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Nutritional evaluation of rain tree (*Samanea saman*) pod and its incorporation in the diet of rohu (*Labeo rohita* Hamilton) larvae as a non-conventional feed ingredient

S. C. RATH, K. C. NAYAK, K. N. MOHANTA, C. PRADHAN, P. V. RANGACHARYULU
S. SARKAR AND S. S. GIRI

Fish Nutrition and Physiology Division, Central Institute of Freshwater Aquaculture, Kausalyaganga - 751 002 Bhubaneswar, Odisha, India
e-mail: scrathcifa@yahoo.com

ABSTRACT

Rain tree (*Samanea saman*) pod (RTP) is identified as a good source of protein (25.2%) and energy (20.4 kJ g^{-1}) for carp feed. In the present study, RTP as a non-conventional feed ingredient was tested for the first time in carp feeds. Five iso-nitrogenous (25% crude protein) feeds were formulated by incorporating RTP at 0 (F1), 100 (F2), 200 (F3), 300 (F4) and 400 (F5) g kg^{-1} as co-ingredient, partially substituting groundnut oil cake and rice bran. Feeds were fed to *Labeo rohita* larvae *ad libitum* for 30 days in FRP flow-through tanks. The survival of the larvae was higher (87 – 90%) in F1- F4, but it reduced significantly (61%, $p < 0.05$) in F5. Weight gain (%) and specific growth rate (SGR) were significantly higher ($p<0.05$) in F4 and F5 groups and food conversion ratio (FCR) was significantly lower ($p<0.05$) in F4. Protein efficiency ratio (PER) and net protein utilisation (NPU) were found significantly higher ($p<0.05$) in F4. The results indicated that RTP can be incorporated at 300 g kg^{-1} substituting 25% groundnut oil cake and 42% rice bran in the diet of *L. rohita* larvae without any adverse effect on growth, survival and nutrient utilisation.

Keywords: *Labeo rohita,* Rain tree pod, *Samanea saman,* Safe level incorporation

Introduction

The global freshwater fish production as estimated during 2010 was 35.0 million t (FAO, 2010). Freshwater fish production relies on commercial feeds, farm made feeds or on fresh feed supplements. The commercial aquafeed production was 29.5 million t during 2008 and the projected production in 2020 is 71 million t at annual percent rate (APR) of 11% (Tacon *et al*., 2011). The projection is exclusive of the farm made feeds used for carp production in Asian countries. Ayyappan and Ali (2007) reported that 97% of carp production in India is solely using farm made feed. Whatever may be the feed types, less competitive and cost effective ingredients are the keys for production of sustainable aquafeeds Hasan *et al.* (2007) opined that the protein supplement in aqua feeds is mostly met by incorporation of either fish meal or plant based ingredients. Plant proteins are used as dietary protein source for carps in farm made feeds. Soybean meal is the common source of plant protein used in compounded aquafeeds followed by oilcakes (Barman and Karim, 2007; Manomaitis, 2009. Incorporation of soybean or cakes of edible oil seeds as ingredients is becoming too competitive and costly especially for farm made feed formulation of carps. It is therefore imperative to search new non-conventional plant feed resources for formulating cost effective carp feeds.

Rain tree (*Samanea saman*) is a fast growing tropical tree under the family Fabaceae. The fruit (pod) is well known as cow tamarind or monkey pod and often browsed by ruminants (Devendra,1989). A full grown tree can produce 500 - 600 kg pods in a year. Dry ripe pods can be stored for long period under normal condition. Sporadic preliminary reports are available on the food value of rain tree pod (RTP) (Thole, *et al.* 1992; Esuoso, 1996; Cruz, 2003; Idowu *et al*., 2006). Indiscriminate use of plant derived materials in fish feed is limited due to presence of a wide variety of anti-nutritional substances and it is wise to evaluate both nutritional and anti-nutritional factors before the ingredient is used in the feeds (Francis *et al*., 2001). Rohu (*Labeo rohita*), is one of the most preferred species of Indian major carps, considered as the principal component of carp polyculture in Indian sub-continent. It is also cultivated as single species aquacrop in freshwater tanks and ponds. To our knowledge, no literature is available on the use of RTP in formulating carp diets. Therefore, the objective of the present study was to generate information on nutritive value, anti-nutritional factors, acceptability and safe level of incorporation of raw RTP in carp feeds. An attempt was made to incorporate RTP as a co-ingredient to substitute the comparatively costly groundnut oil cake and rice bran in larval diet of *L. rohita*.

Materials and methods

As information available on the nutritional value of RTP is scanty, the whole pod, only seeds and pod without seeds were analysed separately to generate nutritional information. Rain tree pods (RTP) were collected by hand picking from the canopy of adult tree from in and around the Central Institute of Freshwater Aquaculture (CIFA), Bhubaneswar India. The pods were sun dried and milled to powder. Rain tree seeds (RTS) were collected by peeling out the RTP. A single pod contains about 8-12 seeds of 0.3-0.5 g each. The pod without seeds (PWS) was also collected and powdered separately. All the three powdered ingredients (RTP, RTS and PWS) were analysed for their chemical composition *viz.,* crude protein, ether extract, ash and crude fibre and were subjected to analysis of important antinutrient factors by qualitative methods. As RTS contained good amount of oil, its fatty acid profile was also analysed.

Chemical analysis

Dry matter was obtained by drying the samples at 105^{0}C for 24 h. Crude protein (CP) was estimated by Kjeldahl's method (N x 6.25) and the ether extract (EE) by Soxhelt method using petroleum ether (boiling point 60–80^{0}C). Ash content was estimated by ignition of sample at 550^{0}C for 3 h in a muffle furnace (AOAC, 1990).

Screening for secondary metabolites

Rain tree pod is a newly identified non-conventional plant resource for carp feeds. Therefore the secondary metabolites of whole pod (RTP), only seeds (RTS and pod without seed (PWS) were assessed separately before incorporation as feed ingredient. Aqueous and n-hexane extracts of RTP, RTS and PWS were prepared and screened for major secondary metabolites (tannin, saponin, alkaloids, steroids, terpinoids, resins, glycosides and flavonoids as described by Evans (2000) and Harbone (1998).

Fatty acid analysis

Total lipids were extracted from 15 g powdered rain tree seed using (2:1 v/v) chloroform-methanol mixture containing 0.01% BHT (Folch *et al.,* 1957). The weight of lipids was determined gravimetrically after evaporation of the solvent. Fatty acid methyl esters (FAME) were prepared by acid-catalysed transesterification of total lipids according to the method of Christie (1982). Fatty acid methyl esters were separated by a gas chromatograph equipped with flame-ionisation detector (Shimadzu GC-2010, Kyoto, Japan) on a DB-25 capillary column (20 m×0.10 mm I.D., 0.10 μm J&W Scientific, Santa Clara, CA, USA). The fatty acids were identified using fatty acid methyl ester (FAME) standards. Area percentage normalised values for the fatty acids were taken as weight percentage.

Test feeds for rearing L. rohita

Five isonitrogenus (25% CP) feeds (F1-F5) were formulated incorporating raw RTP meal at 0, 100, 200, 300, and 400 g kg^{-1} along with groundnut oilcake, rice bran, wheat flour and vitamin and mineral premix as co-ingredients procured from the local market. The feed mix was powdered and pulverised. Feed pellets were made with portable pelletiser and oven dried at 60^{0}C overnight. Pellets were ground and sieved to particle size of 50-80 μm.

Larval rearing and feed evaluation

Hatchery produced larvae of *L. rohita* were used for the experiment. The experiment was conducted in the wet laboratory facility of CIFA, Bhubaneswar. Flow through FRP tanks (45 l) with a flow rate of 0.5 l per min were used for rearing the fish. Five days old *L. rohita* larvae (0.002 g) were reared in triplicates, 200 in each tanks for 30 days. Each tank was aerated with aquarium aerator. The water quality parameters in each of the experimental tanks were monitored on daily basis. The larvae were fed *ad libitum* thrice daily with the test feeds. Unconsumed feed, was siphoned out 2 h following each meal, to calculate the feed intake. Thirty percentage water was exchanged on a daily basis while collecting the uneaten feed and fecal matter. Routine water quality parameters *viz*., temperature, pH, transparency, total alkalinity, hardness, un-ionised ammonia and dissolved oxygen were analysed (APHA, 1989) at regular intervals. On termination of experimental feeding, the *L. rohita* fry were sacrificed and nutritional indices were determined by standard procedures (AOAC, 1990). Data were analysed using one way ANOVA (Snedecor and Cochran, 1967) and difference between means were tested using Duncan's multiple range test. On 30th day of the experiment, fry were harvested and batch weighed. The weight gain (%) , specific growth rate (SGR), food conversion ratio (FCR), protein efficiency ratio (PER), net protein utilisation (NPU) and survival rate (%) were calculated as:

$$\text{Weight gain (\%)} = \frac{\text{(Final weight – Initial weight)}}{\text{Initial weight}} \times 100$$

$$\text{(SGR)} = \frac{\text{ln Final weight – ln Initial weight}}{\text{Days of experiment}} \times 100$$

$$\text{FCR} = \frac{\text{Feed consumed (dry weight)}}{\text{Live weight gain (wet weight)}}$$

$$\text{PER} = \frac{\text{Live weight gain}}{\text{Protein consumed}}$$

$$\text{NPU (\%)} = \frac{\text{Protein gain in carcass}}{\text{Protein intake in food}} \times 100$$

$$\text{Survival rate (\%)} = \frac{\text{Initial no. of larvae – Final no. of fry}}{\text{Initial no. of larvae}} \times 100$$

Results

The water quality parameters recorded in the experimental tanks were: temperature 28-31°C; transparency 25-28 cm; total alkalinity ($CaCO_3$) 90-110 mg l^{-1}; Hardness 80-96 mg l^{-1}; un-ionised ammonia <0.1 mg l^{-1}; Dissolved oxygen 3 - 7 mg l^{-1} and pH 7.5-8.5. The comparative chemical composition of raw RTP, RTS and PWS is presented in Table 1. RTP and RTS were found rich in protein than that of PWS. Crude protein percentage of RTS (42.56) was significantly ($p<0.05$) higher than RTP (25.25) and PWS (19.44). Similarly, ether extract percentage was significantly higher ($p<0.05$) in RTS in comparison to RTP and PWS. Alkaloids, saponin, tannin, flavonoids and resins were detected as phytochemicals in RTP, RTS and PWS by qualitative analysis (Table 1). Fatty acid profile of *Samanea saman* seed oil is given in Table 2. Ingredients and chemical composition of all the five test feeds are summarised in Table 3. The feeds were iso-nitrogenus with 240-250 g protein kg $^{-1}$ diet. The incorporation of RTP in the experimental feeds replaced the groundnut oil cake at 8, 16, 25 and 33% and rice bran at 14, 28, 42 and 56% in F2, F3, F4 and F5, respectively. Survival, growth, nutrient utilisation, weight gain and whole body composition of *L. rohita* larvae under different test feeds are presented in Tables 4. It is observed that, up to 30% incorporation level of RTP (F1- F4) there was significantly ($p<0.05$) higher survival (87-90%) as compared to 40% (F4) inclusion, where the survival was only 61%. The SGR (% day^{-1}) was statistically similar in F4 (9.7) and F5 (9.8) and significantly higher ($p<0.05$) than the other groups. Weight gain (%) was significantly higher ($p<0.05$) in larvae fed F4 and F5 diets than the other diets. The FCR was lowest in larvae fed diet F4 and highest in F5. The PER and NPU were also significantly higher ($p<0.05$) in F4 among all the dietary groups. The final whole body protein content remained constant

Table 2. Fatty acid profile of *Samanea saman* seed oil

Name	Fatty acids	Weight %
C10:0	Capric acid	0.73
C13:0	Trideconoic acid	3.27
C15:0	(Pentadecanoic acid) pentadecanoic acid	0.69
C18:0	(Stearic acid) octadecanoic acid	7.59
C20:0	(Arachidic acid) eicosanoic acid	6.71
C22:0	Behenic acid	8.56
C24:0	(Lignoceric acid) tetracosanoic acid	2.50
∑SFA		30.04
C14:1	(Myristoleic acid) tetradecenoic acid	1.45
C16:1	(Palmitolic acid) hexadecenoic acid	0.81
C18:1n9	(Oleic acid) octadecanoic acid	20.74
C20:1	(Gadoleic acid) eicosenoic acid	1.68
∑MUFA		24.68
C18:2n6	(Linoleic acid) octadecadienoic acid	40.20
C20:3n6	(Dihomo-c-linolenic acid) eicosatrienoic acid	1.24
∑PUFA n-6		41.43
C18:3n3	(Alpha-linolenic acid) octadecatrienoic acid	2.85
∑PUFA n-3		2.85

Table 1. Chemical composition of RTP, RTS and PWS meal (g kg $^{-1}$ dry matter basis)

Parameters	Rain tree pod (RTP)	Rain tree seed (RTS)	Pod without seeds (PWS)
Moisture	107.5 ± 1.3^{c}	52.3± 0.6^{b}	12.4± 1.8 ^{a}
Crude protein	252.5± 2.0^{b}	425.6± 2.0^{c}	194.4± 1.3^{a}
Ether extract	17.7± 0.2^{b}	105.2± 0.2^{c}	7.5±0.3^{a}
Ash	41.4± 0.4^{b}	36.2±0.2^{a}	47.7± 0.6^{c}
Crude fibre	122.0± 0.2^{b}	69.3± 0.4^{a}	137.1± 1.5^{c}
Nitrogen free extract	566.4± 2.^{b}	363.7± 2.2^{a}	613.3±1.3^{c}
Total tannin *	+++	++++	++
Saponin*	+++	++	+++
Resins *	++++	++	++++
Alkaloid *	++++	+	++++
Flavonoid *	++	++	++

*Qualitative estimation (+ less presence,++ moderate presence, +++ high presence). The data are mean ± SE , Values bearing different superscripts in a row differ significantly ($p<0.05$).

Table 3. Ingredients and chemical composition (g kg $^{-1}$ dry matter basis) of the experimental feeds of *L. rohita* larvae

Ingredients	Larval feeds				
	F1 (0% RTP)	F2 (10% RTP)	F3 (20% RTP)	F4 (30% RTP)	F5 (40% RTP)
RTP	0	100	200	300	400
GNOC	600	550	500	450	400
RB	360	310	260	210	160
Vitamin-mineral pre-mix*	20	20	20	20	20
Wheat flour	20	20	20	20	20
Chemical composition					
Crude protein	247.0± 1.3 ^{a}	251.8± 2.6 ^{b}	245.3± 1.1 ^{a}	244.8± 1.3 ^{a}	244.1± 1.9 ^{a}
Ether extract	91.8± 1.1 ^{e}	82.4± 0.6 ^{d}	74.5±1.2 ^{c}	70.0± 0.9 ^{b}	68.2± 0.6 ^{a}
Crude fibre	82.6± 1.0 ^{a}	83.1± 0.1 ^{a}	87.6± 0.6 ^{b}	92.1± 0.6 ^{c}	96.6 ± 0.2 ^{d}
Ash	77.5±0.1 ^{c}	78.0± 0.1 ^{c}	74.2± 1.0 ^{a}	73.6±0.4 ^{a}	75.6± 0.3 ^{b}
Nitrogen free extract	501.2±0.1	502.5± 0.4	518.1 ± 0.7	519.5±0.1	522.1 ± 0.9

*Supplevite-M (Jeco Vet Chem Pvt. Ltd, Mumbai, India). Each 1 kg of Supplevite-M contains: Vitamin A: 200000 IU, Vitamin D_3: 40000 IU, Vitamin B_2: 0.8 g , Vitamin E: 300 IU, Vitamin K: 400 g, Calcium panthonate: 1 g, Nicotinamide: 4 g, Vitamin B_{12} : 2.4 mg, Choline chloride: 60 g, Calcium: 300 g, Manganese: 11 g, Iodine: 0.4 g, Iron: 3 g, Zinc: 6 g, Copper: 0.8 g, Cobalt: 0.18 g. The data are mean ± SE : Values bearing different superscripts in a row differ significantly (P<0.05).

Table 4. Survival, growth and nutritional indices of *L. rohita* larvae fed different test diets

Parameters	Larval feeds				
	F1 (0 % RTP)	F2 (10 % RTP)	F3 (20 % RTP)	F4 (30 % RTP)	F5 (40 % RTP)
Survival %	90.83±0.73 ^{c}	88.67±0.44 ^{bc}	88.00±0.87 ^{b}	87.00±0.58 ^{b}	61.50±1.04 ^{a}
Weight gain (mg)	33.26 ± 0.44 ^{a}	33.44 ± 0.62 ^{a}	32.44 ± 0.86 ^{a}	35.72 ±0.64 ^{b}	36.76 ±0.88 ^{b}
Weight gain (%)	1663.11±22.01 ^{a}	1667.48±17.61 ^{a}	1622.33±17.07 ^{a}	1786.65±16.65 ^{b}	1838.06±23.83 ^{b}
SGR (% day $^{-1}$)	9.56±0.11 ^{a}	9.57±0.03 ^{a}	9.49±0.06 ^{a}	9.79±0.07 ^{b}	9.88±0.04 ^{b}
FCR	2.36±0.12 ^{bc}	2.09±0.04 ^{ab}	2.36±0.15 ^{bc}	1.93±0.15 ^{a}	2.58±0.09 ^{c}
PER	1.84±0.09 ^{a}	2.04±0.04 ^{ab}	1.85±0.10 ^{a}	2.29±0.19 ^{b}	1.74±0.06 ^{a}
NPU (%)	16.07±0.61 ^{a}	16.67±0.33 ^{a}	16.64±0.89 ^{a}	20.80±1.77 ^{b}	15.42±0.55 ^{a}

The data are mean ± SE : Values bearing different superscripts in a row differ significantly (p<0.05).
SGR: specific growth rate; FCR: feed conversion ratio; PER: protein efficiency ratio; NPU: net protein utilisation

(p>0.5) up to 20% RTP incorporation level (F1 to F3) and thereafter, it increased significantly (p<0.05) in F4 and F5 (Table 5). There was a linear increase in final whole body ether extract content with increase in dietary RTP levels up to 30% and declined thereafter (Table 5). The final whole body ash content was significantly higher (p< 0.05) at lower level (0-10 %) of RTP incorporation. There was no definite trend in whole body moisture content with increase in dietary RTP levels.

Discussion

The physico-chemical parameters of water were found within the permissible range for carp seed rearing (Jena and Das, 2005). The whole pod (RTP), only seed (RTS) and pod without seed (PWS) were analysed to generate information regarding their nutritive value. However, only RTP meal was incorporated in the test diet as it was available in bulk. The nutritive value of RTP and its performance in animal feed have been evaluated by Oduguwa *et al.* (2000); Hosmani *et al*. (2005); and Idowu *et al.,* 2006). It has been reported that rain tree pods can be incorporated very well in the ration of ruminants to reduce the feed cost (Hosmani *et al.,* 2005). So far, no information is available on use of RTP (*Samanea saman*) as feed ingredient for fish, particularly in carps. Crude protein content of the RTP as reported by earlier authors varied widely from

Table 5. Final whole body composition (g kg $^{-1}$ dry matter basis) of *L. rohita* larvae fed with different test diets

Parameters	Larval feeds				
	F1 (0% RTP)	F2 (10 % RTP)	F3 (20 % RTP)	F4 (30 % RTP)	F5 (40% RTP)
Moisture	858.0± 1.7 ^{c}	864.7± 1.8 ^{b}	850.9± 2.3 ^{a}	853.0± 1.4 ^{ab}	857.6± 0.7 ^{b}
CP (Crude protein)	637.8± 0.8 ^{a}	637.4± 0.8 ^{a}	634.7± 0.4 ^{a}	645.1± 1.4 ^{b}	648.6± 1.6 ^{c}
EE (Ether extract)	137.5± 1.2 ^{a}	146.9± 0.9 ^{b}	156.6± 1.5 ^{c}	167.4± 0.9 ^{d}	145.6± 1.0 ^{b}
Ash	193.5± 0.9 ^{c}	194.5± 0.2 ^{b}	176.2± 1.6 ^{a}	163.6± 0.8 ^{a}	175.8± 1.6 ^{a}

The initial whole body moisture, CP, EE, and ash contents (g kg $^{-1}$ dry mater basis) were 890.6 ± 0.5, 622.3 ± 0.5, 75.0 ± 1.4, and 277.9 ± 1.2, respectively. The data are mean ± SE; Values bearing different superscripts in a row differ significantly (p<0.05).

19-28% (Esuoso, 1996; Idowu *et al*., 2006; Babayemi *et al.*, 2010). In the present study, the CP and NFE value of RTP obtained were 25% and 56% respectively and therefore, it can very well be used as a source of protein and energy in fish feed. The amino acid profiles of RTS as studied by earlier workers showed a high tryptophan (16.24%) and lysine (14.98 %), however methionine and proline were the limiting amino acids (Esuoso, 1996). In this present feed trial, few other protein sources in addition to the RTP was used to provide the essential amino acids. The lipid content of pod and seed was reported in the range of 1-3% and 11-12% respectively (Thole *et al.,* 1992; Esuoso, 1996; Cruz, 2003). In the present study, lipid level of 1.7% in RTP and 10.5% in RTS was observed. Oil of RTS had 30% of saturated fatty acids (SFA), 25% of mono unsaturated fatty acids (MUFA), 41% of n-6 poly unsaturated fatty acids (PUFA) and 3% of n-3 PUFA (Table 2). Esuoso (1996) reported that the lipid of RTS contained nine fatty acids out of which unsaturated acids accounted over 90% of the total fatty acid content. In the present study, unsaturated fatty acid was 69% of the total fatty acid content with 40% of linoleic acid (LA). LA as precursor, is metabolised to arachidonic acid (ARA, 20:4n-6). In the metabolic pathway of freshwater fish including carps, delta-5 desaturase and elongase utilise linoleic acid to form arachidonic acid (AA). This generates a family of derivatives collectively called eicosanoids which includes prostaglandins, thromboxanes, leukotriens, epoxides and oxylipins (Poudyal *et al.,* 2011). Role of the above derivatives in management of several inflammatory diseases has been well documented (Kapoor *et al*., 2006).

The presence of phytochemicals *viz.*, tannin, saponin, alkaloids, resins and flavonoids, which were detected in our study by qualitative analysis is in agreement with the finding of other workers (Prasad *et al.,* 2008; Kumar *et al*. 2009; Obasi *et al.,* 2010; Ukoha *et al*., 2011). Though tannin in higher concentrations reduce feed intake and affect protein and carbohydrate digestibility, it is reported to be useful in lower concentrations (Mukhopadhyay and Ray, 1999). Tannin at higher concentration affects protein utilisation, as it binds to lysine and makes it unavailable to monogastric animals and fish (Francis *et al.*, 2001). In the present study, as the whole body protein and NPU increased significantly in F4 and F5, probably tannin is not a limiting factor for protein utilisation even at 40% dietary inclusion level of RTP. Fish feed containing higher levels of alkaloids reduces the feed intake (Glencross *et al.,* 2006). In our study, the feed intake was unaffected with increase in RTP levels. Francis *et al.* (2005) summarised the growth promoting effect of saponin and mentioned about its antifungal and antioxidant property in the feed. Complex formation between saponin and other antinutrients can lead to inactivation of the toxic effects of both the substances. Siddhuraju and Becker (2001) incorporated mucuna seed meal (*Mucuna prurient*, Fabaceae) in the diet of common carp as a non-conventional ingredient and did not find any significant growth reduction at 13% incorporation level. Hossain *et al.* (2001) incorporated 10% of *Sesbanea aculeate* seed meal to the diet of common carp and tilapia without compromising growth. Thole *et al.* (1992) replaced rice bran with RTP in the feed at 20% incorporation level and fed heifers for ten months without any adverse effect on growth. Presently RTP is considered as an un-priced and non-conventional commodity. Ground nut oil cake and rice bran are too costly for carp feed, which were replaced by RTP at 25% and 42% respectively. Mohanty *et al.* (1996) reported survival rate of catla larvae at 77% in a feed more or less similar to F1 diet with 26% crude protein. Basavraj and Antony (1997) reported 98% survival of carp larvae in 21 days experiment with conventional feed (groundnut oil cake and rice bran mixure, 1:1) at stocking density of 1500 nos. in 25 m^3. Jena *et al.* (1998) in a pond experiment for 15 days rearing of *L. rohita* spawn with above conventional feed reported the survival (%), SGR, FCR and PER as 45, 26.92,3.97 and 1.04 respectively. There was a linear increase in growth and a progressive decline in survival in F4 and F5. Similar observation has been reported by Sahu *et al*., (2007) in nursery rearing of *Labeo calbasu*. However the present study revealed that there is scope to use 300 g raw RTP per kg of rohu larval feed without compromising survival and growth.

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