The Open Access Israeli Journal of Aquaculture – Bamidgeh

THE INFLUENCE OF MULTIPLE MEALS ON THE GASTRIC EVACUATION RATE IN RAINBOW TROUT *(ONCORHYNCHUS MYKISS)*

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Abstract

Rainbow trout, *Oncorhynchus mykiss* (mean weight 70.8±1.0 g SEM) raised in the Sea of Marmara (Turkey), were used for a preliminary gastric evacuation study. After being starved 72 hours, three groups of 110 fish, each, were fed *ad libitum* once, twice or three times in a single day. Ten fish from each group were withdrawn and killed in an anesthetic solution during each sampling at 0, 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30 hours following the last feeding. Gastric material was removed from the fish and dried for gastric evacuation modelling. Gompertz and logistic models (with fixed asymptotes) best explained the data. The gastric emptying rate of the trout offered a single meal (Group A) was faster than those fed two (Group B) or three (Group C) meals while the emptying patterns of Groups B and C were similar to each other. The time required to evacuate 95% of the gastric material from the first meal was estimated as 54.3, 68.0 and 67.8 h for Groups A, B and C, respectively, according to the Gompertz equations (with a fixed asymptote).

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Introduction

Well-designed nutrition strategies are required to maximize fish growth with a minimum of financial input and pollution. One of the major factors influencing growth in fish is the evacuation rate of the digesta from the stomach which is mainly affected by the feeding level and frequency (Elliott, 1976; Fänge and Grove, 1979; Vahl, 1979; Talbot, 1985). Despite that regulation of feed intake in animals is not adequately understood, the cardiac stomach as an important regulator of appetite has been the subject of numerous works on mammals (Kallogeris et al., 1983; Wirth and McHugh, 1983; Rayner, 1992; Mayer, 1994) and fish (Grove et al., 1978; Fletcher, 1984; Sims et al., 1996; Tekinay and Güner, 2001; Tekinay and Davies, 2002). These studies have generally demonstrated that the stomach emptying rate and the voluntary feed intake correspond, i.e., input rate = output rate (Bromley, 1994).

Most gastric evacuation experiments conducted on fish were based on a single meal, although single meals after starvation periods do not reflect the natural feeding rhythm and gastric evacuation pattern of most wild and farmed fish species. Multiple-meal experiments have demonstrated that the administration of a second or third meal speeds up the evacuation of the first meal and exaggerates the natural gastric evacuation slope. Multiple meal models were used successfully to predict the evacuation of multiple meals in brown trout (Elliott, 1975), perch (Persson, 1981), roach (Persson, 1982) and coho salmon (Ruggerone, 1989).

The objective of the present study was to preliminarily examine the influence of multiple meals on the gastric evacuation rate in rainbow trout raised in sea water.

Materials and Methods

Three hundred and thirty rainbow trout (*Oncorhynchus mykiss* Walbaum 1792, mean weight 70.8±1.0 g SEM) raised on a private farm (Akbas Trout Farm, Erdek, Balıkesir, Turkey) in the Sea of Marmara (salinity 22‰) were used for the gastric evacuation study. Three groups of 110 fish, each, were randomly allocated to net cages (4 x 4 x 5 m). The experimental diet (Cagatay Feed Mill, Ecobio, Turkey; 4 mm diameter extruded feed, 45% protein, 20% lipid, 20 MJ digestible energy) was offered three times daily (9:00, 13:00, 17:00) until all fish reached near-satiation during a two-week adaptation period. The water temperature ranged 17.5-18.3°C and dissolved oxygen ranged 6.7-7.5 mg per l.

Preceding the day of the experiment, fish were deprived of food for 72 hours to ensure that their last meal had been completely evacuated. On the following morning (8:15-9:00), each group (cages A, B and C) was fed until the fish accepted no more feed (approximately 45 min). Cages B and C were fed again at noon (13:00-13:45) and cage C was fed a third time in the afternoon (16:45-17:30). The time the last feeding ended was counted as 'time 0' for each group. Ten fish from each group were sacrificed at time 0; an additional ten fish from each group were sacrificed every three hours afterwards, i.e., 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30 h after time 0. The fish were killed following prolonged immersion in ethyl p-amino benzoate (Benzocaine, Sigma Chemical Co. Ltd, Poole, UK; 1g dissolved in 100 ml of ethanol, added to fresh water at a concentration of 5 ml per l). The body weights of the sampled fish were recorded and paper plugs were placed into the buccal cavity of the trout to prevent regurgitation. The fish were placed in a freezer (-45°C) for up to six hours to solidify the stomach contents and facilitate their removal without any loss (Talbot, 1985). The contents of the excised stomachs were carefully removed, weighed and dried at 105°C until a constant dry weight was obtained. The percent of food remaining in the stomach was determined by dividing the dry weight of the food remaining by the dry weight of the food fed and multiplying by 100.

The mean weights of the experimental fish and the mean percents of food remaining in the stomach at each sampling for each group were subjected to analysis of variance and the Duncan multiple range test ($p < 0.05$; Steel and Torrie, 1960) using the statistical software package, Statgraphic (Manugistics Inc.,

Rockville, MD, USA). Coefficients of variation (CV) were calculated for these two parameters by dividing the standard deviation by the mean of the respective parameter and multiplying by 100.

Linear, square root, exponential, Gompertz and logistic equations were fitted to the evacuation data to find the expression that best described the relationship between the percent of food remaining in the stomach and the time after the last feeding (for discussion on the efficacy of models see Medved, 1985; Ruggerone, 1989; Bromley, 1994). The parameters of the linear model were estimated by standard least squares regression. The Marquardt search algorithm (Marquardt, 1963) of Statgraphics Version 3.1 was used to obtain estimates of the parameters of the non-linear equations. The evacuation models were assayed by comparing the coefficients of determination (r^2), standard errors of the regression (SER), *y*-intercepts and residual plots.

Results

Following the three-day starvation period, the trout consumed 3.3 g (Group A), 3.9 g (Group B) and 4.2 g (Group C) feed per 100 g body weight in the first meal (8:15-9:00). In the second meal (13:00-13:45), the feed intakes of Groups B and C were 1.7 and 1.9 g feed per 100 g body weight, respectively. The last feed intake, offered at 16:45-17:30 to Group C only, was 0.5 g per 100 g body weight. The total feed intakes for the day were 3.3, 5.6 and 6.6 g feed per 100 g body weight for Groups A, B and C, respectively. The amounts of gastric content recovered immediately after the last meal (time 0) were 2.8, 4.5 and 4.9 g per 100 g body weight for the respective groups.

There were no significant differences (*p*>0.05) between the mean weights of the trout, which were 69.8±3.4, 70.3±3.3 and 72.1±3.3 SEM g for Groups A, B and C, respectively. Similarly, the CVs (16.0%, 15.6% and 14.8%) of the fish weights did not significantly differ (*p*>0.05).

There was a six-hour delay between ingestion and the start of stomach evacuation in all treatments. The CV of food remaining in the stomach varied from 16.7 (time 0) to 34.5 (time 21 h) in Group A, from 14.1 (time 0) to 31.7 (time 27 h) in Group B and from 13.4 (time 12 h) to 34.8 (time 15 h) in Group C.

The various models (linear, square root, exponential, Gompertz and logistic) used to describe the relationship between the percent of food remaining in the stomach and the time after feeding are shown in Table 1. Intercepts nearest 100, the lowest residual mean sum of squares (RMS) and the highest r^2 indicated the best fit. The Gompertz and logistic equations explained the data set better than the linear, square root and exponential models for all the groups and are depicted in Figs. 1, 2 and 3 for Groups A, B and C, respectively. Although the Gompertz and logistic models best fit the data, the lowest asymptotes in both models were higher than 0. Therefore both models were re-fitted with the lowest asymptote fixed at 0.

The gastric emptying rate of trout offered only one meal (Group A) was apparently faster than those of Groups B and C which were similar to each other. At the last sampling time (30 h), 39.6% of the initial meal remained in the stomach of fish in Group A, whereas approximately half (49.6% and 50.6%) of the initial meal was still present in the stomachs of fish in Groups B and C. The time required to empty 95% of the digesta from the cardiac stomach, as predicted by the Gompertz and logistic models, is given in Table 2. For each stage of emptying the stomach, the calculated times deviated considerably between the two models.

Discussion

Groups B and C consumed significantly more than the fish in Group A, which may have been possible due to distension of the cardiac stomach wall in the fish in Groups B and C. This theory was supported by Grove and Holmgren (1992) who declared that the stomach wall of a rainbow trout stretches by reflex relaxation to increase its receptive capacity after ingestion.

Variations in feed intake between trout were observed in all groups at time 0. The CVs of the food intakes in Groups A, B and C

Table 1. Models* fitted to gastric emptying data for trout fed one (Group A), two (Group B) or three (Group C) meals during one day.

Group		Linear	Square root	Exponential	Gompertz	Gompertz with a fixed asymptote	Logistic	Logistic with a fixed asymptote
A	Parameters	a = 107.9 b = 2.41	a = 10.51 b = 0.15	a = 4.74 b = 0.038	a = 68.4 b = -9.3 c = -0.15	a = -4.91 b = -0.084	a = 61.93 b = -0.28 c = -16.4	a = -0.135 b = -23.66
	r^2	62.4	63.1	61.3	80.5	80.0	80.7	78.7
	RMS	322	1.18	0.08	303	310	302	327
	y-intercept	107.9	110.46	114.43	100.0	99.26	99.4	96.06
B	Parameters	a = 105.4 b = 1.90	a = 10.34 b = 0.11	a = 4.70 b = 0.030	a = 75.25 b = -4.7 c = -0.09	a = -4.26 b = -0.065	a = 58.91 b = -0.18 c = -18.83	a = -0.116 b = -27.61
	r^2	52.7	53.4	52.5	73.1	73.0	73.0	72.2
	RMS	296.0	1.0	0.1	294.0	292.0	295.0	294.0
	y-intercept	105.4	106.92	109.95	99.32	98.59	98.1	96.09
C	Parameters	a = 104.5 b = 1.94	a = 10.30 b = 0.12	a = 4.70 b = 0.03	a = 71.70 b = -4.40 c = -0.09	a = -3.93 b = -0.064	a = 58.78 b = -0.18 c = -17.86	a = -0.11 b = -27.00
	r^2	55.6	54.9	52.1	74.8	74.56	74.8	73.64
	RMS	275.0	1.0	0.1	275.0	271.0	274.0	264.0
	y-intercept	104.5	106.1	109.9	99.1	98.0	97.7	95.2

S_t = percentage of dry food remaining in stomach at time 't'; a, b and c are fitted parameters; RMS = residual mean square.

* Linear: $S_t = a - bt$

Square root: $S_t = (a - bt)^2$

Exponential: $S_t = a \cdot exp^{-bt}$

Gompertz: $S_t = 100 - a \cdot exp^{b \cdot exp^{-c \cdot t}}$

Gompertz with a fixed asymptote: $S_t = 100 - 100 \cdot exp^{a \cdot exp^{bt}}$

Logistic: $S_t = 100 - a/1 + exp^{b(t+c)}$

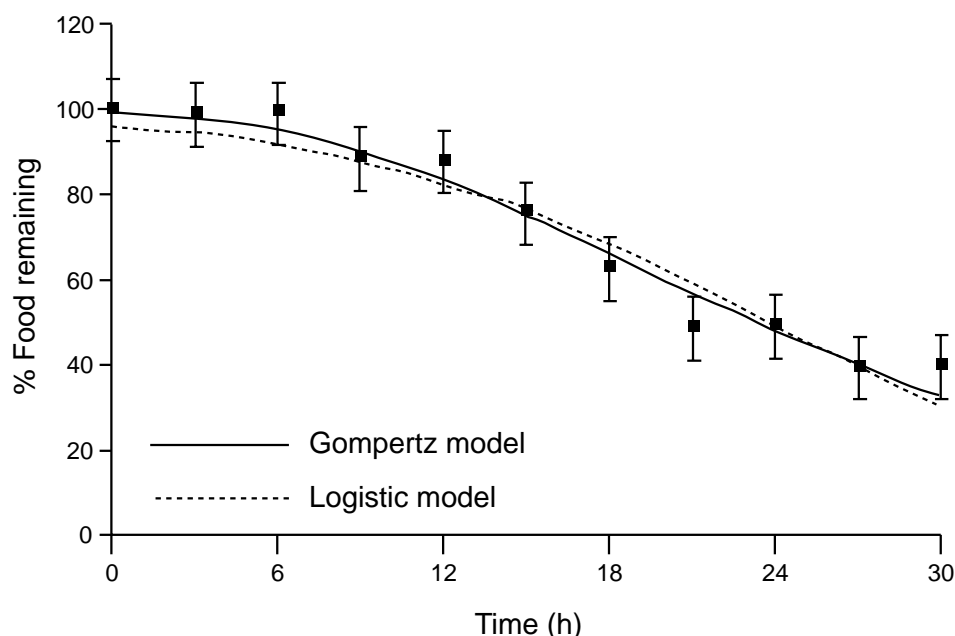Logistic with a fixed asymptote: $S_t = 100 - 100/1 + exp^{a(t+b)}$

Fig. 1. Gastric evacuation in trout fed a single meal (Group A). The rate of gastric evacuation was described by a Gompertz model with a fixed asymptote: $S_t = 100 - 100exp^{-4.91exp^{-0.084t}}$, $r^2 = 80.0$, and by a logistic equation with a fixed asymptote: $S_t = 100 - 100/1 + exp^{-0.135(t - 23.7)}$, $r^2 = 78.7$, where 'S_t' denotes the percent of stomach contents at time 't', n = 110. Bars denote the standard error of the mean.

were 16.7%, 14.1% and 18.0%, respectively. The relatively low CVs were probably due to the use of satiation feeding regimes. It was reported that restricted feeding strategies result in high variation in feed intake of fish (Jobling and Koskela, 1996).

There was a 0.5 g difference between the amounts of recorded feed intake and recovered digesta at time 0 in fish in Group A. This was likely due to the use of a different sample size for estimating the feed intake, i.e., feed intake was calculated for all 110 fish in the treatment while recovered digesta was measured in only ten fish at time 0. Large day-to-day variations in feed intake by fish have been shown previously. However, it was impossible to observe differences in feed consumption in this study due to the serial slaughter technique employed.

The mass of food in the stomachs of the fish in each group remained almost constant

for about six hours following the last meal. This lag before the movement of the digesta through the rest of the alimentary tract is largely due to the time required to liquefy the pellets and reduce the diameter of the nutrient particles to enable them to pass through the duodenum. This delay has been reported in rainbow trout (Windell et al., 1969; Grove et al., 1978; Tekinay, 1999) and other fish species (Fletcher, 1984; Basimi and Grove, 1985; Bromley, 1987). In the present study, examination of RMS values, *y*-intercepts and r^2 indicated that the Gompertz and logistic growth curve models most appropriately described the gastric evacuation since they took into consideration this lag as well as the steep decline and levelling off of the amount of digesta in the cardiac stomach, earlier shown by Medved (1985). Linear models could not be fitted to the gastric evacuation data because the amount of digesta cleared
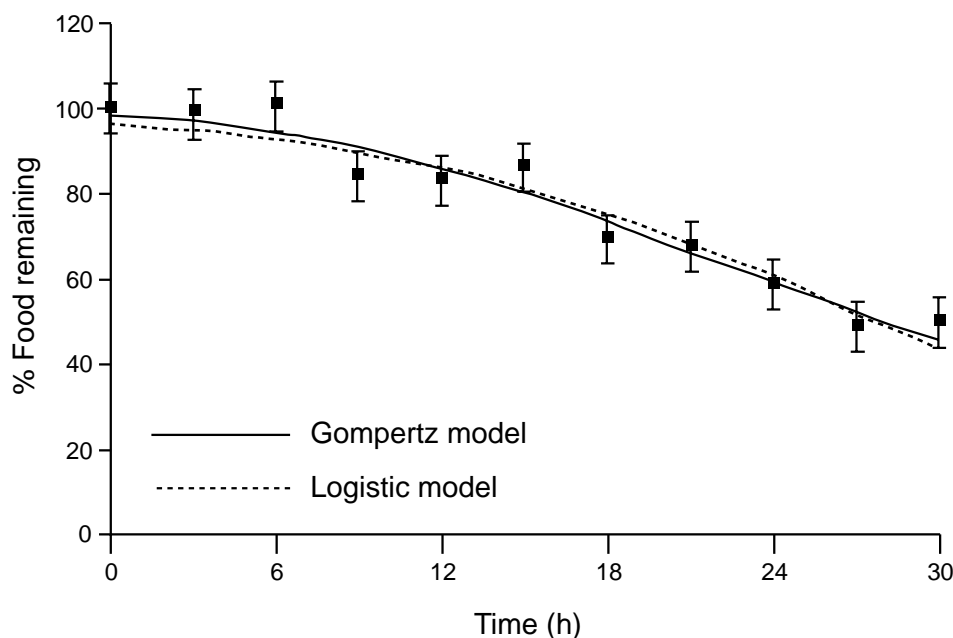
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Fig. 2. Gastric evacuation of the first meal in trout fed two meals (Group B). The rate of gastric evacuation was described by a Gompertz model with a fixed asymptote: $S_t = 100 - 100exp^{-4.26exp^{-0.065t}}$, $r2 = 73.0$, and by a logistic equation with a fixed asymptote: $S_t = 100 - 100/1 + exp^{-0.116(t - 27.6)}$, $r2 = 72.2$, where 'S_t' denotes the percent of stomach contents at time 't', n = 110. Bars denote the standard error of the mean.

per time unit was not constant. Neither the square-root nor the exponential function explains the emptying data, because evacuation did not begin immediately after the meal. The estimated *y*-intercepts in the fitted square root and exponential models were quite higher than the initial size of the meal (100%).

The gastric emptying rate of the trout in Group A was apparently faster than in Groups B and C. The rates in Groups B and C were similar to each other even though Group C consumed 18% more feed than Group B. These results are in accordance with Persson (1984), Ruggerone (1989) and Elliott (1991) who demonstrated that evacuation rates were faster in fish fed a single meal than those fed two or three different meals. The decrease in emptying rate after double or triple meals may be related to negative feedback processes caused by the evacuation of energy-rich food into the upper intestine (Jobling, 1986).

The sampling period in the present study was too short to observe the entire gastric evacuation period of the fish. The percent of ingested feed remaining in the stomach at the final sampling time (30 h) ranged 39.6-50.6%. When calculated according to the Gompertz model, the time required to empty the stomach differed from that calculated according to the logistic model. Since samplings did not continue until the stomach was completely emptied, it is impossible to determine which model is preferable. According to the Gompertz model, the time required to clear 95% of the food from the stomach was considerably longer than that found by Grove et al. (1978) and Tekinay and Güner (2001). Tekinay and Güner (2001) stated that the time required for 95% evacuation ranged 38.2-42.2 h in 186.2 g rainbow trout held in fresh water at 15°C. In an earlier work, the total time required to evacuate mealworms or pellets
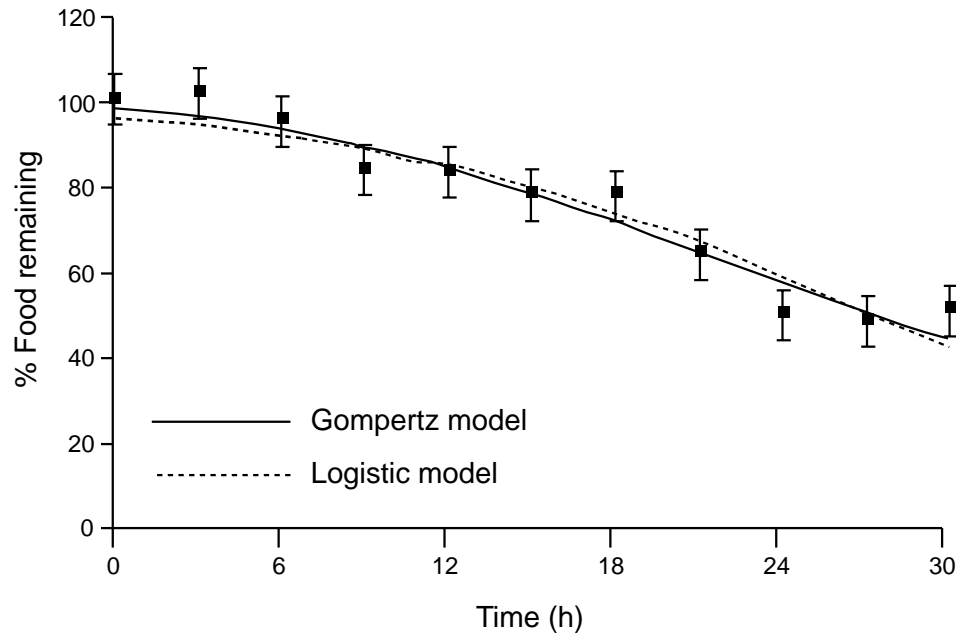
Fig. 3. Gastric evacuation of the first meal in trout fed three meals (Group C). The rate of gastric evacuation was described by a Gompertz model with a fixed asymptote: $S_t = 100 - 100exp^{-3.93exp^{-0.064t}}$, $r^2 = 74.6$, and by a logistic equation with a fixed asymptote: $S_t = 100 - 100/1 + exp^{-0.111(t - 27.0)}$, $r^2 = 73.6$, where 'S_t' denotes the percent of stomach contents at time 't', $n = 110$. Bars denote the standard error of the mean.

Table 2. Hours required to evacuate stomach contents of fish fed once (Group A), twice (Group B) or three (Group C) times during the one-day experiment, calculated according to the best fitted models.

Model	Treatment	25%	50%	75%	95%
Gompertz	A	15.0	23.3	33.8	54.3
with fixed	B	17.3	27.9	41.5	68.0
asymptote	C	16.3	27.1	40.8	67.8
Logistic	A	15.5	23.7	31.8	45.4
with fixed	B	18.1	27.6	37.1	53.0
asymptote	C	17.1	27.0	36.9	53.5

from the stomach of 116.7 or 132 g rainbow trout, respectively, held at 15°C was approximately 36 h (Windell and Norris, 1969). Similarly, Grove et al. (1978) found that total clearance of food from 90 g trout fed at 1% of their body weight and held at 15°C required 36 h, also faster than results in our study. One possible reason for the slow evacuation rate might be the use of nutrient-dense extruded feed in our trial. It was reported by Hilton et al.

(1981) and De Silva and Owoyemi (1983) that extrusion improves feed utilization and that carbohydrate digestion may result in slowing the stomach emptying rate. The salinity of the water could be another factor that influenced the evacuation pattern. More detailed investigation is required to test the influence of extruded feeds and water salinity on gastric motility in rainbow trout.

Our gastric evacuation study was conducted following a 72-hour starvation period to assure complete clearance of the gastrointestinal canal in the fish before beginning the study. However, preprandial or postprandial starvation periods are known to affect the subsequent feed intake and emptying rate. Talbot et al. (1984) showed that preprandial starvation of Atlantic salmon fry for 48 h caused fish to consume a larger meal than preprandial feeding. They also reported that postprandial starvation lowered the evacuation rate of the same species. It can therefore be suggested that gastric evacuation of fish exposed to different starvation periods (i.e., 12, 18, 24, 30, 36 h, etc.) be studied to mimic the actual feeding activity of fish in production conditions.

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