# Effects of short-time fasting and feeding frequencies within 24 hours on histology, cholecystokinin and trypsin enzyme activities of digestive organs in juvenile black bream, *Megalobrama pellegrini (Tchang, 1930)*

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## Abstract:

To study the regulation and feedback mechanism of cholecystokinin and trypsin in black bream, *Megalobrama Pellegrini (Tchang, 1930)* 60 days after hatching under 15 days short-term fasting and different feeding frequencies within 24 hours during the same period, M. Pellegrini (wet weight 183.75±61.16 mg, total length 20.74±4.08 mm) developed in a recirculating aquaculture system were selected as the subject. In the short-term fasting trial, the body weight, trypsin, and CCK of the feeding control group (FCG) were higher than those of the fasting test group (FTG). Trypsin and CCK in FTG reached the lowest value on the ninth day and the CCK content reached the highest value on the 11th day. A negative feedback regulation of CCK and trypsin had not been found in this trial. The degree of damage to intestinal chorionic epithelial cells was higher than that of hepatopancreas, and detachment of epithelial cells and the striatal border was the main damage. In the 24-hour daily rhythm experiment, juvenile fish were randomly assigned to (A) once feeding, (B) twice feeding, (C) three times feeding, (D) fasting. CCK showed a minimum value at 1:00$^{+}$ in group A, while a peak occurred at night in group B\C\D and a maximum value in group C, and a single satiety stimulus can lead to increased hunger. The four treatment groups had an apparent closed-loop regulation while the control point of the fasting group (D) shifted forward to the next day. Different feeding frequencies in a single day had no direct effect on the long-term fluctuation of CCK and trypsin daily rhythm. Feeding three times a day was beneficial to the individual growth of juvenile *M. Pellegrini*, a better growth results may be produced in the long term. The purpose of this study was to provide reference data for the daily feeding of *M. Pellegrini* juveniles in intensive culture, in order to promote the increase of production and income of *M. Pellegrini* artificial culture.

**Keywords:** Black bream juvenile; CCK; Trypsin; Fasting; Histology

## Result:

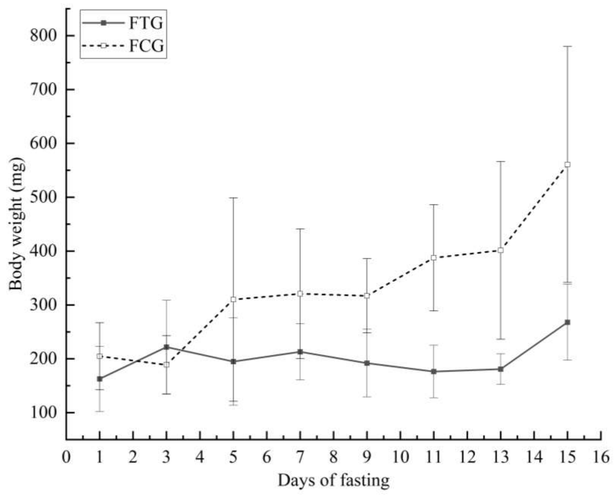


Fig1. The body weight ( mg ) of FTG and FCG within 15 days of fasting test is shown in the line chart. Every morning at 9 : 00, 13 : 00 and 17 : 00 according to the conventional breeding, a total of 3 times. The data were expressed as mean±standard deviation ( n = 3 individuals, n = 3 cans for all groups ). $P < 0.05$ indicated that the difference was statistically significant. The dotted line indicated FCG and the solid line indicated FTG. The growth rate and specific growth rate during the experiment were ( ' -T 'represents FTG, ' -C 'represents FCG ) :

WRG(%) = (FBW-IBW) /W0 SGR(%) =[(lnFBW - lnIBW) / t]×100%

WRG-T=64.56% SGR-T=3.32%

WRG-C=173.8% SGR-C=6.72%

Including FBW is the final weight of the juveniles. IBW is the initial weight of the juveniles and 't' is the time.

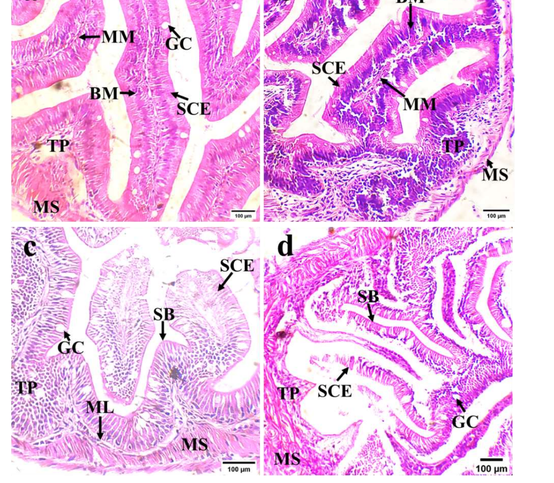


Fig5. The first day of the fasting trial intestinal (middle and anterior intestinal tract) corresponds to figure a, figure b is the ninth day of fasting intestinal slices, figure c is the 13th day of fasting intestinal slices, and the 15th day of fasting intestinal slices was shown in figure d. HE staining, microscope eyepiece (× 40) observation.

MS: muscular, SCE: simple columnar epithelium, ML: mucous layer, BM: basement membrane, TP: tunica propria, SB: striated border, MM: muscularis mucosae, GC: goblet cell

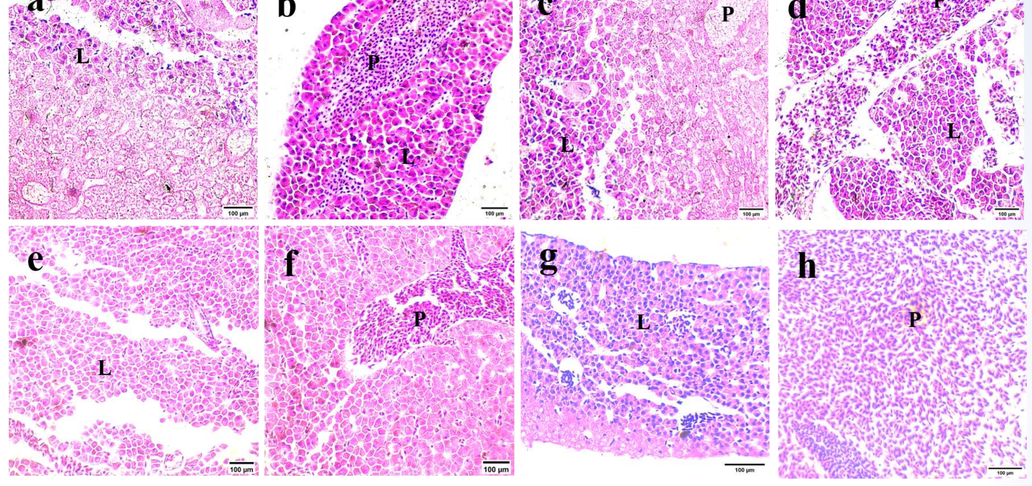


Fig6. Hepatopancreas sections on days 1 and 3 of fasting are shown in a and b. Hepatopancreas sections on days 9 and 11 are shown in c and d. Hepatopancreas sections on day 13 of fasting are shown in e and f. Hepatopancreas sections on day 15 of fasting are shown in g and h. HE staining, microscope observation (× 40).

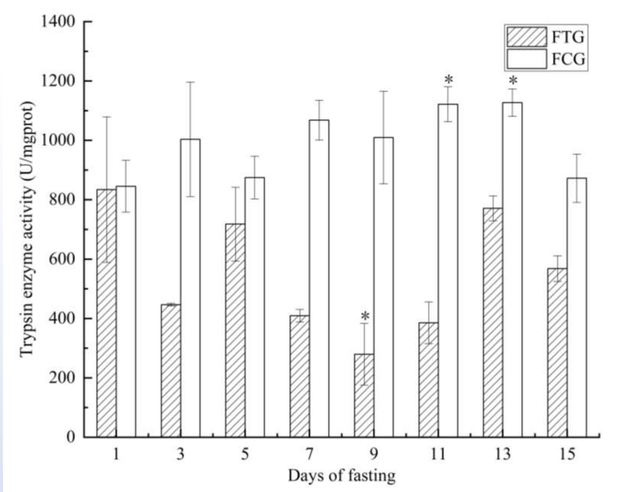


Fig2. The trypsin activity (U/mgprot) of FTG and FCG of the fasting study. The grey stripe indicates the experimental group and the white stripe indicates the control group (feeding at 9:00, 13:00, and 17:00, three times a day as usual). Data are presented as mean±standard deviation (n = 3 individuals, all feeding treatments n = 3 tanks) and $P < 0.05$ is used, "*" represents the significant difference within a group.

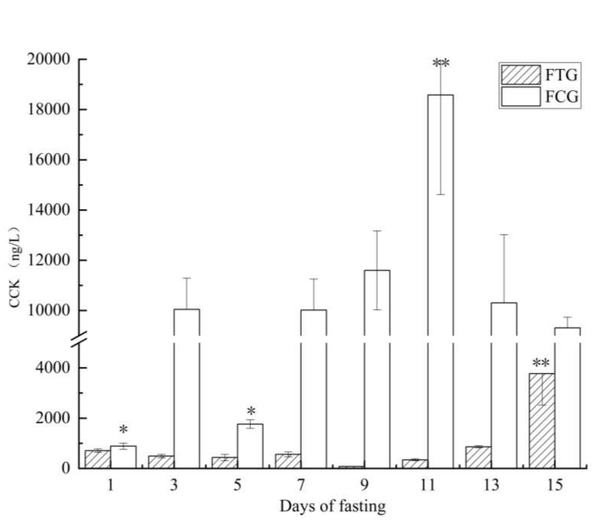


Fig3. CCK levels in the experimental and control groups of the fasting study. The grey strip indicates the experimental group and the white strip indicates the control group (feeding at 9:00, 13:00, and 17:00, three times a day as usual). Y-axis coordinates were processed with breakpoints between 5000 and 9000 scales. Data are presented as mean±standard (n = 3 individuals, all feeding treatments n = 3 tanks), and $P < 0.05$ is used, "*" represents the significant difference within a group, ' ** ' indicates an extremely significant difference.

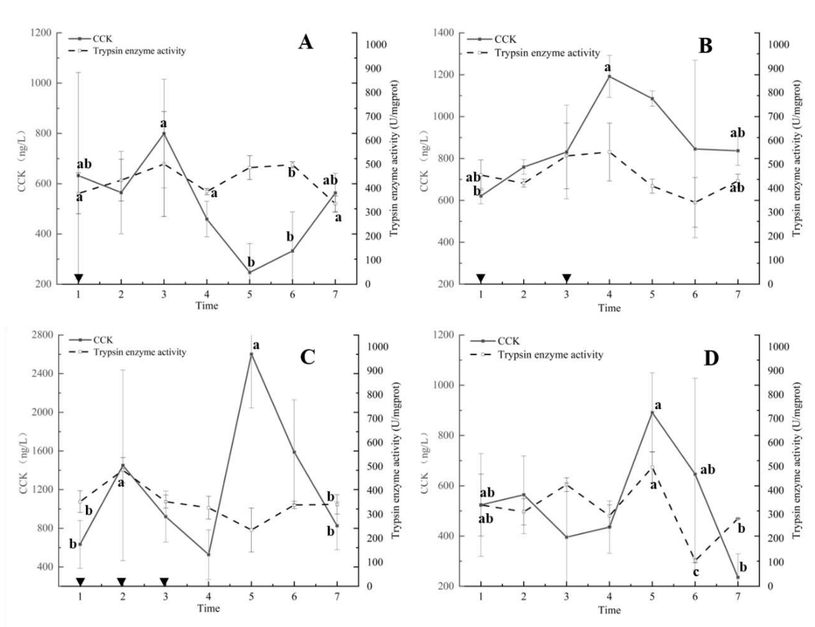


Fig7. The line graph of changes in CCK and trypsin within 24 hours. In the four daily rhythm experimental groups. "▼" indicates the feeding point, A has one feeding point, B has two feeding points, C is the normal feeding control group and has three feeding points, and D is the fasting trial group, of which 1, 2, 3, 4, 5, 6 and 7 correspond to 9:00, 13:00, 17:00, 21:00, 1:00 $^{+1}$, 5:00 $^{+1}$ and 9:00 $^{+1}$, respectively. There are three sets of Y-axes in each graph and data are presented as mean±standard. Different superscripts indicate a significant difference in multiple comparisons ($P < 0.05$)

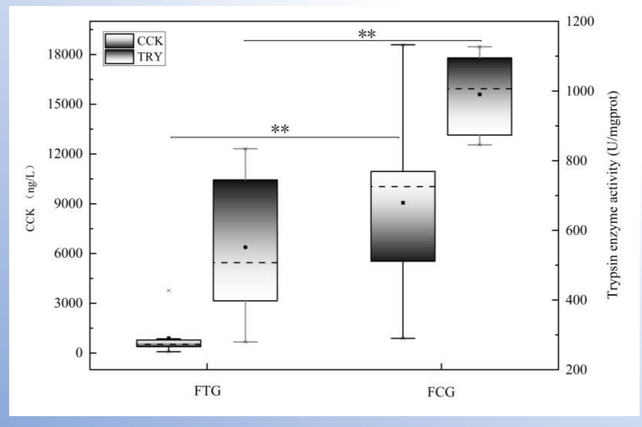


Fig4. CCK content and trypsin activity of FTG and FCG are statistically analyzed between groups. Dark grey represents CCK content, light grey represents trypsin activity (TRY). Data are presented as mean±standard and $P < 0.05$ is used, ' ** ' indicates an extremely significant difference

## Conclusion:

In the short-term fasting trial, there was no obvious negative feedback phenomenon between CCK and trypsin, and the damage to the digestive organs was relatively mild. It can be inferred that the 15-day fasting was not enough to cause *M. pellegrini* to produce real hunger. The daily rhythm experiment in the same period showed that CCK and trypsin had a negative feedback regulatory loop in the circadian rhythm of *M. pellegrini*, and feeding three times a day may produce better growth effects and a single feeding in the morning may induce hunger earlier than fasting throughout the day. Based on the two trials, it was speculated that during the rapid development of juvenile *M. pellegrini*, the fluctuation of CCK and trypsin activity in each day had no obvious relationship with the rhythm during the highly growth level for a long time.