www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences 14: 921-928 (2014) DOI: 10.4194/1303-2712-v14_4_11

Feeding Frequency Differently Affects Post Prandial Patterns of Plasma Glucose, Insulin and Insulin-like Growth Factor I in European Sea Bass (*Dicentrarchus labrax*)

Maria Messina[1,*], Joan Sànchez-Gurmaches[2], Isabel Navarro[2], Joaquim Gutierrez[2], Daniela Bertotto[3], Emilio Tibaldi[1], Francesca Tulli[1]

[1] University of Udine, Department of Food Science, Via Sondrio 2, 33100 Udine, Italy.
[2] University of Barcelona, Department of Physiology, Diagonal 645, Barcelona, Spain.
[3] University of Padova, Department of Comparative Biomedicine and Food Science, Agripolis, Legnaro, Italy.

* Corresponding Author: Tel.: +39.0432 558194; Fax: +39.0432 558199;
E-mail: maria.messina@uniud.it

Received 15 May 2014
Accepted 5 December 2014

Abstract

The aim of this study was to investigate the effects of feeding frequency on post-prandial pattern of circulating glucose, insulin and IGF-I (insulin-like growth factor-I) in European sea bass (*D. labrax*). Fish (average body weight 72.1 ± 2 g) were randomly allocated in 12 tanks, reared in a closed thermo regulated recirculating system and fed a commercial diet at a fixed ration (1.5% body weight) for 7 weeks. Six fish groups were fed the ration in one meal a day (08:^{30} am), while six fish groups were offered the same ration in two daily meals (08:^{30} am and 04:^{30} pm) according to a complete random design. Plasma insulin mean level was affected by the splitting up of the ration (14.4 *vs* 15.2 ng/ml, $P<0.05$, one meal and two meals/day, respectively) and showed a bimodal profile depending on the meal administration. The level of circulating glucose was slightly affected by feeding pattern with an increase of glycaemia after each meal. The daily pattern of plasma IGF-I did not appear related to meal administration. Results show that post prandial plasma glucose, insulin and IGF-I are differently affected by the meal administration pattern in sea bass due to their physiological role in maintaining the internal homeostasis.

Keywords: Feeding response, hormonal regulation, meal, growth, glycaemia.

Introduction

In teleost fish feeding triggers a response in the nervous and endocrine systems which modulate the distribution of nutrients and energy. Among the hormones, insulin and insulin-like growth factor-I (IGF-I) play a central role in metabolism and growth. Insulin is a pancreatic hormone while systemic IGF-I is synthesized by the liver and circulates in plasma bound to its binding proteins. Insulin and IGF-I are structurally related and their effects on metabolism and growth are mediated, at cellular level, by specific tirosine kinase receptors (Mommsen, 1998).

The response of insulin to food intake has been studied in cultured teleost species such as European sea bass (Perez *et al*., 1988), browntrout (Navarro *et al*., 1993) and striped bass (Papatryphon *et al*., 2001) and during food deprivation (Navarro and Gutiérrez, 1995). Differently to mammals under physiological conditions, where insulin is the primary hormone responsible for blood glucose homeostasis during the post prandial state, insulin production in carnivorous fish is only slightly affected by dietary glucose being in a more potent way in response to a dietary aminoacid intake, mostly arginine and lysine (Plisetskaya *et al*., 1991; Mommsen *et al*., 2001; Andoh, 2007) likely due to their low ability to utilize dietary carbohydrates.

IGF-I and its binding proteinsare produced as a consequence of the bound of growth hormone to its hepatic receptorand are part of the somatotropic axis which regulates body growth; thehormonal status of this axis seems to be nutritionally regulated in teleost fish (Pérez-Sanchez *et al*., 1995; Gomez-Requeni *et al*., 2004). Inthe European sea bass, the effects of dietary carbohydrate on plasma insulin and IGF-I has been examined six hours after feeding (Enes *et al*, 2010). The response of these circulating hormones to a glucose load in this species indicates that both insulin and IGF-I may contribute to glucose homeostasis (Enes *et al*., 2011), nevertheless, the postpandrial profile of these plasma peptides has not been analyzed in detail. Circulating IGF-I levels show species-specific differences and are affected by exogenous growth hormone (GH), stress conditions and seasonal changes (Pérez-Sanchez *et al*., 1994; Silverstein *et al*., 2000; Beckmann *et al*., 2004; Dyer *et al*., 2004; Vega-Rubin de Celis *et al*., 2004). Also, fasting diminishes the circulating IGF-I pattern due in part to a developed liver resistance to plasma GH

(Pérez-Sanchez *et al*., 1994; Pierce *et al*., 2005; Small and Peterson, 2005).

Differently from the above mentioned studies, in which single hormones and/or seasonal variations in their circulating levels were investigated, daily changes in plasma insulin and IGF-I concentration have been poorly studied in teleost fish in response to changes in feeding pattern. The effect of fasting and re-feeding has been recently investigated in coho salmon (Shimizu *et al*., 2009) and Mozambique tilapia (Fox *et al*., 2009), showing significant post-prandial increase in circulating levels of insulin, IGF-I and glucose.

The aim of the present trial was to investigate theeffects of feeding frequency on the post prandial pattern of plasma glucose, insulin and IGF-Iin reared European sea bass (E. sea bass) one of the most important fishes in Mediterranean marine aquaculture.

Materials and Methods

Fish, Diet and Experimental Conditions

Juveniles of E. sea bass were obtained from a commercial fish farm (Panittica Pugliese, Torrecanne, Br, Italy) and transported to the facilities of the Department of Food Science (University of Udine, Italy). Sea bass (average body weight, 72.1±2 g), were reared in 0.3 m^3 circular tanks in a thermo regulated recirculating water system. Water temperature was set at 23.7±0.5°C, salinity was 23 psu and a 12D/L photoperiod was maintained during the experimental period. Fish were randomly distributed among 12 homogeneous groups (23 fish/tank). After 3 weeks acclimation to the rearing conditions, fish were fed according to the experimental condition for 47 days. Six fish groups were fed a commercial diet (Ecolife, Biomar, Italia; crude protein of 48%, crude lipid of 18%, starch 12.9%) at a fixed daily ration of 1.5% biomass in two daily meals (08:^{30} am and 04:^{30} pm) (M2), which represent the usual feeding frequency, and six fish groups were offered the same ration in one daily meal (8.30 am) (M1) according to a complete random design. The fixed ration has been chosen to obtain fish at the same nutritional status. Fish were group-weighed at the beginning and at the end of the trial under moderate anaesthesia with an alcoholic solution of clove oil (5%).

Metabolite and Hormone Assays

Blood samples were withdrawn from the caudal vein, after moderate anaesthesia from 5 fish per group. The time of sampling was 0, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 8.5, 9.0, 10.0, 12.0, 14.0 and 24.0 hours after the morning meal. Different group/tank has been used at each sampling time to minimize the stress of the animals. The blood sample was collected in heparinised tube, centrifuged at 2000 g for 15 min at 4°C and plasma was frozen at -20°C until analysis.

Total plasma IGF-I was determined on samples previously extracted according to Shimizu *et al*. (2000) by a microtitre solid phase radioimmunoassay (RIA) validated for sea bass. Immunoplate (96-well Optiplate, Perkin Elmer Life Science) was coated with 100 µl/well of polyclonal anti rabbit serum raised in goat, diluted 1:1.000 in acetate buffer (sodium acetate 0.15 M, pH 9) and incubated overnight at 4°C. The second day the plate was washed twice and incubated with 200 µl/well of anti barramundi IGF-I polyclonal antiserum (GroPep, Adelaide, Australia), diluted 1:20.000, overnight at 4°C. The third day plate was washed twice with 200 µl/well of washing buffer. Series of standards of recombinant barramundi IGF-I (bIGF-I) obtained in *E. coli* (GroPep, Adelaide, Australia), ranging from 10 ng/ml to 0.156 ng/ml, and of extracted samples were diluted with10 mM PBS (pH 7.4) containing 0.02% protamine sulphate, 0.05% tween 20and 0.02% sodium azide (IGF-I buffer) to a final volume of 100 µl and added to the wells. The tracer was recombinant bIGF-I prepared according to Salacinski *et al.* (1981). 50 pg/10µl/well of ^{125}I-bIGF-I (Specific activity = 176.1 µCi/µg) diluted in the IGF-I buffer were added to each well. Plate was incubated overnight at 4°C. The next day plate was washed twice with 200 µl/well of washing buffer and radioactivity counted by the Top Countγ-counter (Packard, Connecticut, USA). The intra- and inter-assay CV% was 7.6 and 12.7 respectively. The sensitivity of the assay, defined as the dose of hormone at 90% binding, was 0.1 ng/ml. The rabbit polyclonal anti-barramundi serum showed cross-reactivity with sea bass extracted plasma samples with a displacement curve parallel to the barramundi standard (Figure 1). Regression curve for parallelism was $y = 0.8921x + 5.5413$ ($R^2 = 0.996$) and for recovery was $y = 0.7141x + 14.339$ ($R^2 = 0.922$).

Plasma insulin levels were measured by RIA using bonito insulin as standard and rabbit anti-bonito insulin as antiserum, according to Gutierrez *et al*. (1984). Insulin was not measured in all samples due to insufficient plasma. Plasma glucose was analysed by an enzymatic commercial kit according to the indication of the manufacturer (PKL, Genova, Italy).

Statistics

Plasma data were analysed by two-way ANOVA considering number of daily meals (M1, M2) and hours after the first meal as main factors. As a significant interaction was observed between number of daily meals and hours after the meal, data are presented per each main factor. If appropriate, Duncan's test was applied to the data means ($P<0.05$). Data processing was performed using the SPSS/PC Statistical package (SPSS Inc release 17.0).

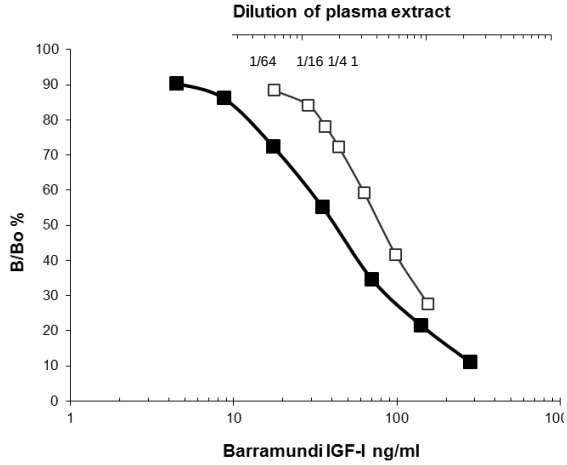

Figure 1. Binding inhibition curves of recombinant barramundi IGF-I (■)and (□) acid-ethanol extracted plasma sample serial dilution (▲). Values are means of duplicate determinations.

Results

European sea bass juveniles fed a commercial diet for 47 daysat the same ration (1.5% body weight) supplied in one meal (complete ration/meal, M1) or two meals (half ration/meal, M2) per day exihibited a final average body weight of 101.9±2.2 in M1 and 105.5±2.5 in M2 (P>0.05) respectively andthe survival observed for each group was 100%.

Feeding fish the complete ration in one meal/day or divided in two meals/daysignificantly affected the mean value of plasma insulin in M1 and M2 (14.4 *vs* 15.3 ng/ml, P<0.05) but did not affect the mean values of plasma glucose and IGF-I over a 24 hr period. The daily patterns of plasma glucose, insulin and IGF-I are reported in Figure 2.

Plasma levels of glucose in M1 and M2 significantly increased in response to feed intake within 1 hour after the first meal and remained essentially similar during the sampling time. A significant effect of feeding one or two meals/dayon circulating glucose could be observed 4 hours after the second meal, when the level in M2 reached its highest value (7.1-9.7 mmol/L, for M1 and M2 respectively, P<0.05) (Table 1).

As stated in Table 2, circulating insulin increased2 hours and 1 hr after feeding respectively in M1 and in M2, and in fish fed twice a day always remained higher than in fish fed once a day. Plasma insulin levels significantly decreased 2 hours (M1) and 4 hours (M2) after the first peak value. In addition, one hour after the second meal plasma insulin of M2 group showed a significant peak value, while at the same sampling time M1 fish exhibited the lowest value (12.6 ng/ml). Significant differences between M1 and M2 groups could be observed 6 (13.5 *vs*14.8 ng/ml, P<0.05) and 9 hours (12.6 *vs*16.3, P<0.05) after the first meal, the latter value was also triggered by the second meal intake (Table 2).

Considering IGF-I in M1 significantly higher levels were found 1 hour after the meal with no differences at later sampling points except 8, 14 and 24 hours after the meal (Table 3). In M2, IGF-I concentrations were similar until 8 hours after the first meal when a significant drop was observed with the lowest value 30 minutes after the second meal, and subsequently recovered. The effect of meal size on circulating IGF-I levels between M1 and M2 fish groups was significantly different at 6 (78.5 vs 66.2 ng/ml, P<0.05), 8.5 (70.3 vs 46.4 ng/ml, P<0.05), 10 (81.4 vs 62.6 ng/ml, P<0.05) and 14 (49.6.5 vs 65.9 ng/ml, P<0.05) hours after the morning meal.

Discussion

Feeding the same ration in one or two meals/day over 47 days did not affect the final body weight of sea bass suggesting that the two patters of fed administration were equally well accepted by fish. Although in mammals, increasing the feeding frequency may improve bioenergetic efficiency (Towhidi *et al*., 2010), it was not the objective of the present study to analyze the effects on conversion and growth rates since longer experimental trials should be designed for that purpose.

The pattern of post prandial glycemia observed in the present trial is similar to that found in sea bass fed commercial diets (Gutierrez *et al*., 1984) and more recently by Enes *et al*. (2010), with a moderate peak in plasma glucose concentration between one and two hours after the meal mainly as a response to carbohydrate intake. These results are also in agreement with those of Gouveia and Davies (2004) who demostrated the ability of sea bass to digest carbohydrates and assimilate glucose from dietary corn starch/yellow destrin (2:1, 21.23% dry matter). Feeding the whole ration or half a ration in one meal seems to trigger, in a similar way, the increase of glucose concentrationsbeginning 30 minutes after the meal, and the amount of dietary carbohydrates in both cases maintains similar values of glycemia, apart from at the sampling time 12 when in M2 group the effect

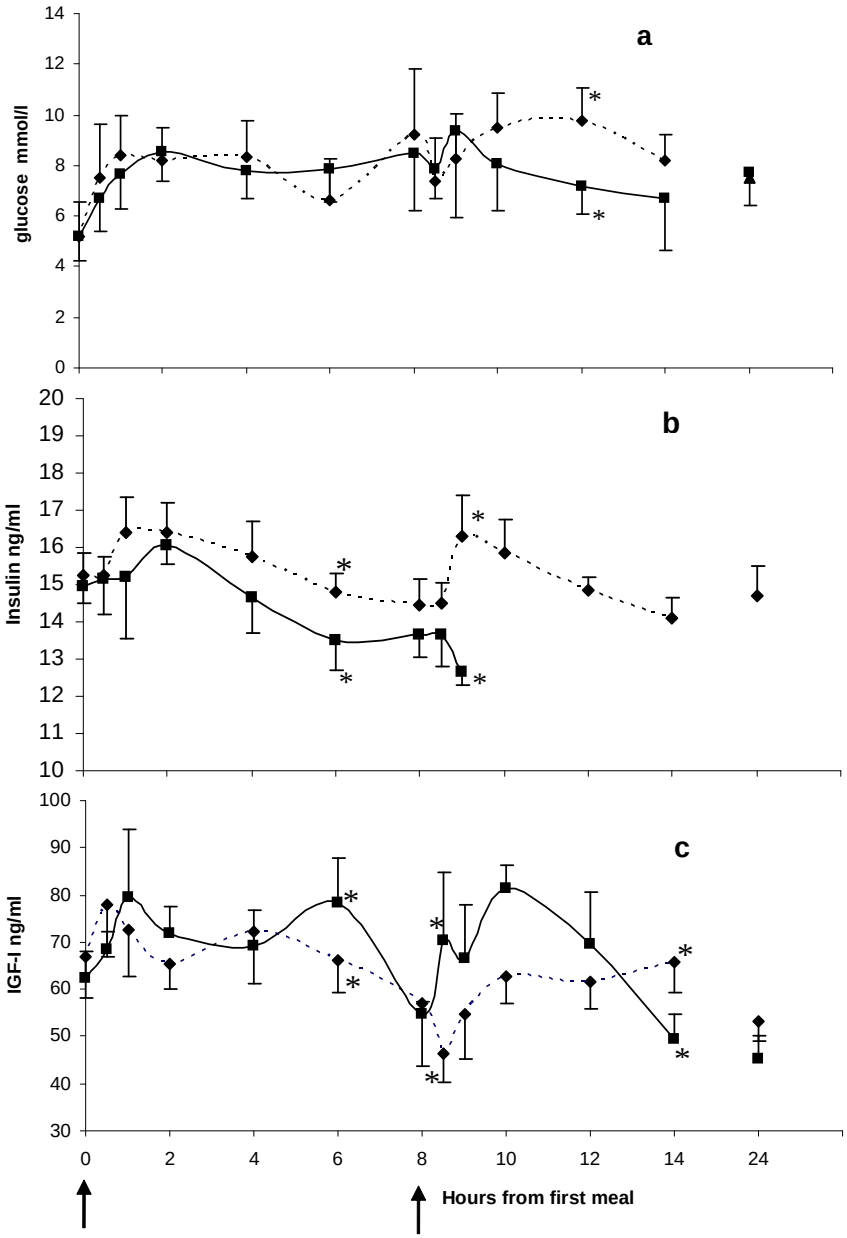

Figure 2. Pattern of circulating glucose (a), insulin (b), and IGF-I (c) in sea bass fed one (M1) or two meals (M2) per day. In abscissa hours after first meal are expressed while arrows indicate the meal time (8.30 am and 4.30 pm). ■ and solid line represents M1 and ▲and dashed line represents M2. Values are mean ± SD. An asterisk indicates significant ($P<0.05$) differences within time between M1 and M2 groups.

of the second meal is showed and higher levels of glycemia are reached. These results suggest that glycemia in E. seabass is not strictly related to the carbohydrates content of a single meal, but it should be related to the amount of carbohydrates in the diet (Enes *et al*., 2010) and to the hormonal control of glucosehomeostasis.

In vertebrates, plasma insulin level is affected by two major components such as circadian rhythm and food intake. The former phenomenon has been well described in mammals (la Fleur, 1999; Froy, 2007). In fish, a circadian rhythm has been observed in the plasma insulin of sea bass fed a natural diet (Gutierrez *et al*., 1984) while the effects of feeding on plasma insulin profile have been studied in trout by Navarro *et al*. (1993), whereinsulin concentration increased 1 hr after the meal to promote uptake of glucose into liver and skeletal muscle and started to decrease 8-11 hours post feeding. In the present trial, the differences in plasma insulin profile could be due to dietary carbohydrate assimilation and to the effect of nutrients other than glucose. In carnivorous fish the insulinotropic action of carbohydrates is less important than that of amino acids, in particular arginine and lysine, and insulin plays its most important role related to growth and amino acid metabolism (Mommsen and Pliseskaya, 1991; Mommsen *et al*., 2001). Thus, in E. seabass fed once

Table 1. Effect of sampling time and number of meals per day on plasma glucose in European sea bass

Hours from first meal	Glucose (mmol/L)	
	M1	M2
0	5.2±1.0[c]	5.2±1.4[c]
0.5	6.7±1.3[bc]	7.5±2.1[ab]
1	7.6 ±1.3[ab]	8.4±1.6 [ab]
2	8.6 ±1.2[ab]	8.2±1.3[ab]
4	7.8 ±1.1[ab]	8.3±1.5[ab]
6	7.9 ±1.2[ab]	6.6±1.6[bc]
8	8.5 ±2.2[ab]	9.2±2.5[ab]
8.5	7.9 ±1.1[ab]	7.4±1.7[a]
9	9.3 ±3.4[a]	8.3±1.7[ab]
10	8.0 ±1.8[ab]	9.5±1.4[a]
12	7.1 ±1.1[abc]	9.7±1.3[a]
14	6.7±2.0[bc]	8.2±1.1[ab]
24	7.7±1.3[ab]	7.4±0.1[ab]

M1= one meal/day, M2 = two meals/day (n=5)
Values are presented as mean ± SD
[a,b,c] Mean values in the same column with different superscript letters are significantly different within a group (P<0.05).

Table 2. Effect of sampling time and number of meals per day on plasma insulin in European sea bass

Hours from first meal	Insulin (ng/ml)	
	M1	M2
0	14.9±0.5 [b]	15.2±0.6[bcd]
0.5	15.1±1.0 [b]	15.3±0.5[bcd]
1	15.2±1.7 [b]	16.4±0.9 [a]
2	16.0±0.5 [a]	16.4±0.8 [a]
4	14.7±1.0[bc]	15.7±0.9[abc]
6	13.5±0.8[cd]	14.8±0.5[cde]
8	13.6±0.6[cd]	14.4±0.7[ef]
8.5	14.1±0.9[bc]	14.5±0.5[ef]
9	12.6±0.3 [d]	16.3±1.1[ab]
10		15.8±0.9[abc]
12		14.8±0.4[cde]
14		14.1±0.6 [f]
24		14.7±0.8[def]

M1= one meal/day,
M2 = two meals/day (n = 5)
Values are presented as mean ±SD.
[a,b,c,e,f] Mean values in the same column with different superscript letters are significantly different within a group (P<0.05).

Table 3. Effect of sampling time and number of meals per day on plasma IGF-I in European sea bass.

Hours from first meal	IGF-I (ng/ml)	
	M1	M2
0	62.1±8.9[de]	66.9±5.8[abc]
0.5	68.3±11.1[bcd]	78.1±4.1[a]
1	79.5±10.0[ab]	72.7±14.3[ab]
2	71.8±5.5[abcd]	65.5±5.8[abcd]
4	69.1±11.0[abcd]	72.1±7.9[ab]
6	78.5±7.1[abc]	66.2±9.5[abc]
8	54.9±13.1[f]	56.9±2.7[de]
8.5	70.3±6.3[abcd]	46.4±14.4[e]
9	66.5±9.5[cde]	54.9±11.7[cde]
10	81.4±5.5[a]	62.6±4.9[bcd]
12	69.7±5.7[abcd]	61.5±10.8 [bcd]
14	49.6±6.6[f]	65.9±5.1 [abcd]
24	45.3±4.8[f]	53.4±4.2[de]

M1= one meal/day,
M2 = two meals/day (n = 5)
Values are presented as mean ±SD.
[a,b,c,e,f] Mean values in the same column with different superscript letters are significantly different within a group (P<0.05).

or twice/day for 47 days, plasma insulin relative increases are similar and not directly proportional to the amount of the ingested food, but increasing always shortly after feeding, reflecting the physiological pattern of this pancreatic hormone (Mommsen and Pliseskaya, 1991). In mammalian species increasing fed frequency decrease the insulin elevations changes quickly after feeding, but the average daily value is higher with higher feeding frequency (Mineo *et al.*, 1990; Vicari *et al.*, 2008). In a similar way, the average value of insulin was higher in M2 in sea bass, and it appears that the double stimulation of food can maintain higher levels during the day, but in this case maintaining the same magnitude of increases after a meal. Whether this pattern of fed administration could help the uptake of nutrients by the insulin dependent tissues deserves further investigation.

The validation of a microtitre solid phase RIA for E. sea bass IGF-I is presented in this study. The antiserum has already been shown to be highly specific for different fish species IGF-I (Dyer *et al*., 2004). Moreover, the amino acid sequence of E. sea bass IGF-I [GenBank accession number: AAV91771] is 92–94% identical to that of barramundi (Kinhult, 1996, cited by Moriyama *et al*., 2000; Vega-Rubin de Celis *et al*., 2004) confirming the high cross-reactivity between E. sea bass IGF-I and barramundi antiserum. Compared with other RIAs (Dyer *et al*., 2004), the assay showed great sensitivity to and specificity for E. sea bass IGF-I.

As in mammals, in teleost fish the circulating levels of IGF-I are regulated by the nutritional status depending on food ingestion and diet composition (Duan and Hirano, 1992; Niu *et al*., 1993; Gomez-Requeni *et al*., 2004). Long-term feeding (4-7 months) and different feeding levels, have significantly increased circulating IGF-I levels coupled with higher weight gainin coho salmon (Beckmann *et al*., 2004). In sea bream a biphasic pattern of plasma IGF-I was observed relating to the season (the lowest level in March and the highest in July) with a significant parallelism between plasma IGF-I levels and specific growth rate. Moreover, increasing ration level from 0 to 2.7 g/100 g body weight/day resulted in an increase of circulating IGF-I in sea bream over a 7 weeks period (Pérez-Sánchez *et al*., 1994).

To our knowledge this is the first time that a daily post prandial pattern of plasma IGF-I in E. sea bass has been evaluated. In the present triala window of 24 hours has been explored to describe the effects of feedingthe same ration in one or two meals on plasma IGF-I levels. The daily pattern of circulating IGF-I seems to be only partially affected by thesize of the meal. Other factors not directly related tothe single meal could be considered such asan intrinsic rhythm of the somatotropic axis, already described in other teleost species (Ebbesson *et al*., 2008; Sing *et al*., 2009), since a biphasic increase along the day was observed in both experimental groups.

Our data are consistent with Enes *et al*. (2010) thatreported a significant increase in plasma IGF-I concentration at 6 hoursin comparison to 24 hours after the morning meal in sea bass. Moreover, plasma insulin and IGF-I in salmon are reported to increase after a meal, but while insulin pattern is associated with short term response to food intake, the IGF-I patternseems to dependon long-term nutritional status (Shimizu *et al*., 2009). Mean plasma IGF-I levels of juvenile catfish have been assessed by Small (2005) on fish sampled every 2 hours over a 24 hours cycle. He obtained significantly higher IGF-I level at 0.5 hr after the meal without differences at later sampling point.

The fluctuations of the plasma IGF-I values that we obtained in sea bass suggest that the choice of the sampling time is determinant to describe the post prandial fish response. In any case, it would be interesting to understand if the fluctuations of plasma IGF-I on a daily basis could be recognized as a trigger by the fish.

In conclusion, our data on the post prandial endocrine response in European sea bass describe the different role of insulin and IGF-I in the regulation of glucose homeostasis. Moreover, it seems that European sea bass is able to adapt to different meal regimes without adverse effect on fish growth and with different minimal effects on plasma glucose, insulin and IGF-I.

Acknowledgements

This work was supported by Department of Food Science, University of Udine and by AGL2009-12427 to J. Gutiérrez.

References

Andoh, T. 2007. Amino acids are more important insulin otropins than glucose in a teleost fish, barfin flounder (*Veraspermoseri*). General and Comparative Endocrinology, 151: 308-317. doi: 10.1016/j.ygcen. 2007.01.015

Beckman, M., Shimizu, M., Gadberry, B. and Cooper, K.A. 2004. Response of the somatotropic axis of juvenile coho salmon to alteration in plane of nutrition with an analysis of the relationship among growth rate and circulating IGF-I and 41 kDa IGFBP. General and Comparative Endocrinology, 135: 334-344. doi:10.1016/j.ygcen.2003.10.013

Duan, C. and Hirano, T. 1992. Effects of insulin-like growth factor-I and insulin on the in-vitro uptake of sulphate by eel branchial cartilage:evidence for the presence of independent hepatic and pancreatic sulphation factors. Journal of Endocrinology, 133: 211-219. doi:10.1677/joe.0.1330211

Duan, C. 1998. Nutritional and developmental regulation of insulin-like growth factors in fish. Journal of Nutrition, 128: 306-314.

Dyer, A.R., Upton, Z., Stone, D., Thomas, P.M., Soole, K.L., Higgs, N., Queen, K. and Carragher, J.F. 2004.

Development and validation of a radioimmunoassay for fish insulin-like growth factor-I (IGF-I) and the effect of aquaculture related stressors on circulating IGF-I levels. General and Comparative Endocrinology, 135: 268-275.
doi:10.1016/j.ygcen. 2003.10.002

Ebbesson, L., Bjoernsson, B.T., Ekstroem, P. and Stefansson, S.O. 2008. Daily endocrine profiles in parr and smolt Atlantic salmon. Comparative Biochemistry and Physiology, 151: 698-704.
doi: 10.1016/j.cbpa.2008.08.017

Enes, P., Sanchez-Gurmaches, J., Navarro, I., Gutierrez, J. and Oliva-Teles, A. 2010. Role of insulin and IGF-I on the regulation of glucose metabolism in European sea bass (*Dicentrarchus labrax*) fed with different dietary carbohydrate levels. Comparative Biochemistry and Physiology, 157: 346-353.
doi: 10.1016/J.cbpa.2010.08.006

Enes, P., Panserat, S., Kaushik S. and Oliva-Teles A. 2011. Dietary carbohydrate utilization by European Seabass (*Dicentrarchus labrax L.)* and gilthead sea bream (*Sparus aurata* L.) juveniles. Review in Fishery Science, 19 (3): 201-215.
doi: 10.1080/10641262. 2011.579363

Fox, B.K., Breves, J.P., Hirano, T. and Grau, E.G. 2009. Effects of short- and long-term fasting on plasma and stomach ghrelin, and the growth hormone/insulin-like growth factor I axis in the tilapia, *Oreochromis mossambicus*. Domestic Animal Endocrinology, 37: 1-11.
doi: 10.1016/j.domaniend.2009.01.001

Froy, O. 2007. The relatioship between nutrition and circadian rhythms in mammals. Frontiers in Neuroendocrinology, 28: 61-71.
doi: 10.1016/j.yfrne. 2007.03.001

Gomez-Requeni, P., Mingarro, M., Calduch-Giner, J.A., Médale, F., Martin, S.A.M., Houlian, D.F., Kaushik, S. and Pérez-Sánchez, J. 2004. Protein growth performance, aminoacid utilisation and somatotropic axis responsiveness to fish meal replacement by plant protein sources in gilthead sea bream (*Sparus aurata*). Aquaculture, 232: 493-510.
doi:10.1016/S0044-8486(03)00532-5

Gouveia, A., Davies, S. J., 2004. Modulation of the Post-Prandial Plasma Glucose Profile in Juvenile European Sea Bass Dicentrarchus labrax Fed Diets Varying in Starch Complexity. Journal of the World Aquaculture Society, 35 (3), 392-400.

Gutiérrez, J., Carrillo, M., Zanuy, S., Planas, J., 1984. Daily rhythms of insulin and glucose levels in the plasma of sea bass Dicentrarchus labrax after experimental feeding. General and Comparative Endocrinology, 55(3), 393-397.

La Fleur, S.E. 1999. A Suprachiasmatic Nucleus Generated Rhythm in Basal Glucose Concentrations. Journal of Neuroendocrinology, 11: 643-652.

Mahon, R., Khokiattiwong, S., Oxenford, H. A. 2000. Selectivity of experimental gillnets for fourwing flyingfish, Hirundichthys affinis, off Barbados. Environmental Biology of Fishes, 59(4), 459-463.

Mineo, H., Oyamada, T. and Kato, S. 1990. Effect of feeding on plasma secretin concentrations in sheep. Research in Veterinary Science, 49(2): 157-159.

Mitchell, G.A. 1990. Method of starch analysis. Starch, 42(4): 132-134.

Mommsen, T.P. and Plisetskaya, E.M., 1991. Insulin in fish and agnathans: history, structure and metabolic regulation. Review in Aquatic Science, 4: 225-259.

Mommsen, T.P. 1998. Growth and metabolism. In: D.H. Evans (Ed.), The Physiology of Fishes. Second Edit., CRC Press, Boca Raton: 65-97.

Mommsen, T.P., Moon, T.W. and Plisetskaya, E.M. 2001. Effect of arginine on pancreatic hormone and hepatic metabolism in rainbow trout. Physiological and Biochemical Zoology, 74: 668-678.
doi: 10.1086/32 2924

Moriyama, S., Ayson, F.G. and Kawauchi, H. 2000. Growth Regulation by Insulin-like Growth Factor-I in Fish. Bioscience Biotechnology and Biochemistry, 64(8): 1553-1562.
doi: 10.1271/bbb.64.1553

Navarro, I., Carneiro, M., Párrizas, M., Maestro, J.L., Planas, J. and Gutierrez, J. 1993. Post-feeding levels of insulin and glucagon in trout (*Salmo trutta fario*). Comparative Biochemistry and Physiology, 104: 389-393.
doi: 10.1016/0300-9629(93)90335-2

Navarro, I. and Gutierrez, J. 1995. Fasting and starvation. In: P.W. Hochacka, T.P. Mommsen (Eds.), Metabolic Biochemistry. Elsevier Science BV, Amsterdam: 393-434.

Niu, P., Pérez-Sanchez, J. and Le Bail, P.1993. Development of a protein binding assay for teleost insulin-like growth factor (IGF)-like: relationship between growth hormone (GH) and IGF-like in the blood of rainbow trout (*Oncorhynchus mykiss*). Fish Physiology and Biochemistry, 11: 381-391.

Papatryphon, E., Capilla, E., Navarro, I. and Soares, H.J. Jr. 2001. Early insulin and glucagon response associated with food intake in a teleost, the striped bass (*Morones axatilis*). Fish Physiol. and Biochemistry, 24: 31-39.
doi: 10.1023/A:1011123908446

Perez, J., Zanuy, S. and Carrillo, M. 1988. Effects of diet and feeding times on daily variations in plasma insulin, hepatic c-AMP and other metabolites in a teleost fish *Dicentrarchus labrax* L. Fish Physiology and Biochemistry, 5: 191-197.
doi: 10.1007/BF01874796

Pérez-Sanchez, J., Martí-Palanca, H. and Le Bayl, P.J. 1994. Seasonal changes in circulating growth hormone (GH), hepatic GH-binding and plasma insulin-like growth Factor-I immunoreactivity in a marine fish, gilthead sea bream, *Sparus aurata*. Fish Physiology and Biochemistry, 13: 199-208.
doi: 10.1007/BF0000 4358

Pérez-Sanchez, J., Martí-Palanca, H. and Kaushik, S. 1995. Ration size and protein intake affect circulating growth hormone concentration, hepatic growth hormone binding and plasma insulin-like growth factor I immunoreactivity in a marine fish, gilthead sea bream, *Sparus aurata*. Journal of Nutrition, 125: 546-552.

Pierce, A.L., Shimizu, M., Beckman, B.R., Backer, D.M. and Dickhoff, W.W. 2005. Time course of the GH/IGF-I axis response to fasting and increased ration in chinook salmon (*Oncorhynchus tshawytscha*). General and Comp. Endocrinology, 140: 192-202.
doi: 10.1016/j.ygcen.2004.10.017

Plisetskaya, E.M., Buchelli-Narvaez, L.I., Hardy, R.W., Dickhoff, W.W. 1991. Effects of injected and dietary arginine on plasma insulin levels and growth of Pacific salmon and rainbow trout. Comparative

Biochemistry and Physiology, 98: 165-170. doi:10.1016/0300-9629(91)90595-4

Salacinski, P.R.P., McLean, C., Sykes, J.E.,Clement-Jones, V.V.,Lowry, P.J. 1981. Iodination of proteins, glycoproteins, and peptides using a solid-phase oxidizing agent, 1,3,4,6-tetrachloro-3a,6a-diphenyl-glycoluril (IODO-GEN). Analytical. Biochemistry, 117: 136-146.
doi: 10.1016/0003-2697(81)90703-X

Shimizu, M., Swanson, P., Fukada, H., Hara, A. and Dickhoff, W.W. 2000. Comparison of extraction methods and assay validation for salmon insulin-like growth Factor-I using commercially available components. General and Comp. Endocrinology, 119: 26-36.
doi: 10.1006/gcen.2000.7498

Shimizu, M., Cooper, K.A., Dickhoff, W.W. and Beckman, B.R. 2009. Postprandial changes in plasma growth hormone, insulin, insulin-like growth factor one (IGF-I), and IGF-binding proteins in coho salmon fasted for varying periods. American Journal of Physiology Regulatory Integrative and Comparative Physiology, 297: 352-361.
doi: 10.1152/ajpregu.90939.2008

Silverstein, J.T., Wolters, W.R., Shimizu, M. and Dickhoff, W.W. 2000. Bovine growth hormone treatment of channel catfish: strain and temperature effects on growth, plasma IGF-I levels, feed intake and efficiency and body composition. Aquaculture, 190: 77-88.
doi: 10.1016/S0044-8486(00)00387-2

Sing, A.K., Lal, B. and Pati, A.K. 2009. Variability in the characteristic of ultradian and circadian rhythms in plasma levels of growth hormone in the Indian walking catfish, *Clarias batrachus.* Biological Rhythm Research,40: 211-22.
doi: 10.1080/09291010701875054

Small, B.C. 2005. Effect of fasting on nychthemeral concentrations of plasma growth hormone (GH), insulin-like growth factor I (IGF-I), and cortisol in channel catfish (*Ictalurus punctatus*). Comp. Biochem. and Physiol., 142(B): 217-223.
doi: 10.1016/j.cbpb.2005.07.008

Towhidi, A., Zali, A., Khoshsokhan, A., Khazali, H., Zhandi, M., Rezayazdi, K., Masoumi, R. and Gholami, H. 2010. The effect of feeding frequency on the hormonal profile, carcass characteristics, and feedlot performance in Iranian Holstein calves. Turkish Journal of Veterinary and Animal Sciences, 34(2): 137-142.
doi: 10.3906/vet-0712-5

Vega-Rubin de Celis, S., Gòmez-Requeni, P. and Pérez-Sànchez, J. 2004. Production and characterization of recombinantly derived peptides and antibodies for accurate determinations of somatolactin, growth hormone and insulin-like growth factor-I in European sea bass (*Dicentrarchus labrax*). General and Comparative Endocrinology, 139: 266-277. doi:10.1016/j.ygcen.2004.09.017

Vicari, T., van den Borne, J.G.C., Gerrits, W.J.J., Zbinden, Y. and Blum, J.W. 2008. Postprandial blood hormone and metabolite concentrations influenced by feeding frequency and feeding level in veal calves. Domestic Animal Endocrinology, 34(1):74-88.
doi: 10.1016/j.domaniend.2006.11.002