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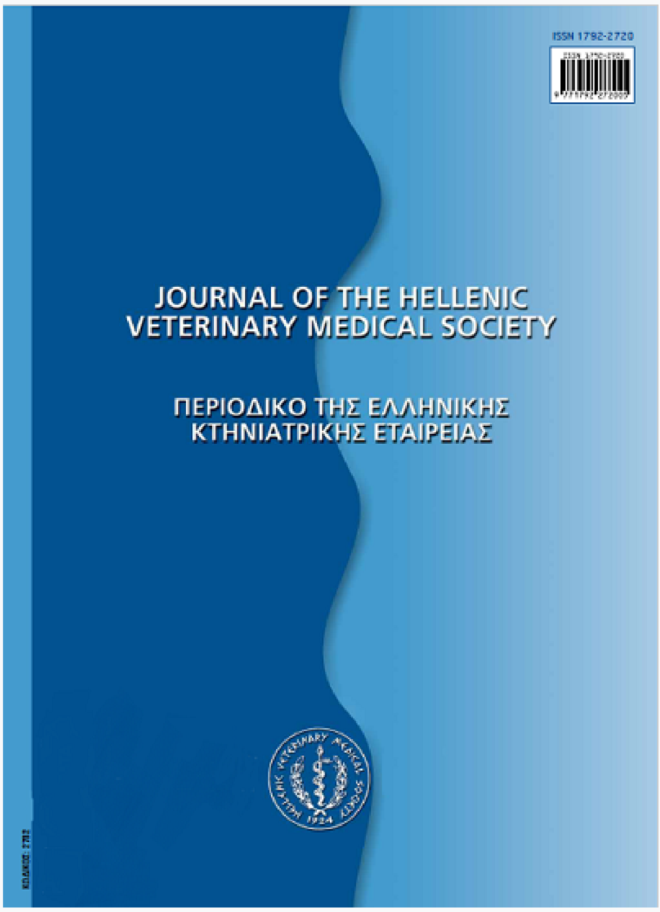

In vitro comparison of hydrolysis of different starches along the digestive tract of teleosts important for aquaculture

E. S. PAPOUTSOGLOU (Ε.Σ. ΠΑΠΟΥΤΣΟΓΛΟΥ), A. R. LYNDON

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Ερευνητική
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In vitro συγκριτική υδρόλυση διαφορετικής προέλευσης αμύλου στον πεπτικό σωλήνα εκτρεφόμενων ειδών ιχθύων

Ε. Σ. Παπουτσόγλου, Lyndon A.R.

ΠΕΡΙΛΗΨΗ. Κατά τη διαδικασία κατάρτισης σιτηρεσίων για εκτρεφόμενους ιχθύες, ιδιαίτερα όσον αφορά στα σαρκοφάγα είδη, δίδεται ιδιαίτερη έμφαση ώστε το επίπεδο και το είδος των παρεχόμενων υδατανθράκων να χρησιμοποιείται για μεταβολικές διεργασίες, έτσι ώστε οι παρεχόμενες με το σιτηρέσιο πρωτεΐνες (που αποτελούν και το ακριβότερο συστατικό) να χρησιμοποιούνται όσο το δυνατόν περισσότερο για την ανάπτυξη. Κατά την παρούσα εργασία μελετήθηκε *in vitro* η επίδραση της προέλευσης του αμύλου του σιτηρεσίου (από γεώμηλα, αραβόσιτο, σόργο, σίτο, όρυζα, καθώς και ενός υποπροϊόντος της βιομηχανίας της ζυθοποιίας-brewer's spent grain, παρασκευασμένου από σίτο και κριθή με δύο διαφορετικές τεχνικές) στη δραστικότητα των ολικών καρβοϋδρασών (ανάλυση ολικών καρβοϋδρασών σε pH 7.6 με χρήση ρυθμιστικού διαλύματος κιτρικού οξέος και φωσφωρικού άλατος, με άμυλο ως υπόστρωμα και επώαση για 4h στους 37° C) κατά μήκος του γαστρεντερικού σωλήνα του λαβρακιού, *Dicentrarchus labrax* (σαρκοφάγο είδος του οποίου η τροφή περιλαμβάνει ακόμα και άλλους ιχθύες, n=10, 116.7±30.0 g), της τσιπούρας *Sparus aurata* (σαρκοφάγο είδος με έμφαση στα ασπόνδυλα, n=10, 105.0±13.5 g) και της τιλάπιας, *Oreochromis aureus* (φυτοφάγο είδος, n=10, 92.0±20.0 g). Κατά τη δειγματοληψία απομονώθηκαν, ζυγίστηκαν, ομογενοποιήθηκαν και μελετήθηκαν το πρόσθιο και οπίσθιο τμήμα εντέρου για όλα τα εξεταζόμενα είδη και ο στόμαχος και τα πυλωρικά τυφλά μόνο στα *S. aurata* και *D. Labrax* (δεδομένου ότι η τιλάπια δεν διαθέτει στόμαχο και πυλωρικά τυφλά). Διάλυμα γλυκόζης χρησιμοποιήθηκε ως standard και τα αποτελέσματα εκφράστηκαν ως mg γλυκόζης παραχθέντα ανά g ιστού ανά λεπτό επώασης. Τα αποτελέσματα στην τιλάπια επέδειξαν σημαντικά αυξημένα επίπεδα υδρόλυσης για το άμυλο γεώμηλων σε σχέση με τα άλλα εξεταζόμενα άμυλα, ενώ τα επίπεδα υδρόλυσης για το άμυλο σόργου και όρυζας ήταν σημαντικά αυξημένα απ'ότι στο σίτο και στο υποπροϊόν της βιομηχανίας της ζυθοποιίας Α, αλλά όχι και στον αραβόσιτο. Επίσης, για κανένα εξεταζόμενο άμυλο δεν υπήρξε διαφορά στα επίπεδα υδρόλυσης ανάμεσα στο πρόσθιο και το οπίσθιο τμήμα του εντέρου της τιλάπιας. Στην τσιπούρα τα επίπεδα υδρόλυσης ήταν υψηλότερα για το άμυλο σόργου ($P>0.05$) και χαμηλότερα για το υποπροϊόν της βιομηχανίας της ζυθοποιίας D ($P<0.05$), ενώ τα επίπεδα υδρόλυσης ήταν ενδιάμεσα για το σόργο και στο υποπροϊόν της βιομηχανίας της ζυθοποιίας Α. Επί-

School of Life Sciences, Heriot-Watt University, Riccarton EH14 4AS, Edinburgh, Scotland, UK

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In vitro comparison of hydrolysis of different starches along the digestive tract of teleosts important for aquaculture

Papoutsoglou E.S., Lyndon A.R.

ABSTRACT. The *in vitro* hydrolysis of different starches (potato, rice, wheat, corn, sorghum), as well as an intermediate product of malting process (brewer's spent grain), by blue tilapia, *Oreochromis aureus*, gilthead sea bream, *Sparus aurata* and European sea bass, *Dicentrarchus labrax*, were compared (total carbohydrase assay, 37° C, pH 7.6, 4 hours incubation). Obtained results (significantly higher hydrolysis levels obtained for potato, rice and sorghum starch) suggest that starch source and associated properties (starch granule size, amylose content) and modifications applied may have significant difference on its digestion by fish digestive carbohydrases. Furthermore, the importance of brewer's spent grain as a low-cost carbohydrate source and feed component, in relation to α-glucosidase adaptive response to dietary carbohydrate, is also considered significant.

Key words: fish, digestion, enzymes, amylase, starch, diet formulation

School of Life Sciences, Heriot-Watt University, Riccarton EH14 4AS, Edinburgh, Scotland, UK

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σης, υπάρχουν διαφοροποιήσεις στα επίπεδα υδρόλυσης του υποπροϊόντος της βιομηχανίας της ζυθοποιίας D στο πρόσθιο και οπίσθιο τμήμα του εντέρου. Στο λαβράκι παρατηρήθηκαν υψηλότερα επίπεδα υδρόλυσης για το άμυλο σόργου και όρυζας ($P<0.05$) μόνο στο οπίσθιο τμήμα του εντέρου και όχι στο πρόσθιο τμήμα του εντέρου και τα πυλωρικά τυφλά. Από τα αποτελέσματα προκύπτει ότι οι διαπιστωμένες από τη βιβλιογραφία διαφορές ανάμεσα στους αμυλόκοκκους (μέγεθος, περιεκτικότητα σε αμυλόζη κ.α.) των διαφόρων εξεταζόμενων αμύλων είναι πιθανώς ικανές να οδηγήσουν σε διαφορετικά επίπεδα πέψης σε συνδυασμό με το είδος του ιχθύος και την περιοχή του γαστρεντερικού σωλήνα. Επίσης, το υποπροϊόν της ζυθοποιίας αποδείχθηκε *in vitro* ότι είναι μια άριστη και φθηνή πηγή υδατανθράκων διαφορετικών μοριακών βαρών και πολυπλοκότητας. Τα αποτελέσματα της παρούσης εργασίας, σε συνδυασμό με πρόσφατες παρατηρήσεις, οι οποίες συσχετίζουν τα παρατηρούμενα υψηλότερα επίπεδα συγκεκριμένων καρβοϋδρασών, όπως η γλυκοσιδάση (ένζυμο που υδρολύει τους δισακχαρίτες) με υψηλότερα επίπεδα παρεχόμενων υδατανθράκων στο σιτηρέσιο, υποδεικνύουν, πιθανώς, την αυξημένη σημασία των υδατανθράκων κατά την κατάρτιση σιτηρεσίων για εκτρεφόμενους ιχθύες. Συνεπώς, η επιλογή του κατάλληλου, για κάθε είδος ιχθύος, υδατάνθρακα (προέλευση, μοριακό βάρος, κόστος παραγωγής, παρουσία αντιδιαιτητικών παραγόντων και κόστος επεξεργασίας) πιθανώς να είναι μια σημαντική και σαφώς περισσότερο πολύπλοκη διαδικασία απ' ότι ήταν μέχρι τώρα γνωστό κατά τη διαδικασία κατάρτισης σιτηρεσίων για Τελεόστεους ιχθύες, ιδιαίτερα για τα σαρκοφάγα είδη.

Λέξεις ευρετηρίασης: ιχθύς, πέψη, ένζυμα, αμυλάση, άμυλο, υδατάνθρακες, κατάρτιση σιτηρεσίων

INTRODUCTION

The use of carbohydrates in artificial diets for intensively reared fish species has gained interest over the last 30 years, and the issues identified to affect their efficient digestion, mainly concerning carnivorous species, involve factors, such as dietary carbohydrate molecular weight, inclusion level, source and processing method, interaction of carbohydrates towards digestibility of other nutrients and vice versa, as well as activity levels of digestive carbohydrases in relation to all the above (Vonk and Western 1984, Hofer and Sturmbauer 1985, Smith 1989, Steffens 1989, Wilson 1991, 1994, Carneiro et al. 1994, Catacutan and Coloso 1995, Jobling 1995, Kuz'mina 1996, Grisdale-Helland and Helland 1997, Hillestad et al. 2001, Papoutsoglou and Lyndon, 1998a,b, 2002, 2003, 2004, 2005).

The dietary carbohydrate kind and source used in practical fish feeds (e.g. monosaccharides and disaccharides or polysaccharides, cereal starches), as well as the processing method used in practical fish feeds is considered important. It is very likely to be affected by economical reasons, the choice of cereal or plant used to as an additional protein source (e.g. wheat, corn, rice, barley, sorghum, soybean, lupin, peas, rapeseed) and its respective processing methodology (Raven and Walker 1980, Wilson 1991, Tacon 1993, Wilson 1994, Aksnes 1995, Hemre and Hansen 1998, Regost et al. 1999, El-Sayed et al. 2000, Storebakken et al. 1998, 2000), especially in current fish feeds, where protein portion originating from fish meal is continuously reduced.

Carbohydrates (mainly in the form of alginic acid, guar gum, carboxyl methyl cellulose, starch and dextrins) also have the physical function of texturising manufactured feeds and act as a binder in the formulation of pellets, while cereal grain products are used as "fillers" to complete feed formulae. Formulae for expanded pellets often contain up to 50% of whole cereal grains, to achieve the floating properties (Watanabe 1988, Wilson 1991, 1994, Pillay 1993), affecting digestive carbohydrase activity, total food passage time and carbohydrate digestibility.

In general, cereal starch is the most widely used carbohydrate in practical fish rearing. The effects of its composition and processing on feed utilisation and fish performance are little understood and seem to vary within species (Wilson 1994).

Starch digestibility is affected by fish species and feeding habits, dietary inclusion level (Bergot and Breque 1983, Kim and Kaushik 1992, Brauge et al. 1994), processing method (Steffens 1989, Pillay 1993, Suarez et al. 2002), starch source and starch amylose/amylopectin ratio (Degani et al. 1986, Steffens 1989, Takeushi et al. 1990, Bergot 1991, Arnessen and Krogdahl 1991, Swarz and Kirchgessner 1991, Garcia Gallego et al. 1994).

In practical aquaculture, carbohydrate sources used include cost-effective options, such as milled cereals (e.g. wheat), which offer gluten as a source of protein, starch and dextrins as a source of carbohydrate and even a pellet binder (Storebakken et al. 1998, 2000).

This study aims to identify possible advantages of certain cereal starches, while by using spent grain from malting process as a carbohydrate source, to investigate the possibility of using a by-product of the brewing industry as an alternative source of carbohydrates for aquaculture. Although the use of carbohydrates by omnivorous and herbivorous species is well documented and advantageous for their intensive rearing, it is also our aim to use the information presented towards improving carbohydrate inclusion and digestion by carnivorous species.

MATERIALS AND METHODS

Experimental animals

Blue tilapia, *Oreochromis aureus* (92.0±20.0 g, mean ± S.D., n=10), gilt-head sea bream, *Sparus aurata*

(105.0±13.5 g, n=10) and European sea bass (116.7± 30.0 g, n=10), *Dicentrarchus labrax,* were used, maintained in the recirculated facilities of the Animal Production Department of the Agricultural University of Athens, Greece.

Dissection and Analysis

Dissection of gut was applied in ice and gut tissues were distinguished to anterior and posterior intestine (all examined species), stomach and pyloric caeca (only in *S. aurata* and *D. labrax*), were weighed and measured, homogenized using ice-cold distilled water, centrifuged at 30,000 x g for 15 min, and the supernatant was used to produce samples diluted ten-fold with ice-cold distilled water, which were stored at -40°C. Samples were further diluted five/ten-fold (whichever appropriate) prior to analysis to make samples which were used for the analyses.

Hydrolysis of Starch

For the hydrolysis of starch, 1% soluble starch (0.5 ml), buffer (1.0 ml, 0.1M citrate- 0.2M phosphate buffer at pH 7.6) and enzyme extract (0.3 ml) were placed in 10-ml conical digestion tubes. The mixture was then incubated for 4 hours at 37°C and aliquots (0.3 ml) were diluted to 1.0 ml, placed in test tubes, and assayed for reducing sugars according to the Somogyi-Nelson assay (Robyt and Whelan 1968). Glucose was used as standard. Activity of total carbohydrase was expressed as mg glucose produced per g tissue per min.

Preparation of Soluble Starch

Soluble starch (1g) was made into a paste with methanol (3 ml) and added to distilled water (150 ml) at 100°C. Boiling was then continued for 15-20 min to give a final volume of 100 ml (Clark et al. 1984).

Starches from different sources

The examined starches include rice starch (Sigma 7260), wheat starch (Sigma 5126, unmodified), corn starch (Sigma S 4126, unmodified regular corn starch: approx. 73% amylopectin – 27% amylose), sorghum starch and potato starch (Sigma S 4251). High amylose starch (B.D.H., now Merck) was also used. The examined starches were made soluble using a 1% solution for substrate, apart from spent grain, where a 2% solution was used.

Brewer's Spent Grain - Spent Grain A

The procedure followed to obtain this product was relatively exceptional in order to obtain furanone ring structures. Following germination of barley and wheat seeds, soaking in water took place and steeping for 66h at 16°C; then stewing at 70°C for 4 h, then drying in kiln at 110°C for 90 min, in order to caramelise and liquify starch, producing a dark coloured product. Mash was then produced by grinding up in mill, soaking in water at 65°C for 1h, then filtering with filter paper. The spent grain remained in the filter paper. Drying for 24h at 55-60°C, grinding with a pestle and mortar reduced spent grain to a fine powder.

Spent grain D

This procedure was similar to that followed in a grain distillery (90% wheat, 10% barley malt). After germination of wheat seeds at 16°C for 48h, followed the heating at 121°C for 85 min, cooling to 62°C and barley malt addition. Then mash at 62°C for 1h and drying in kiln at 55°C for 24h. The next stage involved grinding and soaking in water at 65°C for 1h and filtering. Spent grain was what remained in the filter paper. Drying for 24h at 55-60°C, grinding with a pestle and mortar reduced spent grain to a fine powder.

Data analysis

The digestive enzyme assays were performed in order to investigate whether digestive hydrolysis along the digestive tract of each examined species differed significantly (at the 95% confidence level) regarding the origin of experimental starch used. One-way ANOVA was performed using *STATGRAPHICS for Windows 2.1* (Statistical Graphics Corp.).

RESULTS

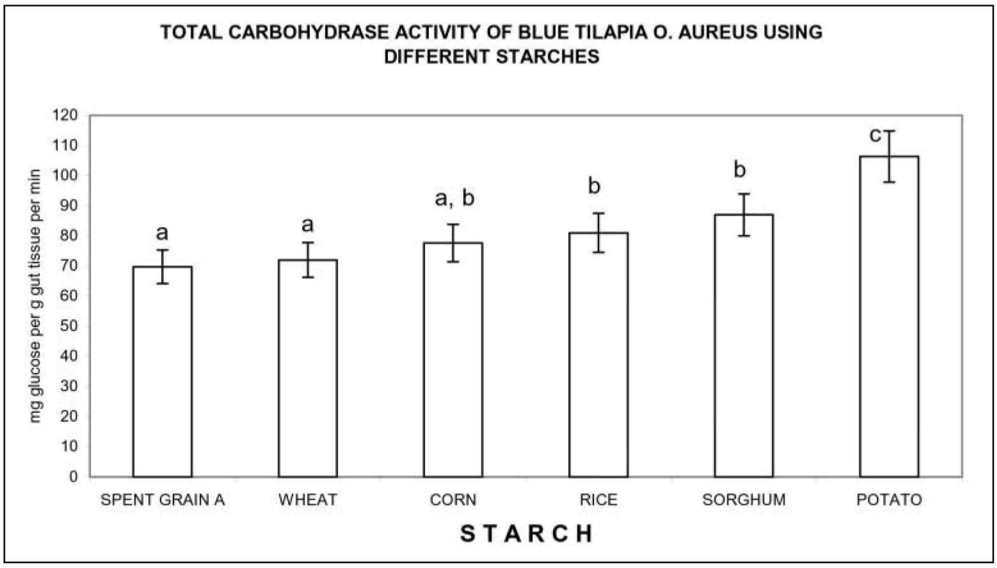
Total carbohydrase activity of *O. aureus* acting on starch from wheat, corn, rice, sorghum, potato and spent grain (Figure 1), demonstrates that potato starch exhibits significantly highest values ($P < 0.05$), while sorghum and rice starches demonstrate significantly higher values than spent grain and wheat ($P < 0.05$). However, there is no significant difference between values of spent grain, wheat and corn, corn and rice or rice and sorghum ($P > 0.05$).

Total carbohydrase per g activity, along the intestine of *O. aureus* for each of the examined starches (Figure 2), demonstrates that potato starch exhibits highest activity and spent grain A the lowest ($P < 0.05$). Furthermore, there is no significant difference in activity between anterior and posterior intestine of *O. aureus* for any of the examined starches. And, although there are differences in activity between the examined starches, the proportion of each part of the intestine of *O. aureus* remains constant-approximately 22% for anterior and 78% for posterior intestine.

Activity of different gut sections of *S. aurata* on different starches (Figure 3) demonstrates that sorghum starch exhibits highest activity and spent grain D the

Εικόνα 1. Δραστικότητα ολικών καρβοϋδρασών στο έντερο της τιλάπιας (*O. aureus*) χρησιμοποιώντας άμυλο από σίτο, αραβόσιτο, ρύζι, σόργο, πατάτα, καθώς και το υποπροϊόν της βιομηχανίας της ζυθοποιίας-brewer's spent grain A.

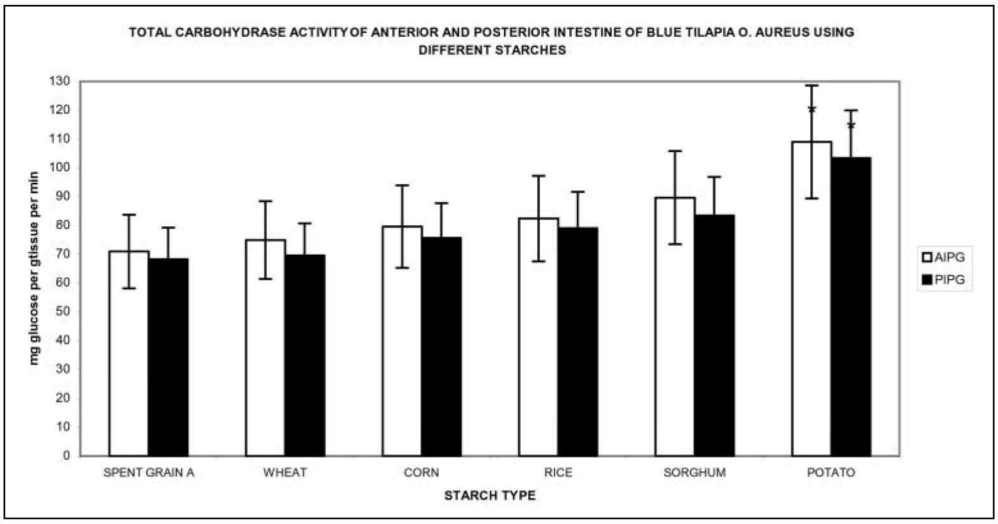
Figure 1. Total carbohydrase activity of *O. aureus* gut extracts (means ± s.d.) on starch from wheat, corn, rice, sorghum, potato and spent grain A.



Ίδια γράμματα υποδεικνύουν μη στατιστικώς σημαντική διαφορά (One-way ANOVA, P <0.05)
Same letters indicate no significant difference (One-way ANOVA, P <0.05)

Εικόνα 2. Δραστικότητα ολικών καρβοϋδρασών στο πρόσθιο **(AIPG)** και οπίσθιο **(PIPG)** τμήμα του εντέρου της τιλάπιας (*O. aureus*) χρησιμοποιώντας το υποπροϊόν της βιομηχανίας της ζυθοποιίας-brewer's spent grain A και άμυλο από σίτο, αραβόσιτο, ρύζι, σόργο και πατάτα.

Figure 2. Total carbohydrase per g activity (means ± s.d.) in anterior and posterior intestine of *O. aureus* for spent grain A, wheat, rice, corn, sorghum and potato starch (**AIPG, PIPG:** anterior and posterior intestine per g tissue activity).



Ο αστερίσκος υποδηλώνει στατιστικώς σημαντική διαφορά (One-way ANOVA, P<0.05)
Asterisk denotes significant difference (One-way ANOVA, P <0.05)

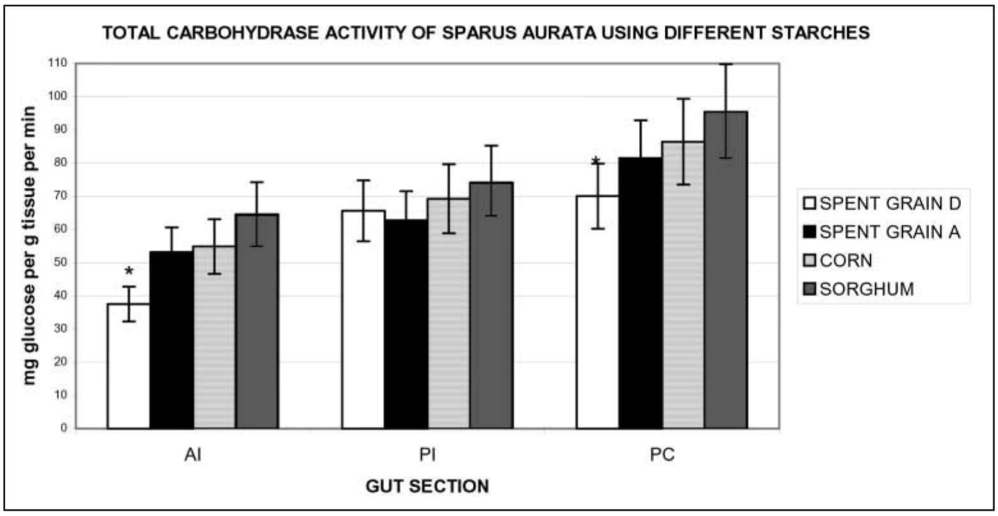
lowest, but differences are not significantly different (P > 0.05). Activity levels along the digestive tract demonstrate that, although activity for spent grain D is significantly lower in anterior intestine and pyloric caeca of *S. aurata* (P<0.05), it is comparable for posterior intestine.

Total carbohydrase activity of *D. labrax* on different starches (Figure 4) exhibits no significant differences between the different starches and hydrolysis rate levels are similar for different gut sections. However, in rice and wheat the activity in posterior intestine is significantly lower comparatively to corn and high amylase starch.

DISCUSSION

The most important findings in this study include the observation that starch source and associated properties (starch granule size, amylose content) and modifications applied may have significant difference on its digestion by fish digestive carbohydrases. Furthermore, the importance of brewer's spent grain as a low-cost carbohydrate source and feed component, in relation to α-glucosidase adaptive response to dietary carbohydrate, is also considered significant.

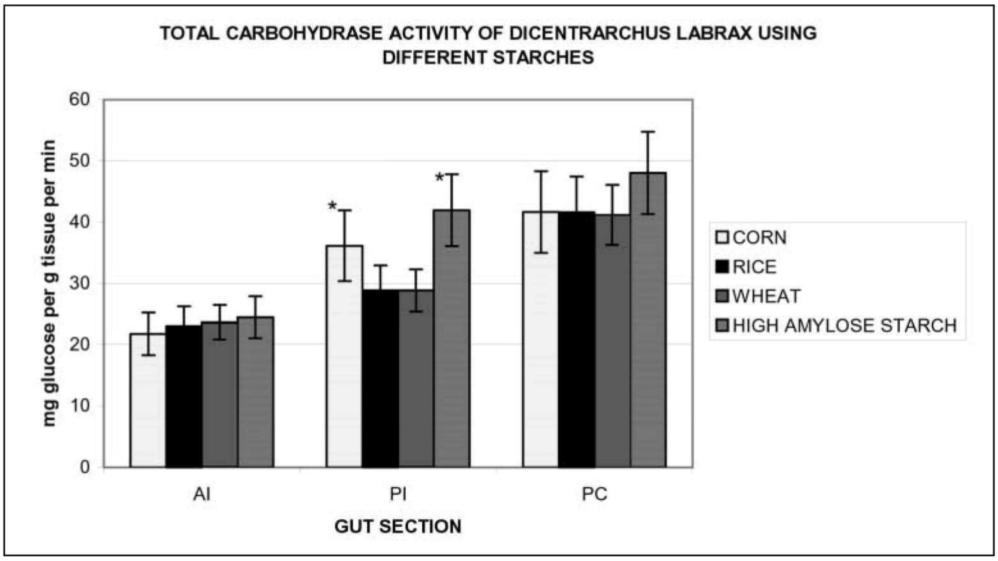
The values obtained for *O. aureus*, *S. aurata* and *D. labrax* for different starches represent how available is



Εικόνα 3. Δραστικότητα ολικών καρβοϋδρασών στο πρόσθιο **(AI)** και οπίσθιο **(PI)** τμήμα του εντέρου, καθώς και τα πυλωρικά τυφλά **(PC)** της τσιπούρας (*S. aurata*) χρησιμοποιώντας άμυλο από αραβόσιτο, σόργο και τα υποπροϊόντα της βιομηχανίας της ζυθοποιίας-brewer's spent grain A και D.

Figure 3. Total carbohydrase activity (mean±s.d.) of different gut sections of *S. aurata* (**AI**: anterior intestine; **PI**: posterior intestine; **PC**: pyloric caeca), on starch from corn, sorghum and spent grain A and D.

Asterisk denotes significant difference (One-way ANOVA, $p<0.05$)
Ο αστερίσκος υποδηλώνει στατιστικώς σημαντική διαφορά (One-way ANOVA, $P<0.05$)



Εικόνα 4. Δραστικότητα ολικών καρβοϋδρασών στο πρόσθιο **(AI)** και οπίσθιο **(PI)** τμήμα του εντέρου, καθώς και τα πυλωρικά τυφλά **(PC)** του λαβρακιού (*D. labrax*) χρησιμοποιώντας άμυλο από αραβόσιτο, ρύζι, σίτο και άμυλο με υψηλό ποσοστό αμυλόζης.

Figure 4. Total carbohydrase activity (mean ± s.d.) of different gut sections of *D. labrax* on starch from corn, rice, wheat and high amylose starch (**AI**: anterior intestine; **PI**: posterior intestine; **PC**: pyloric caeca).

Asterisk denotes significant difference (One-way ANOVA, $p<0.05$)
Ο αστερίσκος υποδηλώνει στατιστικώς σημαντική διαφορά (One-way ANOVA, $P<0.05$)

the starch to the digestive carbohydrases of different fish species to attack on, within the 4 hours of incubation. Increased levels of total carbohydrase activity indicate that a given starch is comparatively more easily available for hydrolysis than others, therefore more likely to be digested and efficiently utilised. Initially, starch hydrolysis proceeds only through action of α-amylase, but subsequently disaccharidases such as α-glucosidase, also take part, in order to complete digestion to glucose. Therefore, the enzymes involved, using starch as substrate, are mainly α-amylase and α-glucosidase. The reported values, thus, are a result of a combination of factors, mainly temperature, starch granule size and composition (amylose/amylopectin ratio), and starch modification.

The relatively high values observed for rice and sorghum starch hydrolysis observed in *O. aureus, S. aurata* and *D. labrax* could be partially explained by the small starch granule size (5-6μm and 15μm, respectively). Rice and sorghum are currently used in countries where they are cultivated in commercial scale (Asia, Africa) as a carbohydrate source and a feed component (e.g. grain meals, such as sorghum grain/flour, sorghum brewery waste meal, rice groats, rice bran with germ, rice polishings) (Raven and Walker 1980, Tacon 1993). In fact, the most common cereal by-product used in warmwater aquafeeds for omnivorous/herbivorous fish species has been rice bran, due to its low cost and ready availability in most developing countries.

The relatively low values obtained for corn in comparison to sorghum, observed mainly in *O. aureus*, could be explained by the high amylopectin content (73%) of the starch used and that no modification was applied. However, many varieties of corn starches and meals are available (e.g. corn grain/flour, corn gluten feed, corn gluten meal, corn distiller's dried grain and solubles) (Raven and Walker 1980, Tacon 1993), and in general, corn meal is widely used as a carbohydrate source for aquaculture species (Degani et al. 1986, Steffens 1989, Takeushi et al. 1990, Wilson 1991, Tacon 1993, Garcia Gallego et al. 1994).

The low values observed for wheat may be explained by the unmodified variety of wheat starch used and the relatively large size of its starch granules, although wheat is generally well accepted and widely used in aquaculture as a carbohydrate source (Raven and Walker 1980, Degani et al. 1986, Steffens 1989, Takeushi et al. 1990, Bergot 1991, Arnessen and Krogdahl 1991, Tacon 1993, Garcia Gallego et al. 1994).

The high values obtained for potato starch cannot be explained by the starch granule size, because most of potato starch granules are almost ten times larger than those of rice, although there could be smaller granules also present. These high values are probably due to the high amylose content of potato starch. Potato starch was suggested to provide better growth than either glucose or fructose in *Salmo trutta* and *O. keta* (Steffens 1989), but this report has more to do with utilisation rather than digestion of carbohydrates.

It is also important to note the difference observed between *O. aureus* and *D. labrax* and *S. aurata* regarding the total carbohydrase levels observed along their digestive tract regarding the different starch sources. In *O. aureus* no difference was observed between anterior and posterior intestine when using different starches. However, in *S. aurata* the total carbohydrase levels obtained for brewer's spent grain were higher for posterior intestine and lower for anterior intestine and pyloric caeca. In *D. labrax*, total carbohydrase posterior intestinal levels for rice and wheat starch were comparatively lower to those for corn and high amylose starch (BDH). These results indicate a different response of posterior intestine digestive carbohydrases in *D. labrax* and *S. aurata* in comparison to anterior intestine and pyloric caeca regarding the source of starch. Considering the fact that posterior intestine is mainly responsible for activity of α-glucosidase, it is plausible that the different amylose/amylopectin ratio of different starches leads to a slightly different response of α-glucosidase.

Values obtained for brewer's spent grain (made of barley and wheat) are relatively lower, but are not significantly different to those of wheat and corn starch, which are cereal starches widely used (Degani et al. 1986, Takeushi et al. 1990, Bergot 1991, Swarz and Kirchgessner 1991, Garcia-Gallego et al. 1994, Storebakken et al. 1998). The importance of observing no difference in starch hydrolysis when comparing a lower-cost cereal by-product of the malting process with highly expensive, purified and modified starches is significant regarding the cost of dietary carbohydrate that can be efficiently digested and utilised, especially for carnivorous fish. Furthermore, no action of antinutritional factors was observed in vitro for any examined species.

Furthermore, brewer's spent grain, as an intermediate product of malting, contains polysaccharides, oligo- and di-saccharides, and proteins apart from carbohydrates, as well as digestive carbohydrases and proteases, making it a valuable addition to an animal feed. In several countries spent grain is actually used as a domestic animal feed. Since the procedure for its production is performed as a stage of the brewing process, production cost is low and there is potential for its use on a large scale as a feed component for aquaculture species. Also, the fact that brewer's spent grain contains oligo- and di-saccharides makes it an appealing, low-molecular weight, digestible carbohydrate source, as the mechanism of α-glucosidase secretion and levels seems to respond adaptively to dietary disaccharide increase, and comparatively more than α-amylase levels do (probable hereditory pre-adaptation), especially in carnivorous teleosts. Inclusion of brewer's spent grain or similar feedstuffs (e.g. sorghum brewery waste meal, corn distillers grains with solubles, barley brewers grains) (Tacon 1993) could significantly improve carbohydrate digestion and utilisation in fish rearing.

Summarising, it could be argued that starch source and associated properties (starch granule size, amylose content) and modifications applied may significantly differ regarding starch digestion by fish digestive carbohydrases. Furthermore, the importance of brewer's spent grain as a low-cost carbohydrate source and feed component, especially in relation to α-glucosidase adaptive response to dietary carbohydrate, is also considered significant for improving carbohydrate digestion and utilisation in carnivorous teleosts.

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