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Effect of Dietary Carboxymethyl Cellulose Level in Diets with different Levels of Protein and Carbohydrates on Digestibility, Growth, and Survival Rate of Tiger Shrimp *(Penaeus monodon Fab.)*

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ABSTRACT : The purposes of this study were to evaluate the effect of carboxymethyl cellulose (CMC) in feed with different protein and carbohydrate content on protein and carbohydrate digestibility, growth, and survival rate of tiger shrimps (*Penaeus monodon*). A factorial pattern with a completely randomized basic design and two factors were used. The first was the difference in CMC in the feed, namely 0%, 5% and 10%, while the second was the different protein-carbohydrate levels, namely P 30% - C 40% and P 40% - C 30%. The results showed that the treatments produced similar responses in survival rates of tiger shrimps. The interaction between differences in protein-carbohydrate and CMC content in the feed had a significant effect with $p<0.05$ on protein and carbohydrate digestibility as well as the biomass growth. Protein and carbohydrate digestibility and absolute growth of biomass at treatment P 40% - C 30% - CMC 0% was the lowest and significantly different from other treatments. Based on protein and carbohydrate digestibility, growth, and survival rates, the feed containing 30% protein, 40% carbohydrates and 5% CMC was the most suitable for tiger shrimps production.

KEYWORDS: carboxymethyl cellulose, growth, protein and carbohydrate digestibility, protein-carbohydrate, survival rate, tiger shrimp (*Penaeus monodon)*

I. INTRODUCTION

Currently, the aquaculture of tiger shrimp (*Penaeus monodon* Fab.) in Indonesia competes with vannamei shrimp (*Litopenaeus vannamei*). Although the price is slightly higher, tiger shrimp is considered to has a more difficult rearing process. Nevertheless, the shrimp is a native Indonesian commodity that needs to be developed. The Provincial Government of South Sulawesi is working in line with the country's agenda of restoring the fame of tiger shrimps. From 2015 to 2018, the production in South Sulawesi decreased from 1.6 tons to 1.0 tons, but in 2019, it increased to 1.8 tons (Anonimus, 2019). Given that the rearing system is carried out intensively in ponds, feed is one of the strategic component that determine the success of the business. Consequently, approximately 60% of the total production costs is used for the purchase of feed (CJDEP, 1996). Therefore, in intensive rearing, one of the important activities to control is feeding management. Improper feeding cause problems, which might lead to a decrease in the water quality. This is because organic matter derived from uneaten feed and feces, as well as other metabolic wastes, contain high protein in the form of ammonia which enters the waters. Without an adequate water quality management system, this causes a decrease in water quality, which in turn triggers the emergence of diseases and mass death. Protein is the largest component in shrimp feed and the most expensive compared to other raw materials. Lante *et al.* (2015) showed that protein content of 40% produced the best efficiency and survival in rearing tiger shrimps. However, the use of extremely high protein potentially leads to high feed prices. Koshio *et al.* (1993) on juvenile kuruma shrimp showed that the higher the protein content of the feed, the greater the ammonia-N excretion produced into the waters. The results showed that juvenile kuruma shrimp fed with the protein content of 21.0%, 31.4%, 41.6%, and 50.3%, secreted ammonia-N at 31.2, 61.7, 102.3, and 114.8 ug/g/hour, respectively. Hence, the protein content in the feed needs to be limited, and optimized only for growth. Meanwhile, energy requirements are fulfilled from other sources, including carbohydrates. Sulaiman *et al.* (2020) stated that carbohydrates are a cheap source of energy, but the shrimp's ability to utilize carbohydrates is limited. This is presumably caused by three factors namely: (1) low carbohydrase enzyme that plays a role in carbohydrate hydrolysis, (2) low plasma glucose regulation caused by insulin deficiency, and (3) low activity of enzymes that play a role in glucose metabolism, namely the activity of hepatic glycolytic and gluconeogenesis enzymes (Shiau, 1997).

There are several ways to increase the ability of shrimp to utilize carbohydrates, including the addition of crude fiber to the feed. Shiau (1997) studied the effect of CMC (Carboxymethylcellulose) on the use of carbohydrates (dextrin) in red sea bream. The results indicated that the addition of CMC increases weight gain and feed efficiency. Its role is thought to be related to the delay in glucose absorption from dextrin. With the increasing ability of shrimp to utilize carbohydrates, it is expected that energy needs are adequately fulfilled, while protein is optimized for growth. Shrimps fed with a lower protein diet are also expected to produce lower amounts of ammonia-N both from the decomposition of uneaten feed, feces, and protein catabolism into the waters.Consequently, the principle of environmentally friendly aquaculture is achieved, while the negative effect on the environmental conditions of rearing, namely by replacing the role of protein with other energy sources (Sparring effect) such as carbohydrates is overcome. Information on the role of cellulose in increasing the ability of tiger shrimps to utilize carbohydrates is limited, consequently, these problems need to be studied and investigated. Therefore, this study aims to evaluate the effect of CMC in the feed with different protein and carbohydrate contents on protein and carbohydrate digestibility,growth and survival of tiger shrimp.

II. MATERIALS AND METHODS

A.Time and Place of the study. This study was conducted from June to September 2021.Research was carried out at Research Institute for Coastal Aquaculture and Fisheries Extension (RICAFE), Maros, South Sulawesi, Indonesia. Proximate analysis was carried out at the Animal Feed Chemistry Laboratory, Faculty of Animal Husbandry, Hasanuddin University, while the amino acid analysis was performed at the Integrated Laboratory of Bogor Agricultural University.

B. Tools and Materials : 1.Tested Shrimp. The tiger shrimps at PL 30 stage were used in this study. Previously, the shrimps were reared from PL20 to PL 30 for adaptation to feed and the rearing condition.

2. Experimental diets. The feed used was crumble-shaped and the ingredient composition is presented in Table 1, while the proximate analysis results of each treatment is presented in Table 2.

Table 1. Ingredient composition of experimental diets

	Composition (%)		
	P30% - C40% - CMC0%	P30% - C40% - CMC5%	P30% - C40% - CMC10%
Fish meal	25	25	25
Shrimp head flour	5	5	5
Soybean meal	30	25	20
Maize meal	8	8	8
Bran flour	30	30	30
Fish oil	1	1	1
Vitamin mineral mix	1	1	1
CMC	0	5	10
	P40% - C30% - CMC0%	P40% - C30% - CMC5%	P40% - C30% - CMC 10%
Fish meal	45	45	45
Shrimp head flour	5	5	5
Soybean meal	25	20	15
Maize meal	8	8	8
Bran flour	15	15	15
Fish oil	1	1	1
Vitamin mineral mix	1	1	1
CMC	0	5	10

Table 2. Proximate composition of the experimental diets

Treatments	Water (%)	Crude protein (%)	Lipid (%)	NFE (%)	Fiber (%)	Ash (%)	Energy (Kcal/g)
P30%-C40%-CMC0%	11.01	31.18	11.10	40.95	3.04	13.73	4.40
P30%-C40%-CMC5%	11.45	30.30	12.23	41.22	6.14	10.11	4.47
P30%-C40%-CMC 10%	11.86	29.09	12.77	40,69	8.17	9.28	4.43
P40%-C30%-CMC 0%	11.40	41.86	10.10	31,01	3.13	13.90	4.49
P40% -C30% -CMC 5%	12.10	40.51	10.59	31.03	6.30	11.57	4.46
P40%-C30%- CMC 10%	12.50	39.76	10.62	31.37	8.18	10.07	4.44

Remarks: 1. Except for water, all fractions are expressed in dry matter, 2. NFE: Nitrogen Free Extract

The shrimps were fed with 10% biomass a day with a feeding frequency of 4 at 05.00, 11.00, 17.00, and 22 00. The amount of feed was adjusted every 10 days based on the body weight.

3.The water media for rearing. The water media used in this study was obtained from drilled wells at the Maros RICAFE pond and deposited in a reservoir for 4 days. Furthermore, the water was pumped and transferred to a container through a filter, then sterilized using 20 ppm chlorine, and neutralized using 10 ppm sodium thiosulfate. Salinity of the water ranged from 27 – 30 ppt.

4. Recearch Container. The containers used were plastic buckets with a volume of 30 L filled with 25 L of water and 20 shrimps.

5. Feces collection. Feces collection were carried out after feeding by siphoning. and filtered using filter paper. Feses were collected in plastic container volume 50 ml then stored in the freezer

6.Experimental design. This study was conducted with the experimental method in the laboratory using a factorial completely randomized design with two factors and three replications. The first factor was CMC level, while the second was different protein and carbohydrate levels in the feed. The treatments were as follows:
The first factor : CMC level in the feed 0%, 5%, and 10%
The second factor : Feed with 30% protein and 40% carbohydrate (P 30% - C 40%)
Feed with 40% protein and 30% carbohydrate (P 40% - C 30%)

The Observed Variables

1. Protein and carbohydrate digestibility

The shrimp were initially fed at satiation and the feces were collected. For the measurement of digestibility, 0.7% Cr_2O_3 was added into the feed as an indicator of digestibility. Protein and carbohydrate digestibility was calculated using the formula by Takeuchi (1988) as follows:

$$\text{Protein digestibility (\%)} = 100 - \left[100 \times \frac{\%\ Cr_2O_3 \text{ in feces} \times \%\ \text{protein in feces}}{\%\ Cr_2O_3 \text{ in feed} \times \%\ \text{protein in feed}} \right]$$

$$\text{Carbohydrate digestibility (\%)} = 100 - \left[100 \times \frac{\%\ Cr_2O_3 \text{ in feces} \times \%\ \text{carbohydrate in feces}}{\%\ Cr_2O_3 \text{ in feed} \times \text{carbohydrate in feed}} \right]$$

2. Absolute growth of biomass (g) = Final weight biomass – Initial weight biomass

$$\text{3. Survival rate (\%)} = \frac{\text{Initial stocking} - \text{Dead shrimp}}{\text{Initial stocking}} \times 100$$

4. Feed quality
Feed quality was evaluated based on the amino acid composition.

Data analysis. To evaluate the effect of treatments on protein and energy digestibility, growth, and survival rate of tiger shrimps, an analysis of variance was used. When the treatment has a significant effect, then it was continued with the W-Tukey test. Feed quality was analyzed descriptively according to tiger shrimp needs

Results

Protein and carbohydrate digestibility are presented in Table 3

Tabel 3.Protein and carbohydrate digestibility of tiger shrimps (*Penaeus monodon* Fab) fed by different level of protein, carbohydrate and CMC.

Treatments	Protein digestibility	Carbohydrate digestibility
P30%-C40%-CMC0%	58.48±0.02[a]	54.26±0,52[a]
P30%-C40%-CMC5%	64.54±3.72[b]	61.16±0,44[b]
P30%-C40%-CMC10%	63.29±1,08[b]	62.23±2,29[b]
P40%-C30%-CMC0%	63.16±0.98[b]	62.22±4,19[b]
P40%-C30%-CMC5%	63.18±0.07[b]	64.80±2,03[b]
P40%-C30%-CMC10%	63.19±0.20[b]	64.13±1,49[b]

Description: The same letter in the same column shows results that are not significantly different ($p>0.05$)

Analysis of variance showed that feed with different protein-carbohydrate content, as well as CMC and the interaction between the two, had a significant effect ($p<0.05$) on protein and carbohydrae digestibility. The W-Tukey test showed that protein and carbohydrate digestibility in treatment P 30% - C 40% - CMC 0% was the lowest and significantly different ($p<0.05$) compared to other treatments. The interaction between protein-carbohydrate levels and CMC on protein digestibility is presented in Figure 1, while the carbohydrate digestibility is presented in Figure 2.

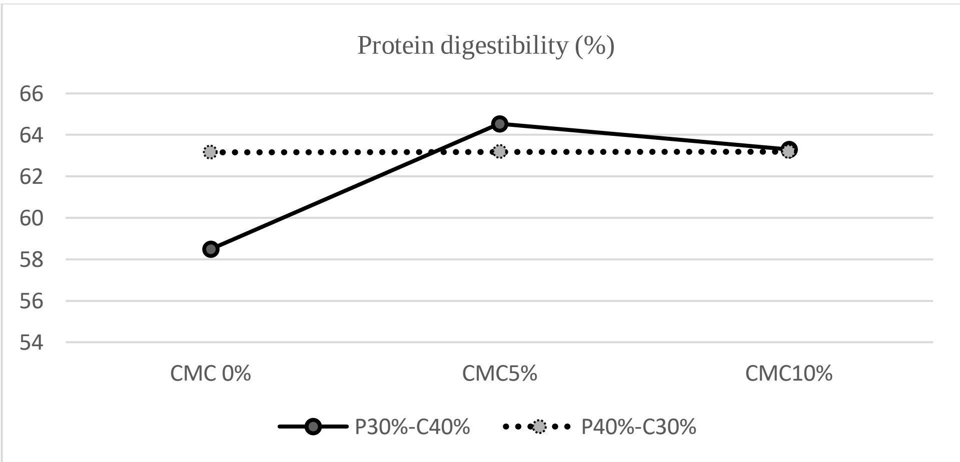

Figure 1. The interaction of protein-carbohydrate and CMC levels to the protein digestibility of *P.monodon*

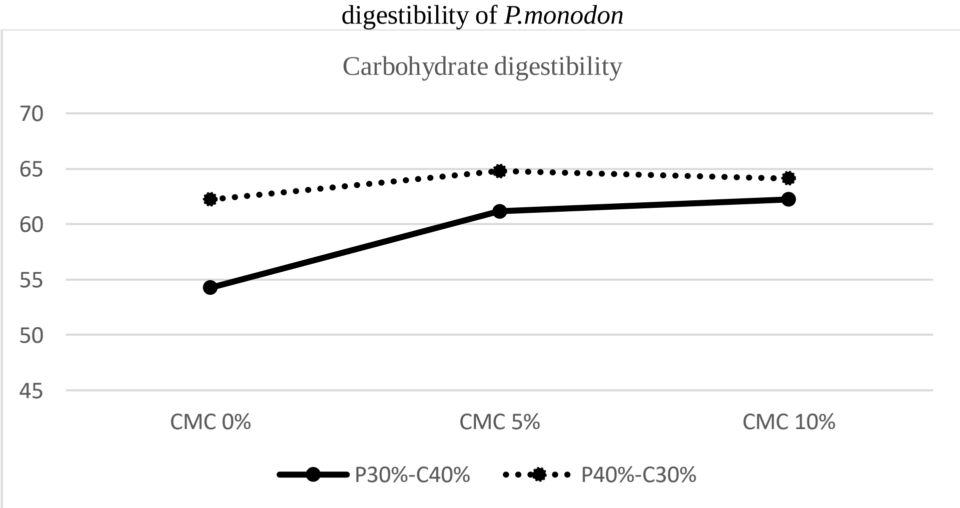

Figure 2. The interaction of protein-carbohydrate and CMC levels to the carbohydrate digestibility of *P.monodon*

Absolute growth of biomass and survival rate of tiger shrimp (*Penaeus monodon* Fab.) are presented in Table 4.

Table 4. Absolute growth of biomass and survival rate of tiger shrimps (*Penaeus monodon* Fab) fed by different level of protein, carbohydrate and CMC.

Treatments	Absolute growth of biomass (g)	Survival rate (%)
P30% - C40% - CMC 0%	2.21±0.56[a]	80.00 ± 8.16[a]
P30% - C40% - CMC 5%	3.52± 0.86[ab]	90.00 ± 10.80[a]
P30% - C40% - CMC 10%	4.61± 0.39[b]	88.33 ±4.08[a]
P40% - C30% - CMC 0%	4.14±0.42[b]	80.00 ±4.08[a]
P40% - C30% - CMC 5%	4.66±1.07[b]	78.33±4.71[a]
P40% - C30% - CMC 10%	4.86±0.05[b]	86.67±10.80[a]

Description: The same letter in the same column shows results that are not significantly different ($p>0.05$)

Analysis of variance showed that feed with different levels of protein-carbohydrates contents, and CMC as well as the interaction between the two had no effect ($p>0.05$) on the survival rate of tiger shrimps, but significantly affected the biomass growth with $p<0.05$. Furthermore, the W-Tukey test showed that the absolute biomass in the treatment P 30%-C 40% - CMC 0% was the lowest and indicated no significant differences ($p>0.05$) compared to P 30% - C 40% - CMC 5%, but differed significantly with $p<0.05$ from other treatments. The absolute biomass growth at P30% - C40% - CMC 5%, P30% - C 40% - CMC 10%, P40% - C 30% - CMC 0%, P 40% - C 30% - CMC 5%, and P 40% - C-30% - CMC 10% were not significantly different. Meanwhile, the interaction between protein-carbohydrate content and CMC on the absolute growth of biomass is presented in Figure 3.

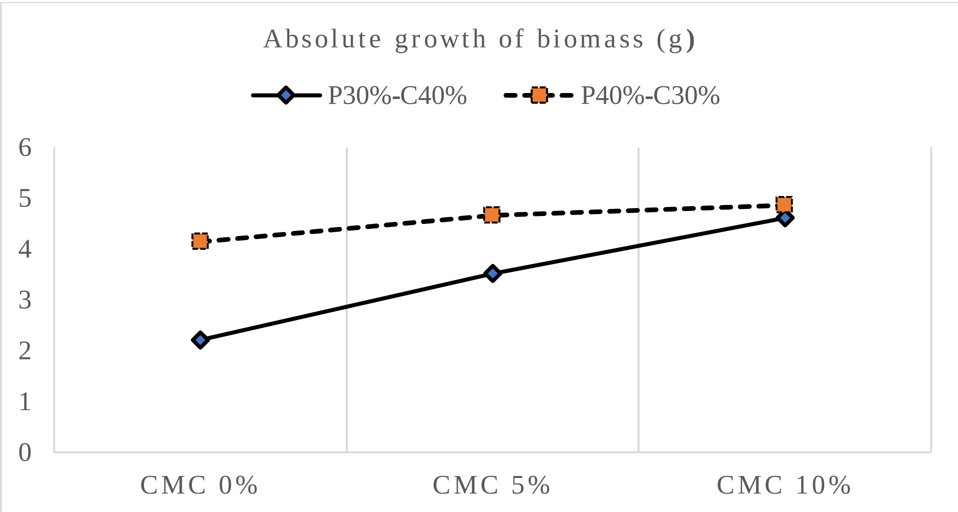

Figure 3. The interaction of protein-carbohydrate and CMC levels to the absolute growth biomass (g) of the *P. monodon*

The amino acid composition of the feed is presented in Table 5.

Table 5. Amino acid composition of feed (% in feed)

Amino acid	A	B	C	D	E	F	Tiger shrimp requirement (% in feed)	References
Aspartic acid	3.98	3.57	4.09	4.76	4.58	4.15		
Glutamic acid	6.76	6.09	7.16	8.21	7.90	6.99		
Serine	1.42	1.29	1.47	1.54	1.59	1.38		
histidine*	1.19	1.10	1.34	1.33	1.18	0.69	0.8	Millamena *et al* (1999)
Glycine	2.41	1.90	2.60	2.79	2.61	2.23		
Threonine*	1.67	1.54	1.85	2.06	1.68	1.05	1.4	Millamena *et al* (1997)
Arginine*	3.03	2.69	3.00	3.40	3.33	2.97	1.9	Chen *et al* (1992)
Alanine	2.64	2.32	2.78	2.97	2.95	2.64		
Tyrosine	1.46	1.34	1.48	1.70	1.76	1.52		
Methionine*	0.81	0.74	0.92	1.18	1.06	1.09	0.9	Millamena *et al* (1996)
Valine*	2.41	2.16	2.46	2.75	2.51	2.13	1.35	Millamena *et al* (1996)
Phenylalanine*	2.05	1.87	2.07	2.33	2.23	1.93	1.4	Millamena *et al* (1999)
Isoleucine*	2.05	1.85	2.03	2.39	2.17	1.81	1.0	Millamena *et al* (1999)
Leucine*	3.40	3.30	3.37	3.77	3.60	3.11	1.70	Millamena *et al* (1999)
Lysine*	3.35	2.64	3.23	3.52	2.66	2.90	2.1	Millamena *et al* (1998)
Tryptophane*	0.16	0.15	0.15	0.18	0.19	0.18	0.2	Millamena *et al* (1999)
	38.80	34.73	40.01	44.96	42.00	36.78		

Description: *Essential amino acids

A.: P30% - C40% - CMC 0%, B: P30% - C40% - CMC 5%, C: P30% - C40% - CMC 10%

D: P40% - C30% - CMC 0%, E: P40% - C30% - CMC 5%, F: P40% - C30% - CMC 10%

III. DISCUSSION

Based on the analysis of the essential amino acid content in the feed for all treatments, only tryptophan was relatively lower. According to Millamena *et al.* (1999), the amino acid requirement for tryptophan in the feed is 0.2%, while the value obtained in this study ranged from 0.15 to 0.19%. Based on these results, CMC increases the ability to utilize carbohydrates by improving feed digestibility. Besides, absorption also depends on the time of contact between the nutrient and the absorbing epithelium. The longer the contact between nutrients and hydrolase enzymes, the higher the digestibility of the feed (Shiau *et al.*, 1997). Sun *et al.* (2019) reviewed the effect of crude fiber in a practical diet on feed utilization and antioxidant capacity of Loach *(Misgurnus anguilicaudatus)*. The results showed that the fiber content significantly affected the feeding rate (FR), feed conversion rate (FCR), and protein efficiency ratio (PER). Crude fiber content also has a significant effect on loach digestibility. Moreover, digestibility showed an increasing trend before decreasing, with the best crude fiber content of 5.52% - 5.65%. Digestibility is an important index for evaluating feed absorption and utilization in the Taiwanese Loach. This is because a suitable fiber content functions as a diluent to expand the contact area between food ingredients and digestive enzymes, there by improving the digestion and absorption of nutrients.

The lowest absolute biomass growth was found in P 30% - C 40% - CMC 0% and was significantly different compared to other treatments. This is in line with the protein and carbohydrate digestibility measurements where the treatment also produced the lowest result and was significantly different from others. An increase in the digestibility of feed leads to higher absorption of nutrients and energy, thereby increasing growth. Morita *et al.* (1982) investigated the effect of CMC on the growth and feed efficiency of red sea bream. CMC was incorporated at 0%, 3%, 6%, 9%, and 12% in the feed, while dextrin was used as the carbohydrate source. At 10% dextrin content, the highest growth and feed efficiency were obtained at 6% CMC followed by 20% dextrin content at 9% CMC, and 30% dextrin at 12% CMC. According to Shiau *et al.* (1991), tiger shrimps measuring 0.9 g need 40 - 44% protein. The results obtained in this study show that the use of 5% CMC in the feed reduced the protein requirement to 30%. Furthermore, the limitation of shrimp in utilizing carbohydrates is caused by low digestibility and low regulation of plasma glucose concentration (Shiau, 1997).

Goulart *et al.* (2017) examined the effect of Dietary Fiber Concentrates (DFCs) on growth performance, gut morphology, and hepatic metabolic intermediates in Jundia *(Rhamdia guelen).* The results indicate that DFCs have great potential and are a beneficial dietary supplement. In addition, supplementation with DFCs promoted improvement in heights of intestinal villi. The higher the intestinal villi, the better the digestion and nutrient absorption, which has a positive effect on animal performance. Zhou *et al.* (2010) also observed improvements in microvilli height of red drum *(Sciaenops ocellatus).*Kamarudin *et al.* (2017) investigated the effects of dietary crude fiber level on survival, growth performance, and feeding efficiency of barb hybrid. The whole-body proximate composition, intestinal short-chain fatty acids, and liver glycogen were also measured. The results showed that a diet containing 5 – 10% fiber, 35% carbohydrates, 30% protein, and 4% lipid was recommended for the culture of fingerling tropical lemon fin barb hybrid.

Moreover, Gao *et al.* (2020) examined the effect of gelatin or carboxymethyl cellulose supplementation during the pellet preparation on feed quality, intestinal ultra structure, and growth performance of gibel carp *(Carassius gibelio).* The results showed that supplementation with 10 g/kg CMC produced the highest intestinal microvilli increase and was significantly different compared to 30 g/kg. Based on these results, it was concluded that CMC is applicable as a supplement in feed to improve feed quality without negatively affecting the growth performance of gibel carp.

IV. CONCLUSION

The results showed that the different levels of protein, carbohydrates, and CMC in the feed produced similar responses to the survival rate of tiger shrimps. Lowest apparent protein and carbohydrate digestibility and absolute biomass growth of the shrimp was found in the feed with a level of protein 30%, carbohydrate 40%, and CMC 0%. Based on the results, the feed containing protein 30%, carbohydrates 40%, and CMC 5% is considered the best for rearing tiger shrimp.

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