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MORPHOMETRIC ALTERATIONS IN HEPATOCYTES AND ULTRASTRUCTURAL DISTRIBUTION OF LIVER GLYCOGEN IN PACU (*Piaractus mesopotamicus* HOLMBERG, 1887) DURING FOOD RESTRICTION AND REFEEDING

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ABSTRACT

The morphometric alterations in hepatocytes and the ultrastructural distribution of tissue glycogen in pacu (*Piaractus mesopotamicus*) were studied following food restriction and refeeding. Fish (200-300 g) were allocated to control and experimental groups. The experimental group was sampled after 0, 2, 7, 30 and 60 days of food restriction and after 7 and 30 days of refeeding. The control group, which was fed daily, was sampled on the same days. The morphometric results were analyzed by ANOVA in a 2x7 (feeding x days) factorial design and the averages compared by the Tukey test. Transmission electron microscopy showed liver glycogen mobilization during food restriction. The levels of glycogen did not return to normal after up to 30 days of refeeding. There was a decrease in the cytoplasmic area and volume after seven days without food whereas changes in the nuclear area and volume appeared after two days of food restriction. Recovery of the nuclear and cytoplasmic area and volume occurred after 7 days and 30 days of refeeding, respectively. These results indicate that liver glycogen supplies at least part of the energy requirement during food restriction in juvenile pacu. Thirty days of refeeding were not enough to re-establish the pre-restriction carbohydrate levels, probably because of the extra energy demand associated with the high metabolic rate that occurred during the compensatory process of refeeding at elevated ambient temperatures. However, the recovery seen in the morphometric parameters of the hepatocytes indicated a functional re-adjustment of the liver stimulated by the restored food supply.

Key words: Fish, food restriction, glycogen, hepatocyte, refeeding

INTRODUCTION

The peculiar pattern of energy metabolism in fish allows them to survive long periods of natural starvation [2]. This ability has been attributed to a low metabolic rate or to the activation gluconeogenesis [13]. Glycogen is the main carbohydrate reserve in liver and muscle. Hepatic glycogen plays a fundamental role in blood glucose homeostasis [10] and is mobilized during stressful conditions such as starvation [8,13,20]. In fish, the energy source during starvation varies among species, with some using mainly glycogen while others use lipids or proteins [8,19,20]. Energy mobilization during prolonged food restriction may provoke marked alterations in tissue structure. In hepatocytes, starvation leads to a decreased cellular area and volume, the appearance of a collagen fiber network, iron particle accumulation, changes in nucleus shape and position, reduced intercellular spaces and

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cell disorganization. Additionally, the cytoplasm shows a low affinity for stains, the nucleus assumes a darker coloration, and glycogen and lipid reserves diminish [1,3,21,23].

Energy mobilization during food restriction may be affected by other factors, including ambient temperature [15]. Independent of the effect of temperature in different species, the mobilization of energy-providing substrates occurs to support the body's requirements, and the use of lipids, protein and glycogen leads to cellular modifications in fish tissues [2,4,18,21,23]. The re-establishment of feeding after restriction stimulates the use of food and the replenishment of energy deposits in somatic tissues [4,5,20]. The liver cell alterations provoked by starvation are also reversible [4,11,23].

In this study, we assessed liver glycogen mobilization in pacu (*Piaractus mesopotamicus*) subjected to food restriction and refeeding by evaluating hepatocyte morphometry and the ultrastructural distribution of hepatic glycogen.

MATERIAL AND METHODS

The experiments were done from October 95 to January 96 at the Aquaculture Center at UNESP, Jaboticabal, in the state of Sao Paulo. Juvenile pacu (*Piaractus mesopotamicus*), weighing 200-300 g, were assigned to control and experimental groups. The experimental group was sampled on days 0, 2, 7, 30 and 60 of food restriction and on days 7 and 30 of refeeding. The control group was fed daily, once a day, and sampled on the same days. Seven days before the first sampling, the fish were stocked in tanks with flowing water to allow for adaptation. An extruded commercial ration (24% crude protein) was supplied in an amount corresponding to 3-5% body weight throughout the experiment. The mean water temperature monitored daily was 24.8°C in the morning and 27.2°C in the afternoon.

At each sampling, the fish were quickly anesthetized with benzocaine (1 g/15 L) and the liver then collected through a ventral incision.

Transmission electron microscopy (TEM)

Tissue fragments were fixed in Karnowsky solution, post-fixed in 1% osmium tetroxide [14] and processed for TEM. The sections were examined using a JEOL-100C electron microscope.

Light microscopy

Liver fragments were fixed in Bouin solution at 4°C for 20-24 h and then processed by routine histological methods prior to embedding in paraffin. Sections 5 µm thick were stained with hematoxylin and eosin (HE) and examined in light microscopy in association with a Kontron Elektronik Image Analyzer (Videoplan). In one slide from each fish, the area and volume of the cytoplasm and nucleus of 30 hepatocytes were measured and the averages calculated. The sections were photographed using a Nikon Alphaphot-2 YS2 photomicroscope. The morphometric results were analyzed by ANOVA in a 2x7 (feeding x days factorial design), with seven repetitions (n=7 fish) and the averages were compared using Tukey's test. The level of significance was set at 5 % [22].

RESULTS

The hepatocytes of both groups of fish contained a large number of glycogen particles in the cytoplasm on day 0 of the experiment (Figs. 1A, B). After two days of food restriction, the number of glycogen particles was still elevated (Fig. 1C) but gradually decreased as the food restriction continued (Figs. 1D-F). The glycogen level remained low, even after 7 (Fig. 1G) and 30 (Fig. 1H) days of refeeding.

Figure 2 A-H shows the histological appearance of pacu liver sections at various intervals during the experiment. Morphological analysis revealed three lobes surrounded by a thin layer of connective tissue. The hepatocytes were aligned in cords interspersed by sinusoidal capillaries which converged on a terminal hepatic vein. These cells were polygonal with one or two central nucleus, an evident nucleolus and biliary ducts, but did not form lobules. Exocrine pancreatic tissue (hepatopancreas) was present and was also surrounded by connective tissue but was separated from the liver cells by sinusoids containing blood vessels, both at the periphery and in interior of the tissue.

There was a significant difference ($P<0.05$) in the area and volume of the cytoplasm and nucleus of hepatocytes from the control and food restricted groups (Table 1). There was a decrease ($P<0.05$) in the area and volume of the cytoplasm in food restricted fish from 7 days onwards while the nuclear area and volume were smaller ($P<0.05$) after 2 days. Seven days of refeeding were sufficient to re-establish the nuclear area and volume in food restricted fish to the levels seen in control fish. However, the cytoplasmic area and volume returned to prerestriction values only after 30 days of refeeding.

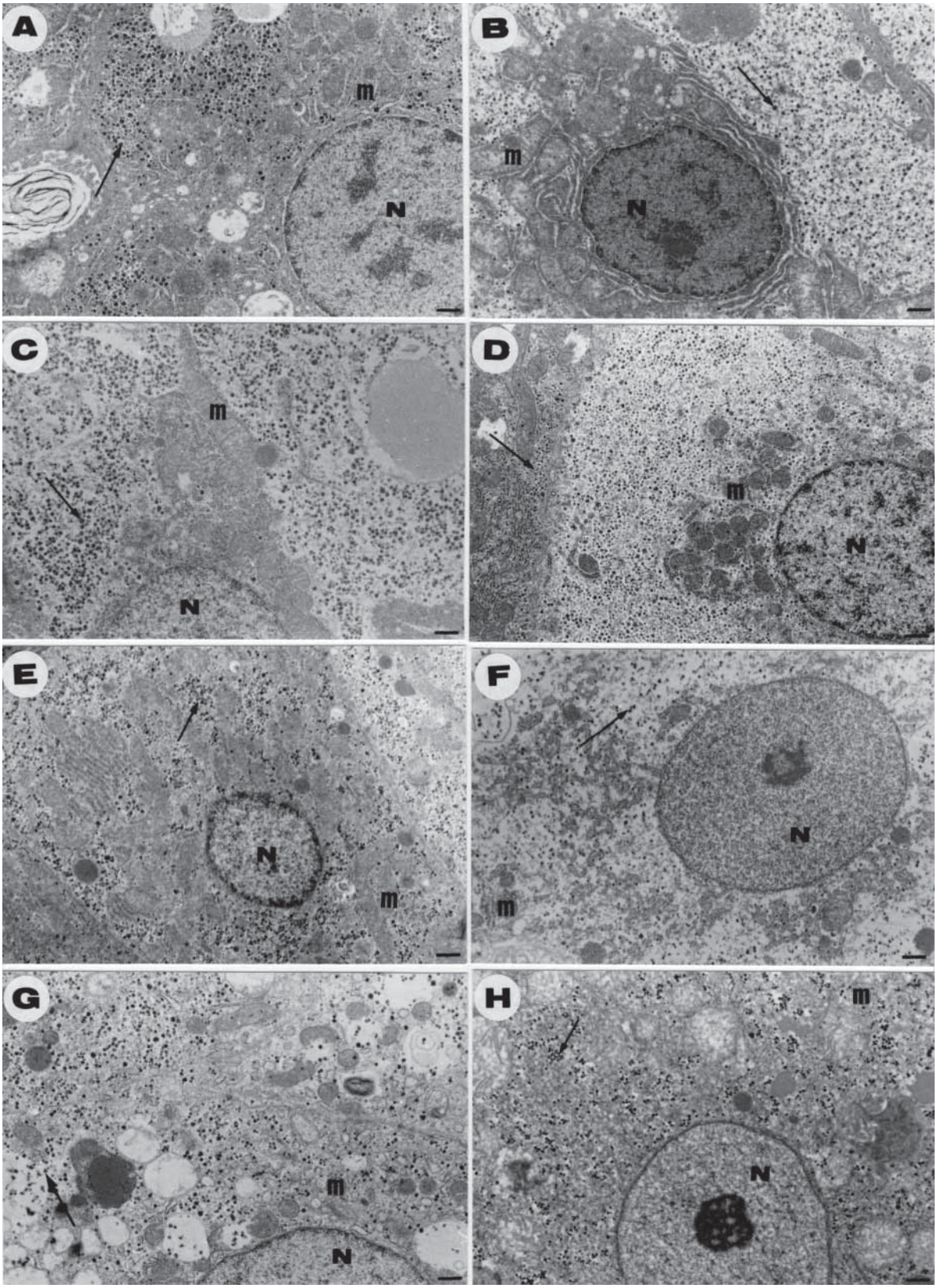

Figure 1. Electron micrograph of juvenile pacu (*P. mesopotamicus*) hepatocytes. Day 0 (**A** – control and **B** – experimental), day 2 (**C**), day 7 (**D**), day 30 (**E**) and day 60 (**F**) of food restriction and day 7 (**G**) and day 30 (**H**) of refeeding. N= nucleus; m= mitochondria; → = glycogen. Bar = 3 μm.

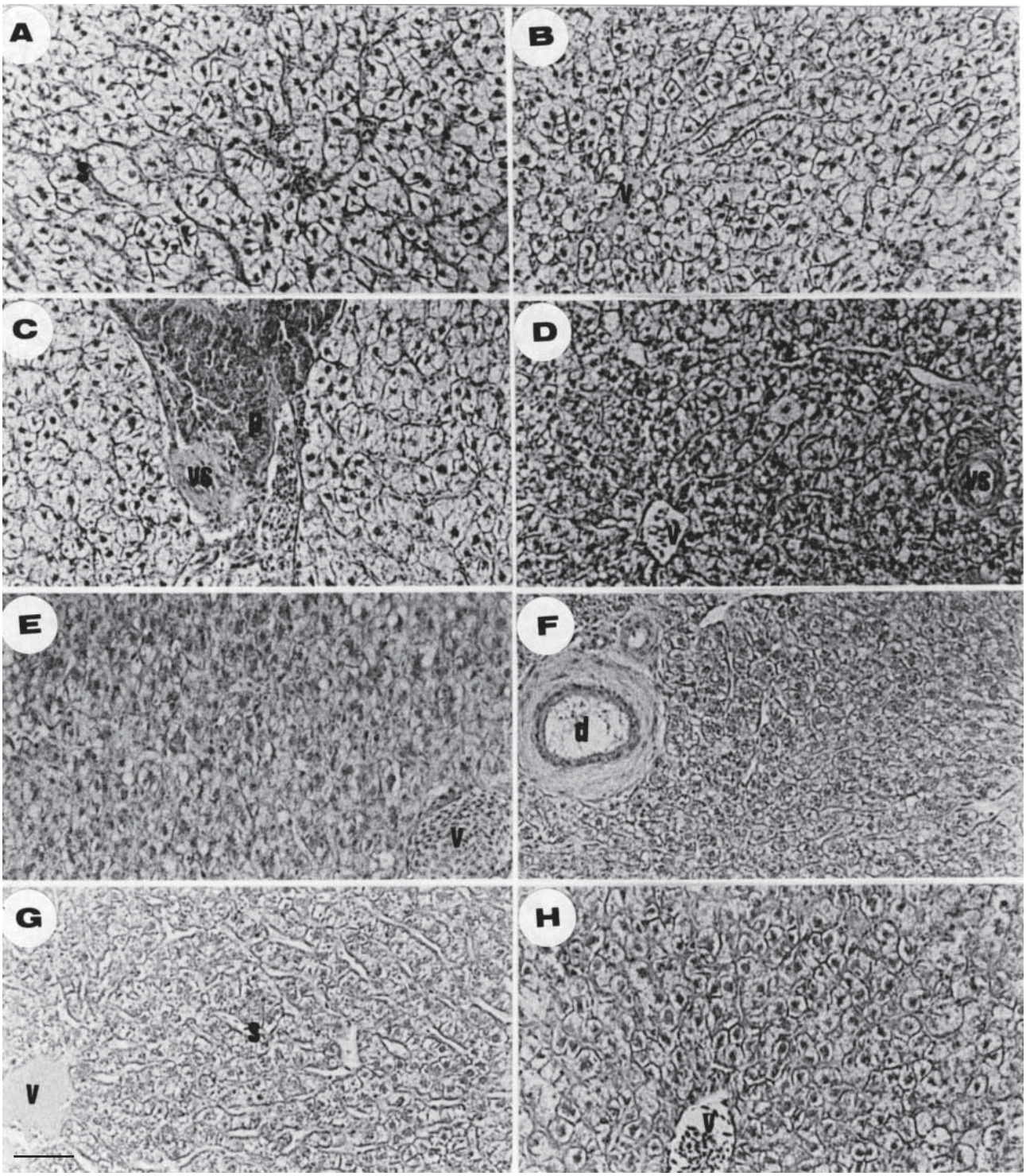

Figure 2. Photomicrograph of juvenile pacu (*P. mesopotamicus*) hepatocytes. Day 0 (**A** – control and **B** – experimental); day 2 (**C**), day 7 (**D**), day 30 (**E**) and day 60 (**F**) of food restriction and day 7 (**G**) and day 30 (**H**) of refeeding. s – sinusoid capillaries, d – biliary duct; p – hepatopancreas, v – terminal hepatic vein, vs – blood vessel. HE, Bar = 1000 µm for all panels.

Table 1. Average values for cytoplasmic area (CA), nuclear area (NA), cytoplasmic volume (CV) and nuclear volume (NV) in *P. mesopotamicus* hepatocytes.

Variable	Group	Food restriction (days)					Refeeding (days)		C.V. **
		0	2	7	30	60	7	30	
CA	Control	133.8 Ab*	158.6 Aab	165.1 Aa	152.1 Aab	140.2 Aab	137.5 Ab	137.4 Ab	
(μm^2)	Experimental	146.7 Aa	159.7 Aa	143.1 Ba	96.4 Bbc	76.7 Bc	114.4 Bb	141.4 Aa	11.4
NA	Control	12.5 Aab	13.6 Aa	12.2 Aab	12.3 Aab	11.8 Abc	10.4 Ac	11.1 Abc	
(μm^2)	Experimental	12.3 Aa	12.4 Ba	10.9 Babc	10.7 Bbc	9.6 Bc	9.5 Ac	11.8 Aab	8.6
CV	Control	14.5 Ab	17.3 Aab	18.0 Aa	16.9 Aab	16.0 Aab	14.9 Ab	14.7 Ab	
(μm^3)	Experimental	15.7 Aa	17.3 Aa	15.3 Bab	10.5 Bcd	8.6 Bd	12.4 Bbc	15.2 Aab	12.4
NV	Control	1.34 Ab	1.53 Aa	1.36 Aab	1.36 Aab	1.30 Abc	1.16 Ac	1.21 Abc	
(μm^3)	Experimental	1.34 Aab	1.40 Ba	1.22 Babc	1.22 Bbc	1.09 Bc	1.04 Ac	1.27 Aab	8.7

* Averages followed by the same lower case letters in the lines and upper case letters in the columns do not differ significantly (Tukey test). **Coefficient of variation (%).

DISCUSSION

During food restriction, the growth rate of fish is reduced and energy is re-directed to the maintenance of metabolism [12]. Liver glycogen in juvenile pacu was mobilized from the seventh day of food restriction onwards. Refeeding for up to 30 days was not enough to restore the glycogen reserves to the levels seen in control fish. The compensatory process of physiological reorganization after food restriction, which includes body growth [20], may have consumed most of the food energy available during refeeding, thereby preventing glycogen deposition in the liver.

The energy requirement may have been exacerbated because the study was conducted in spring and summer, the hottest seasons of the year (mean morning and afternoon temperatures of 24.8ºC and 27.2ºC, respectively). Indeed, the effects of food restriction in summer are more severe than in winter, because of the higher metabolic rate [24]. Similar results were reported by Souza *et al.* [20] based on biochemical analysis in the same species. Leatherland [9] and Storch and Juario [23] also obtained similar results in *Oncorhynchus kisutch* and *Chanos chanos*, respectively. In *Macquaria ambigua* [5], the levels of liver glycogen recovered totally after 30 days of refeeding in experiments done at temperatures below 20ºC.

Histologically, the appearance of the liver tissue agreed with that of *Leuciscus idus* [18], *Spauratus auratus* [16] and *Hydrocynus forskahii* [7]. However, unlike in *Micropogon undulatus* [6] and *Salmo salar* [17], there was no lobule formation in the hepatic parenchyma.

The hepatocyte cytoplasmic and nuclear area and volume decreased, probably because of decreased anabolic and increased catabolic processes during food restriction. This would agree with the reduced levels of tissue glycogen. After 30 days of refeeding, the morphometric parameters returned to control values, indicating a functional adjustment in the liver stimulated by the renewed food supply. The lack of glycogen deposition suggested that this energy substrate was being used for body maintenance to replace somatic catabolism and support fish growth [20,24]. Alterations in liver cells provoked by starvation have been reported to be reversible thereby permitting *de novo* formation of glycogen deposits and recovery of the organ size [4,8,11,23].

The pronounced depletion of liver glycogen provoked by 60 days of food restriction suggested that this carbohydrate provided part of the energy requirements during starvation. Other studies [8,20] have indicated the use of lipids and proteins as an additional source of energy. Although the compensatory processes during the refeeding may be influenced by an elevated ambient temperature, the recovery of the morphometric parameters in liver cells indicated that *P. mesopotamicus* did not lose its capacity for physiological adjustment after the nutritional deprivation. This finding

corroborates the statement by Weatherley and Gill [24] that fish have developed this ability to recover in response to environmental oscillations.

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