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Original article

Response of hexokinase enzymes and the insulin system to dietary carbohydrates in the common carp, *Cyprinus carpio*

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Abstract – The response of the common carp to diets with varying amounts of digestible starch, provided either as pea meal (LP, HP, 30 and 46% peas, respectively) or as cereal (LW, HW, 30 and 46% wheat, respectively), was studied and compared with the response to a carbohydrate-free protein-rich diet (CF). Here we focused on the utilisation of dietary carbohydrates by examining the relationship between dietary starch intake, hepatic hexokinase activities, circulating insulin and muscle insulin receptor system. Plasma glucose concentration and hepatic high Km hexokinase (glucokinase, GK) activity were not affected by the content of digestible starch, but 6 h after feeding enzyme activity was higher in the fish fed carbohydrate diets. Similarly, low Km hexokinase (HK) activity was also higher in the fish 24 h after feeding. Fat gain and protein retention were significantly improved by increased digestible starch intake, especially in the HP group, which in turn, presented the highest plasma insulin levels. Glycogen stores were moderately increased by the ingestion of digestible starch. The number of insulin receptors was greater in the CF group than in fish on carbohydrates, except the HP group. Our results confirmed that the common carp uses dietary carbohydrates efficiently, especially when there are provided by peas. This efficiency might be related to the enhanced response of postprandial insulin observed in the HP group.

glycaemia / insulinemia / hexokinases / fish / insulin muscle receptors

Abbreviations
GK: glucokinase or high Km hexokinase; HK: low Km hexokinase; GLUT: glucose transporter; TKA: tyrosine kinase activity.

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1. INTRODUCTION

Omnivorous fish such as the common carp, *Cyprinus carpio* use dietary starch more efficiently than carnivorous fish like salmonids [1]. A high glucose load either through diets rich in digestible starch or by glucose injection results in prolonged hyperglycaemia in carnivorous fish [2–4]. The mechanisms that may explain the distinct capacity of species to metabolise glucose are not fully understood. Fish increase insulin secretion in response to glucose challenge; however, the key steps in glycemia regulation that are controlled by insulin remain to be elucidated. In mammals, the phosphorylating glucose reaction catalysed by glucokinase (GK) is a key rate-limiting step in the removal of glucose from the circulation by the liver [5]. After some years of controversy on the existence of an inducible GK in teleost, the presence of a GK-like glucose-phosphorylating enzyme has been reported in fish [6, 7], and the GK gene has been cloned from the liver of rainbow trout, the common carp and gilthead seabream [8, 9]. In addition, the activity of hexokinase (HK) can also be nutritionally regulated in fish [10–12].

In mammals, an increase either in glycaemia or insulinemia induces GK gene expression, enhances the activity of this enzyme and promotes glucose uptake [13]. The main factors involved in the induction of GK are still unknown in fish. Omnivorous fish, such as carp, have a potentially higher tissue response to insulin than carnivorous species, such as rainbow trout; skeletal muscle insulin receptors are more numerous and have a higher affinity for insulin in carp than in trout [14]. Nevertheless, the relation between HK activities and the insulin receptor system has not been analysed in this species.

Here we examined some of the major steps in glucose homeostasis in response to dietary carbohydrate in the carp. We studied the consequences of adaptation to diets that contain graded amounts of starch on circulating levels of insulin, and the effect on muscle receptors in relation to glucose-phosphorylating activities and energy stores.

2. MATERIALS AND METHODS

2.1. Experimental diets and growth trials

Four experimental diets (Tab. I) containing carbohydrates provided either as wheat or as peas were formulated with constant levels of lipids (≈ 12%) and proteins (crude protein around 36%) according to the requirements of the species. The diets contained 30 or 46% of extruded wheat or extruded peas in order to provide graded amounts of starch of two origins (LW and HW for the diets with low and high levels of wheat, LP and HP for those with peas; Tab. I). Besides supplying starch, peas are also a protein source used in fish diets as a partial replacement of fishmeal. A carbohydrate-free diet (CF) was also formulated (Tab. I) to study the metabolic response to the practical absence of dietary carbohydrate in comparison with the response to different levels and sources of starch.

Each experimental diet was hand distributed to triplicate groups of fish, twice a day to near satiation (visual observation of the first refusal of feed) and the quantity of food delivered to each tank was recorded. Fish were reared for 10 weeks in experimental facilities in which the water temperature was maintained at 24 ± 1 °C (UTAD, Vila Real).

Fifteen fish were sampled at the beginning of the experiment for body composition analysis and 6 fish per tank at the end. Fish were killed by an excess of anaesthesia (ethylene glycol monophenyl ether) and were kept frozen until analysis which was performed on the groups of fish. Fish from each tank were ground together; an aliquot was analysed for dry matter content and the remaining part was freeze-dried. For digestibility measurements, an automatic faecal collection procedure [15] was used; faecal samples were freeze-dried before analysis.

Table I. Composition of the experimental diets LW (30% flaked wheat), HW (46% flaked wheat), LP (30% extruded peas), HP (46% extruded peas) and CF (carbohydrate-free).

Diets	LW	HW	LP	HP	CF
Ingredients ($g \cdot Kg^{-1}$)					
Flaked wheat[1]	300	460	0	0	0
Extruded pea[2]	0	0	300	460	0
Fish meal	210	320	110	210	860
Soybean meal	355	90	440	180	0
CPSP[3]	0	0	0	0	50
Cod oil	75	70	90	90	30
Mineral mix[4]	10	10	10	10	10
Vitamin mix[4]	30	30	30	30	30
Binder	20	20	20	0	20
Analytical composition (digestible energy in $MJ \cdot kg^{-1}$ dry matter and nutrients as $g \cdot kg^{-1}$ dry matter)					
Energy	16.1	17.0	16.5	17.2	18.7
Protein	292	291	306	297	578
Lipid	116	115	113	119	126
Starch	161	238	137	210	< 1

[1] Cofna, France (starch content: 56.9 % DM).
[2] Aquatex, Sotexpro, France (starch content: 51.7 %DM).
[3] Fish soluble protein concentrate, Sopropêche, France.
[4] According to NRC (1993).

The analyses of fish, faeces and experimental diets were done following standard procedures: dry matter after drying in an oven at 110 °C for 24 h, crude protein (as N × 6.25) by the Kjeldahl method after acid digestion, energy in an adiabatic bomb calorimeter (IKA), fat by petroleum ether extraction (Soxtherm) after hydrolysis. The starch content was measured following the method described by Thivend et al. [16]. The data allowed the calculation of daily gain in protein and fat. Protein retention efficiency was calculated as: (protein gain/digestible protein intake) × 100.

2.2. Tissue sampling and analyses

Blood and tissue sampling was done at the end of the growth trial. On the day of sampling, fish received a single meal which corresponded to 75% of the mean daily feed intake (usually distributed as two meals a day). Nine fish from each treatment were killed by a blow to the head, 6 and 24 h after feeding. Blood was sampled from the caudal vein. Plasma collected after blood centrifugation (7000 rpm, 10 min) was divided into two fractions, which were kept frozen until analysis for glucose and insulin, respectively. The whole liver and a part of white muscle from the right anterior dorsal fillet were quickly excised, cut into small pieces and frozen in liquid nitrogen for further studies, except one piece of each liver sample which was immediately analysed for HK and GK activities and protein content. All experiments were conducted in compliance with the European Community regulations on the treatment of experimental animals, and the researchers were authorised by the French and Catalonian governments to perform animal experiments (No. 5228 and 214/97, respectively).

Plasma glucose levels were measured using an automatic glucose analyser (Beckman II, USA) and plasma insulin levels by radioimmunoassay using bonito insulin as a standard and rabbit anti-bonito insulin as the

antiserum [17]. Hepatic glycogen contents were determined using the modified method of Murat and Sefarty [18] and protein contents following Bradford [19]. HK and GK activities were measured in crude liver homogenates from all the fish sampled, using the method described in Panserat et al. [20]. The reaction was initiated by 1 mmol·L^{-1} glucose and 100 mmol·L^{-1} glucose for measuring low-Km HK and total HK activity measurements, respectively. GK activity was estimated by subtracting the rate of NADPH formation at 340 nm in the presence of 1 mmol·L^{-1} glucose from that measured with 100 mmol·L^{-1} glucose. One unit of the enzyme activity was defined as the amount that phosphorylates 1 µmol glucose·min^{-1}.

2.3. Muscle insulin receptors

Partial purification of solubilised insulin receptors from white muscle sampled 24 h after feeding was performed at 4 °C by affinity chromatography on wheat-germ agglutinin (WGA) bound to agarose [21]. The glycoproteins obtained were measured according to Bradford [19].

Binding assays were performed using the method described by Párrizas et al. [21]. A volume of 30–40 µL of the WGA eluate (approximately 30 µg glycoproteins) was incubated for 14–16 h at 4 °C with increasing concentrations of cold hormone (from 0 to 100 nmol·L^{-1}, final dilution) and the radio labelled ligand (Human Tyr A14 ^{125}I-monoiodoinsulin, specific activity: 2000 Ci·mmol^{-1}) as a tracer (25 pmol·L^{-1}). Semi-purified receptors were precipitated by the addition of bovine γ-globulin and polyethylene glycol, followed by centrifugation at 14 000 × *g* for 7 min at 4 °C. Binding data were analysed in Scatchard plots and only the high-affinity, low-capacity binding sites were taken into account.

Tyrosine kinase activity (TKA) was measured following James et al. [22] with minor modifications. Receptor glycoproteins (15 µL) were first incubated for 14–16 h at 4 °C in the absence (basal) or presence of insulin (60 nmol·L^{-1} final concentration). Receptors were then incubated with 50 µmol·L^{-1} [γ-^{32}P] ATP for 10 min and with synthetic substrate poly (Glu:Tyr) 4:1 added in a final concentration of 0.25 mg·mL^{-1} for 30 min. The reaction was stopped by transferring samples to filter papers which were washed, dried and counted in a scintillation counter.

2.4. Statistical analyses

The values are given as means with standard deviations. Statistical analyses were performed using the General Linear Model procedure of the Statistical Analysis System (SAS Institute, Cary, NC). Probabilities of differences between means were considered significant when the *P* values were lower than 0.05.

Growth performance, feed efficiency, fat and protein gain and protein retention values obtained from the groups of fish were analysed by one-way analysis of variance. The tank was used as the experimental unit for these statistical analyses. The effects of dietary treatments, sampling time and interactions between these two factors on the data obtained from the individual fish were analysed by two-way analysis of variance. When the interaction was significant ($P < 0.05$), the effects of the diets and sampling time were analysed separately. The effect of sampling time was assayed by the Student *t* test. The effect of the diets was analysed by one-way analysis of variance; when it was significant ($P < 0.05$), the means were compared using the Newman-Keuls multiple range test. The effect of dietary treatments on insulin receptor characteristics was tested by a one-way analysis of variance followed by the Newman-Keuls multiple range test when relevant ($P < 0.05$).

3. RESULTS

3.1. Growth and nutrient utilisation

At the end of the 10-week feeding trial, carp on the HW and HP diets had significantly higher body weight gain than those in the

Table II. Growth performance, feed efficiency, fat and protein gain and protein retention in the common carp fed the experimental diets for 10 weeks[1].

	LW	HW	LP	HP	CF
Initial body weight (g)	35.1 ± 0.9	35.2 ± 0.8	35.3 ± 0.6	35.2 ± 0.4	35.1 ± 1.0
Final body weight (g)	79.5 ± 4.8^{c}	101.6 ± 9.9^{ab}	91.4 ± 3.2^{b}	120.6 ± 9.9^{a}	24.9 ± 15.1^{a}
Feed intake (g DM·kg^{-1}·d^{-1})[1]	14.0 ± 0.9^{a}	12.4 ± 0.9^{bc}	13.2 ± 0.1^{ab}	11.0 ± 0.5^{c}	11.9 ± 0.1^{bc}
Feed efficiency[2]	0.78 ± 0.07^{c}	1.12 ± 0.15^{b}	0.95 ± 0.04^{bc}	1.42 ± 0.12^{a}	1.33 ± 0.09^{a}
Fat gain (g·kg^{-1}·d^{-1})	1.25 ± 0.07^{b}	1.45 ± 0.14^{a}	1.34 ± 0.05^{ab}	1.45 ± 0.04^{a}	0.64 ± 0.13^{c}
Protein gain (g·kg^{-1}·d^{-1})	1.64 ± 0.17^{b}	2.07 ± 0.27^{ab}	1.90 ± 0.14^{ab}	2.27 ± 0.23^{a}	2.32 ± 0.05^{a}
Protein retention (% digestible intake)	40.1 ± 1.9^{c}	57.4 ± 6.1^{b}	46.7 ± 3.4^{bc}	69.1 ± 6.7^{a}	33.7 ± 0.2^{c}

The experimental diets were LW (30% flaked wheat), HW (46% flaked wheat), LP (30% extruded peas), HP (46% extruded peas) and CF (carbohydrate-free).
[1] DM: dry matter.
[2] Feed efficiency = wet weight gain/dry feed intake.
Values are means ± SD (n = 3 groups except with CF diets where n = 2). The tank was used as the experimental unit.
On each line, means with distinct superscripts are significantly different ($P \leq 0.05$).

LW and LP groups and a similar weight to those on the CF diet. Nevertheless, the HW, HP and CF groups had lower feed intakes (Tab. II). Feed efficiency was enhanced by increasing the dietary level of the two carbohydrate sources. The highest values were found for the diet containing 46% extruded peas (HP), and were close to CF. Increasing the dietary level of carbohydrates resulted in a significant improvement of dietary protein utilisation as reflected by high protein retention and daily protein gain. The highest value was obtained with the HP diet. An increase in body fat gain (more than twofold) was also observed in fish fed digestible starch compared to those deprived of carbohydrates (Tab. II).

3.2. Hepatic glycogen stores and hexokinase activities

There were significant ($P < 0.05$) interactions between dietary treatments and sampling time on these three parameters.

Hepatic glycogen contents were higher in the carp fed digestible starch than in those on the CF diet ($P < 0.05$; Tab. III) and the levels tended to be higher in the HW and HP groups. Glycogen levels were thus not related to the sources of carbohydrates.

The activity of hepatic HK (Tab. III) measured at 6 h was not significantly affected by the treatments, but tended to be higher in the HP group. The activity of this enzyme remained almost constant 24 h after ingestion of the carbohydrate diets, while the activity in fish on the CF diet declined 24 h after feeding ($P < 0.05$). The values of hepatic GK activity were similar in the four groups of carp on carbohydrates and significantly ($P < 0.05$) lower in those fed the CF diet. In the former groups, the GK activity tended to be higher at 6 than 24 h after feeding but the differences were not significant.

3.3. Plasma glucose, insulin levels and muscle insulin receptors

Plasma glucose levels ranged between 3.4 and 5.3 mmol·L^{-1}. There was not a significant effect of neither dietary treatment nor sampling time on glycemia ($P > 0.05$; Fig. 1). Nevertheless, the observation of digestive tract contents after blood sampling ensured us that all the fish sampled had consumed the diets.

Table III. Glycogen content and activities of low-Km hexokinase (HK) and glucokinase (GK) in the liver of carp, 6 and 24 h after feeding the experimental diets for 10 weeks.

	LW	HW	LP	HP	CF
Glycogen ($mg \cdot g^{-1}$) 6 h	34.1 ± 9.8^{a}	38.1 ± 5.0^{a}	29.5 ± 9.3^{a}	36.4 ± 6.7^{a}	10.5 ± 3.1^{b}
Glycogen ($mg \cdot g^{-1}$) 24 h	37.5 ± 12.7^{a}	$50.9 \pm 11.4^{a*}$	38.9 ± 15.9^{a}	41.5 ± 9.6^{a}	$21.7 \pm 6.1^{b*}$
HK ($mU \cdot mg^{-1}$ prot) 6 h	4.09 ± 1.61	4.81 ± 0.90	5.14 ± 1.86	6.57 ± 1.91	4.05 ± 0.67
HK ($mU \cdot mg^{-1}$ prot) 24 h	4.25 ± 0.90^{a}	4.12 ± 0.95^{a}	4.46 ± 0.79^{a}	4.42 ± 0.95^{a}	$2.32 \pm 0.35^{b*}$
GK ($mU \cdot mg^{-1}$ prot) 6 h	1.65 ± 0.46^{a}	2.54 ± 1.23^{a}	2.31 ± 0.68^{a}	2.98 ± 0.92^{a}	0.47 ± 0.25^{b}
GK ($mU \cdot mg^{-1}$ prot) 24 h	1.21 ± 0.56^{ab}	1.03 ± 0.44^{ab}	1.12 ± 0.44^{ab}	1.69 ± 0.56^{a}	0.64 ± 0.48^{b}

The experimental diets were LW (30% flaked wheat), HW (46% flaked wheat), LP (30% extruded peas), HP (46% extruded peas) and CF (carbohydrate-free). The values are means ± SD, $n = 9$. Due to significant ($P < 0.05$) interactions between dietary treatments and sampling time, the effect of these two factors were tested separately. On each line, means with distinct superscripts are significantly different ($P \leq 0.05$). * Indicates a significant difference ($P \leq 0.05$) between values of each parameter measured at 6 h and 24 h after feeding.

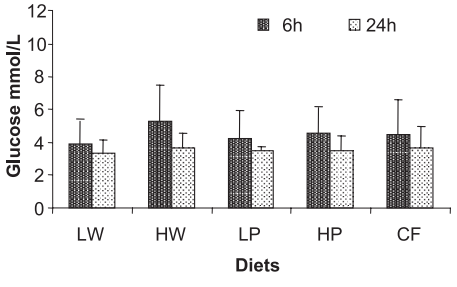

Figure 1. Plasma glucose levels in the common carp 6 and 24 h after administration of the experimental diets LW (30% flaked wheat), HW (46% flaked wheat), LP (30% extruded peas), HP (46% extruded peas) and CF (carbohydrate-free). Each bar represents means ± SD, $n = 9$.

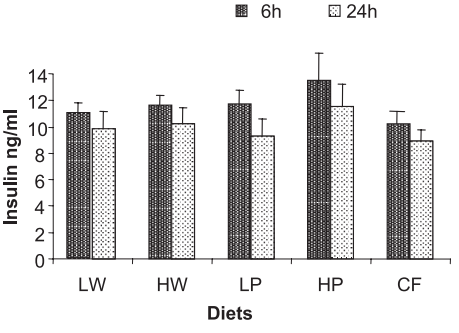

Figure 2. Plasma insulin levels in the common carp 6 and 24 h after the administration of the experimental diets LW (30% flaked wheat), HW (46% flaked wheat), LP (30% extruded peas), HP (46% extruded peas) and CF (carbohydrate-free). Each bar represents means ± SD, $n = 9$. There was a significant effect of the dietary treatment ($P < 0.0001$) and of sampling time ($P < 0.0001$) but not a significant interaction between these two factors ($P = 0.3$).

Plasma insulin levels were significantly affected by both the dietary treatments ($P < 0.0001$) and the time after the meal ($P < 0.0001$). The highest insulin levels were found in carp on the HP diet and the lowest values in the CF group (Fig. 2).

The number of receptors (Ro) was significantly ($P < 0.05$) higher in the CF group than in those fed digestible starch, except the HP group. The dissociation constant (Kd) and insulin-induced TKA were not affected by the treatments (Tab. IV).

4. DISCUSSION

We analysed the effects of the diets containing two distinct sources and levels of carbohydrate on glucose utilisation in the carp, and we studied whether the glucose phosphorylating capacities, post-prandial insulin and its binding to muscle tissue could

Table IV. Characteristics of the insulin receptors in white muscle of carp fed the experimental diets for 10 weeks.

	LW	HW	LP	HP	CF
%Bsp/30 µg protein	2.17 ± 0.30^{ab}	1.77 ± 0.20^{b}	2.43 ± 1.19^{ab}	3.24 ± 0.97^{ab}	4.42 ± 1.13^{a}
R_0 (fmol·mg^{-1} prot.)	48.7 ± 21.8^{b}	48.9 ± 20.8^{b}	61.1 ± 4.5^{b}	100.7 ± 45.4^{ab}	148.1 ± 35.7^{a}
Kd (nmol·L^{-1})	0.39 ± 0.14	0.38 ± 0.11	0.43 ± 0.22	0.41 ± 0.08	0.51 ± 0.12
TKA	280 ± 52	282 ± 58	283 ± 23	265 ± 33	283 ± 58

% Bsp: specific binding of insulin on semi-purified membrane receptors (30 µg eluted glycoproteins).
R_0: number of insulin receptors.
Kd: constant of dissociation.
TKA: insulin-induced tyrosine kinase activity; the values are percentages of basal activity.
The experimental diets were LW (30% flaked wheat), HW (46% flaked wheat), LP (30% extruded peas), HP (46% extruded peas) and CF (carbohydrate-free). The values are means ± SD, $n = 4$. On each line, means with distinct superscripts are significantly different ($P \leq 0.05$).

contribute to the dietary glucose utilisation in this species.

Glucose and insulin plasma levels and enzyme activities 24 h after feeding have been considered as control values to be compared with 6 h postfeeding. Previous studies have demonstrated that 24 h pancreatic hormone and glucose levels can be considered as basal values [23]. In contrast, the values at the time of feeding could be affected by neural or gastrointestinal factors and thus cannot be considered suitable control values [24]. Regarding the enzyme activities, hexokinase values 24 h after feeding have also been considered as basal in other fish studies [9, 11, 25].

The absence of changes in plasma glucose levels 6 h after receiving carbohydrate-rich diets indicates that glucose homeostasis is maintained after adaptation to experimental diets. Earlier studies in the carp [26] showed that intake of a diet with 25% digestible starch provokes only a moderate rise in glycaemia 2 h after feeding, with values being maintained above 5.5 mmol·L^{-1}. Phosphorylation of glucose into glucose-6-phosphate, the first step of glucose metabolism, is regulated by the whole family of hexokinase enzymes [27]. Owing to its particular affinity for glucose, GK plays a significant role in glucose homeostasis in mammals by regulating both glucose uptake and output [5, 28]. In the present study, glycaemia was maintained stable after carbohydrate intake even with a weak induction of GK activity, in comparison with the CF group. In fact, the induction of GK activity after feeding was much lower than that found in mammals [29] and even in carnivorous fish, gilthead seabream or trout [20, 25]. On the contrary, hepatic HK activity in the carp was higher than that found in trout [11]. HK working at low glucose concentration could be sufficient to phosphorylate dietary glucose and to promote the progressive use of glucose-6-phosphate as a substrate for glycolysis in the carp. The hepatic HK activity in fish on carbohydrates was still high 24 h after feeding, indicating that a more prolonged activation of this enzyme could maintain glucose homeostasis, without the need for the induction of GK. Nevertheless, no differences were observed in hepatic HK activity at 24 h between the groups fed various levels of carbohydrates or between the groups of different origin. The determination of whether this pattern of HK activation (a low induction of GK and high activity of HK) is a characteristic of omnivorous species may help us to elucidate glucose regulation in fish.

In mammals and fish, increases in hepatic GK activity are related to the induction of GK gene transcription [20, 29, 30]. In mammals, the transcription of this gene is stimulated by insulin. Nevertheless, in

trout or perch, hepatic GK activity increases with the presence of dietary carbohydrates independently of the levels of plasma insulin, which are elevated after ingestion of either a carbohydrate or a carbohydrate-free diet [11, 12]. Similarly, in the carp, postprandial insulin levels were higher in the HP group but were not accompanied by increases in GK activity. These results indicate that in fish, insulin could be necessary but not sufficient to induce GK activity.

Insulin acts in peripheral tissues, mainly skeletal muscle, by binding to membrane receptors and promoting the entry of glucose into cells, thus regulating glycaemia. In mammals, exposure to insulin provokes a down-regulation of the number of insulin receptors in target tissues, as a mechanism of auto-inhibition of hormone response [31, 32]. In trout, an up-regulation of receptors in white muscle occurs in response to increased insulin levels induced by carbohydrate-rich diets [33, 34]. This up-regulation was considered an adaptation to improve the efficiency of glucose utilisation, in response to excess dietary carbohydrate, especially in carnivorous fish, such as trout, which have few muscle receptors [33]. The present study was the first to test the effect of a long- term feeding trial on insulin binding characteristics in the carp. A lower number of receptors was found in the fish fed digestible starch, which could be an example of the down-regulation of muscle insulin receptors by dietary carbohydrates. In contrast to salmonids, carp may not need to increase tissue sensitivity to insulin, at least with the levels of dietary starch tested here.

Dietary glucose carbons are efficiently used by carp as energy substrates, since changing part of fish protein with a high level of carbohydrates in the diet did not impair growth performance. Increasing digestible starch intake also results in increased fat deposition, suggesting that more dietary glucose-carbons are directed towards lipogenesis [35]. In addition, increasing a digestible starch supply significantly improved protein retention suggesting an increased glucose utilisation for energy production that leads to a reduction in protein breakdown. Proteins supplied in excess in the diet are utilised by fish for energy production, which explains why protein retention was lower in the carp fed the protein-rich diet, the CF group. Improvement of protein retention was especially evident in the HP group, which, in turn, presented higher levels of plasma insulin and body weight gain than fish on the other diets. Some authors have described a positive correlation between insulin and growth rate in fish [36] seems that, on the contrary to what is generally observed in salmonids, in which insulin is stimulated predominantly by amino acids rather than glucose [36], carp show an efficient insulin response to carbohydrate diets. So, taken together, the groups with a higher content of carbohydrates (HW, and especially HP), had higher plasma insulin levels than the CF group.

Neither GK activation nor an increase in the number of insulin receptors seem to be responsible for this efficiency in dietary glucose utilisation for growth. On the contrary, the relatively high activity of HK could contribute to stimulating the glycolytic pathway, as reported in rat hepatocytes that overexpress either HK I or GK [37], and thus prevent an elevated accumulation of glycogen in the liver.

On the basis of our results, we conclude that GK activity is not the major regulator of glucose homeostasis in the carp and that the activity of low Km HK is more relevant in carbohydrate utilisation in this species. The incorporation of starch at the higher level tested in this study is adequately utilised by the carp, especially when it is provided by peas rather than wheat.

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