

## Effects of High Dietary Protein and High Energy Density on Gastric Evacuation and Return of Appetite in the Rainbow Trout, *Oncorhynchus mykiss*

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**Abstract:** Rainbow trout (*Oncorhynchus mykiss*) were fed diets containing three different concentrations of crude protein (56.3%, 51.7% and 45.8%) and digestible energy (21.3, 20.3 and 18.8 MJ/kg), and the effects of dietary protein and energy level on gastric evacuation and return of appetite times were investigated. Gastric evacuation determinations were achieved by slaughtering 8 fish every 6 hours until no residue was found in the cardiac stomach of fish. Return of appetite experiments were conducted by re-feeding groups of trout every 4 or 6 hours following the first feeding. The data obtained from each process was modelled by regression analysis and compared statistically. For gastric evacuation modelling, square root models gave the best fits for the data set under examination. According to the square root fit for evacuation of all diets, no significant difference ( $P > 0.05$ ) was evident. One first-order and two sigmoid equations were used for the description of return of appetite data. Return of appetite slopes of all diets were also not observed to be significantly different ( $P > 0.05$ ). Regardless of the models employed, a very close relationship was apparent between appetite revival and gastric evacuation times in rainbow trout. The results indicated that gastric emptying time could be a major factor in the feed intake regulation whilst dietary protein and energy concentrations are likely to have a minor influence on the control of feeding.

**Key Words:** Feed intake, high-protein diets, high-energy diets, gastric evacuation time, return of appetite, rainbow trout

### Gökkuşığı Alabalıklarında (*Oncorhynchus mykiss*) Yüksek Yem Proteini ve Enerjisinin Mide Boşalımı ve Yem Alma İsteğine Dönüş Süreleri Üzerine Etkileri

**Özet:** Üç farklı ham protein (% 56.3, % 51.7 ve % 45.8) ve sindirilebilir enerji (21.3, 20.3 ve 18.8 MJ/kg) konsantrasyonunda formüle edilmiş araştırma yemleri, gökkuşığı alabalıklarına (*Oncorhynchus mykiss*) yedirilerek, yem proteini ve enerjisinin, balıkların mide boşalımı ile yem alma isteğine dönüş süreleri üzerine etkileri araştırılmıştır. Mide boşalım süresi, yemlemenin bitimini müteakip ( $t=0$  saat) midenin tamamen boşalınca kadar ( $t=6, 12, 18, 24, 30, 36$  saat) 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 takiben her 4 veya

6 saatte yemlenmesi sayesinde ölçülmüştür. Her iki denemelerin dataları, regresyon analizi yardımıyla modellendirilerek istatistiki olarak karşılaştırılmışlardır. Her üç yemle beslenen alabalıkların mide boşalım süreleri en iyi şekilde üç karakök (square root) eşitliği ile açıklanmış ve aralarında önemli derecede farklılık gözlenmemiştir ( $P>0.05$ ). Yem alma isteğine dönüş süreleri de sırasıyla iki adet 'sigmoid' ve bir adet birinci mertebe (first order) eşitlik ile tanımlanmış; yine birbiriyle aralarında istatistiki bir farklılığa rastlanmamıştır ( $P>0.05$ ). Diğer taraftan her yem ile beslenen balıkların mide boşalım ve yem alma isteğine dönüş süresi arasında çok yakın bir ilişki tespit edilmiştir. Sonuçlara göre; mide boşalım süresinin alabalıkların yem alma isteğini regüle eden önemli bir faktör olabileceği, diğer taraftan, yem protein ve enerjisinin (araştırmada kullanılan sınırlarda) yem tüketimi kontrolünde etkin bir rol oynamadığı iddia edilmiştir.

**Anahtar Sözcükler:** Yem tüketimi, yüksek proteinli yemler, yüksek enerjili yemler, mide boşalım zamanı, yem alma isteğine dönüş süresi, gökkuşağı alabalığı

## Introduction

The prediction of the gastric evacuation and return of appetite times in cultured fish is essential since one of the most significant considerations in aquaculture is to determine the appropriate feeding frequency and optimum ration size. If fish are fed continuously, then not only will uneaten feed be lost but also the environment will be polluted (1). Furthermore, considerable amounts of dry matter may escape from gastric and intestinal digestion and assimilation following feeding in excess (2, 3). This claim has not been sufficiently examined in rainbow trout. It is obvious that the quantification of the rate of evacuation of a meal from the cardiac stomach and comparison of this pattern with the time at which appetite returns can provide considerable information towards understanding the processes of digestion and optimizing feeding regimes for farmed fish (4, 5). Information on evacuation rates with the knowledge of the type and quantity of prey obtained from the stomach of wild fish has been widely used to estimate the feeding rates of fish populations (6, 7, 8). The influence of both digestible energy and digestible protein density on gastric evacuation rates and return of appetite in trout remains to be explored. Therefore, the objectives of the present investigation are the quantification of gastric evacuation and return of appetite times and the relationship between these parameters in rainbow trout fed diets of different energy and nutrient densities. It is hypothesized that if fish are allowed to eat as much as their energy requirement, then there will be significantly different gastric evacuation times in fish fed diets of varying energy densities. Similarly, appetite revival time in fish fed energy dense diets may be significantly longer than in those fed lower energy diets.

Furthermore, gastric evacuation modelling has also been the centre of discussion for over two decades as to whether linear, square root or exponential equations best describe the evacuation pattern in salmonids (9, 10, 11, 12). Therefore, the present study was also directed at providing further information through comparison of certain models.

## Materials and Methods

### Experimental Fish and Holding Facilities

Seventy-two rainbow trout, *Oncorhynchus mykiss* (mean weight  $185.0 \pm 12.0$  g SEM) for the subsequent return of appetite analysis and 180 trout ( $186.2 \pm 15.1$  g SEM) for the gastric evacuation determinations were supplied by a local fish farm (Mill Leat Trout Farm, Ermington, Devon, UK) and acclimatized to the aquarium conditions for three weeks.

### Test Diets

Formulation and chemical composition of experimental diets are presented in Table 1. Manufacturing technique of the experimental diets was as outlined in (5).

Ingredients	Diet 1	Diet 2	Diet 3
LT Fish Meal <sup>1</sup>	66.5	55.5	45.6
Blood Meal <sup>2</sup>	3.0	3.0	3.0
Poultry Meat Meal <sup>3</sup>	8.3	8.3	8.3
Extruded Wheat Meal <sup>4</sup>	-	14.0	24.0
Fish Oil <sup>5</sup>	18.5	15.0	12.1
Vitamin/Mineral Premix <sup>6</sup>	2.5	2.5	2.5
$\alpha$ - Cellulose <sup>7</sup>	0.2	0.7	3.5
Binder <sup>7</sup> (CMC*)	1.0	1.0	1.0
<i>Nutrient Analysis</i> <sup>8</sup>			
Crude Protein (% DM)	56.3	51.7	45.8
Crude Lipid (% DM)	25.5	22.2	19.0
Crude Ash (% DM)	11.3	10.4	9.2
NFE9 (% DM)	7.0	15.7	26.1
Digestible Protein (DP) (%)	52.1	47.2	41.7
Digestible Energy (DE)(MJ kg <sup>-1</sup> )	21.3	20.3	18.8
DP/DE Ratio (g DP MJ <sup>-1</sup> DE)	24.4	23.2	22.2

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

1. Low-temperature fish meal, Norsea Mink, LT 94. Donated by 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.
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 in separate groups. Following a 72-hour starvation period, fish were fed each respective diet for about 45 minutes until all fish reached apparent satiation (13, 14). This was determined by monitoring the bottom of the tanks where 1-2 feed pellets remained. After removing and weighing residual feed, the amount of feed consumed was recorded. Fish were fed each respective diet again to apparent satiation 4 hours after the 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 hours and the same procedure was repeated for subsequent time periods of 8h, 12h, 24h, 30h and 36h. 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 completing return of appetite measurements, the fish used for return of appetite experiments together with those provided for the gastric evacuation investigation were pooled. Sixty fish were placed in each of the three tanks and were fed for one week on the respective diets prior to post-mortem analysis of the stomach contents.

The sampling procedure was the same as that detailed in (5). In summary, 8 fish from each six treatments were sacrificed following feeding all groups of fish with respective diets. After weighing sampled fish, paper plugs were placed in the buccal cavity of the trout following weighing and measuring individually to prevent regurgitation of digesta. Digesta from each fish were carefully recovered and analysed as explained in (5).

### Statistical Analysis

Return of appetite and gastric evacuation data were subjected to analysis of variance (ANOVA) and the multiple range test ( $P < 0.05$ ) of Duncan (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 following equations were fitted where necessary:

$$S_t = S_0 \bar{n} k^*t \quad (\text{Linear}) \dots\dots\dots(1)$$

$$S_t = (S_0 \bar{n} k^*t)^2 \quad (\text{Square root}) \dots\dots\dots(2)$$

$$S_t = S_0 \bar{n} e^{k^*t} \quad (\text{Exponential}) \dots\dots\dots(3)$$

$$RA = 1 / (a + b * e^{-k^*t}) \quad (\text{Sigmoid}) \dots\dots\dots(4)$$

$$RA = a * (1 - e^{-k^*t}) \quad (\text{First Order}) \dots\dots\dots(5)$$

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

## Results

Following comparative stomach content analysis for the gastric evacuation modelling, square root models gave better fits than did linear and exponential equations for the data set under examination. Fitted models were compared by multiple regression analysis (Table 2). In order to choose the best fit, the minimum residual mean sum of squares (RMS), intercepts nearest to 100 and consequently the highest  $r^2$  were taken into account. Minimum RMS and the highest  $r^2$  for the evacuation of all treatments were obtained in the square root model. According to the square root fit for evacuation of Diets 1, 2 and 3, no significant difference ( $P > 0.05$ ) at a 95% confidence level was evident. First-order and sigmoid equations were used for the description of return of appetite data (Table 2). Although both models fitted well, a first-order equation resulted in a better fit due to the lower residuals mean sum of squares in Diet 3. The return of appetite data of Diets 1 and 2 were marginally better explained by sigmoid models (Table 3). The return of appetite slopes of Diets 1, 2 and 3 were not observed to be significantly different ( $P > 0.05$ ) (Table 2).

Treatments	Gastric Evacuation <sup>1</sup>		Return of Appetite <sup>2</sup>	
	ANCOVA <sup>3</sup> d.f. (3:108)		ANCOVA d.f. (3:46)	
	f	P	f	P
D.1 & D.2	0.6	>0.05	0.0	>0.05
D.1 & D.3	0.0	>0.05	2.0	>0.05
D.2 & D.3	0.4	>0.05	1.6	>0.05

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

<sup>1</sup> The fitted square root model  $St = (S_0 - k \cdot t)^2$ , where ' $St$ ' is the percentage of meal remaining in the cardiac stomach at time ' $t$ '.

<sup>2</sup> The fitted first order model  $RA = a(1 - e^{-k \cdot t})$ , where ' $RA$ ' is the percentage of feed consumed.

<sup>3</sup> Significant differences at the 95 % confidence level ( $P < 0.05$ ) in shape of slopes determined by multiple regression analysis (ANCOVA).

Gastric evacuation and return of appetite models for D.1, D.2 and D.3 are presented in Figures 1, 2 and 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°C) is presented on the same graph. One first-order and two sigmoid equations described the appetite revival data of experimental groups (Figures 1, 2 and 3, respectively). There was always a significant increase in feed intake ( $P < 0.05$ ) at time 4h in all groups of trout. The feed intake of all groups at times 30h and 36h was not significantly different ( $P > 0.05$ ). However, the appetite return patterns of the groups displayed some variances. For instance, fish fed Diet 1 and Diet 2 did not increase their feed intake significantly ( $P > 0.05$ ) between times 6h and 12h.

The times required for 95% of appetite return were predicted as 31.0, 32.4 and 27.9 hours for D.1, D.2 and D.3 treatments, respectively (Table 3), according to the fitted first-order equations. Primarily, a significant evacuation ( $P < 0.05$ ) was observed every 6 hours until the sampling time of 30h in all groups and no considerable difference ( $P > 0.05$ ) was detected in evacuation pattern between time 30h and time 36h for all treatments.

The evacuation time of 95% of the digesta from the cardiac stomach was calculated as 42.2 hours for trout fed D.1, 39.7 hours for trout fed D.2 and 38.3 hours for trout fed D.3 (Table 3). Regardless of the models employed, an almost 100% relationship was apparent between appetite revival and gastric evacuation rates in rainbow trout fed D.1, D.2 and D.3, respectively. These relationships are presented in Table 4.

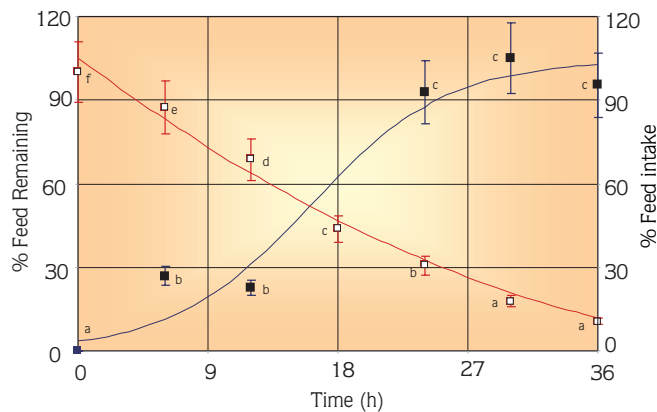


Figure 1. Percentages of stomach evacuation (□) and return of appetite (■) in trout fed D1. Stomach evacuation rate was described by a square root model;  $S_t = (10.26 - 0.19 \cdot t)^2$ ,  $R^2 = 0.90$ , where 'S<sub>t</sub>' denotes percentage stomach content at time 't', n = 56. Non-linear regression model for return of appetite (Sigmoid);  $FI = 1 / (0.0096 + 0.28 \cdot e^{-0.21 \cdot t})$ ,  $R^2 = 0.95$ , 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  $\pm 5$  standard error of the mean.

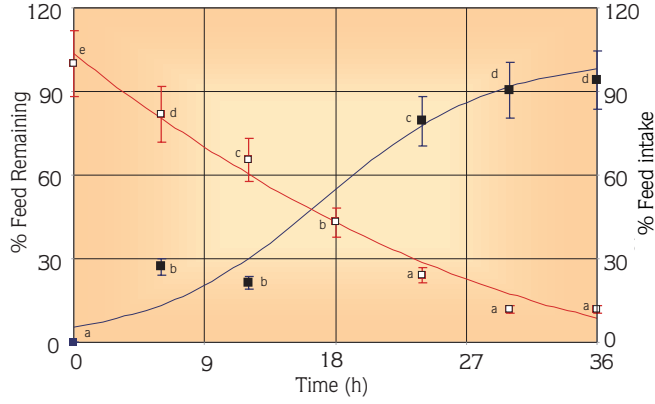


Figure 2. Percentages of stomach evacuation ( $\square$ ) and return of appetite ( $\blacksquare$ ) in trout fed D.2. Stomach evacuation rate was described by a square root model;  $S_t = (10.17 - 0.2 \cdot t)^2$ ,  $R^2 = 0.87$ , where 'St' denotes percentage stomach content at time 't',  $n = 56$ . Non-linear regression model for return of appetite (Sigmoid);  $FI = 1 / (0.0098 + 0.18 \cdot e^{-0.17 \cdot t})$ ,  $R^2 = 0.96$ , Where, 'FI' represents percentage feed intake or appetite return at time 't',  $n = 24$ .

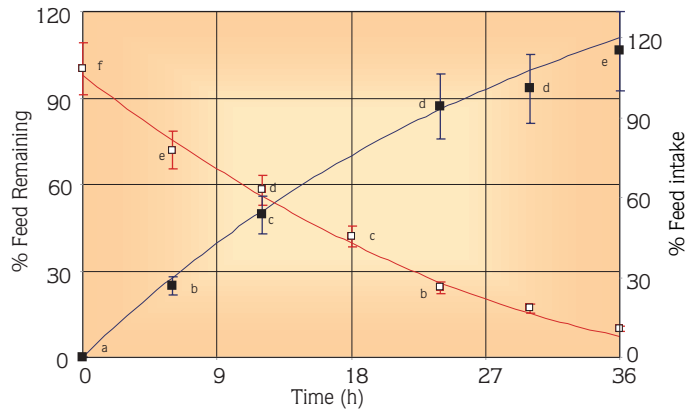


Figure 3. Percentages of stomach evacuation ( $\square$ ) and return of appetite ( $\blacksquare$ ) in trout fed D.3. Stomach evacuation rate was described by a square root model;  $S_t = (9.89 - 0.2 \cdot t)^2$ ,  $R^2 = 0.86$ , Where 'St' denotes percentage stomach content at time 't',  $n = 56$ . Non-linear regression model for return of appetite (First Order);  $FI = 167.73 \cdot (1 - e^{-0.03 \cdot t})$ ,  $R^2 = 0.97$ , where 'FI' represents percentage feed intake or appetite return at time 't',  $n = 24$ .

Calculated times (h) for gastric evacuation (%)					
Model	Treatments	25	50	75	95
Square Root	D.1	8.4	16.8	27.7	42.2
	D.2	7.6	15.5	25.9	39.7
	D.3	6.2	14.1	24.5	38.3
Calculated times (h) for appetite revival (%)					
Model	Treatments	25	50	75	95
Sigmoid	D.1	10.6	15.7	20.6	27.2
	D.2	10.5	16.9	23.1	32.4
	D.3	6.2	11.7	16.7	22.3
First Order	D.1	6.5	13.9	22.3	31.0
	D.2	7.6	15.8	24.6	32.4
	D.3	5.4	11.8	19.8	27.9

1. Calculations are based on the fitted square root models.
2. Calculations are based on the fitted first-order and sigmoid models.

Diet	Model <sup>1</sup>	a	b	K	R <sup>2</sup>	RMS
D.1	Sigmoid	0.009	0.29	-0.07	1.0	0.7
	Square Root	2.04	-	0.1	0.98	0.2
	Linear	-6.1	-	1.29	0.97	44.8
D.2	Sigmoid	0.009	0.2	-0.05	1.0	0.6
	Square Root	2.14	-	0.09	0.99	0.1
	Linear	-6.42	-	1.14	0.97	1.0
D.3	Sigmoid	0.008	0.009	-0.045	0.99	11.0
	Square Root	-2.16	-	1.2	1.0	0.3
	First-Order	211.2	-	-0.007	0.98	25.3

<sup>1</sup>Coefficients derived from the fitted sigmoid,  $RA=1/(a+b \cdot e^{-k \cdot GE})$ , linear,  $RA=a+k \cdot GE$ , first order,  $RA=a \cdot (1-e^{-k \cdot GE})$  and square root functions,  $RA=(a+k \cdot GE)^2$ , where 'RA' is the return of appetite (% Feed Intake) and 'GE' is gastric evacuation (%).

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

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

## Discussion

Gastric evacuation pattern appeared to exert the main influence on the return of appetite in rainbow trout, as compared to systemic factors such as circulating plasma nutrients and metabolites. The gastric evacuation times (GET) of treatments were described by three square



root equations. The exponential, rectilinear and surface area models of stomach emptying described in the literature, and the square root equations of evacuation pattern described in this study agree in that the evacuation rate is fast initially and slows down with time as the amount of the digesta in the cardiac stomach declines (12, 16, 17, 18). This is a general phenomenon for pelleted feeds which are quickly broken down to a chyme-like consistency as was also observed by (8) in turbot, *Scophthalmus maximus*.

Calculated times for the evacuation of 95% digesta from the cardiac stomach varied between 38.3 hours (D.3) and 42.2 hours (D.1), which were lower than the values (40-50 hours for 200 gram trout at 15°C) presented by (1). It appears from the present investigation that it was unlikely that a 200 gram fish evacuated its stomach in 50 hours, and thus the diagram represented by (1) is not always applicable. In contrast to the present findings, (1) reported 36 hours as the total clearance of 1% bw capsules from the stomach of a 90-gram rainbow trout at 15°C. This value may not be comparable since the fish sizes and meal intakes used in the latter study were smaller than the ones employed in the present study.

Square root models applied for the gastric evacuation data provided a rational approach in that the distension of the cardiac stomach wall is more important than the surface area of the digesta in the regulation of stomach emptying as previously described by (19) and demonstrated by (20) in *Limanda limanda* and (21) in *Scophthalmus maximus*. However, it cannot be stated that the fitted square root equations are exclusive since the differences between the RSM (residuals of mean square) and  $r^2$  of the linear, exponential and square root models were marginal. Actually, the choice of a model for stomach emptying cannot be made on a biological basis alone. Even if all the factors were known, a biologically based model would be very complex (22). Therefore, comparing different evacuation rates of fish fed diets of different quality would provide more information towards the understanding of digestive physiology rather than trying to standardise certain models (for discussion, see 23).

From the results, it is clear that a change in dietary digestible energy content ( $21.3 \pm 18.8$  MJ kg<sup>-1</sup>) does not affect gastric evacuation times in rainbow trout. The gastric evacuation slopes of D.1, D.2 and D.3 were not significantly different ( $P > 0.05$ ).

It also suggests that rainbow trout maintain a uniform rate of dry matter consumption. In this regard, (12) and (24) 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 dry matter or energy. Furthermore, (25) and (26) suggested that certain receptors situated in the upper intestine may monitor the total, digested or metabolizable energy level, and consequently this information can modulate feed consumption according to the diet quality.

Satiation times (40-50 minutes) for all groups of trout were quite similar in this experiment irrespective of the diet quality. This is in accordance with (13), (3) and (1) for rainbow trout and (27), (28) and (29) for other salmonids. The similar satiation times observed for trout

offered different nutrient and energy dense diets could support the claim that rainbow trout eat to maintain a constant dry matter intake. In this manner, (30) suggested that the pyloric part of the stomach in rainbow trout is not affected whilst the cardiac part of the stomach distends following a satiation meal. This may indicate that the amount of delivered digesta from the cardiac stomach to the upper intestine is approximately constant since the pyloric part of the stomach is unaffected by the mass of the digesta in the cardiac stomach. However, little is known of the mechanisms and which neurons and endocrine cells play a modulatory role in this process. Similar evacuation rates derived from trout fed D.1, D.2 and D.3 may be due to similar digestible energy of test diets; however, rainbow trout increase their feed intake when the energy content of the diet is diluted (1, 31, 32). A similar finding was documented in goldfish (*Carassius carassius*) (33), turbot, (*Scaphthalmus maximus*) (34) and plaice (*Pleuronectes platessa*) (11).

It has been suggested that energy concentration is more important than specific nutrients in the control of feed intake (19, 25). On the other hand, similar gastro-intestinal evacuation rates in other fish fed different dietary energy concentrations have also been reported. For instance, the sand dab, (*Limanda limanda*) (20), tilapia, (*Sarotherodon mossambicus*) (35), cod (*Gadus morhua*) (36) and more recently dogfish (*Scyliorhinus canicula* L.) (26) did not demonstrate a significant response when offered diets of different energy and nutrient densities. Therefore, it appears that macro- and micronutrients are interrelated and should be investigated together.

A very high relationship between gastric evacuation and return of appetite was found following data plotting according to first-order, linear and square-root equations (Table 4). This high correlation indicated that rainbow trout adjusted feed intake so that stomach capacity was at near-maximum fullness. Therefore it may be that gastric tension receptors were the main regulatory factors in relation to the amount of digesta in the cardiac stomach in the short term. Consequently, the appetite of trout was controlled by the gastric emptying of a meal in a weight-dependent manner in the short term. In this context, the nutritional status and history of the experimental fish are also important points to be considered. For instance, the results of the present study were derived from fish starved 72 hours and fed a single satiation meal. Therefore we have limited our discussion according to the constraints of the study as undertaken.

This investigation confirms the general view of (2) and (1) that stomach evacuation time is an important feature in the modification of feeding behaviour in rainbow trout. Gastric distension is likely to be a major factor in the short-term satiety of trout whilst the energy density of the meal may be a less significant component. Dietary digestible energy concentration appears to be important in the long-term regulation of feed intake. If the basis of appetite regulation is dependent on the bulk of food, then the bulk density effect of dietary carbohydrates may influence appetite in trout.

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