## Pepsin activity and pepsinogen mRNA expression during protein digestion in gilthead seabream juveniles.

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Pepsin is activated from its precursor, the pepsinogen, at low gastric pH environment. In fish with stomach, low pH values are eventually attained during the postprandial period. Previous results indicated that meal timing and frequency affected the daily acidification rhythm. These gastric pH variations may affect the potential digestive capacity in relation to the pepsin activity. In this study we have examined the daily pepsin activity pattern in gilthead seabream *Sparus aurata* juveniles (5-7 g wet weight) maintained under 12L/12D photoperiod and three different feeding regimes: A) one daily meal at 9:00, B) two meals at 9:00 and 17:00, respectively, and C) continuous feeding during the light period, from 9:00 to 21:00. In all cases the same amount of food was supplied. Specifically we have determined the pepsin activity at the actual working pH and compared with the gene expression of mRNA codifying pepsinogen in samples taken periodically during a 24 h cycle.

The results revealed that the feeding protocol affected significantly the rhythm of gastric pH and the pepsin activity pattern. In the regimes A (only one daily meal) and B (two meals), the pepsin activity peaked few hours after the meals, although the evening meal in B produced a higher peak. In the regime C (continuous feeding) the peak occurred in the middle of the feeding period. Lowest total pepsin activity was observed in regime A, and the highest activity with the regimen C. In contrast, the estimated pepsinogen available and the pepsinogen gene expression were practically constant along the daily cycle, with the exception of a maximum in the expression observed just before the morning meal in both regimes A and C. We concluded that two meals or continuous feeding allow a better and prolonged gastric digestion and that the process is mainly regulated by the proton pump at translation level.



Pepsin activity at the actual gastric pH under the three feeding regimes

*This work was financed by projects Aquagenomics CDS2007-00002 and AGL2007-64450-C02-01/02 (MICINN, Spain, and FEDER/ERDF).*