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Some Observations on Gastric Emptying in the Black Sea Whiting, *Merlangius merlangus euxinus*, (N. 1840) Fed on Natural Prey

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Abstract: In this study a new perspective on the feeding physiology of whiting, *Merlangius merlangus euxinus*, N. 1840 was investigated. The effects of the skin of the prey, double feeding and prey type on the gastric emptying rate (GER) of whiting fed on natural prey, namely anchovy (*Engraulis encrasicholus*) and mussels (*Mytilus galloprovincialis*), were investigated. Return of appetite was also studied. It was found that in laboratory conditions, whiting (48.0±8.30 g, n=12) had consumed about 10.2% of its body weight in mussels and return of appetite was found to be approximately 30 h. The effect of the skin of the anchovy on the gastric emptying rate (GER) in whiting was found to be significant. Skinned anchovy was emptied from the stomach in 30 h whilst unskinned anchory was emptied in 34 h. It was observed that the effect of the prey type, namely anchovy and mussels, was significant such that the gastric emptying rate of anchovy was 0.063 g/h whilst that of mussels was found to be 0.075 g/h. The reason was thought to be due to the different biochemical composition of the prey items used. In the double feeding experiments, it was found that the mussels given 4 h after the first meal of anchovy, slowed down the emptying process of the first meal. On the other hand, the emptying of the prey items used in double feeding experiments was slower than those used in the single feeding.

Key Words: Black Sea, Whiting, Return of Appetite, Gastric Emptying Time (GET).

Doğal Yemle Beslenen Karadeniz Mezgit, *Merlangius merlangus euxinus*, (N.1840)'inde Sindirim Üzerine Bazı Gözlemler

Özet: Araştırma mezgitlerde (*Merlangius merlangus euximus*, N.1840) beslenme fizyolojisi üzerine bir çalışmayı kapsamaktadır. Bu araştırmada doğal yem olan hamsi (*Engraulis encrasicholus*) ve midye (*Mytilus galloprovincialis*) ile beslenen mezgitlerde gıda doyum noktası ve sindirim oranını etkileyen parametrelerden yem derisi, iki farklı doğal yem ve ardıl yemlemenin sindirim oranı üzerine etkisinin araştırılması amaçlanmıştır. Mezgitlerde (48.0±8.30 g, n=12) günlük maksimum midye tüketim oranının vücut ağırlıklarının %10.2'si kadar olduğu ve bu değere 30. saatte ulaştığı saptanmıştır. Sindirim oranı üzerine yem olarak tüketilen balık derisinin etkili olduğu kanıtlanmıştır. Derisiz bir hamsinin sindirimi 30 saat iken normal bir hamsinin sindirimi 34 saat sürmüştür. İki doğal yemin (hamsi ve midye) sindirim oranı üzerine etkisi araştırılarak; midyenin 0.075 g/saat lık sindirim oranına, hamsinin ise 0.063 g/saat lık sindirim oranına sahip olduğu gözlenmiştir. Bu oranlardaki farklılık hamsi ve midyenin farklı biyokimyasal içeriklere sahip olmasından kaynaklanmaktadır. İlk yem olarak verilen hamsiden 4 saat sonra kullanılan midyenin verildiği ardıl yemlemede, ikinci yemin birinci yemin sindirimini yavaşlattığı bulunmuştur. Verilen yemlerin tek ve ardıl yemlemedeki sindirimi karşılaştırıldığında, ardıl yemlemedeki sindirimin tek yemlemedeki sindirime nazaran daha yavaş olduğu bulunmuştur.

Anahtar Sözcükler: Karadeniz, Mezgit, Gıda Doyum Noktası, Sindirim Zamanı (GET).

Introduction

Estimates of gastric evacuation rates are needed for most models of daily ration and food consumption in the wild. Food consumption rates of fish, and factors which govern the feeding rate, are of interest in studies of fish production, the ecology of fish populations and even the behaviour of fish (1). With the current interest in multispecies fisheries and total ecosystem management, it is essential to understand the role of the fish predators within the ecosystem (2, 3). As a part of this, it is necessary to quantify the feeding habits and daily ration of such species (4-10). Such studies eventually provide important information on interspecific feeding interactions, such as competition and predation (11).

The aim of this paper is to increase the present information of the process of gastric emptying in the

whiting. Although a wide variety of species have been examined in some detail, results from whiting are scattered, were mainly carried out on fish from the North Sea and are not always in agreement. For example Bromley (12), Robb (13) and Seyhan (10) disagree about the effect of increasing meal size on the gastric emptying rate. Gastric emptying studies were carried out by many authors (10, 12, 13, 15, 16) for the whiting found in different parts of the world. In the Black Sea however, no study of comparable quality has been attempted.

Since gastric emptying is a complicated process and its rate is known to show fluctuations in response to changes in a number of biotic and abiotic factors; the results obtained are also sensitive to the design of the experiment. Therefore, in this laboratory study, the rate of emptying in small whiting fed on natural prey, namely anchovy, *Engraulis encrasicholus* and mussels, *Mytilus galloprovincialis* was studied. Gastric emptying curves were drawn based on observations of stomach contents collected at frequent time intervals. Using single meals, the Gastric Lavage Technique was applied to study fluctuations in gastric emptying rate with changes in prey type. The interaction of double meals and the effect of the skin of the anchovy on gastric emptying rates were also investigated.

Materials and Methods

Whiting (*Merlangius merlangus euxinus*) ranging in weight from 32-59g were caught by rod and line off Çamburnu-Trabzon (Eastern Black Sea) and were acclimated to the laboratory tank conditions, in a normal light-dark cycle (12 hours each), for four weeks prior to use. All individuals were placed in a holding tank where they were fed every day on finely chopped pieces of prey species which were to be used in the experiments. Before the experiments, each fish was first weighed and then transferred to the experimental aquaria where each one was held separately in the compartments. These fish were given a further 7 days to acclimate to the conditions of the experiment. Prior to each experiment all fish were deprived of food for 3 days as done by Basimi and Grove (8), Seyhan (10) and Robb (13) to ensure complete emptying of the alimentary tract. The temperatures stated in the text reflected natural fluctuations, and remained within 11-16°C.

Return of Appetite Following a Satiation Meal

After being deprived of food for 3 days, twelve individual fish (48±8.30 g) were given mussels from a preweighed supply until food was refused. This stage was reached after between 1 and 3 minutes depending on the individuals. The return of appetite was then measured by offering the fish more food, after a predetermined deprivation interval (4, 8, 12, 24, 30, 36 or 48 h). Every fish was tested at each deprivation time. The experiment was carried out at 11-13°C.

Effect of Skin on Gastric Emptying and Double Feeding Interaction in Small Whiting

Seyhan (10) has stated that one of the factors which might affect the gastric emptying in whiting is the skin of sprat (*Sprattus sprattus*). Therefore at this stage, 2 experiments were performed at 15-16°C to see whether it was the case for anchovy. First, the emptying of a single anchovy (2.29±0.12 g, n=12) in the small whiting was investigated. Second the emptying of skinned anchovy (2.31±0.07, n=12) fed to small whiting (47±9.70, n=12) was studied. In each experiment, four hours after the first feeding one or two fish, depending on the availability of experimental animals, were sampled and lightly anaesthetised in approx 0.006 g/l MS222, Tricaine Methanesulfonate of aerated seawater as done by Seyhan (10) and Foster and Pulsed (17). Samplings were then carried out at different time intervals until the first empty stomach was observed (10, 12, 21). Gastric lavage technique is an efficient way of recovering the stomach contents since it was tested that the difference between the residuum provided by gastric lavage and serial slaughter is not significant (10, 17) except for in some cases where prey items were at an early stage of digestion and could not be reached out the oesophagus (10). In this case forceps were used to remove the prey items. The stomach contents were measured using gastric lavage (10, 16, 18). A polythene tube 0.5 cm in diameter, attached to a hand pump, was inserted through the mouth and pharynx to the distal region of the cardiac stomach and the contents were gently flushed out with seawater (10). The wet weight of the blotted recovered items was recorded to the nearest 0.001 g.

In order to investigate the effect of the prey type on gastirc emptying in small whiting (48±0.03 g, n=7) another experiment was performed in which mussels (2.16±0.03, n=7) were used as prey in contrast to the previous experiment in which the emptying of unskinned anchovy was studied. The min temperature was 16°C throughout the experiment.

Next, the interaction of two meals was investigated. Whiting were first fed one anchovy (2.09±0.07, n=12) and 4 h later (calculated from the end of the anchovy meal) they were fed an additional meal consisting of exactly the same amount of mussels (2.1 g). Two fish

were sampled soon after the mussels were ingested and the others thereafter at regular intervals. The food remains were obtained using gastric lavage and separated for weighing according to whether they were from anchovy or mussels. The temperature was 15°C.

Data Analysis

Gastric emptying in whiting was best described by a linear rather than a curved function (10, 13). In all experiments therefore, a Linear Evacuation Model was used for the pattern of the emptying of the stomach contents. Data were fitted using wet mass (g) since it is the quantity measured for the food consumption computations (6, 10, 13, 16). The Gastric Emptying Rates (GER, g/h) were estimated as slopes of the regression lines, for each data set. The data sets were tested using the General Linear Model (GLM).

Results

Return of Appetite After a Single Satiation Meal

Following the deprivation time (3 days) each fish was fed until satiation. The average satiation meal for the size of whiting studied (48±8.30 g, n=12) was 4.90±2.99 g. (approx. 10% body weight, bw.) at 11-13°C. The return of appetite after a single satiation meal for the small size of whiting is given in Figure 1. It was reported that the relationship between food ingested and deprivation time was best described by a linear function for the whiting (10). That was also the case for the present study although a high variation among individuals was observed. In addition the excepted amount of food evacuated from the whiting's stomach was estimated using the Gastric Evacuation Model proposed for the whiting (10).

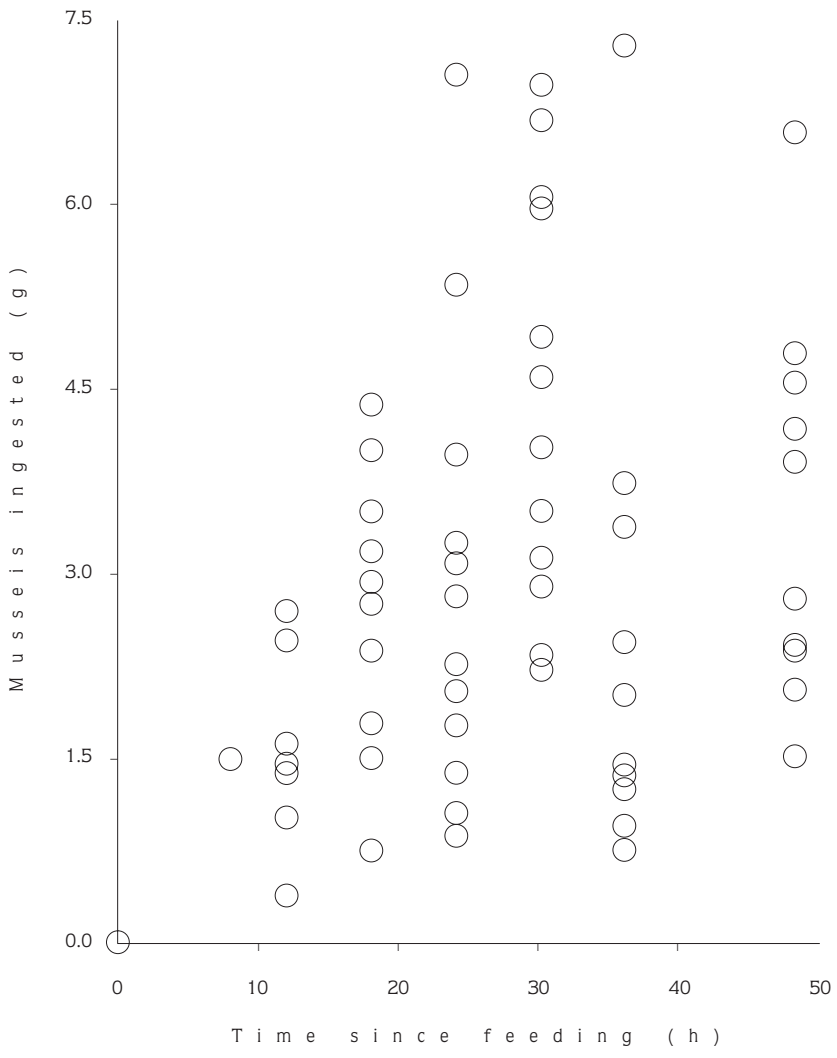

Figure 1. Return of appetite for small whiting (48±8.30, n=12) after a single of mussels.

Effect of Skin on Gastric Emptying and Double Feeding Interaction in Small Whiting

Since it is well known that the presence of well formed resistant skin of the sprat (*Sprattus sprattus*) and butterfish (*Peprilus triacanthus*) is the major limiting factor on gastric digestion in whiting (10, 19). An experiment was performed to test this phenomena for anchovy and the results obtained from the emptying of a single skinned sprat together with an unskinned one are shown in Figure 2. As seen in this figure, the decrease in the stomach content with time was best described by a linear function. The overall emptying process showed that gastric emptying of the unskinned anchovy was completed in 34 h whilst the skinned anchovy was in 30 h corresponding to the gastric emptying rates of 0.063 gh^{-1} and 0.071 gh^{-1} respectively. This difference was found to be significant (GLM, $p<0.05$).

Comparison of Evacuation Rates of Anchovy and Mussels In Single and Double Meal Experiments

Two different natural prey, anchovy and mussels, used at 15°C to determine the effect of prey type on the gastric emptying rates showed variation such that the gastric emptying rate of mussels increased by about 19%, corresponding to shorter gastric emptying times (28 and 34 h for mussel and anchovy respectively). It was found to be significant (GLM, $P<0.05$). The time course of the emptying of the anchovy and mussels at 15°C is shown in Figure 3.

In the previous experiment, whiting had been deprived of food for 3 days, so that residua of the previous meal could not interfere with the observations. However under field conditions, fish will digest organisms which enter the partly-filled stomach and this may lead to

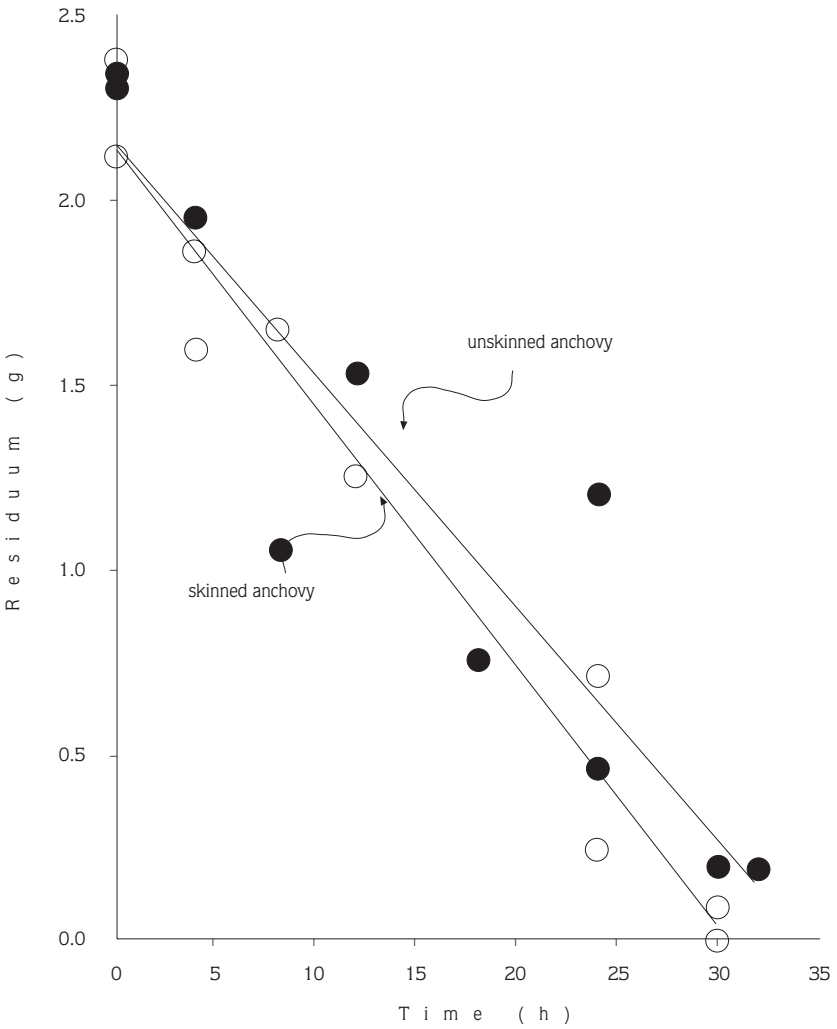

Figure 2. Effect of skinned and unskinned anchovy on gastric emptying in small whiting at 15°C.

altered digestion rates (10). Therefore the emptying rates of two consecutive meals of approx. 2.20 g each, fed to small sized whiting 4 hours apart, were tested. The anchovy was presented first, followed by the mussels so that residua of each prey could be recognised. The emptying patterns for both the anchovy and the mussels since the first feeding under single and double feeding regimes, are given in Figures 4 and 5. Table 1 shows the Gastric Emptying Rates (GER) that followed. A significant change in the GER of both anchovy and mussels was observed (GLM, $P<0.003$ and $P<0.01$ for anchovy and mussels respectively). As seen in Figure 4, a sudden decrease in the residuum of the predigested anchovy was somewhat slower resulting in a considerably later completion of the emptying of all the anchovy. The same effect was also observed in the emptying process of the mussels as gastric emptying time (GET, h) of mussels under double feeding regime increased.

Discussion

Potential Biases in the Results

For the study in the laboratory, one of the limiting factors on the result is the availability of healthy animals to be studied. The technique being employed also effects the result. In this series of experiments, the designs of the experiments adopted were strongly constrained by the availability of the fish. However, the results obtained in this study were checked using the previously published information (10). It was seen that there has been no difference especially for the gastric emptying rates for similar prey items (e.g., sprat used by Seyhan (10) and anchovy used in this study). The other potential bias is the handling, anaesthetisation and stomach flushing process. This possibility would be difficult to dismiss totally, as all fish used in the experiment were kept and fed for more than 2 weeks and during this time they were not used in any other experiment. Therefore this was expected not to unduly affect the fish since they fed soon after recovery and appeared healthy and behaved normally.

Experiments using the single meal technique the decline in stomach content weight with time do not take into account the fact that the evacuation rate will decrease at low levels of stomach fullness. This occurs in response to the increased proportion of indigestible material in the stomach. If the time between feeding and examination is such that some of the fish have emptied their stomach then the estimation of the evacuation rate will be biased (12). If this is not allowed for either in the design of the experiment or in the analysis of the data, then underestimates of evacuation rates and exaggerated curvilinearity can be obtained (20). Therefore in this study as soon as fish with an empty stomach was encountered, sampling ceased.

Factors Affecting Gastric Emptying in Small Whiting

It has been reported that the gastric emptying in whiting is best described by a linear function (10, 12, 13, 16). However data collected by gastric lavage showed quite high variability. Different individuals of approximately the same weight examined under the same conditions often showed food residues about ±30% of the average contents recovered from the stomach. That finding is in agreement with the previous study performed by Seyhan (10) who worked on the same species, Irish Sea whiting: *Merlangius merlangus.*

Seyhan (10) reported evidence that natural diets may reflect a combination of 2 factors:

a) the relative size of stimulation to the stomach (% distension) and,

b) the existence of protective, fibrous skin which may resist digestion.

Feeding type	Temperature (C°)	GER (g/h) Normal	GER (g/h) Fixed	R^2	P	n
Single Feeding						
Anchovy	15	0.063±0.008		0.86	0.001	11
Mussels	16	0.075±0.007		0.94	0.001	8
Double Feeding						
Anchovy (overall)	15	0.047±0.007	0.063	0.82	0.001	9
Anchovy after 2nd feeding	15	0.030±0.004	0.057	0.84	0.001	9
Anchovy before mussels ingested	15	0.161±0.020	0.186	0.97	0.015	4
Mussels	15	0.047±0.009	0.057	0.76	0.002	9
Overall (anchovy+mussels)	15	0.095±0.018		0.81	0.002	9

Table 1. Gastric empying rates of anchovy and mussels under single and double feeding regimes and its statistical parameters (GER: Gastric Emptying Rate; R2: Regression Coefficient; P: Probability; n: Number of points on which the regression analyses were performed).

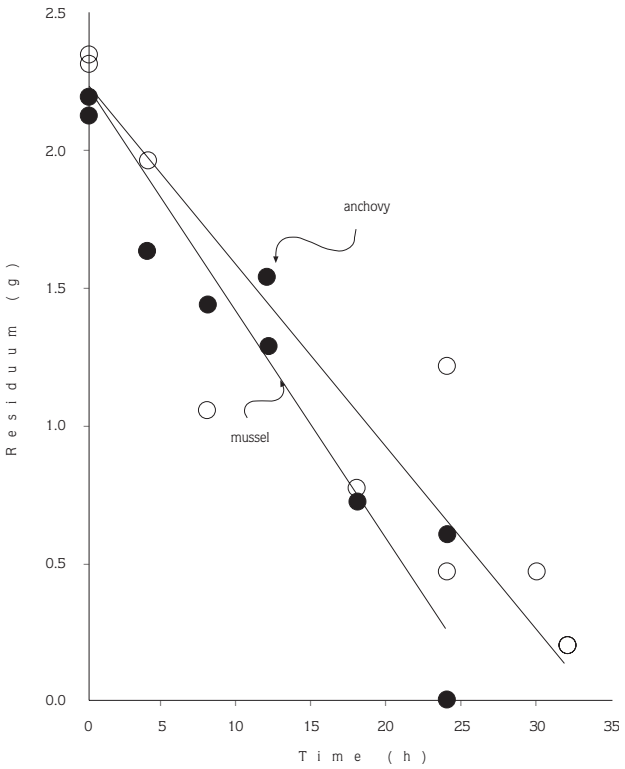

Figure 3. The effect of prey type on gastric emptying in small whiting at 15°C.

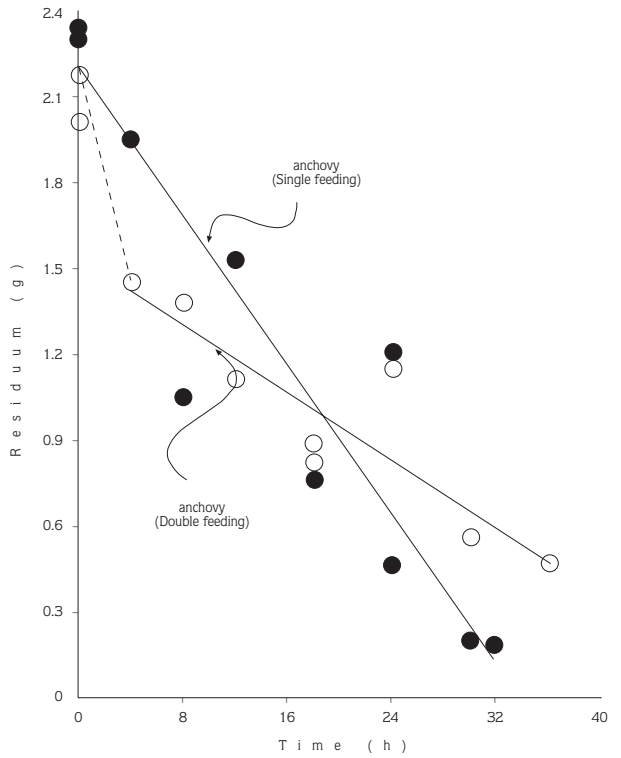

Figure 4. The effect of subsequent meal mussels on the emptying of the first meal (anchovy) at 15°C (The dotted line indicates the rapid decrease in residuum soon after the ingestion of the second meal).

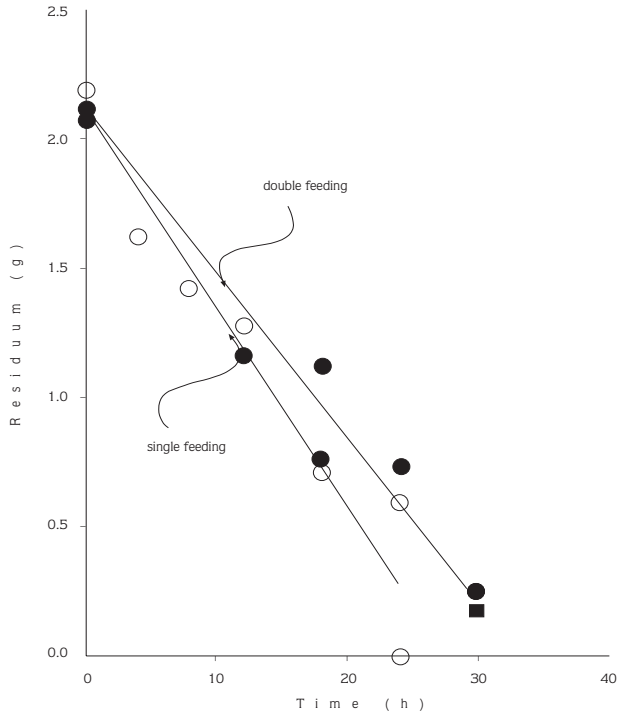

Figure 5. The effect of single ond double feeding on the emptying of mussels whiting (Solid square is the value which was not used in the regression 20).

In this study, therefore "**b**" was tested using anchovy as a prey type which has similar characteristic features with the sprats used by Seyhan (10). The result has showed that the skin is one of the factors affecting gastric emptying in fish feeding on a natural diet.

The chemical composition, including indigestible natural material of food organisms or artificial diets, is an important variable in gastric emptying studies. Many factors are important, whether related to the feeding habits of natural fish populations or fish culture practice. There is an increasing body of evidence showing that energy content affects gastric emptying in fish (21-24). It was indicated that an increase in energy content of a given prey type leads to an increase in the time required to empty the stomach. In this study two prey items namely, anchovy and mussels were evacuated at different rates. That was expected since the energy content of anchovy was 193 kcal/100 g (25) whilst that of mussels was stated to be approximately 85 kcal/100 g (26).

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