



Protein and lipid metabolism adjustments in silver catfish (*Rhamdia quelen*) during different periods of fasting and refeeding

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Received: September 15, 2016 – Accepted: February 21, 2017 – Distributed: October 31, 2018

Abstract

The fish may experience periods of food deprivation or starvation which produce metabolic changes. In this study, adult *Rhamdia quelen* males were subjected to fasting periods of 1, 7, 14, and 21 days and of refeeding 2, 4, 6, and 12 days. The results demonstrated that liver protein was depleted after 1 day of fasting, but recovered after 6 days of refeeding. After 14 days of fasting, mobilization in the lipids of the muscular tissue took place, and these reserves began to re-establish themselves after 4 days of refeeding. Plasmatic triglycerides increased after 1 day of fasting, and decreased following 2 days of refeeding. The glycerol in the plasma oscillated constantly during the different periods of fasting and refeeding. Changes in the metabolism of both protein and lipids during these periods can be considered as survival strategies used by *R. quelen*. The difference in the metabolic profile of the tissues, the influence of the period of fasting, and the type of reserves mobilized were all in evidence.

Keywords: fasting, lipids, protein, refeeding, silver catfish.

Ajustes no metabolismo de proteínas e lipídios no jundiá (*Rhamdia quelen*) durante diferentes períodos de jejum e realimentação

Resumo

Os peixes podem sofrer períodos de privação de alimentos ou de fome, que produzem mudanças metabólicas. Neste estudo, jundiás machos adultos foram submetidos a jejum períodos de 1, 7, 14 e 21 dias e realimentação 2, 4, 6, e 12 dias. Os resultados demonstraram que a proteína do fígado foi esgotada depois de um dia de jejum, mas restabeleceu após 6 dias de realimentação. Após 14 dias de jejum, ocorreu a mobilização dos lipídios no tecido muscular sendo que estas reservas começaram a re-estabelecer-se após 4 dias de realimentação. Os triglicérides plasmáticos aumentam após um dia de jejum, e diminuíram após 2 dias de realimentação. O glicerol no plasma oscilou constantemente durante os diferentes períodos de jejum e realimentação. As alterações no metabolismo de proteína e lipídios durante estes períodos podem ser consideradas uma estratégia de sobrevivência utilizada pelo *Rhamdia quelen*. Sendo que a diferença no perfil metabólico tecidual bem como a influência do período de jejum e o tipo de reserva a ser mobilizada foram observadas neste estudo.

Palavras-chave: jejum, lipídios, proteína, realimentação, jundiá.

1. Introduction

In subtropical climate zones of the world, fish species used for aquaculture must grow rapidly during the summer and withstand low temperatures in the winter season. In this context, a promising fish species for aquaculture is the South American catfish, *Rhamdia quelen*

(Teleostei: Pimelodidae), that exhibits high prolificacy and fast weight gain, particularly in the warmer months of the year (Gomes et al., 2000). Due to these characteristics, *R. quelen* fish have been intensively cultured, and have even been used as a model to improve the management

of several other species of fish in the Pimelodidae family (Barcellos et al., 2001).

R. quelen, as with other fish species, may experience periods of food deprivation or starvation—both *in natura* (due to limited food availability in winter and the reduction in food intake associated with life-cycle phenomena, e.g., spawning migration) and also in aquaculture (due to pre-harvest fasting, as well as shipping management and feeding regimes, which restrict availability of food) (Mackenzie et al., 1998; Souza et al., 2003; Camargo and Urbinati, 2008). Natural or artificial fasting periods produce a number of hormonal and biochemical changes, including reduction in muscle glycogen and mobilization of fat and protein, which may impair fish meat quality (Sigholt et al., 1997; Thomas et al., 1999).

Fish use food primarily to meet energy requirements for maintaining vital processes and restoring tissue catabolism (Hepher, 1988). Fasting induces a complex series of changes that regulate biological functions, and its ultimate consequences are highly dependent upon the fish species and age, as well as on experimental conditions, e.g., water temperature, photoperiod, and pre-fasting diet (Love, 1980; Weatherley and Gill, 1987; Blasco et al., 1991). Hematological studies on *R. quelen* are present in the literature (Fukushima et al., 2012) and Nilo Tilápia (Jeronimo et al., 2011), also on the feeding by jundiá (Brandão-Gonçalves and Sebastien, 2013) however studies that can elucidate the influence of fasting on metabolism are rare. Therefore, studies on metabolism in relation to different periods of fasting is of paramount importance since it will show which metabolic adjustments will be necessary to maintain the homeostasis of the species, showing which reserves will be mobilized or not for their survival. Barcellos et al. (2010) have described alterations in the carbohydrate metabolism of *R. quelen* during different periods of fasting and refeeding. In this study, endocrinal alterations were observed as being responsible for an increase in the levels of cortisol, a hormone which undergoes change in situations of stress and causes a number of physiological modifications in the metabolism of carbohydrates, proteins and lipids.

As such, this study aims to evaluate the use of protein and lipid reserves in *R. quelen* fish as energy sources, following different periods of food deprivation and refeeding.

2. Material and Methods

2.1. The fish

Six-month-old *R. quelen* males weighing 375.9 ± 43.9 g were kept under natural photoperiod conditions in two 6,200-L plastic tanks with water change ($10\% \text{ day}^{-1}$) prior to distribution to experimental tanks. They were fed twice a day (at 10 a.m. and 4 p.m.) with 42% extruded crude protein ($3400 \text{ kcal kg}^{-1} \text{ DE}$), corresponding to 5% of their body weight. Water temperature and dissolved oxygen concentrations (YSI 550A oxygen meter; Yellow Spring Instruments, Yellow Springs, OH, USA) were monitored. The pH values (Bernauer pH meter, Indaial, SC, Brazil),

total ammonia-N (colorimetric test), total alkalinity, and hardness were also measured.

2.2. Experimental design

The fish were divided into 5 experimental groups. Group I was fed on a normal diet (control group). The fish in the other groups were subjected to a period of fasting for 1 day (group II), 7 days (group III), 14 days (group IV), and 21 days (group V). All the fish were refed at the end of each fasting period. Each group had four 3000-L concrete tanks with 20 fish in each one (stocking density of approximately 2.4 g L^{-1}). The fish were kept in the tanks over a 5-day period, as described in previous reports using the same species (Barcellos et al., 2006, 2010). During the experimental period, the water quality parameters determined were as follows: temperature, $24.6 \pm 0.08 \text{ }^\circ\text{C}$; dissolved oxygen, $6.2 \pm 0.01 \text{ mg L}^{-1}$; pH, 7.3 ± 0.01 ; total hardness and alkalinity, 60 and $65 \text{ mg L}^{-1} \text{ CaCO}_3$, respectively. NH_4 and NO_2 content did not exceed 0.5 mg L^{-1} .

Since a dark environment is preferred by *R. quelen*, all the tanks were covered with a layer of thin black plastic to prevent stress caused during management of adjacent tanks, but light was not totally excluded. This situation it is similar replicates the natural habitat of this species, i.e., river bottoms with high turbidity (Gomes et al., 2000; Barcellos et al., 2009).

During the refeeding period, the fish were fed to near to satiation (verified by observation of their feeding behavior) via a small hole in each black plastic cover. Faeces and food residues were not siphoned off, in order to prevent stress caused during management. The experiment was conducted during the summer months, with an average temperature of $24.6 \pm 0.08 \text{ }^\circ\text{C}$.

2.3. Sampling

Blood samples were obtained from the control group and from groups II, III, IV, and V after 1, 7, 14, and 21 days of fasting, respectively 12 fish (3 from each of the 4 tanks) were randomly captured using a pen net. During the refeeding period, a further 12 fish (3 per tank) from each group were sampled at the time of refeeding, and thereafter on days 2, 4, 6, and 12. At the end of each fasting/refeeding period, the fish were captured and anaesthetized with MS222 (300 mg L^{-1} , Argent Redmond, WA, USA). Blood samples were then drawn from the caudal vessels using heparinized syringes. Following this, the fish were killed by means of spinal section, and liver and muscle tissue was removed, weighed, placed on ice, frozen in liquid nitrogen and stored at $-20 \text{ }^\circ\text{C}$ for later analysis. The blood samples mentioned above were then centrifuged ($3000 \times g$, 10 min), and the plasma was stored at $-25 \text{ }^\circ\text{C}$ for later analysis.

2.4. Blood and tissue analysis

Total lipids were extracted using a 2:1 ratio of chloroform to methanol solution, and were quantified by means of the sulfo-phospho-vanillin method (Folch et al., 1957). Purpose-made kits were used to measure plasma triglyceride concentrations (Triglycerides Liquiform; Labtest, Lagoa Santa, MG, Brazil) and plasma glycerol

levels (R-Biopharm, Darmstadt, Germany). Protein levels in the liver and muscle tissues were determined according to the Bradford (1976) method.

2.5. Statistical analysis

The mean \pm SEM value of each group was calculated using the GraphPad InStat 3 statistical package (GraphPad Software, San Diego, CA, USA) and compared by one-way analysis of variance (ANOVA), followed by the Tukey test. Statistical significance was accepted as being $p < 0.05$. The Hartley test was carried out to verify the homogeneity of variance, and normality was verified using the Kolmogorov-Smirnov test. Log-transformation was performed where necessary, and non-transformed data are presented in the tables below.

All the procedures involving animals were conducted in compliance with the guidelines approved by the Committee on Research Ethics and Animal Welfare of the Universidade de Passo Fundo (UPF). The authors declare that there is no conflict of interest in this regard.

3. Results

In the liver (Table 1) of the catfish, protein reserves were reduced ($p < 0.05$) in groups II and III after 1 and 7 days of fasting respectively, group IV (14 days), and group V (21 days).

In groups III and IV, these values were similar to those of the control group. The change in protein reserves in the liver in all groups persisted until the sixth day of re-feeding, and then began to increase ($p < 0.05$) in group II and group III. In the other groups (IV and V), where the fasting period was longer, protein reserves were not restored during the 12-day re-feeding period.

In contrast, muscle proteins remained unchanged during the fasting period (Table 2). However, a decrease ($p < 0.05$) in muscle protein was observed in group III during different re-feeding periods.

The different fasting periods did not significantly alter reserves of liver lipids (Table 3). However, during the re-feeding of group III (7 days of fasting) and group IV (4 days of fasting) there was an increase in lipid reserves in this tissue from the 4th day of re-feeding in group III, and from the 12th day of re-feeding in group IV.

In the muscle tissue (Table 4), a decrease was observed ($p < 0.005$) in the lipid reserves from the 14th day (group IV) of fasting. After 4 days of re-feeding, these reserves returned to values similar to those of the control group (group I).

Plasma triglyceride levels (Table 5) increased significantly in group II (after 1 day of fasting), but fell again after 2 days of re-feeding. In group III (7 days of fasting), triglyceride levels increased after 4 days of re-feeding, a

Table 1. Liver protein content (mg g⁻¹ tissue) after different fasting periods and during re-feeding.

		Experimental groups				
		I (control)	II (1d)	III(7d)	IV (14d)	V (21d)
Fasting	0d	0.40 \pm 0.06A				
	1d		0.03 \pm 0.01cC			
	7d			0.26 \pm 0.01cB		
	14d				0.28 \pm 0.01AB	
	21d					0.31 \pm 0.03AB
Re-feeding	2d	0.43 \pm 0.01	0.13 \pm 0.03c	0.27 \pm 0.01c	0.33 \pm 0.04	0.25 \pm 0.02
	4d	0.46 \pm 0.06	0.16 \pm 0.01bc	0.27 \pm 0.01c	0.28 \pm 0.04	0.28 \pm 0.02
	6d	0.47 \pm 0.05	0.19 \pm 0.08b	0.33 \pm 0.01b	0.29 \pm 0.01	0.22 \pm 0.08
	12d	0.43 \pm 0.04	0.55 \pm 0.09a	0.78 \pm 0.02a	0.31 \pm 0.09	0.23 \pm 0.07

Small letters in the columns indicates statistical differences between the different sample days within the same group and the capital letters indicates statistical differences between the different days of fasting ($P < 0.05$; $n = 12$).

Table 2. Muscle protein content (mg g⁻¹ tissue) after different fasting periods and during re-feeding.

		Experimental groups				
		I (control)	II (1d)	III(7d)	IV (14d)	V (21d)
Fasting	0d	0.652 \pm 0.08				
	1d		0.605 \pm 0.042b			
	7d			0.600 \pm 0.05a		
	14d				0.497 \pm 0.073b	
	21d					0.591 \pm 0.02a
Re-feeding	2d	0.657 \pm 0.08	0.495 \pm 0.011b	0.112 \pm 0.009b	0.494 \pm 0.02ab	0.492 \pm 0.04a
	4d	0.69 \pm 0.08	0.622 \pm 0.083b	0.171 \pm 0.04b	0.425 \pm 0.015b	0.485 \pm 0.02a
	6d	0.63 \pm 0.09	0.68 \pm 0.05b	0.134 \pm 0.05b	0.433 \pm 0.01b	0.520 \pm 0.02a
	12d	0.67 \pm 0.08	0.565 \pm 0.05b	0.211 \pm 0.09b	0.468 \pm 0.08b	0.512 \pm 0.02a

Small letters in the columns indicates statistical differences between the different sample days within the same group ($P < 0.05$; $n = 12$).

Table 3. Total liver lipid content (mg g⁻¹ tissue) after different fasting periods and during re-feeding.

		Experimental groups				
		I (control)	II (1d)	III(7d)	IV (14d)	V (21d)
Fasting	0d	20.1 ± 5.5				
	1d		26.6 ± 11.5			
	7d			16.2 ± 0.6b		
	14d				12.7 ± 3.3b	
	21d					30.1 ± 7.3
Re-feeding	2d	19.9 ± 5.9	32.8 ± 14.2	14.1 ± 1.3b	10.1 ± 3.8b	30.7 ± 8.3
	4d	19.8 ± 5.6	19.2 ± 5.1	24.5 ± 6.1a	9.7 ± 2.2b	25.7 ± 5.3
	6d	18.9 ± 4.9	19.0 ± 7.0	20.2 ± 4.3a	9.3 ± 5.5b	23.0 ± 4.2
	12d	21.1 ± 5.8	18.5 ± 6.8	19.1 ± 5.8a	21.5 ± 4.0a	20.0 ± 5.1

Small letters in the columns indicates statistical differences between the different sample days within the same group ($P < 0.05$; $n = 12$).

Table 4. Total muscle lipid content (mg g⁻¹ tissue) after different fasting periods and during re-feeding.

		Experimental groups				
		I (control)	II (1d)	III(7d)	IV (14d)	V (21d)
Fasting	0d	6.2 ± 1.4 A				
	1d		8.8 ± 2.4 A			
	7d			6.5 ± 2.6 A		
	14d				2.1 ± 0.2Bb	
	21d					1.1 ± 0.08Bc
Re-feeding	2d	6.1 ± 1.5	15.2 ± 7.3	12.4 ± 4.1	3.2 ± 0.9b	1.2 ± 0.1c
	4d	5.9 ± 1.1	11.7 ± 6.8	11.8 ± 1.9	8.2 ± 2.1a	3.3 ± 1.1b
	6d	6.9 ± 2.1	10.6 ± 6.0	9.5 ± 4.1	10.1 ± 1.9a	7.0 ± 1.1a
	12d	6.6 ± 1.8	10.0 ± 5.7	7.4 ± 3.3	7.7 ± 1.7 a	7.1 ± 2.1a

Small letters in the columns indicates statistical differences between the different sample days within the same group and the capital letters indicates statistical differences between the different days of fasting ($P < 0.05$; $n = 12$).

Table 5. Plasmatic triglycerides (mg mL⁻¹) after different fasting periods and during re-feeding.

		Experimental groups				
		I (control)	II (1d)	III(7d)	IV (14d)	V (21d)
Fasting	0d	158.5 ± 20.2B				
	1d		748.9 ± 92.5aA			
	7d			210.1 ± 32.5bB		
	14d				117.7 ± 7.1bB	
	21d					212.1 ± 53.8B
Re-feeding	2d	145.7 ± 21.1	388.4 ± 67.8b	274.8 ± 36.6b	133.1 ± 9.8b	338.7 ± 101.2
	4d	158.2 ± 22.1	222.5 ± 26.9b	322.4 ± 53.3ab	222.1 ± 36.5a	314.1 ± 38.9
	6d	160.0 ± 58.7	318.8 ± 92.1b	325.5 ± 56.6ab	167.7 ± 9.1ab	375.4 ± 89.9
	12d	155.5 ± 21.4	235.1 ± 65.1b	500.1 ± 65.8a	247.1 ± 21.1a	531.1 ± 77.1

Small letters in the columns indicates statistical differences between the different sample days within the same group and the capital letters indicates statistical differences between the different days of fasting ($P < 0.05$; $n = 12$).

phenomenon which was similar to the effect observed in group IV (14 days of fasting).

During the different periods of fasting an oscillation in plasma glycerol was observed (Table 6). There was a significant increase after 1 day of fasting (group II), but when the fasting period was longer, as in group III (7 days) and group IV (14 days), the values of plasma metabolite decreased ($p < 0.005$) to values similar to those of the control group. However, when fasting lasted for 21 days (group V), plasma glycerol values began to

increase again, just as in group II. During the refeeding periods, a different response in each group was observed (there was a decrease in values in groups II and III, and an increase in group IV).

4. Discussion

Studies evaluating the mobilization of reserves during fasting have reported that protein was the last nutrient to be used up (Black and Love, 1986; Bines, 1999; Hervant et al.,

Table 6. Plasma glycerol (g L⁻¹) after different fasting periods and during re-feeding.

		Experimental groups				
		I (control)	II (1d)	III(7d)	IV (14d)	V (21d)
Days Fasting	0	0.010 ± 0.002B				
	1		0.100 ± 0.014aA			
	7			0.024 ± 0.007bB		
	14				0.010 ± 0.002bB	
	21					0.148 ± 0.060A
Re-feeding	2	0.008 ± 0.003	0.015 ± 0.004b	0.028 ± 0.002b	0.070 ± 0.024ab	0.084 ± 0.010
	4	0.010 ± 0.001	0.034 ± 0.011b	0.014 ± 0.002ab	0.090 ± 0.022ab	0.056 ± 0.021
	6	0.009 ± 0.003	0.028 ± 0.007b	0.043 ± 0.003a	0.110 ± 0.033a	0.036 ± 0.020
	12	0.007 ± 0.001	0.019 ± 0.009b	0.029 ± 0.002ab	0.070 ± 0.012ab	0.058 ± 0.030

Small letters in the columns indicates statistical differences between the different sample days within the same group and the capital letters indicates statistical differences between the different days of fasting ($P < 0.05$; $n = 12$).

1999; Caloin, 2004). However, in the present study, we have demonstrated that protein mobilization is the primary energy source in food-deprived adult *R. quelen*. This is an unusual pattern, and represents a significant finding, since fish may experience limited food availability and a reduction in food intake, both *in natura* and in aquaculture, e.g., during pre-harvest fasting, shipping management, and feeding regimes that restrict food availability (Mackenzie et al., 1998; Souza et al., 2003; Camargo and Urbinati, 2008). Like all animals, fish need protein, fat, carbohydrates, vitamins, minerals, and other dietary elements to satisfy their physiological needs for growth and reproduction (Hertrampf and Piedad-Pascual, 2000).

A marked reduction in liver protein was observed during the different fasting periods, and mobilization of liver protein reserves began only 1 day after fasting. According to Rossi et al. (2015), the liver is the tissue which is most active metabolically in the mobilization of energy stocks to satisfy physiological needs. Kim et al. (2014) have also observed a decrease in plasma protein in the olive flounder during starvation. This initial reduction in protein reserves demonstrates the high susceptibility of this species to situations of food restriction. However, when this fasting period is longer (14 days and 21 days), mobilization appears to decrease.

No significant difference was found in muscle protein levels, suggesting that, in the case of catfish, these reserves are not mobilized during a 21-day fasting period.

Another factor to consider is the type of tissue in which the protein is stored and the length of fasting to which the fish are exposed. This demonstrated a different metabolic profile of muscle tissue in relation to fasting.

Other species of fish also use muscle protein as their primary fuel source, such as the European eel (*Anguilla anguilla Linnaeus*), goldfish (*Carassius auratus Linnaeus*), and plaice (*Pleuronectes platessa Linnaeus*), as reviewed by Czesny et al. (2003). However, the length of time between the beginning of the fasting period and protein mobilization for these species was generally of several days. Furthermore, this mobilization was shown to begin after 8 days of fasting in common carp (*Cyprinus carpio Linnaeus*) (Blasco et al., 1992), 21 days in sea bream (*Sparus aurata Linnaeus*)

(Power et al., 2000), and 50 days in salmon trout (*Salmo trutta fario*) (Navarro et al., 1992). Thus species is a key determining factor in the length of time required for protein mobilization following fasting. In addition, protein catabolism in rainbow trout (*Oncorhynchus mykiss* Walbaum) is active after 28 days of fasting, as indicated by high levels of transaminases (Kullgren et al., 2010).

Following the initial protein mobilization observed in the present study, protein liver stores were re-established within 6 days of refeeding, whilst with 12 days of refeeding nominal protein values are superior to control group values. This suggests a possible increase in protein reserves to provide for future fasting situations. For example, sea bream protein reserves were reestablished after 7 days of refeeding (Power et al., 2000). According to McCue (2010), successful strategies for maintaining homeostasis during periods of fasting are developed via mobilization of endogenous physiological energy sources, such as proteins, lipids and carbohydrates, which can respond to demand and ensure survival.

Despite the fact that numerous studies have highlighted the biological importance of lipids as a source of energy in fish (Stirling, 1976; Reinitz, 1983; Alliot et al., 1984), the liver lipid reserves of *R. quelen* were not significantly modified during the fasting period. This may be explained by the primary mobilization of liver protein at the beginning of fasting. However, following a longer period of fasting (14 days), the muscular reserves of lipids are mobilized, thus increasing levels of plasmatic triglycerides. According to Furné et al. (2012), the mobilization of hepatic reserves of lipids is related to species, the tissue of the reserves, and the strategies via which a particular species can mobilize other energy sources.

Triglycerides are formed by sterification of a glycerol molecule with 3 fatty acids (FA), and represent the main type of fat that constitutes lipid reserves (Van den Thillart and Van Raaij, 1995). The first step in their use as an energy source involves hydrolysis of triglycerides into FA and glycerol. The model for distribution and transport of lipids in fish consists of rapid and/or slow release in function of the chain length (Sheridan, 1988). Thus, the high triglyceride and glycerol levels found at the beginning

of the fasting period demonstrate rapid energy release into the circulation in order to maintain homeostasis. Several studies have demonstrated that plasma free FA levels increase in fish subjected to fasting regimens (Lewis and Epple, 1984; Machado et al., 1988, 1989; Shimeno et al., 1990; Sheridan and Mommsen, 1991; Souza et al., 2000; Figueiredo-Garutti et al., 2002). The elevation in plasma glycerol levels observed in the present study suggest that *R. quelen* mobilized glycerol from lipid stores (mesenteric, muscle, belly flap, and liver), as has been observed in other fish species (Albalat et al., 2006; Farbridge et al., 1992; Navarro et al., 1992).

Fatty acid mobilization during a fasting period is essential for control of glucose levels. The increase in free FAs could competitively inhibit glucose mobilization by peripheral tissues and the release of glucose by the liver. In turn, higher fatty acid uptake by the liver stimulates lipid metabolism, glucose homeostasis, and lipid gluconeogenesis (Souza et al., 2000).

Based on the literature and our results, we can conclude that length of fasting period and species-related metabolic differences were determining factors for the type of reserves mobilized. Barcellos et al., (2010) observed that *R. quelen* mobilizes its glycogen reserves after 7 days of fasting. These data reinforce those reviewed by Love (1980); certain fish species mobilize proteins as their primary energy source (Butler, 1968; Narasimhan and Sundararaj, 1971); proteins and lipids are mobilized simultaneously (Larsson and Lewander, 1973), whereas other species maintain protein levels by mobilizing fat and/or glycogen (Inui & Oshima, 1966; Kamra, 1966; Ince and So, 1984).

In conclusion, changes in the metabolism of proteins and lipids in relation to different periods of fasting and refeeding were observed as constituting survival strategies used by *R. quelen*. Results showed that protein is an important source of energy during shorter fasting periods, whilst lipids are mobilized during longer ones. The metabolic activity of the liver as one of the principal tissues involved in satisfying energy needs during periods of restricted feeding was also evidenced by this study. In addition, there is strong evidence that the *R. quelen* species is extremely capable of replenishing energy reserves following different periods of fasting.

Acknowledgements

This work was supported by grants from FINEP 0106019000 (Financiadora de Estudos e Projetos), Unilasalle (Centro Universitário Lasalle) and Universidade de Passo Fundo (UPF).

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