

## Effects of dietary carbohydrate/lipid ratios on growth, body composition, and nutrient utilization of hybrid catfish (*Pseudoplatystoma reticulatum* x *Leiarius marmoratus*)<sup>□</sup>

*Efectos de la relación carbohidratos/lípidos en el crecimiento, composición corporal y utilización de nutrientes del bagre híbrido (*Pseudoplatystoma reticulatum* x *Leiarius marmoratus*)*

*Efeitos da relação carboidratos/lípidios sobre o crescimento, composição corporal e utilização de nutrientes do bagre híbrido (*Pseudoplatystoma reticulatum* x *Leiarius marmoratus*)*

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### Summary

**Background:** farming of hybrid catfish is relatively recent in Brazil; consequently, it is necessary to develop practical diet formulations for this fish. **Objective:** to evaluate the influence of different carbohydrate/lipid ratios (CHO:L) on growth performance, body composition and nutrient utilization of hybrid catfish (*Pseudoplatystoma reticulatum* x *Leiarius marmoratus*). **Methods:** four isonitrogenous diets were formulated with increasing lipid levels, using the following CHO:L ratios: 1.3:1 (diet 1); 1.1:1 (diet 2); 0.9:1 (diet 3), and 0.8:1 (diet 4). Fish were fed 5% of BW/day (dry-weight basis) in triplicate groups of 6 fish each ( $18 \pm 1.5$  g) for 2 months.

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**Results:** though final weight and absolute weight gain decreased with increasing dietary lipid ( $p < 0.05$ ), there was no significant difference in daily feed consumption among treatments ( $p > 0.05$ ). Moreover, viscerosomatic index and hepatosomatic index showed no statistical difference among dietary treatments. Body lipid increase for fish fed diet 4. Lipid and energy efficiency retentions were higher at 0.8:1 CHO:L group. **Conclusion:** CHO:L ratios around 1.3:1 produced large benefit by best growth performance in the studied hybrid model.

**Keywords:** *feed intake, feed utilization, performance.*

### Resumen

**Antecedentes:** el cultivo del bagre híbrido es relativamente reciente en Brasil, con lo cual se hace necesario desarrollar dietas prácticas para este pez. **Objetivo:** evaluar la influencia de la relación carbohidratos/lípidos (CHO:L) en el crecimiento, composición corporal, y utilización de nutrientes por el bagre híbrido (*Pseudoplatystoma reticulatum* x *Leiarius marmoratus*). **Métodos:** cuatro dietas isonitrogenadas fueron formuladas con niveles incrementales de lípidos, utilizando la siguiente relación CHO/L: 1,3:1 (dieta 1); 1,1:1 (dieta 2); 0,9:1 (dieta 3), y 0,8:1 (dieta 4). Los peces fueron alimentados con 5% de BW/día (en base a peso seco), en triplicado con 6 peces/grupo ( $18 \pm 1,5$  g) por grupo, por un tiempo de 2 meses. **Resultados:** aunque el peso final y la ganancia de peso absoluto disminuyó con el aumento de lípidos en la dieta ( $p < 0,05$ ), no hubo diferencia significativa en el consumo diario de alimento entre los tratamientos ( $p > 0,05$ ). Por otra parte, para el índice viscerosomático e índice hepatosomático no hubo diferencias estadísticas entre los tratamientos. Se ha observado el aumento de los lípidos corporales de peces alimentados con la dieta 4. La eficiencia de retención de lípidos y energética fueron mayores en el grupo 0,8:1 CHO:L. **Conclusión:** la relación CHO:L alrededor de 1,3:1 produce un gran beneficio al mejorar el crecimiento del modelo del pez híbrido estudiado.

**Palabras clave:** *consumo alimenticio, performance, utilización de alimento.*

### Resumo

**Antecedentes:** o cultivo de bagre híbrido com características favoráveis ao crescimento é relativamente recente no Brasil, conseqüentemente é necessária a formulação de dietas práticas para essa espécie. **Objetivo:** se avaliou a influência da relação carboidrato/lipídio (CHO:L) no crescimento, composição corporal e utilização de nutrientes pelo bagre híbrido (*Pseudoplatystoma reticulatum* x *Leiarius marmoratus*). **Métodos:** quatro dietas isonitrogenadas foram formuladas com o aumento dos níveis de lipídios na dieta, utilizando a seguinte relação CHO/L: 1,3:1 (dieta 1); 1,1:1 (dieta 2); 0,9:1 (dieta 3), y 0,8:1 (dieta 4). Os peixes foram alimentados a 5% de BW/dia (base de peso seco), sendo triplicatas de 6 peixes ( $18 \pm 1,5$  g) por grupo por um período de 2 meses. **Resultados:** apesar do peso final e do ganho de peso absoluto decrescerem com o aumento da dieta lipídica ( $p < 0,05$ ), não houve diferença significativa no consumo diário de alimento entre os tratamentos ( $p > 0,05$ ). Contudo, os índices viscerossomático e hepatossomático não demonstraram diferença estatística entre os tratamentos. Foi registrado aumento dos lipídios corporais para os peixes alimentados com a dieta 4. A eficiência de retenção de lipídios e a eficiência de retenção energética foram maiores para o grupo 0,8 CHO:L. **Conclusão:** a relação ao redor de 1,3 produziu grandes benefícios por melhorar o crescimento do modelo de peixe híbrido estudado.

**Palavras chave:** *consumo alimentar, desempenho, utilização alimento.*

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## Introduction

Omnivorous or herbivorous warm-water fish generally tolerate high carbohydrate levels, using them as a source of energy more effectively than carnivorous species. Dietary excess of carbohydrates or lipids can be stored in the form of body lipids (Abimorad *et al.*, 2007). Both carbohydrates and lipids are important non-protein

energy sources for fish, and are commonly incorporated in fish diets to maximize the use of dietary protein for growth. To optimize the formation of muscle tissue in fish, proteins and amino acids must not be diverted to oxidative energy metabolism and, concurrently, the levels of non-protein energy sources (lipids and carbohydrates) must be at concentrations that do not overload the liver and pancreas (Abimorad *et al.*, 2007).

Breeding hybrids with selected or favored characteristics of each parent is one of the goals of animal sciences. Interspecific hybrids (e.g between channel catfish, *Ictalurus punctatus* females and blue catfish, *I. furcatus* males) have been described as the most suitable for culture conditions compared to channel catfish due to better growth, increased resistance to low oxygen levels and diseases, ease of harvest, and higher carcass yield (Dunham *et al.*, 1990). The farming of hybrid catfish (e.g pintado, *Pseudoplatystoma coruscans* x cachara, *P. fasciatum*) started recently in Brazil; consequently, practical diet formulation for this fish is based on the existing diets for other fish species (Martino *et al.*, 2005). Therefore, in order to develop profitable aquaculture systems, the protein-sparing effect of nutrients as lipids and carbohydrates may be effective for improving growth and reducing feed costs.

The omnivorous *Leiarius marmoratus*, popularly known as “Jandiá” and the carnivorous *Pseudoplatystoma reticulatum*, known as “cachara” or “barred sorubim”, are among the largest fishes in the main South American hydrographic basins. The aim of this study was to evaluate the effect of four dietary inclusion levels of crude starch and lipids on growth performance, body composition and nutrient utilization of hybrid catfish (♀ *P. reticulatum* x ♂ *L. marmoratus*).

## Material and methods

### Ethical considerations

This study is in agreement with the ethics principles in animal experimentation, adopted by the Animal Experimentation Ethics Committee of Universidade Federal de Lavras, Brasil (Protocol 028/12 of September 6, 2012).

### Experimental diets

Four test diets were formulated with approximately 43% crude protein (salmon meal, with 65% crude protein and 10% crude lipid). Diets (1, 2, 3 and 4) were formulated to include four different soybean oil levels, respectively (120, 140, 160 and 180 g/Kg) as described in Table 1. Corn flour and wheat flour

were used as carbohydrate sources and soybean oil was used as the source of lipids. The dry ingredients were mixed and distilled water was added until adequate consistency was obtained. The dry ingredients were thoroughly mixed and the oil was added later and mixed until a homogenous mixture was obtained. Then, half of the mixture was cold-pelleted to appropriate sizes. Prepared pellets, through a 2 mm dice were air-dried for 24 h and then oven-dried at 58 °C for other 48 h. All diets were then stored at -5 °C.

**Table 1.** Ingredients and diets composition.

Ingredients (%)	Diets			
	1	2	3	4
Fish meal	55.0	55.0	55.0	55.0
Soybean meal	1.9	1.4	1.9	2.6
Corn gluten meal	6.0	7.0	7.0	7.0
Wheat flour	13.0	12.0	11.0	9.8
Corn flour	10.0	8.5	7.0	5.5
Soybean oil	12.0	14.0	16.0	18.0
Vitamin and mineral premix <sup>1</sup>	2.0	2.0	2.0	2.0
Vitamin C	0.1	0.1	0.1	0.1
<i>Analysis</i>				
Moisture (g/Kg)	60.0	60.8	59.7	58.3
Crude protein (g/Kg)	429	431	435	433
Crude lipid (g/Kg)	192	210	232	246
Ash (g/Kg)	63	58	65	63
Crude fibre (g/Kg)	13	12	12	11
NFE <sup>2</sup> (g/Kg)	243	228	196	198
Gross energy (MJ/Kg)	22.3	22.7	23.3	23.6
CP/GE (g/MJ) <sup>3</sup>	19.2	19.0	18.6	18.3
Carbohydrate:lipid	1.3:1	1.1:1	0.9:1	0.8:1

<sup>1</sup>Percents unless otherwise noted IU, which is given per Kg: vitamin A, 500 000 IU; vitamin D3, 500 000 IU; vitamin E, 25 000 IU; vitamin K3, 0.05; thiamine, 0.125; riboflavin, 0.25; pyridoxine, 0.25; pantothenic acid, 0.5; niacin, 0.5; biotin, 0.0125; folic acid, 0.025; vitamin B12, 0.375; ascorbic acid, 2.8; Co, 0.0025; Cu, 0.2; I, 0.01; Fe, 2.4; Mn, 0.50; Zn, 0.05; Se, 0.01.

<sup>2</sup>NFE = Nitrogen free extract = 1000 – (moisture + CP + CL + ash + CF).

<sup>3</sup>CP/GE = Crude protein (g/Kg)/Gross energy (MJ/Kg).

Diets were formulated with 43% of crude protein (salmon meal, with 65% of crude protein and 10% of crude lipid). Diets 1, 2, 3, and 4 were formulated to include increasing levels of soybean oil (120, 140, 160, and 180 g/Kg, respectively). Corn flour and wheat flour were used as carbohydrate sources and soybean oil was used as a lipid source.

### *Fish and feeding trial*

Seventy-two fish (mean weight  $18 \pm 1.5$  g, and 30 days old) were used. Fish were randomly distributed in 12 tanks (150 L) at a stocking density of 6 fish per tank (triplicate groups of 6 fish). At the start of the trial 15 fish from the stock population were sampled, pooled and frozen for subsequent whole-body composition analysis (see analytical methods).

The fish were previously adapted to be fed during the light period. Fish in each tank were weighed every 15 days, the average weight gain per aquarium was calculated and food availability was recalculated at a fixed rate of 5% body weight of dry feed per day. The trial lasted 60 days and during that period fish were fed twice a day (08:00 and 17:00 h). Protein intake (%) was calculated during the trial. Fish were sampled every 15 days for weight gain during the trial. The presence of uneaten food pellets was checked every day and real feed intake was calculated by difference. Protein intake was also calculated. The water temperature was maintained between 25 and 26 °C. The oxygen level was above 6.5 mg/L, pH: 7.3, the water exchange rate 100% per hour in closed circuit.

At the end of the experiment the fish were starved for 24 h. Then, fish were anaesthetized with clove oil (100 mg/L), individually weighed and frozen until analysis. Viscera was removed from each fish and weighed. Fish whole-body and liver was stored in a freezer at -5 °C for subsequent analysis.

Body composition as moisture, protein, lipid, ash, and body weight was measured to compare growth performance. The measured traits were final weight (g), viscera somatic index (VSI, %), hepatosomatic index (HSI, %), daily feed consumption (DFC, g/Kg), absolute weight gain (AWG, g per fish), lipid efficiency retention (LER %), protein efficiency retention (PER%), energy efficiency retention (EER %), and protein gain (PG). These parameters were calculated as described by Martino *et al.* 2005.

### *Analytical methods*

Moisture, crude protein, lipid, and ash of raw ingredients, experimental diet, and fish composition

were determined by standard methods of the Association of Official Analytical Chemists (AOAC, 1990). Gross energy was determined by adiabatic calorimetry (NRC, 2011).

### *Data Analysis*

Data on proximate analysis and body indices were analyzed using the Statistical Package for the Social Sciences SPSS software (version 11.5, USA). Data were expressed as mean  $\pm$  SEM. One-way ANOVA was used, followed by a Duncan Test for *post hoc* analysis. P-value was fixed at 0.05.

## **Results**

Final weight (FW) and absolute weight gain (AWG) were increased in fish fed diet 1 (ratio 1.3:1 CHO:L,  $p < 0.05$ ) (Table 2). No differences ( $p > 0.05$ ) were observed in HSI or VSI among groups. No significant difference for DFC was found during the 8 weeks of experimentation, but there existed a tendency of reduced feed intake with increasing dietary lipid levels. Survival was 100% in all groups.

No differences were observed in the protein content of fish fed the different CHO:L ratios (Table 3). However, lipid contents of muscle and liver were significantly increased and moisture decreased only for fish fed 0.8:1 (CHO:L) ratio ( $p < 0.05$ ). Moreover, fish fed the 1.1:1 (CHO:L) diet had the least lipid content in muscle and liver ( $p < 0.05$ ). Whole-body ash content was unaffected by treatments ( $p > 0.05$ ).

No significant differences for PER and PG were found (Table 4). On the other hand, fish fed the 0.8:1 (CHO:L) diet had the highest LER ( $126.3 \pm 5.3\%$ ,  $p < 0.05$ ), and the highest EER ( $66 \pm 5.2\%$ ,  $p < 0.05$ ) compared to fish fed the other diets.

## **Discussion**

Hybrid catfish (*P. reticulatum* x *L. marmoratus*) has a tendency to omnivory and it is capable to readily utilize carbohydrate-rich diets. This study demonstrates that carbohydrates could replace lipids as an energy source in the diet of hybrid catfish improving growth performance. Overall, the best

**Table 2.** Growth, viscera somatic index, hepatosomatic index, and diet utilization of hybrid catfish fed the experimental diets<sup>1</sup>.

Diets	IW <sup>2</sup> (g)	FW <sup>3</sup> (g)	VSI <sup>4</sup> (%)	HSI <sup>5</sup> (%)	DFC <sup>6</sup> (g/hg)	AWG <sup>7</sup> (g)
1	18 ± 0.1	194.6 ± 5.8 <sup>a</sup>	9.12 ± 0.3	0.67 ± 0.1	14 ± 0.2	176.4 ± 3.6 <sup>a</sup>
2	18 ± 0.3	181.1 ± 4.3 <sup>b</sup>	9.11 ± 0.4	0.69 ± 0.1	14 ± 0.1	163.1 ± 2.2 <sup>b</sup>
3	18 ± 0.2	185.0 ± 1.5 <sup>b</sup>	8.61 ± 0.3	0.69 ± 0.1	15 ± 0.2	167.0 ± 3.1 <sup>b</sup>
4	18 ± 0.4	184.3 ± 1.2 <sup>b</sup>	8.53 ± 0.2	0.68 ± 0.1	16 ± 0.1	166.2 ± 3.6 <sup>b</sup>

<sup>1</sup>Mean (n = 3) ± SEM.<sup>2</sup>Initial weight.<sup>3</sup>Final weight.<sup>4</sup>Viscera somatic index (viscera weight/body weight) x 100.<sup>5</sup>Hepatosomatic index (liver weight/body weight) x 100.<sup>6</sup>Daily feed consumption [feed intake/(days fed x average body weight)].<sup>7</sup>Absolute weight gain (final body weight - initial body weight).<sup>a,b</sup>Different letters in the same column indicate significant differences (p<0.05) between treatments.**Table 3.** Proximate composition (wet basis; g/Kg) of hybrid catfish carcass<sup>1</sup>.

Diets	Moisture	Protein	Ash	Lipid	
				Muscle	Liver
1	744 ± 1.1 <sup>a</sup>	157 ± 0.1	38 ± 0.6	40 ± 0.9 <sup>a</sup>	39 ± 2.1 <sup>a</sup>
2	741 ± 1.2 <sup>a</sup>	149 ± 0.3	37 ± 0.8	26 ± 0.6 <sup>b</sup>	28 ± 1.2 <sup>b</sup>
3	746 ± 0.9 <sup>a</sup>	145 ± 0.3	35 ± 0.8	49 ± 0.8 <sup>a</sup>	39 ± 1.1 <sup>a</sup>
4	728 ± 1.0 <sup>b</sup>	144 ± 0.2	36 ± 0.5	53 ± 0.7 <sup>c</sup>	47 ± 2.6 <sup>c</sup>

<sup>1</sup>Means of pooled samples of three fish from three different replicate groups (n = 9).<sup>a,b,c</sup>Different letters in the same column indicate significant differences (p<0.05) between treatments.**Table 4.** Nutrient efficiency retention.

Diets	PER <sup>1</sup> (%)	LER <sup>2</sup> (%)	EER <sup>3</sup> (%)	PG <sup>4</sup> (g total/days)
1	115.2 ± 8.8	101.2 ± 4.3 <sup>a</sup>	55 ± 5.4 <sup>a</sup>	77.3 ± 0.6
2	105.1 ± 7.8	102.3 ± 5.1 <sup>a</sup>	51 ± 5.3 <sup>a</sup>	76.2 ± 0.7
3	102.3 ± 7.2	106.7 ± 6.1 <sup>a</sup>	54 ± 5.1 <sup>a</sup>	78.7 ± 0.5
4	100.1 ± 8.3	126.3 ± 5.3 <sup>b</sup>	66 ± 5.2 <sup>b</sup>	77.6 ± 0.4

<sup>1</sup>Protein efficiency retention (PER %) = 100 x [final body weight x final body protein] - [initial body weight x initial body protein]/total protein intake (g).<sup>2</sup>Lipid efficiency retention (LER %) = 100 x [final body weight x final body lipid] - [initial body weight x initial body lipid]/total lipid intake (g).<sup>3</sup>Energy efficiency retention (EER %, based on gross energy) = 100 x [final body weight x final body energy] - [initial body weight x initial body energy]/total energy intake (g)<sup>4</sup>Protein gain (PG, g total experimental days) = [final body weight x final body protein - initial body weight x initial crude protein] days.<sup>a,b,c</sup>Different letters in the same column indicate significant differences (p<0.05) between treatments.

results were obtained with the CHO:L ratio of 1.3:1, corresponding to a CP/GE ratio of 19.2 g/MJ.

It is well known by Brazil fish farmers that hybrid catfish (*P. reticulatum* x *L. marmoratus*) can be fed

on high carbohydrate-containing feeds in intensive culture. The overall growth performance (FW and AWG) of juvenile hybrids fed diets containing 230 g carbohydrate/Kg diet was higher compared with other Brazilian catfish species (*Pseudoplatystoma coruscans*;

Martino *et al.*, 2005). The maximum growth at 230 g carbohydrate/Kg diet are in agreement with the 23% requirement for other catfish species such as the Chinese longsnout (*Leiocassis longirostris*; Tan *et al.*, 2007). Other researchers found that Chinese longsnout catfish achieves the best growth with 1.63:1 CHO:L ratio (Pei *et al.*, 2004), close to the results of the present study. Diets higher to 12% lipid inclusion for common sole (*Selea solea*) juveniles not only led to a substantial decline in performance but also affected gut health (Bonvini *et al.*, 2015). In omnivorous warm-water fish such as channel catfish (*Ictalurus punctatus*), common carp (*Cyprinus carpio*) and tilapia (*Oreochromis niloticus*), dietary carbohydrates, mainly starch, constitute the main non-protein energy source (Wilson, 1994; Vásquez-Torres and Arias-Castellanos, 2012). Since the diet of parental catfish (*Leiarius marmoratus*) in its natural environment consists mostly of low-energy plants (Marciales-Caro *et al.*, 2011), the relative low capacity to utilize dietary lipids in the studied hybrid model is conceivable.

As with other animals, fish control feed intake to meet their energy requirements (Fortes-Silva *et al.*, 2011; Fortes-Silva and Sánchez-Vázquez, 2012). According to Santinha *et al.* (1999) an increase in dietary lipid content leads to a significant decrease in feed intake. However, in the present study no significant difference ( $p > 0.05$ ) in food intake was observed. As mentioned above, there existed a tendency of reduced feed intake with decreasing CHO:L ratios. Because of that, DFC was the same for all treatments. According to Peres and Oliva-Teles (1999), feed intake for a 24% lipid diet seemed to be regulated by protein rather than by energy intake in the European sea bass (*Dicentrarchus labrax*).

Some studies have demonstrated that liver enlargement occurs with carbohydrate increase in the diet as a consequence of glycogen accumulation (Kim and Kaushik, 1992; Hemre *et al.*, 2002). Wang *et al.* (2014) studied the effects of dietary CHO:L ratios on liver histology of juvenile yellow catfish. Results of this study demonstrate that high dietary lipid levels can significantly impair liver function and high dietary carbohydrates can induce hypertrophy of the hepatic cells. In our study, both VSI and HSI and lipid liver content were not affected by the CHO:L ratio. Similar results were reported in “cachara” catfish for VSI and

HSI (Martino *et al.*, 2005) and piracanjuba (*Brycon orbignyanus*) (Borba *et al.*, 2006). Senegalese sole (*Solea senegalensis*) fed diets with 4 and 17% lipids did not affect HSI levels (Borges *et al.*, 2013). Borges *et al.* (2009) reported increased VSI in Senegalese sole fed diets with lipid content above 16%. In the present study, fish fed 1.1:1 CHO:L ratio significantly decreased lipid content in liver and body.

The significant decrease in body water and lipid content of fish fed 0.8:1 CHO:L ratio in the present study suggests that excessive dietary lipids are stored as body lipids. Similar results have also been reported in hybrid striped bass (Nematipour *et al.*, 1992). Moreover, protein efficiency retention (PER) and protein gain (PG) were not correlated with dietary carbohydrate/lipid ratio. However, the highest lipid efficiency ratio (LER) and energy efficiency retention (EER) were observed for 0.8:1 CHO:L ratio, or 246 g/Kg of lipid, indicating that high levels of this nutrient increased lipid deposition in carcass. On the other hand, previous research in southern catfish (*Silurus meridionalis*) showed the best PER when carbohydrate was increased (Fu, 2005).

According to this author, dietary starch has a protein-sparing effect in carnivorous southern catfish. According to Li *et al.* (2013), fingerling blunt snout bream (*Megalobrama amblycephala*) has a better ability to utilize dietary lipids than carbohydrates and also shows a protein-sparing effect of dietary lipid. However, excessive dietary lipids may cause lipid deposition both in muscle and visceral adipose tissue, but not in the liver. Apparently, the carbohydrate/lipid ratios used in this study were not enough to induce differences in body protein deposition. However, considering LER and EER, these results are consistent with hybrid tilapia fed diets with lipid levels similar to those used in this work (Fitzsimmons *et al.*, 1998) and gilthead seabream m (*Sparus aurata*) fed levels between 150 and 209 g/Kg (Santinha *et al.*, 1999). Usually, when diets contain high levels of these compounds, a fraction of them is deposited as fat in the abdominal cavity and carcass (Hemre and Sandnes, 1999). This fact has been demonstrated by studies employing isocaloric diets, where increasing carbohydrate concentrations in detriment of lipids resulted in lower fat deposition in hybrid tilapia (*Oreochromis niloticus* x *Oreochromis aureus*; Chou

and Shiao, 1996) and other species (Erfanullah, 1998; Nankervis *et al.*, 2000).

According to Gao *et al.* (2010), utilization of dietary lipids and carbohydrates was comparatively moderate in grass carp (*Ctenopharyngodon idella*), but the fish were a little more capable of utilizing lipids compared with high carbohydrate intake.

In conclusion, growth, body composition and nutrient utilization by this hybrid are greatly affected by dietary non-protein energy sources. This hybrid has a great capacity to utilize dietary carbohydrates. The best growth performance and the best use of nutrients corresponded to fish fed 1.3:1 CHO:L ratio.

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### Conflicts of interest

The authors declare they have no conflicts of interest with regard to the work presented in this report.

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