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Impact of high dietary carbohydrates on feed evacuation of common carp (*Cyprinus carpio* L.)

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Abstract

A total of 120 young common carp (Cyprinus carpio L.) ranging in weight from 25 g to 39 g were used to study the effect of feeding high carbohydrate diets on feed evacuation. Fish were fed at the rate of 3% body weight on three rations, standard ration SR (37%), and medium carbohydrate ration MCR (42%) and high carbohydrate ration HCR (53%) for 70 days in oval plastic tanks using 4 replicates for each treatment. Pellets enriched with 2% iron powder as a marker were used for additional 21 days for X-ray tracing. Pre-prandial starvation for one day was adopted before feeding the experimental diets and X–raying the fish at six intervals (1, 4, 8, 12, 24 and 48 h) after feeding to estimate the evacuation rate and time. Results showed that high carbohydrate ration was evacuated faster than MCR and SR ration. After 24 hours of feeding, 91 % of the HCR ration was evacuated compared with 84 and 79 % in both SR and MCR ration. Differences between HCR and the other two rations were significant ($P<0.05$).The rate of evacuating HCR ration ranged between 6.7 -7.6 % per hour compared with 5.5 -6.3% per hour for MCR and 5.7-7 % per hour for SR ration. The theoretical evacuation time of HCR reached 26 h. compared with 30-31 h for the MCR and SR rations respectively. The results were discussed in term of the role of inert and indigestible fibrous materials which can accelerate the passage of food in fish alimentary canal. The validity of the radiographic method for estimating the rate of evacuation in omnivores carp was also evaluated.

Introduction

Production in both wild and cultivated fish populations are largely depends on food consumption and the way in which food is utilized within the body. Important aspects of trophic dynamics of fish include appetite, meal size and frequency, rate of gastric evacuation and assimilation efficiency and many other methods have been devised to study these parameters *[1]*. The development of optimum feeding schedule and gastric evacuation time for cultured fish include variables like temperature, season, body size, gut capacity, and metabolic rate. The rate of gastrointestinal evacuation is one of the major determinants of feeding rate *[2]*. Many factors such as temperature, meal size, fish size, dietary composition and feeding frequency will influence gastrointestinal evacuation *[3]*. One of the popular methods is to use the X-radiographic techniques to observe and describe the movement of food items in the alimentary tracts of fish after feeding *[4]* and *[5]*. It has been extensively reported that return of appetite is probably controlled by the stomach evacuation rate in teleostean fish species *[6]*. It was demonstrated by Tekinay and Guner, (2001) *[7]* that the appetite was more dependent on the bulk of the food rather than the dietary composition (energy

and protein composition) in short term feeding. 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 *[8]*. Dietary fiber improved gastric evacuation time of rainbow trout fed purified diets *[9]*. The poor performance of salmonids fed certain types of fiber may result from a combination of factors including poor digestion and faster gastric emptying rates, which in turn affect feed intake and utilization of nutrients *[10]*. The aim of the present investigation is to test the impact of adding more carbohydrate sources to common carp rations on feed evacuation rate and emptying time in gastric and whole alimentary canal.

Materials & Methods

Young common carp (*Cyprinus carpio* L.) ranging in weight from 25g to 39g were used as experimental fish. They were obtained from a local fish farm, near Kirkuk. A total of 120 fish were used during the whole experiment. Upon arrival to the laboratory fish were sorted depending on size, then fish of narrow size range were weighed and distributed randomly among the experimental oval plastic tanks. They were left without feeding for three days for adaptation to their new in-door environment. Feeding with standard ration (control pellets) resumed after adaptation period for five days. The experimental trial continued for 70 days. Fish used for evacuation trials were fed for 21 days on special diets, supplemented with feed markers (Iron Powder), before sampling.

A. Experimental system and design

Twelve plastic tanks were used in this feeding experiment. Each tank contained almost 100 liter of freshwater pumped from a nearby well to accommodate ten fishes. Artificial aeration was maintained for each tank during the whole experiment via electric air generating motor. Water quality parameters such as temperature, pH and dissolved oxygen were monitored weekly using normal thermometer and electric meters. During the experimental water temperature in the rearing tanks varied between 25- 27°C. The recorded levels of dissolved oxygen concentration in the plastic tanks ranged between 7.4- 8.6 mg / L. They were near saturation levels due to the artificial aeration throughout the whole experiment. Water pH values were slightly alkaline and varied in narrow range between 8.1- 8.4. The experimental design consists of three treatments with four replicates each, all arranged randomly in the experimental plastic tanks which were held on iron shelf inside the Fish Lab of the Animal Production Department in the Faculty of Agricultural Sciences at University of Sulaimani. The tanks were cleaned from fish feces and non-consumed feed every alternative two or three days by siphoning via small manual pump and sponge. The original level of water was maintained by replacement of the lost water. The experimental treatments are as follows: The first treatment (Standard Ration SR) was used as a control treatment that contained the low level of carbohydrates (37.04%); the second treatment (Medium Carbohydrates Ration MCR) contains the medium level of carbohydrates (42.74%); the third treatment (High carbohydrates Ration HCR) contains the highest level of carbohydrates (53.11%).

B. Diet formulation

Three different kinds of experimental diets were formulated using the following ingredients: Fish meal, Soya bean meal, Vegetable oil, Wheat bran, Wheat flour, Barley, Corn, Starch and Vitamin-Mineral Premix. Formulation of the experimental diets (SR, MCR and HCR) was done depending on the proximate analysis of the feed ingredients. The ratios of each ingredient are shown in Table (1) for the three feeding rations.

The ingredients were mixed with nearly 30% water to obtain suitable dough then the dough passed through electrical mincer for making pellets. The pellets were dried at room temperature for a few days and then crushed to suitable pellets size. The proximate composition of the experimental rations is shown in Table (2). The three experimental rations were further reformulated for the purpose of supplementing feed markers for evacuation tests using the same manufacturing procedure. Iron powder at the level of 2% was added as a marker to estimate the evacuation rate.

Table -1: Composition (%) of the experimental rations fed to common carp.

Feed stuffs	SR	MCR	HCR
Fish meal	15	15	10
Soya bean meal	35	25	15
Vegetable Oil	5	5	5
Wheat Bran	11	15	15
Wheat Flour	10	10	15
Barley	10	12	15
Corn	10	12	15
Starch	2	4	8
Premix	2	2	2
Cost (ID)	2741	2550	2092

Table -2: Proximate composition (%) of the experimental rations

Components	SR	MCR	HCR
Total Carbohydrates (TC)	37.0	42.7	53.1
Crude Protein (CP)	28.7	25.6	22.7
Ether Extract (EE)	10.6	9.8	8.7
Ash	5.8	5.4	4.1
Moisture (H_2O)	17.9	16.5	11.4
Total	100.0	100.0	100.0
Nitrogen Free Extract (NFE)	27.1	31.9	39.7
Crude Fiber (CF)	9.9	10.8	13.4

C. Feeding Method

The fish were fed twice daily at 9:00 am and 2:00 pm. Feeding rate started with 8% of biomass during adaptation period, then gradually reduced to the satiation level of 3% of body weight according to monitoring the feeding behavior of the fish. Feed rations were applied manually to each tank. Care was taken to provide the ration gradually to allow fish to consume the pellets individually. Fish in all tanks were weighed together every two week. The feed amount was then re-adjusted according to new weights. Fish feces and traces of the remained feed were removed by siphoning to keep the tanks clean.

D. Measurements of Evacuation Rate

Iron powder at the rate of 2% was added to fish diet in the three experimental treatments. Four fish were selected to represent each treatment and fed the iron powder diets at the rate of 5% of their body weight. Adaptation period on the new diets continued for four day in the same laboratory. After adaptation, fish were quickly transported to the Internal Teaching Hospital in Sulaimani City using Styropor box supplied with continuous aeration. They were left without feeding for one day prior to resuming feeding experiment. Feeding started at 09:00 am the next day on the iron powder diets for all treatments at the rate of 5% body weight. Sampling for X-ray radiation was taken at various periods after feeding, namely (1h, 4h, 8h, 12h, 24h and 48h) to cover the food passage time. Anesthetic solution was prepared in alight dose from the powder of the stalk of Carnation flowers and used for short anesthetization of fishes to prevent movement during X-raying. Fish recovered immediately upon returning to the water after x-raying. The unlikely effect of anesthetic on evacuation, if any, is assumed to be similar in all treatments due to similar handling process. The X-raying plates would be used to count the number of iron powder inside the alimentary canal of the fish. Number of iron particles can be expressed as amount of feed using standard curve (Fig. 1), previously prepared from an x-ray chart representing pellets with known weight and number of iron powders (Plate 1).

Median value of gut content with times was found to be nonlinear one. In order to linearize the evacuation curves, the square root transformation was tried:

$$\sqrt{W} = a - bT \quad \text{..... Jobling (1981) } [11]$$

Where W is stomach or gut content (% dry weight of fish) and T is time (h). The square root formula was chosen as it linearized the relationship without the confusion of negative values shown by the log transformation. Linear regression lines were fitted for the relationship between the square root of the median values of gut contents and times by the method of the least square. The percentage evacuated from the initial meal after different periods of time was also calculated. The evacuation rate was estimated as % test meal evacuated per hour for each period after feeding. Evacuation time and meal size were determined from regression line as intercepts with X and Y axis respectively. Evacuation time was also measured by the intercept of the regression line with a horizontal line representing the 0.5 % level of gut contents since they are considered to be empty of food and containing material derived from stomach at this level *[4]*. The experiment was conducted using the (CRD) design and general linear models (GLM) procedure of XLSTAT. Pro.7.5 One way (ANOVA) *[12]*. Duncan's multiple range test was used to compare between means of the experiment treatments.

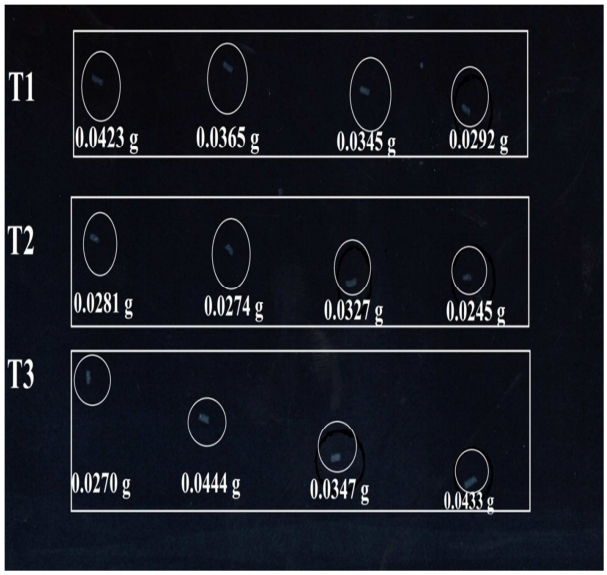

Plate- 1:X-ray radiograph of random samples of pellets with iron powder representing the experimental rations.

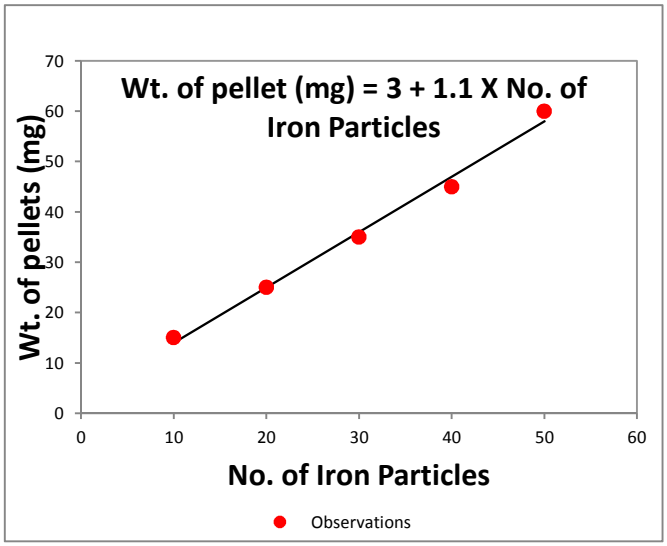

Fig-.1:Standard curve for the linear relationship between pellets weight & number of iron particles.

Results and Discussion

The radiographic method of Talbot and Higgins (1983) *[4]* was adopted to study the evacuation rate of rations with three levels of dietary carbohydrates in common carp. In such method a linear relationship is established between number of iron particles in pellets and pellets weight as seen in Figure (1). Such relationship enables researchers to monitor the decrease in gut contents by weight from counting the number of iron particles appeared in the gut through x-ray photographs. Photographs showing different stages of feed evacuations are shown for the three rations SR, MCR and HCR in plate (2). The validity of this method for the study of feed evacuation in common carp is not at the same level as that shown by Talbot (1985) *[2]* in rainbow trout. The main interference was the complicated coiling system of the common carp gut, compared with the straight gut of rainbow trout. This, as shown in the previous plates caused crumple accumulation of the iron powder particles in the coiled intestine. Moreover, the lack of true stomach in the common carp prevented the calculation of gastric evacuation. Median values of feed evacuation expressed as gut contents, percent evacuation and % evacuation rate per hour are all illustrated in Table (3). It can be seen that the high carbohydrate ration was evacuated faster than MCR and SR ration. After 24 hours of feeding, 91 % of the HCR ration was evacuated compared with 84 and 79 % in both SR and MCR ration. Differences between HCR and the other two rations were significant ($P<0.05$). The rate of evacuating HCR ration ranged

between 6.7 -7.6 per hour compared with 5.5 -6.3 per hour for MCR and 5.7-7 % per hour for SR ration. The explanation of the faster rate shown in the ration with 53% carbohydrates could well be related to the amount of fibers in the HCR ration which act as inert materials acceleration gut motility and evacuation rate. Similar findings were previously reported in rainbow trout fed on fiber-rich rations *[13]* and salt enriched diets by Salman (1987) *[5]*.

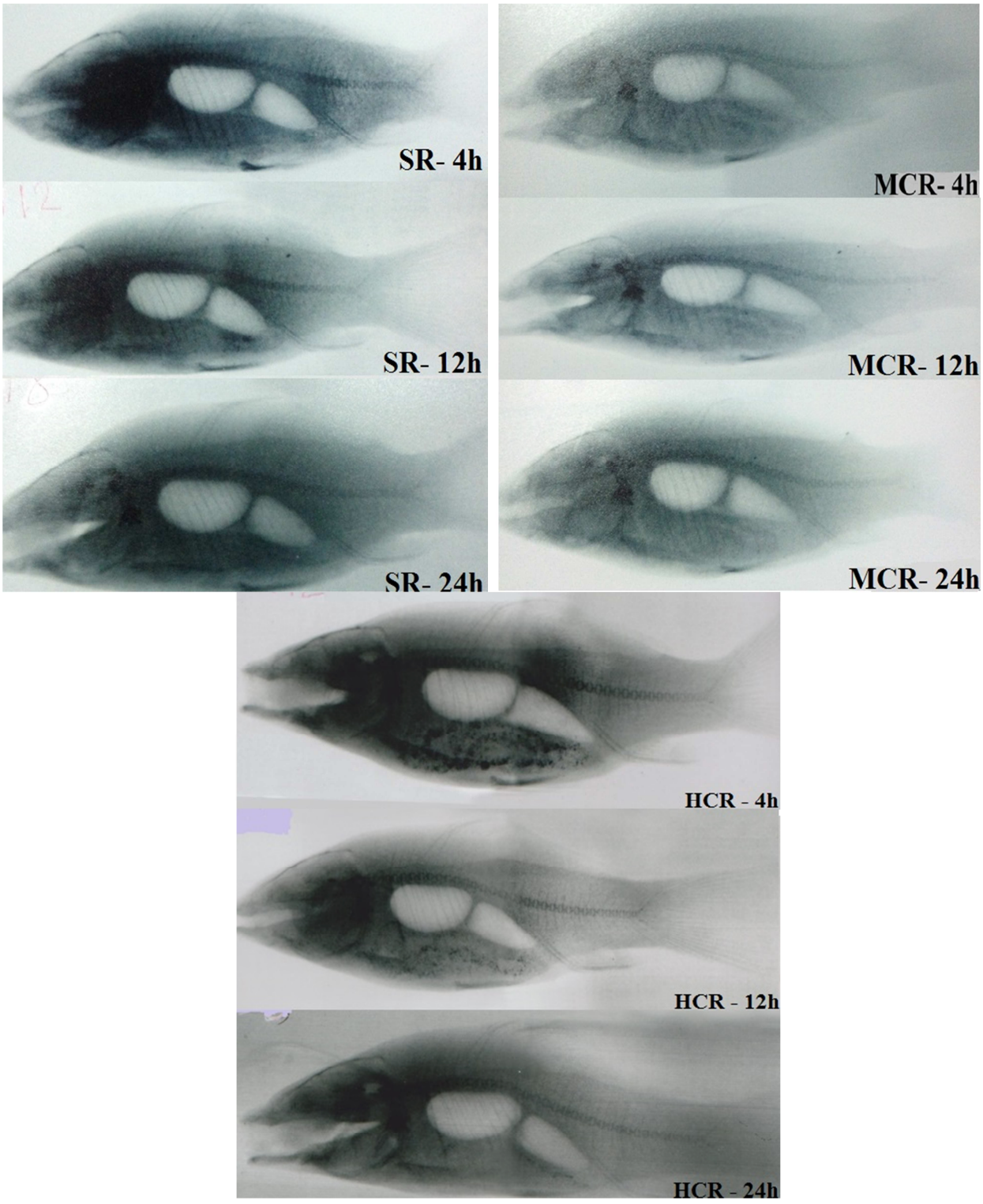

Plate -2: X-ray radiograph of common carp taken at 4, 12 and 24 h after feeding on SR, MCR and HCR.

Gut evacuation data for the three rations are also expressed graphically into an evacuation curve in Figure (2) using the actual gut contents expressed as percentage of dry weight of fish. Square root transformation was adopted to linearized the evacuation curve as suggested by Jobling and Davies (1979) *[14]*. From the linear regression lines of the evacuation curves (Figure 2), calculation of the meal size and the evacuation time was possible by measuring the intercept with x-axis and y-axis respectively. Such data are presented in Table (4). The theoretical evacuation time of HCR reached 32 h. But considering 0.5% gut content as empty gut instead of 0% as suggested by Talbot (1985) *[2]* and Salman (1987) *[5]*, the actual evacuation time for the HCR ration approached 26 hours only. The 0.5% gut contents is considered as amount related to gastric and gut secretions of mucus and enzymatic remains and not as actual food *[2]*. As for the MCR and SR rations, the evacuation time was more than HCR approaching 37-40 h (30-31 h actual times).

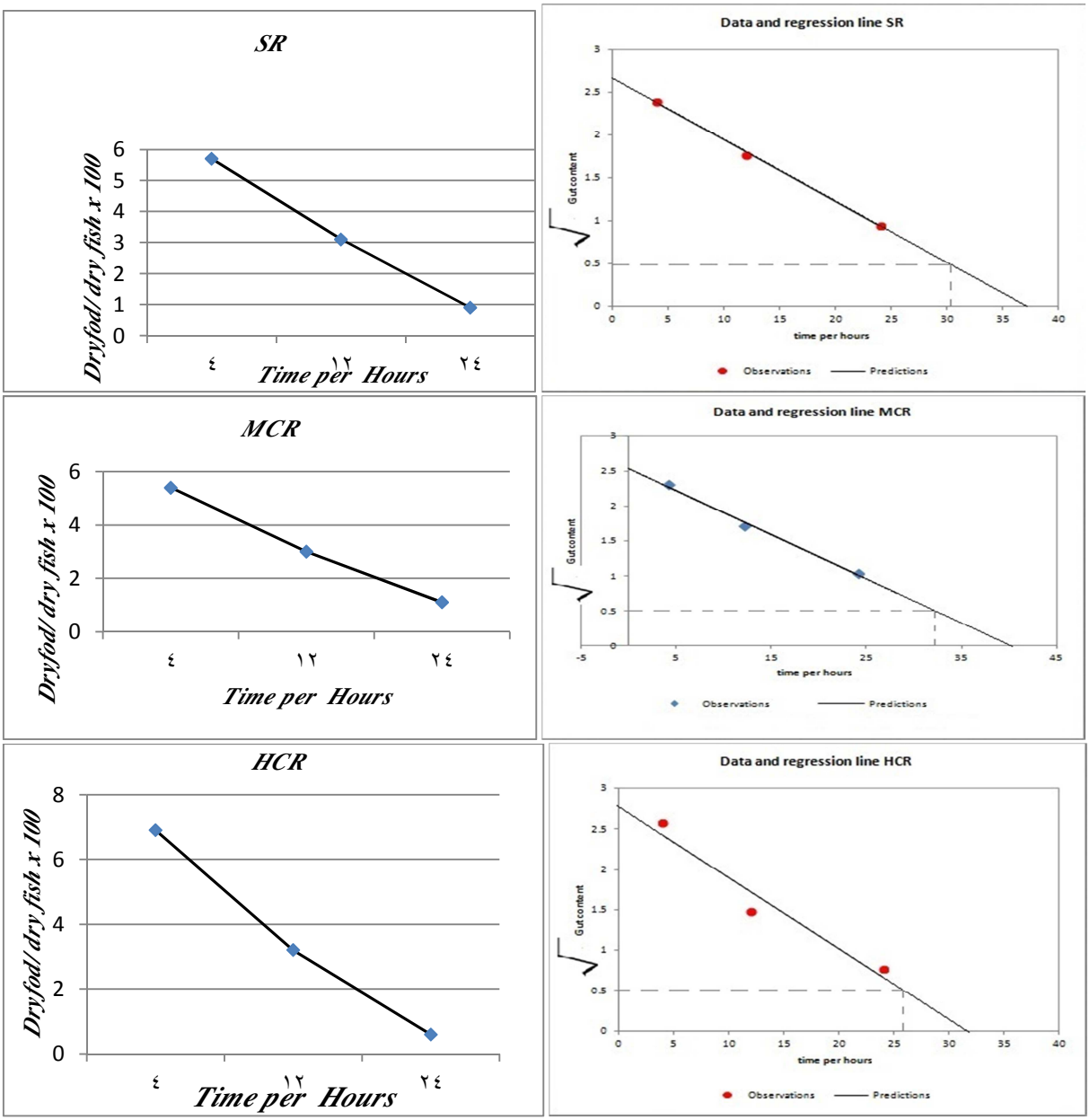

Figure -2: Evacuation curves and regression lines for the three rations (SR, MCR and HCR) fed to young common carp

Table -3: Data representing gut content, % evacuation and % evacuation/hours values in young common carp fed on SR, MCR and HCR.

Dietary treatments		Hours after feeding		
		4	12	24
SR	Gut content	5.7	3.1	0.9
	% Evacuation	0	45.6	84.2
	% Evacuation/ h	0	5.7	7.0
MCR	Gut content	5.4	3.0	1.1
	% Evacuation	0	44.4	79.6
	% Evacuation/ h	0	5.5	6.6
HCR	Gut content	6.9	3.2	0.6
	% Evacuation	0	53.6	91.3
	% Evacuation/ h	0	6.7	7.6

Table -4: Meal size, theoretical & actual evacuation time in common carp

Dietary treatments	Meal size % dry feed/ dry wt. fish x 100	Theoretical Evacuation time (h)	Actual Evacuation time (h)
SR	2.6	37	31
MCR	2.6	40	30
HCR	2.7	32	26

Gastrointestinal or gastric evacuations have been studied in some carnivorous species with well-developed stomach, such as Atlantic salmon *[15]* and rainbow trout *[16]*. The information on the intestinal evacuation in common carp *Cyprinus carpio*, an omnivorous and stomachless fish, are relatively rare. Zhen *et al*. (2009) *[17]* revealed that the intestinal content tended to decrease gradually in grass carp *Ctenopharyngodon idella* until the 12th h with no significant difference was found between two diets with different energy level. Such fast evacuation was not seen in our results as evacuation continued up to 24 hours. This could be related to pre- or post-prandial starvation and differences in meal size. Grass carp have much longer intestine, 2.3-2.5 times to its body length, than carnivorous fish *[18]*. The long intestine could enlarge the surface area of digestion and absorption and it also could lead to shorten the evacuation time. Li *et al.* (1980) *[19]* found in a single daytime, the time used of grass carp for eating is about 20h and it only stops eating in midnight for 4 h in natural environment.

The present results are similar to many other studied fish species, in which gastrointestinal evacuation time normally exceed 24h *[20], [21], [22], [23], [3] and [24]*. Feed evacuation in common carp may be explained by the large surface-to-volume ratio in intestine which makes the progress of digestion and absorption turn evener and smoother than other fish which have stomach and shorter intestine. Despite differences in alimentary canal anatomy and feeding habits, our results are comparable to those reported by Tekinay *et al*. (2003) *[16]* in rainbow trout, *Oncorhynchus mykis.* Regarding the evacuation of carbohydrate-enriched diets, they stated that gastric evacuation rate of the low carbohydrate diet that contained (15%) carbohydrate was significantly slower than the median carbohydrate diet that contained (31%) carbohydrate and high carbohydrate diet with (41%) of carbohydrate contents. In conclusion, dietary carbohydrates up to the level of 53% can cause slight acceleration of food passage throughout stomach and intestine of young common carp.

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