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ORIGINAL ARTICLE

BIOCHEMICAL COMPOSITION OF INTERTIDAL MURICID GASTROPOD *Thais mutabilis* (LINK) IN TRANQUEBAR, NAGAPATTINAM DISTRICT, SOUTHEAST COAST OF TAMILNADU INDIA

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ABSTRACT

The present investigation was aimed on the biochemical composition of molluscan *Thais mutabilis*. From overall investigation showed the lot of changes in biochemical composition of carbohydrate in gonad, digestive gland and foot of selected marine molluscan (*T.mutabilis*).Methods: The gastropods (*T.mutabilis*) were collected from the Tranquebar coast of Nagapattinamduring December 2010 to August 2011. The animal shell was broken and body muscles (expected Gonad, digestive gland and Foot) were dried at 60 ^{o}C in an oven and used for biochemical analysis, such as protein, carbohydrate and lipid. The results of proximate composition showed that the percentage of protein in the tissue was (53.86%), followed by the carbohydrate content (16.85. The results of this research showed that *T. mutabilis*meat is a valuable food with high quality protein.

Key words: *Thais mutabilis*, Protein, Carbohydrate, Lipid

1.INTRODUCTION

The knowledge on the biochemical composition of any edible organism is extremely important, since the nutritive value is reflected in its biochemical components (Nagabushanam and Mane, 1978). Further, according to above authors, a newer species should be recommended for human consumption only after assessing the nutritive value of the species. With regard to its nutritional merits Giese (1969) revealed that investigation on biochemical composition in different body parts will be more meaningful than the whole animal. Pronounced seasonal variations in the biochemical constituents of gastropods were reported by Lambert and Dehnel, 1974; Stickle, 1975; Suryanarayanan and Nair, 1976; Ansari *et al* 1981; Shanmugam, 1987; Maruthamuthu, 1988; Thivakaran, 1988; Ramesh and Ayyakkannu, 1992; Jamila Patterson *et el* 1994; Stella, 1995 and Paul Ravindran, 2003. Though information is available on the biochemical composition of gastropods, detailed and systematic information on the biochemical composition of intertidal prosobranchs is lacking. Based on the above factors an attempt has been made to investigate the biochemical composition of *T. mutabilis*, an intertidal prosobranch. The shellfish and other aquatic organisms suitable for food and feed are of worldwide importance. They are excellent sources of high quality proteins, which are superior to those in meat and poultry. Man lives on land, which occupies a quarter of the surface of the planet, and takes most of his food from the land. Approximately 14% of the animal protein consumed by human beings comes from marine fisheries. Biochemical assays and nutrients play a vital role in physical growth, development, maintenance of normal body function of physical activity and health. The knowledge of the biochemical composition of any edible organisms is extremely important since the nutritive value Biochemical assays and nutrients play a vital role on physical growth and development, maintenance of normal body function physical activity and health. The knowledge of the biochemical composition of any edible organisms is extremely important since the nutritive valueis reflected in its biochemical contents (Nagabhushanam and Mane, 1978). A newer species should be recommended for human consumption only after assessing the nutritive value of the species with regards to its nutritional merits (Xavier, 1996). Even though large numbers of marine gastropods are suitable for human consumption, our knowledge on its nutritive value is fragmentary. Generally proximate composition means percentage composition of five basic constituents such as protein, carbohydrate, lipid, ash and water. The proximate composition varied widely depending on several factors like species, size, sex, maturity, season and feeding regimes. Information on daily dietary intake of nutrients especially

cholesterol is quite important for especially those with cardiovascular problems (Xavier, 1996; Ajaya, 2002). Proteins are fundamental bio molecules in all aspectsof cell structure and function. An increasing demand for good quality of animal protein for the exploding population has led to effective and increasing exploitation of the aquatic resources. Carbohydrates are major sources of energy in all human diets. The ratio of carbohydrate was less when compare to the other nutrients such as proteins and lipids in animal tissues, especially in aquatic animals. Lipids can be defined as substances such as a fat, oil or wax that dissolves in alcohol but not in water.

2. MATERIALS AND METHODS

For the present investigation, Collection of abalone Random live samples of *T.mutabilis* werecollected monthly from the intertidal rocks at Tranquebar coast Nagapattinam basin in the from a depth of 1_3 m during December 2010 to August 2011. The abalone transported to thelaboratory were stocked in a 1.5 ton capacity FRP tank with filtered seawater and kept overnight to allow the clearance of waste materials accumulated in their body. A minimum of 45 specimens were then shucked and dissected to examine the gonad. The soft body weight, gonad weight and digestive gland weight weremeasured on a wet weight basis.To observe the seasonal biochemical composition of foot, gonad and digestive gland, twenty medium sized animals were collected twice every month from the field and were brought to the laboratory, kept in aquarium tanks filled with sea water (32‰) at5° C ± 0.5°C for 24 hours to ensure that the gut contents were released. These animals were dissected and the respective tissues were removed and kept in oven at 60° C for 24 hours. The dried components were brought to constant weight, after which their biochemical composition was estimated both in male and female.

Estimation of Total Protein

A known weight of the dried sample was homogenized in 2ml of 10% TCA which is used as protein precipitant. The homogenate was transferred to centrifuge tube and centrifuged at 3000 rpm for 15 minutes. The supernatant was discarded and the precipitate was used for the estimation of protein by following the procedure of Lowry *et al* (1951) with Folin's Phenol reagent. The results have been expressed in mg of protein per 100 mg of dry tissue material.

Estimation of Total Carbohydrates:

A known weight of dried sample was taken and homogenized in 2ml of 10% TCA in a glass tissue homogenizer. The homogenate was transferred to centrifuge tube and was centrifuged at 3000 rpm for 15 minutes. The supernatant was decanted to test tube. To each test tube, following Carroll *et al* (1956), 10 ml of anthrone reagent (0.05% anthrone in 72% sulphuric acid containing 1.0% thiourea) was added with constant shaking of the test tubes. One holed rubber cork fitted with glass tube served as air condenser, which also served to prevent the entry of water while heating in boiling water bath. The tubes were immersed to the level of the liquid in the tubes and kept for 15 minutes after which they were removed and immersed in cold water bath. On reaching room temperature, the contents of the tubes were transferred to 1cm cuvettes and read at 620 nm in Spectronic 21 after adjusting the system with the reagent blank prepared with 2ml of 10 % TCA. Standard was run along with experiment with 0.1 mg of glucose in 2ml of 10% TCA. The results have been expressed in mg of carbohydrate per 100 mg of dry tissue material.

Estimation of Total Lipid:

The total lipid was estimated gravimetrically using chloroform methanol mixture described by Folch*et al* (1956). To a known weight of dried, powdered sample taken in a test tube, 5ml of chloroform: methanol (2:1) mixture was added and incubated overnight at room temperature after closing the mouth of the test tube with aluminium foil. After incubation, the mixture was filtered using a Whatman No. 1 filter paper. The filtrate was collected in apreweighed 10 ml beaker which was then kept on a hot plate. The beaker with the residue at the bottom was weighed after the chloroform: methanol mixture evaporated and the weight of the empty beaker was subtracted from this to know the weight of the lipid present in the sample. Estimation of protein, lipid and carbohydrate were carried out in dry tissues of the animal and values are expressed as percentage of dry weight (mg / 100mg).

Statistical analysis

Statistical significance was evaluated by using ANOVA followed by Duncan Multiple Range Test (DMRT).

3.RESULTS

The seasonal changes observed in the biochemical composition of the foot, gonad and digestive gland of *T. mutabilis* during December 2010 to August 2011

Fig. 1.Monthly mean variations in the protein content (%) of gonad of male and female*T. mutabilis* during December 2010 – August 2011

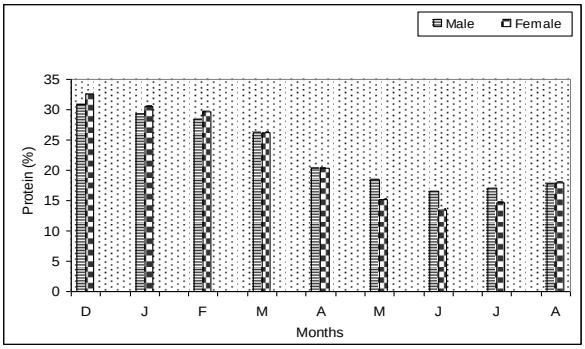
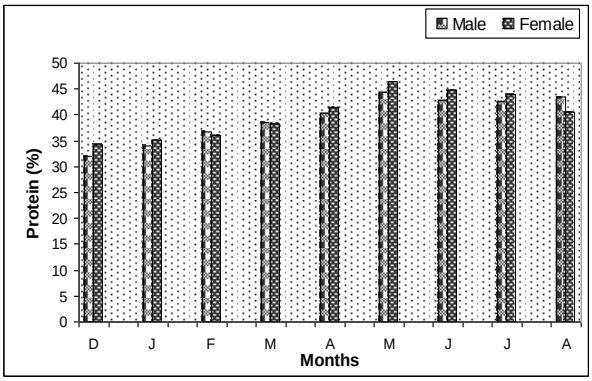


Fig. 2. Monthly mean variations in the protein content (%) of digestive gland of Male and female T. *mutabilis* during December 2010 – August 2011



i.Protein content of Gonad

Male gonad showed maximum protein content (30.8%) during December 2010 and minimum value (16.54%) during June 2011. The maximum protein content of ovary was (32.63%) during December 2010 and minimum (13.52%) during April 2011. The mean protein values of male and female *T. mutabilis*were 22.77% and 22. 32%. The mean protein content of gonad is given.

Fig .3. Monthly mean variations in the protein content (%) of foot of male and female *T. mutabilis* during December 2010 – August 2011

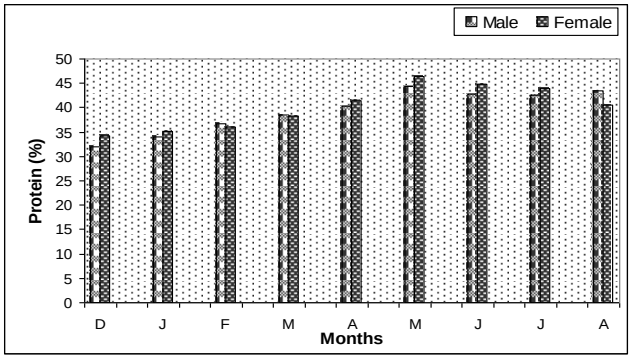


Fig.4. Monthly mean variations in the carbohydrate content (%) of gonad of male and female *T. mutabilis* during December 2010 – August 2011

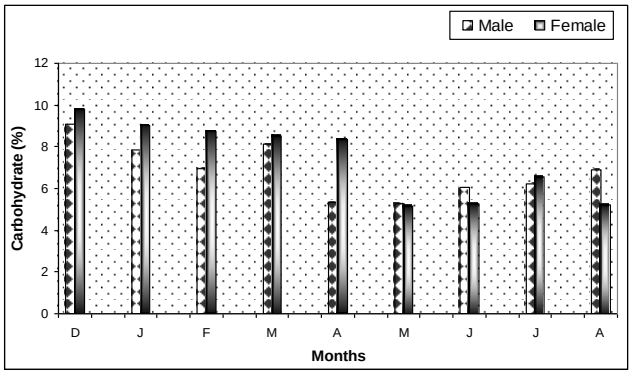


Fig.5. Monthly mean variations in the carbohydrate content (%) of digestive gland of male and female *T. mutabilis* during December 2010 – August 2011

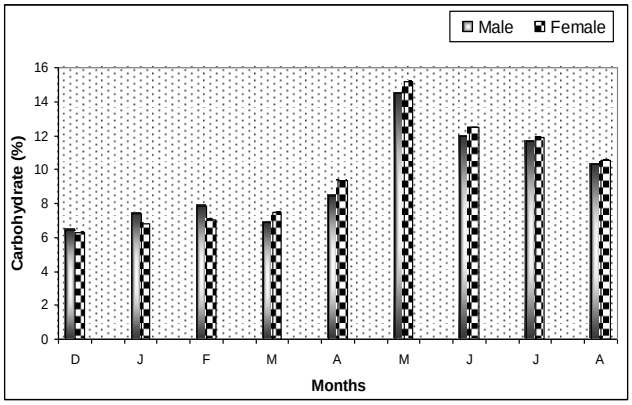
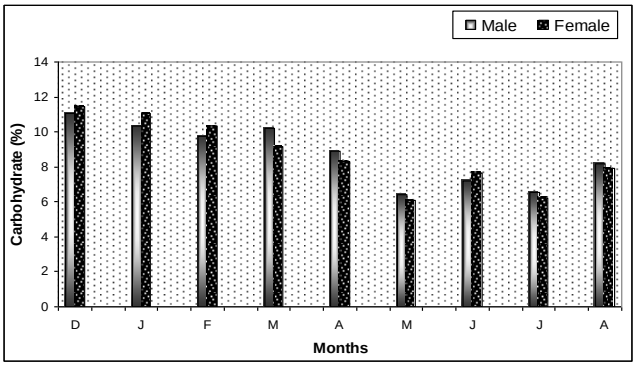


Fig.6.Monthly mean variations in the carbohydrate content (%) of foot of male and female *T. mutabilis* during December 2010 – August 2011



ii.Protein content of Digestive gland

Thedigestivegland of male *T. mutabilis* showed highest value of protein (44.32%) in May 2011 and minimum value (32.08%) during December 2010. The protein content of digestive gland of female was maximum (46.33%) during May 2011 and minimum (34.23%) during December 2010. The mean values of protein content of male and female digestive gland of *T.mutabilis*were 39.43% and 40.11% respectively

iii.Protein content of Foot

In male, the maximum protein content of foot was observed during December 2010 (37.32%) and minimum protein content in May 2011 (32.52%). Female *T.mutabilis* foot showed maximum protein content during December 2010 (38.12%) and minimum value (29.25%) during April 2011. The mean values of protein content of male and female foot of *T.mutabilis*were 34.53% and 33.59% respectively.

i.Carbohydratecontent of Gonad

The highest carbohydrate value in testis of *T.mutabilis* was 9.10% in December 2010 andminimum (5.25%) in May 2011. In ovary, the maximum carbohydrate content (9.83%) was recorded during December 2010 and minimum value (5.18%) in May 2011. The mean carbohydrate values of male and female gonad were 6.86% and 7.40 % respectively.

ii.Carbohydrate content of Digestive gland

In male digestive gland maximum carbohydrate value (14.51 %) was noticed in May 2011 and lowest value (6.45%) in December 2010. In female, highest value of carbohydrate (15.16%) was recorded during May 2011 and minimum during December 2010 (6.29%). The mean carbohydrate values of male and female digestive gland were 9.50% and 9.66% respectively

iii.Carbohydrate content of Foot

The foot of male showed highest carbohydrate content (11.05%) during December 2010 and minimum value (6.41%) in May 2011. In female the foot had maximum carbohydrate (11.47%) during December 2010 andminimum value (6.06%)

Fig.7. Monthly mean variations in the lipid content (%) of gonad of male and female*T. mutabilis* during December 2010 – August 2011

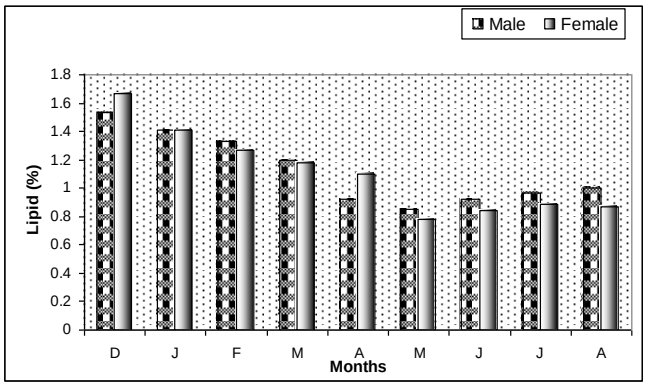


Fig.8. Monthly mean variations in the lipid content (%) of digestive gland of male and female *T. mutabilis* during December 2010 – August 2011

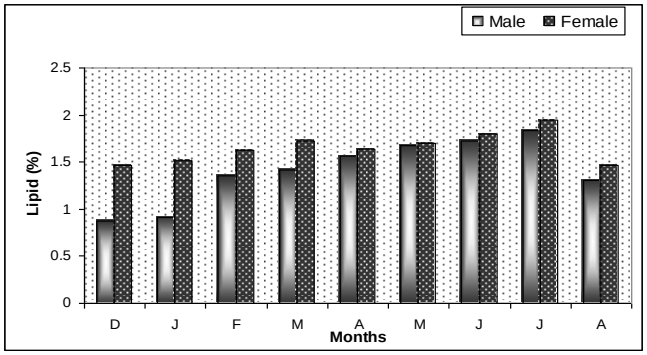
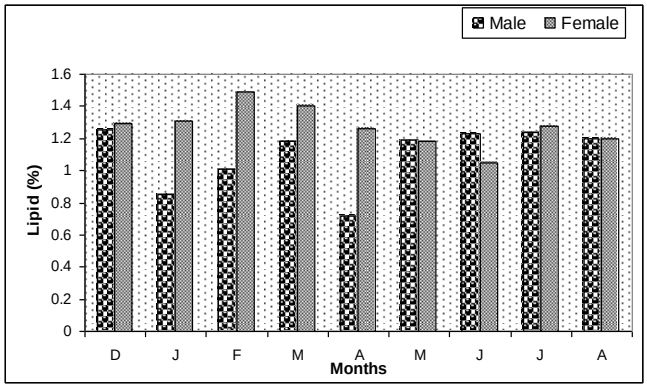


Fig.9. Monthly mean variations in the lipid content (%) of foot of male and female *T. mutabilis* during December 2010 – August 2011



during May 2011. The mean values of foot of male and female were 8.72% and 8.6% respectively.

i. Lipid content of Gonad

The testis of male *T. mutabilis* showed maximum value during December 2010 and minimum value during June 2011 (1.53% and 0.85%) respectively. Similarly female ovary also showed maximum lipid amount during December 2010 (1.67%) and minimum value during May 2011 (0.78 %). The mean values of lipid content of male and female gonad were 1.125% and 1.112% respectively.

ii.Lipid content of Digestive gland

Lipid content of digestive gland varied from a maximum value of 1.68% in May 2011 to minimumvalue of 0.87% in December 2010 whereas in female the maximum value 1.72% and minimum 1.47% were recorded during March 2011 and December 2010 respectively. The mean values of lipid content of digestive gland of male and female were 1.405% and 1.654% respectively.

iii.Lipid content of Foot

Foot of male *T. mutabilis* recorded maximum lipid content (1.25%) during December 2010 and minimum value (0.72%) in April 2011. The female foot also showed maximum lipid content (1.29%) in December 2010 and minimum value (1.05%) during June 2011. The mean values of lipid in male and female *T.mutabilis* were 1.096% and 1.273% respectively.t statistics and ANOVA revealed significant variations between seasons and biochemical composition.

4.DISCUSSION

The purpose of the present study was to gather information on the general biochemical make – up and the seasonal variations in different organs like foot, gonad, and digestive gland. In the present study, a considerable variation was observed seasonally in the biochemical composition of the foot, gonad, and digestive gland. The reproductive cycle also plays a major role in the biochemical composition of the snail, though the intake of food may sometimes interfere to some extent.Similar seasonal variations in the biochemical constituents particularly of the stored materials in various gastropods were observed by several authors (Krishnakumari, 1985; Shanmugam, 1987; Maruthamuthu, 1988; Thivakaran, 1988; Tagore, 1989 and Paul Ravindran, 2003).

The present study on *T.mutabilis revealed* an increasing trend in the biochemical composition from December 2010 to April 2011. This might be due to maturation of gonads. However it was noticed that the biochemical constituents of gonads decreased gradually from May to August 2008, since it is the spawning period.Giese (1969) reported that protein is the dominant organic constituent in molluscs. The protein content in the various body parts of *T. mutabilis*showed the highest value during non breeding season except digestive gland. Such a trend might be due to the fact that the digestive gland serves as the storage organ as quoted by Umadevi (1983); Jayabal (1983); Anandhakumar (1986); Tagore (1989); Ramesh and Ayyakkannu (1992.Besides that, seasonal differences in the availability of food may also affect the protein reserves in various organs. If there was plenty of supply of food throughout the year, food had to be kept in reserve prior to the initiation of gametogenesis (Bhanu, 1980). *T.mutabilis*is a carnivore and in this animal, during ripening of gonads markedly high values of protein contents werenoticed. According to Arevalo (1948) high values of protein contents in any sex are not a year round phenomenon, but may be related to certain physiological states of either intensive feeding or spawning depending on the season. The present study also confirms the fact that the high protein level in the tissues may be due to intensive feeding and maturation of gonads.

Table 1 Level of protein content in male and female *T. mutabilis*

S.No.	Parameter	Mean		Difference in mean	t	df	p
		Male	Female				
1	Gonad	22.77	22.32	0.455	0.602	8	0.000*
2	Digestive gland	39.43	40.11	-0.677	-1.194	8	0.267
3	Foot	34.53	33.59	0.948	1.136	8	0.000*

* Significant at 0.05 level

Table Level of carbohydrate content in male and female *T. mutabilis*

S.No.	Parameter	Mean		Difference in mean	t	df	p
		Male	Female				
1	Gonad	6.863	7.407	-0.544	-1.090	8	0.001*
2	Digestive gland	9.500	9.663	-0.633	-0.638	8	0.000*
3	Foot	8.725	8.686	0.038	0.192	8	0.003*

* Significant at 0.05 level

Table 3 Level of lipid content in male and female *T. mutabilis*

S.No.	Parameter	Mean		Difference in mean	t	df	p
		Male	Female				
1	Gonad	1.125	1.112	0.013	0.363	8	0.000*
2	Digestive gland	1.405	1.654	-0.248	-3.357	8	0.010
3	Foot	1.096	1.273	-0.176	-2.625	8	0.000*

* Significant at 0.05 level

In the present study, carbohydrate values were higher than that of lipid and lower than that of protein values. The present study also showed great seasonal variations of carbohydrate in the tissues of *T. mutabilis.* This could be attributed to the utilization of carbohydrate reserves under favorable conditions as stated by Ansell *et al*, (1973). According to Ansari *et al*, (1981) the carbohydrate of molluscs were mainly composed of glycogen and the changes in the carbohydrate level may be due to accumulation of glycogen at different stages like gametogenesis and spawning. Further, Krishnakumari (1985) reported that the increase in the amount of carbohydrate during prespawning can be attributed to the proliferation of sex cells and decrease in the amount of carbohydrate during postspawning may be due to release of gametes from gonad. In the present study, the carbohydrate content of *T.mutabilis* increased during December 2010 to April 2011 and decreased during the active reproductive periods from May 2011 to August 2011. The present study is in conformity with the reports of Ansari *et al* (1981); Stella *et al* (1992) and Paul Ravindran 2003. The increase of carbohydrate level in the digestive gland in summer strongly suggested that it could be due to optimal conditions like the availability of food etc., as reported in *Thais lamellose* (Stickle, 1975). The high level of carbohydrate in *Morulagranulata*suggested that it may be used during non reproductive physiological needs or may be converted into gonadial lipids.

Compared to protein and carbohydrates the lipids formed only a minor percentage of total biochemical constituent in male and female *T.mutabilis*and hence its fluctuation within the sexes was almost negligible. Among the various body

regions in*T.mutabilis*the maximum lipid content was found in digestive gland (1.83% and 1.94%). Rosoiu *et al* (1985) had investigated that the lipid composition of *Rapanathomasiana*as 2.25%, which perhaps be due to the storage nature of above organ, John (1980) and Jayabal (1984).Giese (1969) suggested that a lipid value of 5% dry weight is a good estimate of structural lipid and it plays a role as reserves. However in the present study no such trends could be recorded.

In the present study, in contrast to the gonad and other body tissues, digestive gland in *T. mutabilis*exhibited a distinct seasonal variation in biochemical constituents. Owen (1966); John (1980) and Jayabal (1984) had stated that the digestive gland acts as a storage site in most of the tropical snails. Similarly the present study also confirmed that variations in the biochemical constituents of the digestive gland indicated that the digestive gland is a probable storage site. The biochemical constituents, in general in all the body parts always appeared to increase during maturing period of gonad, whereas in case of digestive gland, it shows an inverse relationship between peak breeding period and biochemical levels. Giese (1969) also indicated that an inverse relationship exists between these components indirectly evidencing the transfer of the nutrients. Webber (1970) speculated that the foot of *Haliotiscrachemidii*may act as storage depot but in the present study the digestive gland seemed to be the principal storage site followed by foot as reported by Rajakumar (1995) and Stella (1995).

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