)				
Model	Treatments	25	50	75	95
Square Root	D.1	6.9	14.8	25.2	39.0
	D.2	5.4	12.7	22.1	34.6
	D.3	5.4	13.0	22.9	36.0
	Calculated times (h) for appetite revival (%)				
Model	Treatments	25	50	75	95
First Order	D.1	4.7	10.4	17.9	26.2
	D.2	4.8	11.2	20.7	28.8
	D.3	4.6	10.9	20.8	27.5

between 12 h and 18 h. Then, the evacuation rates of the D1 and D3 groups rose significantly (P < 0.05) at 24 h and 30 h. For all treatments, no significant difference (P > 0.05) was detected in evacuation patterns between 30 h and 36 h. The removal time for 95% of the digesta from the cardiac stomach was calculated as 39.0 h for D1, 34.6 h for D2 and 36.0 h for D3 fed trout (Table 3). An almost 100% relationship was apparent between appetite revival and gastric evacuation rates in rainbow trout, irrespective of the diets offered (Table 4).

Discussion

Different levels (181, 350 or 475 g kg^{-1} diets) of a non-nutritive material (α-cellulose) were used for the dilution of dietary energy and other nutrients in the present investigation (Table 1). This component had previously been used for the same purpose in rainbow trout (*Oncorhynchus mykiss*) (6,9,17), chinook salmon (*Oncorhynchus tshawytscha*) (18), channel catfish (*Ictalurus punctatus*) (19,20), plaice (*Pleuronectes platessa*) (21) and turbot (*Scopthalmus maxima*) (22).

Diet	Model[1]	a	b	k	R^2	RMS
	Sigmoid	0.008	0.07	-0.05	0.99	12.1
D1	*Linear*	5.13	-	1.17	1.0	2.7
	First Order	184.8	-	-0.009	0.99	7.0
	Sigmoid	0.01	0.1	-0.05	0.99	9.6
D2	*Linear*	1.83	-	1.0	1.0	2.9
	First Order	172.9	-	-0.008	1.0	8.6
	Sigmoid	0.01	0.1	0.05	0.99	9.3
D3	*Linear*	3.93	-	0.99	0.99	8.9
	First Order	191.1	-	-0.007	1.0	2.7

Table 4. Fitted equations for the relationship between return of appetite and gastric evacuation in rainbow trout.

[1] Coefficients derived from the fitted sigmoid FI = 1/ (a+b* e^{-k*GE}), linear FI = a + k*GE, first-order FI = a*(1-e^{-k*GE}) and square root function RA = (a + k*GE)^2, where 'FI' is the return of appetite (% Feed Intake) and 'GE' is the gastric evacuation (%).

Hilton et al. (9) observed that 10 and 20% of α-cellulose impaired the feeding and growth performance of rainbow trout. They also determined that the gastric evacuation time (GET) in trout (mean weight = 45 g) was lower in high-fibre diets than in the control diet (GET = 782 min), although there was no apparent difference between the 10% (GET = 379 min) and 20% (GET = 412 min) α-cellulose groups. However, they were unable to prove the difference or similarity in the gastric clearance times at a confidence level of 95%.

Bromley and Adkins (17) showed that rainbow trout regulated feed intake on diets including up to 30% α-cellulose so as to maintain protein and energy intake and growth performance at the optimum level. They reported that the feeding control mechanism of trout was operational within 2-4 days from the start of the experiment. Furthermore, these authors explained that the compensatory feeding mechanism broke down when fibre levels increased to 40-50% of the diet, leading to a sharp decline in nutrient intake. However, experimental fish in the present study acclimatised to the diets in 1 week, and no appetite suppression was observed in trout fed diets containing 350 or 475 g kg^{-1} of α-cellulose levels throughout the feed intake experiment.

In this study, increasing the inclusion level of α-cellulose from 181 g kg^{-1} to 350 or 475 g kg^{-1}, (or diluting the digestible energy concentration from 15.5 MJ kg^{-1} to 12.5 or 9.0 MJ kg^{-1}) resulted in a peak in the instantaneous rates of stomach clearance in trout (Table 2). However, in the study by Tekinay and Güner (4), no

noticeable change (P > 0.05) in the rates of gastric evacuation was observed in trout fed diets with varying dietary digestible energy contents (21.3 – 18.8 MJ kg^{-1}). In another piece of research by Tekinay and Davies (5), higher (P < 0.05) rates of gastric evacuation were determined in trout fed diets with 16.1 or 17.3 MJ kg^{-1} digestible energy than in fish fed a diet with 20.2 MJ kg^{-1} digestible energy.

Evacuation times of 95% of the digesta from the cardiac stomach were 42.2 h for trout fed on D1 (21.3 MJ kg^{-1}), 39.7 h for trout fed D2 (20.3 MJ kg^{-1}) and 38.3 h for trout fed D3 (18.8 MJ kg^{-1}) according to the fitted square-root models (4). Moreover, the time required for clearance of 95% of stomach content was 44.8, 37.0 and 35.4 h for low carbohydrate (20.2 MJ kg^{-1}), medium carbohydrate (17.3 MJ kg^{-1}) and high carbohydrate (16.1 MJ kg^{-1}) diets, respectively (5). In the present work, the same parameter was calculated as 39.0 h in the D1 (15.5 MJ kg^{-1}), 34.6 h in D2 (12.5 MJ kg^{-1}) and 36.0 h in D3 (9.0 MJ kg^{-1}) groups (Table 3).

From the above mentioned and present studies, a decrease in evacuation rates, in fish fed relatively high energy diets was apparent, and this could be explained by the feedback signals due to the transportation of energy-dense digesta into the upper intestine or the amino acid receptors in the duedonum performing as a regulatory factor (3,23).

It can be suggested that dietary digestible energy concentration is one of the most important factors in the regulation of feed intake. This is in accordance with Lee

and Putnam (6), Cho et al. (24) and Grove et al. (7), who reported that rainbow trout appeared to regulate feed consumption in order to maintain a relatively constant energy intake when fed practical pellet feeds. In this connection, Grove (25) and Jobling (3) hypothesised that the stomach may release (via neurons or hormonal feedback mechanisms) varying volumes of digesta, such that the intestine receives a constant amount of energy or dry matter. In addition, Jobling and Wandsvik (26) suggested that certain receptors situated in the upper intestine may monitor the total, digested or metabolisable energy level and that consequently this information can modulate feed intake according to the diet quality. There are, however, contradictory studies in which similar gastro-intestinal evacuation rates were observed in fish fed different dietary energy concentrations. For instance, the sand dab, *Limanda limanda* (27), tilapia, *Sarotherodon mossambicus* (28), cod, *Gadus morhua* (29) and more recently dogfish, *Scyliorhinus canicula* L. (30), did not demonstrate a significant response when offered different energy and nutrient dense diets.

In the present study, the experimental fish returned to 95% of appetite approximately 24 h after the initial feeding (26.2, 28.8 and 27.5 h in D1, D2 and D3, respectively) (Table 3). This is fairly close to the findings of Tekinay and Güner (4), who estimated the time for 95% of appetite revival as 27.2, 32.4 and 27.9 h in trout fed diets with 21.3, 20.3 and 18.8 MJ kg^{-1} digestible energy levels, respectively. However, appetite return times of trout in the study by Tekinay and Davies (5), who used diets with 20.2, 17.3 and 16.1 MJ kg^{-1} digestible energy, were some 10 h longer than in the above mentioned and present works.

As was recently observed (4,5), a close correlation between stomach emptying and return of appetite in trout was determined in the present study (Table 4). This substantial evidence suggests that the capacity of the cardiac stomach in rainbow trout plays the primary role in regulation of feed consumption. Emptying is stimulated by stretch-receptors in the stomach wall and by the food contents contained (31-34). Thus, square-root equations explained the evacuation data better than other models, which indicated that distension of the cardiac stomach wall was more significant than the surface area of the digesta in the regulation of stomach emptying as previously reported (4,5,13,28).

In conclusion, this research has confirmed the general belief that the stomach evacuation rate and gastric distension are the most important phenomena in the control of feeding in rainbow trout in the short term. Furthermore, dietary digestible energy concentration (within certain limits) is a significantly important factor in the control of cardiac stomach emptying and consequently feed intake in trout. More detailed studies are required to establish the limits of dietary digestible concentrations resulting in a significant modification in the gastric evacuation rate in trout.

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