Aquaculture Nutrition

doi: 10.1111/anu.12152

Aquaculture Nutrition 2015 **21**; 206–213

Effective use of glucose rather than starch in formulated semimoist diets of common octopus (*Octopus vulgaris*)

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Abstract

The aim of the present work was to test the capacity of *Octopus vulgaris* to use carbohydrates supplied in three diets: a diet without added carbohydrates (diet C0: 500 g kg^{-1} water, 200 g kg^{-1} gelatine, 100 g kg^{-1} egg yolk powder, 50 g kg^{-1} freeze-dried *Sardinella aurita* and 150 g kg^{-1} freeze-dried *Todarodes sagittatus*) and two obtained by substituting 50 g kg^{-1} of *T. sagittatus* by glucose (diet GLU50) or by starch (diet STA50). The most stable and best-accepted diet was STA50 (SFR 1.26%BW day^{-1}) although there were no significant differences in the growth rates obtained with the three diets: 10.12 g day^{-1}, 9.37 g day^{-1} and 11.22 g day^{-1} for C0, GLU50 and STA50, respectively ($P > 0.05$). The feed efficiency indices were better for GLU50, of particular note being the protein productive value of 71.88% and a feed conversion ratio lower than 1. Protein and lipid digestibility were similar in all the three diets (96–98% for proteins and 85–94% for lipids), whereas carbohydrate digestibility was higher in GLU50 (98%) than in C0 (84%) and STA50 (0.33%). The content of carbohydrates increased in muscle and the digestive gland as a consequence of the increased carbohydrates intake.

KEY WORDS: carbohydrates, digestibility, formulated diet, growth, nutrition, *Octopus vulgaris*

Received 17 April 2013; accepted 12 November 2013

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Introduction

The recent developments of formulated feeds for common octopus (*Octopus vulgaris*) have shown reasonable levels of acceptability by the animals. This allows manipulation of the formulations to improve our knowledge of the nutritional requirements for this species (Cerezo Valverde *et al.* 2008, 2013; Quintana *et al.* 2008; García *et al.* 2010; Estefanell *et al.* 2012; Morillo-Velarde *et al.* 2013).

Cephalopods are exclusively carnivorous and, while they commonly use proteins, they rarely use carbohydrates (CH) or lipids as energy source (Lee 1994). However, it also has been seen that they do have the capacity to digest, store and use CH, which provides them with energy, especially for anaerobic burst work (Wells & Clarke 1996), as in the case of catching preys or fleeing from predators, although also for periods of starvation (Morillo-Velarde *et al.* 2011).

The digestive gland is active in digesting carbohydrates. Amylases were detected in the lumen of the digestive channels of cuttlefish (Romijn 1935; Boucaud-Camou 1974) and in digestive gland cells of octopus (D'Aniello & Scardi 1971) and squid (Okutani & Kimata 1964). Caruso *et al.* (2004) described a decreasing gradient of amylases from the salivary glands to the digestive gland in *O. vulgaris* and *Sepia officinalis*.

As regards their storage, carbohydrates have been found in variable and significant amounts in all the tissues of several cephalopod species, including the gonad (21.4 g kg^{-1} dry weight), muscle (31.6 g kg^{-1} dry weight) and digestive gland (34.7 g kg^{-1} dry weight) of *O. vulgaris* (Zamora & Olivares 2004; Rosa *et al.* 2005; Morillo-Velarde *et al.* 2011).

Recently, Morillo-Velarde *et al.* (2011) estimated that the contribution of carbohydrates to daily energy costs is about 9.9% in *O. vulgaris* during short-term starvation: 8.6% from muscle and 1.3% from the digestive gland. Glycogen may also be replenished rapidly and directly from the glucose present in the circulation or from glycogenic amino acids (Hochachka & Fields 1982). According to O'Dor *et al.* (1984), glucose was rapidly catabolized after ingestion and only a small part would be accumulated as glycogen in

the muscles, where it would remain practically immovable during starvation.

The fact that carbohydrates are the cheapest components in feed formulas for aquaculture increases the interest in studying the capacity of animals to use this nutrient when they are incorporated in a formulated diet, especially in the case of species whose metabolism is based on proteins. Morillo-Velarde *et al*. (2012) demonstrated the poor use of CH obtained from pea in *O. vulgaris*, suggesting that starch is not very digestible, perhaps because of the presence of amylase inhibitors in this ingredient. It is probable that the addition of pure starch would not have this inconvenience.

The aim of this work was to test the capacity of *O. vulgaris* to use simple carbohydrates, in the form of glucose, or complexes in the form of starch, by incorporating them in formulated ongrowing diets, recording changes in the digestibility of the diet and nutritional composition and growth of the animals, using the diet format proposed by Morillo-Velarde *et al*. (2012). In consequence, this study attempts to answer the question whether the low levels of carbohydrates in the tissue of octopus are due to their low level in natural diets or are the consequence of the low capacity of octopus to store them.

Material and methods

Experimental animals and acclimation

Common octopus (*O. vulgaris*) were caught in the Mediterranean Sea (Murcia, S.E. Spain) by trawling and kept in 2000 L tanks in the laboratory. The animals were allowed to acclimatize for 2 weeks and were fed with round sardinella (*Sardinella aurita*) and crab (*Carcinus mediterranus*) on alternate days. Subsequently, the animals were placed and kept individually in 262 L circular tanks of a seawater recirculation system with controlled temperature (Model 400 Ti; Air Energy, Heat Pump Inc., Fort Lauderdale, FL, USA) and filtration (mechanical and biological). The tanks contained PVC tubes as shelters and an external net to prevent the animals from escaping. The water temperature was maintained constant 19.11 ± 0.74 °C, which is within the optimal range of temperatures for this species (Aguado Giménez & García García 2002); dissolved oxygen was maintained at above 80% saturation (Cerezo Valverde & García García 2005). Other conditions included a 12L/12D photoperiod, 37 g L^{-1} salinity, pH between 7 and 8, and total ammonia nitrogen (TAN) content below 0.2 mg L^{-1}.

Preparation and water stability of the diets

Three different diets were prepared. A control diet without added carbohydrates (diet C0) consisting of 500 g kg^{-1} water, 200 g kg^{-1} gelatine as binder, 100 g kg^{-1} egg yolk powder, 50 g kg^{-1} freeze-dried round sardinella (*S. aurita*) and 150 g kg^{-1} freeze-dried European flying squid (*Todarodes sagittatus*). The two other diets were similar but substituting 50 g kg^{-1} of *T. sagittatus* by glucose (diet GLU50) or starch (diet STA50).

European flying squid and round sardinella were caught by traditional fishing methods from the same zone as the octopus. European flying squid and round sardinella were cleaned of shell and entrails, and later triturated. All the ingredients were freeze-dried and triturated in a blender to obtain a fine powder (<200 μm), which was vacuum packed. Egg yolk powder, gelatine, glucose and starch of potato were bought in commercial form (Table 1). To prepare the feeds, all the ingredients were mixed in a domestic food mixer (Mycook® 1.8; Electrodomésticos Taurus, S.L. Lleida, Spain). First, the glucose or starch and gelatine were dissolved in water at 40 °C, and subsequently, all the ingredients were added until they were homogenized. The homogenized mixture was allowed to cool to 4 °C in an aluminium mould for 24 h and then freezed at −20 °C until use.

The water stability was determined from the loss of dry matter in three samples of each feed after soaking in water for 24 h. With the data obtained, the mean values of the following indices were calculated: $\mathrm{VDW}\,(\%) = (\mathrm{DW_f} - \mathrm{DW_i})/\mathrm{DW_i} * 100$, which expresses the variation in dry

Table 1 Composition in weight (g kg^{-1}) of diets formulated

	C0	GLU50	STA50
Water	500	500	500
Gelatine[1]	200	200	200
Egg yolk[2]	100	100	100
Round sardinella (*Sardinella aurita*)[3]	50	50	50
European flying squid (*Todarodes sagittatus*)[3]	150	100	100
Glucose[4]	0	50	0
Starch[5]	0	0	50

[1] Granulated Gelatin, Bloom 220, supplied by Productos Sur, S.A. (Pol. Ind. Oeste, San Ginés, Murcia, Spain).
[2] Egg yolk powder, supplied by Avícola San Isidro S.L. (Los Belones, Cartagena, Murcia, Spain).
[3] Freeze-dried ingredients.
[4] Glucose anhydrous, supplied by Guinama S.L.U. (Alboraya, Valencia, España).
[5] Soluble starch from potato, supplied by Panreac Quimica S.L.U. (Castellar del Vallés, Barcelona, España).

weight of the diet after soaking in water, where DW_i and DW_f are the initial and final dry weights, respectively.

$F = DW_i/DW_f$, which represents a correction factor. The dry weight of uneaten diet was multiplied by this correction factor to account for disintegration.

Experimental design

After acclimation, the animals were weighed and sexed being divided into three experimental groups of seven male individuals: group C0 (fed with diet C0), group GLU50 (with diet GLU50) and group STA50 (with diet STA50). All individuals were male to avoid any influence of reproductive processes and were kept individually. The diets were supplied for 42 days (April–June 2012). The mean initial weights were 751 ± 107 g (637–891 g) for group C0, 883 ± 83 g (813–1013 g) for group GLU50 and 876 ± 64 g (753–953 g) for group STA50. Water temperature varied between 17.7 and 21.0 °C during the experimental period (19.1 ± 0.7 °C). The feeds were weighed and provided to satiety, in the form of one cube-shaped piece, corresponding to 5% of the body weight of each individual, and then readjusted to exceed the demands of each individual. The octopus were fed at 09:00 hours, 6 days a week (García García & Cerezo Valverde 2004, 2006), and any remaining food was collected after 24 h using a small net. The remaining food was dried at 105 ± 1 °C for 48 h until constant weight (AOAC 1997; Method no. 930.15).

Sample collection and preservation

A control group (CI) consisted of three individuals were sacrificed at the beginning of the experiment. On the last day of the experiment, all the animals were weighed and anesthetized by immersion in cold seawater before sacrifice conforming to the principles of Directive 2010/63/EU. The individuals of each group were dissected to obtain the digestive gland and carcass (animal excluding digestive gland) of three animals, and the digestive gland and muscle tissue (three arms and part of the mantle) of the other four of each group, along with their respective weights. This procedure was necessary to obtain the proximate composition of whole animals, from the results of the digestive gland and carcass. Each of these parts was triturated and mixed to obtain a homogeneous mixture per animal, which was vacuum packed and frozen at −20 °C until carrying out the biochemical analyses.

The faeces from each group were collected daily through the use of a small net and frozen at −80 °C. These were then lyophilized (Heto, PowerDry LL3000; Allerød, Denmark). All the faeces from each group were collected together to provide a sufficient amount for analysis.

Analytical method

All the diets were analysed in triplicate and octopus samples in duplicate; 1 g sample was used to obtain the moisture, ash and crude protein contents, a 2 g sample for the crude lipid content and a lyophilized 0.05 g sample in the case of carbohydrates. Moisture was obtained by drying at 105 ± 1 °C for 24 h to reach constant weight (AOAC 1997; Method no. 930.15) and ash by incineration at 450 ± 1 °C for 24 h in a muffle oven (HOBERSAL, HD-230). Crude protein was determined by the Kjeldahl method using a conversion factor of 6.25. The total lipid content was obtained using ethyl ether in a SOXTEC AVANTI 2058 (AOAC 1997; Method no 920.39) and total carbohydrates by the method described by Dubois *et al.* (1956). Gross energy and the protein energy ratio (P/E in MJ) were estimated using the Miglavs and Jobling (1989) energy coefficients: protein 23.6 kJ g^{-1}, lipid 38.9 kJ g^{-1} and carbohydrate 16.7 kJ g^{-1}.

Total body proximate composition (TBC) was calculated by adding the content of macronutrients (M) in the digestive gland and in the carcass according to the following equation:

$$\mathrm{TBC}\,(\%) = [((\mathrm{DGW} * \%\mathrm{DGM}) + (\mathrm{CW} * \%\mathrm{CM})) * 100] / \mathrm{BW}.$$

Where TBC is the percentage of macronutrients in the whole animal, DGW is the weight of the digestive gland, CW is the weight of the carcass (animal excluding digestive gland), DGM is the percentage of macronutrients in the digestive gland, CM the percentage of macronutrients in the carcass and BW is the total weight of the whole animal.

Determination of the digestibility

The apparent digestibility coefficients were calculated for the dry matter (ADCDM), protein (ADCPROT), lipids (ADCLIP) and carbohydrates (ADCCH) using the standard equation, according to Maynard and Loosli (1969):

$$\mathrm{ADC} = 100 - (100 * \%\mathrm{Mdiet}\%\mathrm{Mfaeces}) \times (\%\mathrm{Nfaeces}/\%\mathrm{Ndiet}).$$

Where M is the inert marker and N the nutrient. Acid insoluble ash (AIA) was used as inert marker, following the method described by Atkinson *et al.* (1984). For both

the faeces and lyophilized diets, 5 g of sample was used to obtain the moisture content and the acid insoluble ash (AIA), 0.1 g for proteins, 0.2 g for lipids and 0.05 g for carbohydrates, carrying out the analysis in triplicate in all cases.

Parameters calculated and data analysis

All individual octopuses were weighed at the beginning (W_i, initial weight in g) and end of the experiment (W_f, final weight in g). The following indices were calculated: average weight: W_a (g) = $(W_i+W_f)/2$; weight gain: W_g (g) = W_f-W_i; absolute feeding rate: AFR (g day^{-1}) = IF/t; absolute protein feeding rate: APFR (g day^{-1}) = IP/t; absolute lipid feeding rate: ALFR (g day^{-1}) = IL/t; absolute carbohydrate feeding rate: ACFR (g day^{-1}) = IC/t; specific feeding rate: SFR (%BW day^{-1}) = (AFR/W_a)*100; absolute growth rate: AGR (g day^{-1}) = $(W_f-W_i)/t$. Specific growth rate: SGR (%BW day^{-1}) = $(L_n W_f-L_n W_i)*100/t$; feed efficiency: FE (%) = $(W_f-W_i)*100$/IF; feed conversion ratio: FCR = IF/(W_f-W_i); protein productive value: PPV (%) = 100*(Retained protein/IP); lipid productive value: LPV (%) = 100*(Retained lipid/IL); carbohydrate productive value: CPV (%) = 100*(Retained carbohydrate/IC); digestive gland index: DGI (%) = (DGW/W_f)*100, where IP is the ingested protein in g, IL the ingested lipid in g and IC the ingested carbohydrate in g; DGW = digestive gland weight and IF is the feed ingested in g, corrected by taking into account the disaggregation rate in water and calculated according to the following formula:

$$\begin{aligned}\text{IF (wet weight in g)} = {} & (\text{dry feed supplied in g} \\ & - \text{uneaten dry feed in g} * F) \\ & + \text{Moisture feed supplied in g}\end{aligned}$$

where F values were 1.02, 1.72 and 1.14 for the formulated diets C0, GLU50 and STA50, respectively.

The results obtained were expressed as means ± standard deviation (SD). To analyse the differences, a one-way analysis of variance (ANOVA) was carried out. The significant differences obtained between the mean values were analysed by Tukey's test, using a level of significance of $P < 0.05$. A Naperian logarithmic transformation of the indices and content was made before the ANOVA.

Results

The formulated diets had a firm texture before being put into water. Diet C0 lost 1.75%, diet GLU50 lost 41.9% and diet STA50 lost 12.3% of their respective dry weights after 24 h in water, reflecting the greater stability of STA50.

There were significant differences in the content of all the macronutrients analysed in the diets, the content of protein and ash being lower and the content of lipids and carbohydrates higher in the carbohydrates-enriched diets (GLU50 and STA50) than in diet C0 ($P < 0.05$; Table 2). The energy content was similar in all the diets (2487–2551 kJ 100 g^{-1}), whereas the P/E ratio (g MJ^{-1}) was lower in diets GLU50 and STA50 than in diet C0 (Table 2).

All the octopus found the diets acceptable, and there was a 100% survival rate in all the three groups. There were no significant differences in the mean initial weights or mean final weights between the three groups, the W_g being between 393 g (GLU50) and 471 g (STA50) after 42 days of experiment ($P > 0.05$; Table 3). The best SFR was observed in STA50 diet (1.26 ± 0.15%BW day^{-1}). The STA50 group also showed significantly higher absolute feeding rates (AFR, APFR, ALFR, ACFR) than the groups fed with diets C0 and GLU50 ($P < 0.05$; Table 3). The feed efficiency indices (FE, FCR, PPV and CPV) were significantly better for diet GLU50 than for STA50, of particular note being the PPV of 71.88% and conversion indices below 1. Higher values of CPV were observed for diet GLU50 (37.9%), although no significant differences were detected between diets GLU50 and STA50. Both groups showed higher values than C0. Neither were there differences in DGI ($P > 0.05$; Table 3).

The differences in the composition of the faeces of the three groups were significant for all the macronutrients

Table 2 Macronutrient composition (g kg^{-1} dry weight) of diets formulated without carbohydrates added (C0), with 50 g kg^{-1} glucose (GLU50) and with 50 g kg^{-1} starch (STA50)

	C0	GLU50	STA50	*P*
Moisture	533.2 ± 7.3	560.0 ± 1.1	537.6 ± 11.7	*
Crude protein	815.7 ± 6.1	727.0 ± 2.3	711.1 ± 8.7	*
Crude lipid	137.7 ± 6.2	169.9 ± 11.0	171.1 ± 11.2	*
Ash	43.8 ± 0.9	36.4 ± 0.4	37.0 ± 0.3	*
Carbohydrates	19.6 ± 9.4	104.3 ± 27.9	100.8 ± 39.3	*
AIA	0.84 ± 0.04	0.51 ± 0.08	0.86 ± 0.06	*
Energy (kJ 100 g^{-1})	2487	2551	2512	–
P/E (g MJ^{-1})	32.80	28.50	28.30	–

AIA, acid insoluble ash; P/E, protein/energy ratio.
Energy coefficients: protein 23.6 kJ g^{-1}, lipid 38.9 kJ g^{-1} and carbohydrate 16.7 kJ g^{-1} according to Miglavs and Jobling (1989).
Data are expressed as mean ± SD; Values on the same line and different superscripts are significantly different [*$P < 0.05$; n.s. = not significant ($P > 0.05$)].

Table 3 Growth and feed efficiency indices in *Octopus vulgaris* fed formulated diets without carbohydrates added (C0), with 50 g kg^{-1} glucose (GLU50) and with 50 g kg^{-1} starch (STA50)

	C0	GLU50	STA50	*P*
N	7	7	7	
W_i (g)	797 ± 92	899 ± 65	876 ± 64	n.s.
W_f (g)	1222 ± 157	1293 ± 92	1347 ± 72	n.s.
W_g (g)	425 ± 69	393 ± 61	471 ± 40	n.s.
AFR (g day^{-1})	10.10 ± 0.85^a	8.10 ± 1.29^b	13.99 ± 2.14^c	*
APFR (g day^{-1})	3.85 ± 0.32^a	2.70 ± 0.43^b	4.82 ± 0.74^c	*
ALFR (g day^{-1})	0.62 ± 0.05^a	0.61 ± 0.10^a	1.11 ± 0.17^b	*
ACFR (g day^{-1})	0.08 ± 0.01^a	0.37 ± 0.06^b	0.65 ± 0.10^c	*
SFR (%BW day^{-1})	1.01 ± 0.12^a	0.74 ± 0.12^b	1.26 ± 0.15^c	*
AGR (g day^{-1})	10.12 ± 1.63	9.37 ± 1.45	11.22 ± 0.96	n.s.
SGR (%BW day^{-1})	1.02 ± 0.07^a	0.86 ± 0.12^b	1.03 ± 0.08^a	*
FE (%)	100.08 ± 13.38^{ab}	116.98 ± 18.25^a	81.93 ± 15.18^b	*
FCR	1.01 ± 0.13^{ab}	0.87 ± 0.14^a	1.26 ± 0.23^b	*
PPV (%)	45.52 ± 8.44^a	71.88 ± 4.37^b	37.50 ± 6.07^a	*
LPV (%)	17.00 ± 13.38	12.45 ± 24.63	17.08 ± 7.78	n.s.
CPV (%)	6.45 ± 4.28^a	37.89 ± 5.16^b	17.10 ± 7.99^{ab}	*
DGI (%)	6.01 ± 0.81	6.45 ± 1.16	6.73 ± 1.01	n.s.

N, number of animals per treatment; W_i, initial weight; W_f, final weight; W_g, weight gain; AFR, absolute feeding rate; APFR, absolute protein feeding rate; ALFR, absolute lipid feeding rate; ACFR, absolute carbohydrate feeding rate; SFR,. specific feeding rate; AGR, absolute growth rate; SGR, specific growth rate; FE, feed efficiency; FCR, feed conversion ratio; PPV, protein productive value; LPV, lipid productive value; CPV, carbohydrate productive value; and DGI, digestive gland index.
Data are expressed as mean ± SD; values on the same line and different superscripts are significantly different [$*P < 0.05$; n.s. = not significant ($P > 0.05$)].

Table 4 Freeze-dried faeces composition (g kg^{-1} dry weight) and apparent digestibility coefficients obtained with the diets formulated without carbohydrates added (C0), with 50 g kg^{-1} glucose (GLU50) and with 50 g kg^{-1} starch (STA50)

	C0	GLU50	STA50	*P*
Moisture	79.1 ± 3.9^a	32.4 ± 2.9^b	80.3 ± 1.2^a	*
Crude protein	324.8 ± 10.1^a	273.3 ± 2.6^b	137.0 ± 9.3^c	*
Crude lipid	211.4 ± 9.1^a	199.4 ± 2.4^a	74.2 ± 17.6^b	*
Ash	308.5 ± 1.1^a	400.8 ± 1.0^b	153.3 ± 1.2^c	*
Carbohydrates	43.9 ± 50.3^a	46.2 ± 41.6^a	468.7 ± 103.7^b	*
AIA^a	11.73 ± 3.92^a	9.18 ± 0.67^a	4.01 ± 0.44^b	*
ADCCH^b	83.91	97.56	0.33	–
ADCPROT^c	96.09	97.91	95.87	–
ADCLIP^d	84.57	93.48	90.71	–
ADCDM^e	90.52	94.44	78.55	–

AIA, acid insoluble ash; ADCCH, apparent digestibility coefficients of the carbohydrates; ADCPROT, apparent digestibility coefficients of the protein; ADCLIP, apparent digestibility coefficients of the lipids; and ADCDM, apparent digestibility coefficients of the dry matter.
Data are expressed as mean ± SD; values on the same line and different superscripts are significantly different [$*P < 0.05$; n.s. = not significant ($P > 0.05$)].

analysed ($P < 0.05$; Table 4), of particular note being the high content of carbohydrates in the faeces of the diet STA50 (468.7 g kg^{-1}) than the diet C0 (43.9 g kg^{-1}) and GLU50 (46.2 g kg^{-1}; $P < 0.05$). The ADCDM and ADCCH values for diet GLU50 were higher with respect to C0 and STA50, detecting in diet STA50 the lowest value for ADCCH (0.33%). ADCPROT and ADCLIP were similar in the three diets (96–98% and 85–94%, respectively; Table 4).

The nutritional composition of the animals was affected by the diets they consumed (Table 5). In the digestive gland, the groups fed with GLU50 showed significantly higher content of proteins (550.9 g kg^{-1}) than the animals fed diets with STA50 (413.0 g kg^{-1}; $P < 0.05$). Furthermore, the animals fed with GLU50 and STA50 showed significantly higher values of carbohydrates than those fed diet C0. Both the muscle and carcass of animals fed with GLU50 and STA50 diets contained a significantly lower content of lipids and a higher content of CH than animals fed diet C0 ($P < 0.05$; Table 5). As regards the whole animal, differences were only significant for the CH content, which was higher in animals fed GLU50 (48.9 g kg^{-1}) and STA50 (39.4 g kg^{-1}) than those fed C0 (16.9 g kg^{-1}), and for the ash content, which was significantly lower in the animals fed STA50 than those fed GLU50 and C0 ($P < 0.05$; Table 5).

Discussion

In the present study, three diets differing in the quantity and complexity of the CH added were studied, so that any difference in the resulting performance could only be attributed to the CH content. All the three formulated diets showed good acceptability, the highest absolute feeding rates corresponding to diet STA50. The dry weight ingestion values obtained with STA50 (6.47 g day^{-1}) were similar to those obtained by other diets with a similar format (Morillo-Velarde *et al.* 2012) and better than those obtained with extruded diets (Querol *et al.* 2012a,b). This may be because starch increases viscosity and gelatinization at low temperatures (Thomas *et al.* 1998), imparting good stability and texture, while improving the diet's palatability.

As regards the effect of the CH content of the diet on growth, the values were similar for animal fed with control

Table 5 Macronutrient composition (g kg^{-1} dry weight) of the different fractions of *Octopus vulgaris* fed with the diets formulated without carbohydrates added (C0), with 50 g kg^{-1} glucose (GLU50) and with 50 g kg^{-1} starch (STA50)

	C0	GLU50	STA50	*P*
Digestive gland (*N* = 7)				
Moisture	605.7 ± 27.4	634.7 ± 32.6	625.4 ± 18.7	n.s.
Crude protein	506.8 ± 96.6^{ab}	550.9 ± 50.3^{a}	413.0 ± 26.9^{b}	*
Crude lipid	350.7 ± 118.9	299.0 ± 49.5	379.2 ± 99.8	n.s.
Ash	33.7 ± 5.5^{a}	31.7 ± 6.3^{a}	50.2 ± 4.0^{b}	*
Carbohydrates	20.7 ± 5.2^{a}	38.5 ± 9.4^{b}	40.7 ± 10.4^{b}	*
Muscle (*N* = 4)				
Moisture	801.3 ± 4.3^{a}	796.4 ± 6.5^{a}	815.5 ± 5.2^{b}	*
Crude protein	803.5 ± 16.4	779.0 ± 14.9	821.6 ± 46.0	n.s.
Crude lipid	14.7 ± 5.5^{a}	1.9 ± 1.4^{b}	1.8 ± 0.8^{b}	*
Ash	102.7 ± 4.7^{a}	94.5 ± 5.2^{a}	57.6 ± 2.5^{b}	*
Carbohydrates	21.6 ± 4.4^{a}	43.1 ± 12.0^{b}	39.9 ± 5.1^{b}	*
Carcass (*N* = 3)				
Moisture	812.6 ± 3.8	795.5 ± 9.7	802.7 ± 13.7	n.s.
Crude protein	804.8 ± 39.5	750.4 ± 24.7	803.6 ± 39.8	n.s.
Crude lipid	7.4 ± 0.8^{a}	2.6 ± 1.7^{b}	3.0 ± 1.3^{b}	*
Ash	114.3 ± 4.1^{a}	96.2 ± 3.8^{b}	48.7 ± 0.6^{c}	*
Carbohydrates	14.8 ± 4.6^{a}	53.8 ± 6.9^{b}	40.4 ± 13.3^{b}	*
Whole Animal (*N* = 3)				
Moisture	800.8 ± 5.9	785.7 ± 11.3	789.9 ± 12.8	n.s.
Crude protein	778.3 ± 42.1	733.1 ± 21.8	754.2 ± 35.1	n.s.
Crude lipid	33.4 ± 10.1	26.1 ± 17.7	45.8 ± 10.8	n.s.
Ash	104.6 ± 5.2^{a}	89.6 ± 5.1^{a}	55.0 ± 11.1^{b}	*
Carbohydrates	16.9 ± 3.7^{a}	49.2 ± 3.5^{b}	39.7 ± 12.4^{b}	*

Data are expressed as mean ± SD; values on the same line and different superscripts are significantly different [*$P < 0.05$; n.s. = not significant ($P > 0.05$)].

diet (C0, 820 : 20 protein/carbohydrate) and fed with CH-enriched diets (GLU50, 730 : 100 protein/carbohydrate; STA50, 710 : 100 protein/carbohydrate), although ingestion was greater in the case of STA50, suggesting the better feed efficiency of diets C0 and GLU50.

Our results highlight the better feed efficiency obtained with the glucose diet compared with the diet containing starch or with no added carbohydrates. PPV values (71.88%) for GLU50 were higher than those obtained with crab-based diets (28%), mixed diets of fish and crab (33%) and fish (36%) (García García & Cerezo Valverde 2006), suggesting the greater efficiency of CH and a 30–40% sparing dietary protein in the diet. These results were accompanied by high digestibility coefficients in the GLU50 diet, which were similar to those obtained using natural diets of bogue, sardine and crab (Hernández & García García 2004; Mazón *et al*. 2007), but higher than those obtained with other experimental diets (Seiça Neves *et al*. 2010; Morillo-Velarde *et al*. 2012) using the same methodology as in our. In contrast, the octopus had difficulty in digesting and absorbing the starch on diet STA50, as reflected by the high content of the same in the faeces (470 g kg^{-1}). O'Dor *et al*. (1984) observed 98% digestibility of pure glucose in *O. vulgaris*. However, according to our results, when the octopus were fed diet STA50, there was a sharp reduction in the dry matter digestibility coefficient (94% from 79%) and carbohydrates (98% from 0.3%), suggesting that starch is not well digested by the *O. vulgaris* digestive system. Previous studies have pointed to the poor use of pea-derived CH in *O. vulgaris* (Morillo-Velarde *et al*. 2012), which could be due to the presence of certain antinutritional factors and amylase inhibitors (Carmona *et al*. 1991; Trago *et al*. 2000) or the poor digestibility of starch. However, the pure starch used in the present study had no such antinutritional factors and digestibility was still low, suggesting that *O. vulgaris* is not capable of digesting potato starch in the quantities provided (50 g kg^{-1} of diet). It seems, then, that the complexity of the CH and their origin might be important factors that should be keep in mind when designing experimental diets. Whatever the case, even though octopus has a limited capacity to metabolize CH (Boucher-Rodoni 1973; Boucaud-Camou *et al*. 1976), the inclusion of low proportions of glucose in the diet would contribute to sparing proteins supplied.

In the present study, the content of CH in all the tissues analysed was modified as a consequence of the diets supplied. The CH content of the digestive gland, muscle, carcass and whole animal increased as a consequence of increased CH intake, particularly with diet GLU50 (380 g kg^{-1}). Carbohydrate retention was lower with diet STA50 (170 g kg^{-1}) although higher than in C0, suggesting that, despite the poor digestibility of starch, even small quantities of CH may help maintain its levels in tissues. The CH retention capacity of the digestive gland and muscle was also evident in animals fed a mixed crab and fish diet compared with animals starved for 8 days (Morillo-Velarde *et al*. 2011).

The possibility of substituting protein by carbohydrates has been widely studied in fish to attain maximum sparing protein accompanied by maximal growth (Sen 1981; Rao 1987; Erfanullah 1995; Fernández *et al*. 2007; Árnason *et al*. 2009), but this has not been possible in cephalopods because, until now, there have been no suitable feeds available. As in the case of fish diets, protein is the most expensive macronutrient in the diet for cephalopods. For this reason, it is important to establish the protein content in the minimum value that supports maximum growth to reduce the cost of feeding. In this sense, the content of CH in the diet should be established in future studies to optimize the performance of the diet on octopus ongrowing.

Our study demonstrates that *O. vulgaris* is capable of storing CH in its tissues when the CH intake is increased, although in no case did the levels exceed 6% dry weight. The inclusion of 50 g kg^{-1} glucose in the diet meant a substantial sparing protein, so that a deeper knowledge of the optimal protein/carbohydrate ratio could be used to reduce feeding costs and optimize the performance of the diet in *O. vulgaris*.

Acknowledgements

This work was financed by European Regional Development Fund (PO2007-2013 FEDER) and was also partially sponsored by the IMIDA grant programme. We thank Productos Sur S.A. for their advice about the binders used in the study.

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