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Review

Chemoattractants: Their essentiality and efficacy in shrimp aquaculture

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ABSTRACT

Aquaculture serves as a sustainable source of good quality wholesome food and major input is feed. In recent years, considerable quantities of plant sources have been attempted for inclusion in shrimp feed, due to high demand and high cost of fishmeal. Plant-based ingredients are in general poor in attractability and palatability to aquatic species compared to marine sources. It is desirable to develop an economical and nutritious feed that is attractive and palatable for ensuring reduction of feed wastage. Chemoattractants nowadays are unavoidable in commercial shrimp feeds and are included either individually or in combination. The present review revealed that the various marine sources, in particular krill meal would be more effective for aquatic species, compared to those derived from vegetable origin and chemical compounds. In addition to acting as a feed attractant, marine sources serve as a potential fishmeal substitute due to their rich nutritional composition. However, certain biogenic amines present in these marine-based ingredients need to be examined before their use, since these amines when present in high concentrations reduces feed intake by producing undesirable odour. Use of a combination of chemoattractants would give a better effect, rather than using them individually and compounds from plant origin have limited beneficial effects. The present review concludes that incorporation of chemoattractants would be beneficial in formulation of enriched and economical feeds with better attractability and palatability. The selection of suitable attractant and their supplementation at correct proportion is significantly more important to avoid undesirable effects in cultured shrimp. Further field-based research is needed to predict the actual effects of chemoattractants on farmed shrimp and to provide a sustainable base for the expansion of shrimp aquaculture sector, by reducing feed wastage.

Keywords: Aquaculture, Aqua feed, Attractability, Chemoattractants, Marine sources, Palatability

Introduction

Chemoattractants are external stimuli that help to induce movement of chemotactic cells towards the direction of their highest concentration (Yang e*t al.,* 2015). They are mainly being considered as a means of improving feed intake by enhancing the attractability and palatability in aquatic species, in particular crustaceans since they are chemosensory feeders. Chemoattractants are generally certain chemical agents, natural substances, animal-based products and their hydrolysates. The positive effect of chemoattractants on aquatic species is high when used in a combination rather than individually. In a diet formulated with enriched marine proteins such as fishmeal, krill meal, shrimp meal and squid meal, they themselves will act as potential feed attractants or feeding effectors, since in addition to the essential nutrients, these marine ingredients are rich in free amino acids, nucleotides, nucleosides and amines that are readily recognised by the chemosensory system of aquatic species during the process of locating and ingesting feed materials. A diet, primarily containing plant-based ingredients as a sole source of protein would not have similar effects. Therefore, supplementing chemoattractants is likely to enhance growth in the cultured species. Derby *et al.* (2016) reported that chemoattractants influence the different phases of feeding behaviour in aquatic species, in particular shrimp. By stimulating the appetitive phases of arousal, search initiation and locating the feed, chemoattractants enhance the attractability and palatability, by which they not only increase the feed consumption rate and growth, but also reduce the waste production during culture operation. Besides, chemoattractants would also help to compensate reduction in feed intake when the animals are affected by disease or under stress (Boonyaratpalin, 2000). Over the past two to three decades, much attention has been given in evaluating the nutritional requirements of both macro (protein, lipid and carbohydrate) and micro (vitamins, phospholipids, sterols, carotenoids, essential amino acids and fatty acids) nutrients for various aquatic species. In addition, the dietary specifications have also been addressed through various digestibility studies in order to emphasise the efficiency of aquaculture practice and to reduce the downstream environmental impacts around the globe. However, certain myths still persist regarding utilisation of chemoattractants; in particular those mainly derived from chemicals. Hence, the present review article is aimed to elucidate the essentiality and efficacy of various chemoattractants in farmed shrimp species. This would definitely provide baseline information about the necessity of chemoattractants in feed formulation for high performance, economical and nutritious shrimp feed for sustainable aquaculture.

Need for chemoattractants

Commercial shrimp feed formulation primarily relies on marine proteins, in particular fishmeal due to its excellent nutritional traits, higher digestibility and palatability. In addition to the nutritional characteristics, fishmeal limits waste production during culture by enriching nutrient digestibility, which results in reduced environmental pollution, particularly water contamination (Jannathulla *et al.,* 2019). The inclusion of fishmeal in shrimp feed was 19-40% in 2000 and reduced to 11-23% in 2014 and is still expected to reduce to 6% by 2025 due to its uncertain availability and high cost (Salin *et al.,* 2018). Simultaneously, the level of use of plant-derived feed stuffs such as oilseed meals/cake and gluten meals, has increased in commercial feed formulation to compensate fishmeal quantity as they have almost reliable nutrient content and are readily available, sustainable and most economical. However, the feed intake with plant-based products is not at satisfactory levels. Yang *et al.* (2015) suggested that the inferior performance of animals fed higher content of plant proteins would mainly be attributed to their poor attractability and palatability. In addition, deficiency of certain essential nutrients, higher content of fibre fractions and the presence of anti-nutrients would also partly be a reason for the diminished effect of plant-based ingredients (Jannathulla *et al.,* 2018). It is reported that the use of chemoattractants in feeds helps to stimulate the animals' behavioural pattern of feeding by promoting feed detection towards the source (Derby *et al.,* 2016). Mendoza *et al.* (1997) reported that the supplementation of chemoattractants shortened the time period to detect feed by the animals thereby increasing likelihood of ingestion. This faster ingestion would also help to reduce the cost of feed formulation by reducing the level of binder in the diet as it is expensive and also non-nutritious. This positive behavioural response ultimately helps to enhance growth of the cultured species.

In general, based on the source, chemoattractants are broadly classified into two different categories *viz.,* feeding stimulant (helps to initiate feeding) and feeding enhancer (helps in continual feeding). Commonly used chemoattractants in aquafeeds are detailed in Table 1. Grey *et al.* (2009) further classified them as attractants, arrestants and repellents according to their olfactory nature and as incitants, suppressants, stimulants and deterrents according to their gestatory nature. Chemoattractants that fall under the category of olfaction, are mainly chemical effectors, which include amino acids like taurine, glycine, arginine, glutamic acid and alanine, betaine, nucleotides and organic solvents, since they can be detectable even at very low concentration. However, their effects are more successful when used in a combination than when used individually. Harada (1986) suggested certain important chemical characteristics for the materials to be a good chemoattractant. It should be a nitrogen containing compound with low molecular weight and should be water soluble as well as non-volatile in nature. In addition, it should possess either acidic or basic and/or amphoteric properties.

Chemoattractants that come under gestation category, primarily influence contact between the chemoreceptors of the animal and the source (Yang *et al.,* 2015). They are mainly protein rich marine derivatives, which play an important role in behavioural, physiological and electrophysiological response (Ekerholm and Hallberg, 2002). However, they are most expensive components when compared to other conventional feed ingredients. In general, the alkaline and neutral nitrogenous substances like amino acids (glycine, proline, taurine and valine) and their derivatives (betaine) act as potential chemoattractants in carnivorous species, whereas acidic substances, in particular aspartic acid and glutamic acid showed the most positive responses in herbivores. In addition, certain common feed ingredients, those mainly derived from animal origin and their hydrolysates play an important role in attracting and stimulating feeding pattern of aquatic species especially in marine species due to their improved nutritional quality (Harada, 1986). Nutritional composition of various chemoattractants derived from marine source is summarised in Table 2.

Chemoattraction and feeding stimulation in crustaceans

Aquatic species, in particular crustaceans identify their food or feed materials using water-borne chemical signals and orient towards them. Lee and Meyers (1996) categorised the response of aquatic species into five

Table 1. Chemical compounds used as chemoattractants and their responses in crustaceans

Compounds	Responses in crustaceans
L-amino acids	
Arginine	-
Glycine	Moderate
Histidine	-
L-alanine	-
Lysine	Low
Ornithine	Moderate
Threonine	-
Tyrosine	Moderate
Valine	-
Proline	High
Nucleic acids and their derivatives	
AMP	High
Adenine	-
Adenine+cytosine+GMP+guanosine	-
AMP+xanthine+cytosine	High
Cytosine	Moderate
Cytosine+AMP	-
Cytosine+AMP+dGMP	-
Cytosine+AMP+guanine	-
GMP	-
Guanine	-
Guanosine	-
Xanthine	Low
Nitrogenous bases	
Monoethylamine	Moderate
Dimethylamine	-
Trimethylamine	-
R'-aminobutyric acid	High
Trimethylamine oxide	-
Ammonia	-
Pyrrolidine	Moderate
Betaine	-
Monomethylamine	High
Choline	Moderate
Ammonium acetate	Moderate
Trimethylammonium hydrochloride	High

Source: Boonyaratpalin (2000)

different phases to describe and predict their responses to feeding stimuli. They are detection, orientation, locomotion or displacement, initiation of feeding and continuation or termination of feeding. The behavioural pattern and response of crustaceans to chemical stimuli is briefly described in Table 3. In addition, four more categories have been recognised to elucidate the chemotactic behaviour of aquatic species. Among them the most sensitive one is antennule flicking, which is most widely used to address the physiological response of crustacean species according to chemical reception (Pittet *et al.,* 1996). The process of feeding stimulation in general indicated by movements of the mouthparts and periopods helps to detect and reach the feed quickly.

Once the organism get to the feed, the feeding process is initiated with the help of chelipeds and mouthparts, which allow exposing more chemoreceptors to the chemical signals, so that animals continue their feeding behaviour pattern of detection and orientation repeatedly. Finally, animals either continue the feeding or reject the feed materials based on their quality and other characteristics.

Efficacy of chemoattractants in shrimp aquaculture

In general, diet for aquatic species should be chemically attractive. Therefore, supplementation of even a minute quantity of chemoattractants plays a crucial role in inducing detection and feeding, thereby increasing the

Table 2. Nutritional composition of certain chemoattractants from marine source

Particulars	Chemoattractant from marine organisms					
	Krill meal	Squid meal	Shrimp head meal	Squid liver meal	Fish hydrolysate	Shrimp meal
Proximate composition (% as fed basis)						
Crude protein	55.00	75.22	52.95	48.74	17.62	36.80
Crude fat	25.16	4.06	6.43	6.02	2.26	6.81
Crude fibre	3.06	1.31	14.37	5.68	0.08	-
Total ash	8.55	14.89	21.81	7.86	7.83	34.30
Essential amino acids (% as fed basis)						
Arg	3.34	4.56	3.55	3.29	0.93	3.24
His	1.31	2.73	1.43	1.24	0.58	0.80
Ile	2.73	4.27	2.39	2.31	0.69	1.51
Leu	4.22	5.96	3.90	3.49	1.14	2.26
Lys	3.86	6.24	3.57	3.28	1.41	1.76
Met	1.54	2.18	1.29	0.67	0.46	0.99
Phe	2.90	3.51	2.14	2.49	0.67	1.43
Thr	2.38	3.28	2.31	1.83	0.62	1.46
Tyr	3.25	3.12	1.78	1.70	0.50	1.25
Val	3.90	3.90	2.66	2.16	0.79	1.67
Major fatty acids (% of total fatty acids)						
C16:0	26.75	38.67	23.79	25.25	26.19	-
C18:0	1.35	16.01	8.09	6.31	7.52	-
C16:1	9.66	5.91	3.27	6.15	3.54	-
C18:1	14.11	18.23	17.88	11.30	0.04	-
C18:2	1.48	19.60	2.21	12.62	2.21	-
C18:3	1.59	0.25	1.56	0.33	0.44	-
C20:4	0.32	0.25	3.27	2.49	2.65	-
C20:5	17.70	2.22	7.47	10.30	8.41	-
C22:6	8.74	3.94	7.47	12.79	31.42	-
Macro elements (% as fed basis)						
Calcium	1.25	4.36	5.75	0.51	53.00	-
Phosphorus	1.23	1.14	1.39	0.53	1.81	-

Source: Smith *et al.* (2005); Nunes *et al.* (2019)

Table 3. Behavioural pattern and response of crustaceans to the chemical stimuli

Feeding phases	Behavioural response	Description
Detection	1. Antennule flick 2. Antennule wipe 3. Maxilliped beat 4. Dactyl wave 5. Dactyl wipe 6. Head bob	1. Antennules are moved quickly from side to side 2. Antennules are stroked by the maxillipeds 3. Maxillipeds beat rapidly (>10 beats min^{-1}) 4. Dactyls of pereipods are waved slowly in front of the animal (>10 s for 1 wave) 5. Dactyls are strocked by the maxillipeds 6. Anterior part of animal is elevated above substrate
Orientation	1. Dactyl rake 2. Dactyl probe 3. Dactyl dig 4. Turn	1. Dactyls of pereiopods are dragged across the substrate of surface 2. Dactyls of pereiopods are used to probe the substrate 3. Dectyls of pereiopods are used to dig into substrate and body touches substrate 4. Animals turn towards or away from the chemical signals
Locomotion	1. Walk 2. Run 3. Search	1. Animals walk from one location to another 2. Animals run from one location to another 3. Animals search a large area in a deliberate manner
Initiation of feeding	1. Grab 2. Lunge 3. Pounce 4. Hold 5. Taste	1. Animals grab source or grab at source with chelipeds or dactyls 2. Animals spring forward towards the source 3. Animals spring downward touching the source or substrate with thorax 4. Source of chemical signal is held with chelipeds 5. Source of chemical signal is moved to mouth, contacting mouthparts
Continuation of feeding	1. Ingest 2.Reject	1. Source of chemical signal is moved to mouth and ingested wholly or partially 2. Source of chemical signal is dropped and animals ignore it

Source: Lee and Meyers (1996)

rate of ingestion, which ultimately enhances growth of the cultured species. Though use of feed attractants in shrimp was reported as early as 1970's in Japan (Tokunaga *et al.,* 1977), their usage globally was more pronounced after 2000. Willams *et al.* (2005) reported that the growth rate of *Penaeus monodon* fed diets containing shrimp head meal and krill meal as a chemoattractant was 1.66 and 1.68% per day, respectively, while it was only 0.95% per day in the group fed a basal diet that did not contain any attractant. Similarly, about 20% faster growth was observed by Smith *et al.* (2005) when crustacean meal or krill meal was supplemented to same species compared to the control group. Suresh and Nates (2011) found increased attractability in *Litopenaeus stylirostris* reared with fishmeal challenged diet using 20% poultry-byproduct (w/w) with the supplementation of 3% squid liver meal and krill meal. But feed palatability and shrimp growth were found to be high in the groups fed diets with krill meal compared to those fed on squid liver meal. Nunes *et al.* (2011) reported that the supplementation of krill meal increased the nutrient utilisation of meat and bone meal as well as soybean meal by substituting dietary fishmeal completely in *Penaeus vannamei.* The authors also documented that shrimp fed krill meal at the rate of 11% by replacing both fishmeal and lecithin showed comparable growth to those fed a basal diet containing no krill meal in both clear and green water rearing systems. This result is in agreement in certain fishes like Atlantic salmon (Julshamn *et al.,* 2004) and Atlantic halibut (Suontama *et al.,* 2007), where fishmeal could partially be substituted by krill meal without having any deleterious effects on fish growth. This clearly indicates that krill meal is not only an effective feed attractant but also a potent feeding stimulant compared to other chemoattractants. This corroborates with the findings of Derby *et al.* (2016), who reported that searching, probing and grabbing behaviour was very high in *P. vannamei* fed very low concentration of aqueous extract of krill meal (13.3 $\mu g\ ml^{-1}$). However, there is no clear-cut information regarding the growth enhancing effect of chemoattractants, in particular those mainly derived from marine sources.

It is assumed that the fish hydrolysates that are mainly derived by solubilising proteins from the raw materials using certain proteolytic enzymes could be used as potential feeding attractants for various aquatic species. Grey *et al.* (2009) documented that 5% of fish hydrolysates obtained from salmon could serve as viable feeding stimulants in *P. vannamei.* Kolkovski and Tandler (2000) suggested that up to 50% of fish hydrolysates could be included in fish larval feed, whereas higher supplementation did not show any positive effect on shrimp growth and digestibility. The authors stated that this hindered performance with the higher supplementation of fish hydrolysates could be attributed to the reduced activity of protein digestive enzymes (trypsin and chymotrypsin), who also suggested that the higher supplementation of protein hydrolysates is not necessary while formulating high performance shrimp feeds. But in contrast, Cordova-Murueta *et al.* (2002) observed higher growth in *P. vannamei* fed diets supplemented with krill hydrolysate at 3, 9 and 15% over the control group that fed on a diet without krill hydrolysate. Cruz-Surez *et al.* (1987) reported that 1.5% squid protein fractions showed an enhanced growth rate in *P. vannamei, Penaeus indicus* and *L. stylirostris,* whereas *P. monodon* required squid protein fractions at the rate of 6-16% for growth improvement. This difference would probably be due to the variation in the dietary requirement of amino acids between the species and also due to the variation in the dietary content of amino acids. A difference in feeding pattern and food selection between different crustacean species would also be a possible reason for this response (Mendoza *et al.,* 1997).

In addition to feed ingredients of animal origin, various chemical agents are also used as chemoattractants in shrimp feeds with varying degree of success. The important ones are biogenic amines, which includes histamine, cadaverine, phenylethylamine and spermidine. They are organic nitrogenous base containing one or more amine group. They are mainly synthesised by microbial, vegetable and animal metabolism by the process of decarboxylation of amino acids or by amination and transamination of aldehyde and ketones. Mendoza *et al.* (1997) stated that biogenic amines have not only acted as chemoattractants but also triggered the feeding response of the animals as feeding incitants and led animals to continue the feeding process. But in contrast, the impaired growth performance was observed in rainbow trout (Fairgrieve *et al.,* 1994) and Atlantic salmon (Opstvedt *et al.,* 2000) while supplementing certain biogenic amines. Cowey and Cho (1992) observed intestinal damage and reduced feed intake in rainbow trout reared with a diet containing putrescine or histamine. However, Mendoza *et al.* (1997) reported that even 0.2% of cadaverine acted as a potential attractant in *Macrobrachium rosenbergii* fed a diet containing 5% fishmeal. But, later Tapia-Salazar *et al.* (2004) in *L. stylirostris,* failed to show a similar effect even after supplementing 0.46% of cadaverine. However, when it was supplemented with histamine it showed an enhanced growth performance in the species. The authors suggested that the lower performance with cadaverine might be due to poor metabolism of this compound. This clearly indicates that the positive effect of biogenic amines as chemoattractants not only varies based on the quantity of supplementation but is also dependent on the cultured species.

As biogenic amines are foul-smelling compounds, Nunes *et al.* (2006) suggested that determining these compounds in animal tissues and their derivatives is likely to be important. Because they would affect the freshness of the materials when present in high level, thereby influencing the efficacy of the animal materials to be used as chemoattractants. Other chemical agents like betaine, L-amino acids, inosine and finnstim are recognised as chemoattractants in certain cases. Betaine is a derived product of glycine amino acid and is highly soluble in nature. By stimulating olfactory bulb of the aquatic species, in particular fish, betaine induces feed intake and thereby enhances growth. A similar result was observed by Nakajima *et al.* (1989) when using dimethyl-β-propiothetin in gold fish, common carp and crucian carp. Considerable quantity of betaine is present in marine invertebrates and certain microorganisms and some plant-based materials were found to contain betaine in lower amount. However, it has to be supplemented exogenously to show its effect on the species. Earlier studies showed that betaine has been shown to act as a potential dietary feeding chemoattractant in various aquatic species such as carps (Shankar *et al.,* 2008), striped bass (Papatryphon and Soares, 2001), rainbow trout (Tiril *et al.,* 2008), tilapia (Kasper *et al.,* 2002) and *P. indicus* (Jasmine *et al.,* 1993). Rumsey (1991) documented that supplementation of betaine in the diet of rainbow trout spared the dietary requirement of choline and methionine, which indicates that the metabolism of all three components would almost be similar. Penflorida and Virtanen (1996) and Lee and Meyers (1997) reported that there was no preferential feed intake due to the supplementation of betaine in *P. monodon* and crustaceans, respectively. However, they recognised betaine as a potential feed attractant and incitant due to the enhancement effect of betaine on daily feed intake as well as growth. This statement is in agreement with the findings of Hartati and Briggs (1993), who documented that betaine increased the attractiveness of casein-based, semi-purified diet of shrimp. The daily feed intake of shrimp reared with various chemoattractants is reported by Smith *et al.* (2005) (Table 4), who revealed that increasing the inclusion level of chemoattractants increased daily feed intake except squid meal, where the reverse trend was noticed. However, a positive response in feed intake was observed by Holland and Borski (1993) with squid meal in penaeid shrimp. The difference in results observed between the studies might be due to the variation in the nutritional quality of squid meal used. Mendoza *et al.* (1997) documented that there was no consistency in the nutritional composition of natural materials used as a chemoattractant, that are mainly derived from animal origin.

Similarly, it has been reported that L-amino acids, in particular L-alanine, L-glutamic acid, L-arginine and L-glycine have feed attractant properties. However, the effectiveness of L-glutamic acid, L-arginine and L-glycine are less in terms of properties as feed attractants. But they become very effective when used with some other chemical stimulants particularly betaine, glycine and inosine. Polat (1999) reported that in addition to acting as a chemoattractant, these L-amino acids could be recognised as important energy source when supplemented

Table 4. Effect of chemoattractants on daily feed intake of shrimp

Chemoattractants tested	Inclusion level (%)	Daily feed intake (% body weight day^{-1})
Squid meal	1	3.6
	2.5	2.2
	5	1.7
Crustacean meal	1	2.0
	2.5	2.4
	5	3.7
Krill meal	1	0.7
	2.5	0.5
	5	1.0
Fish hydrolysate	0.5	2.7
	1	1.5
	2	4.6
Krill hydrolysate	0.5	1.2
	1	2.0
	2	0.9
Betaine	0.5	0.6
	1	1.0
	2	3.2

Source: Smith *et al.* (2005)

with valine, serine, leucine and isoleucine in fish. Nunes *et al.* (2006) observed the superior effect of commercially available amino acid mix in attracting *P. vannamei* when compared with betaine, indicating that a blend of amino acids would be a better attractant than individual ones. This similar effect has been reported in various aquatic species like puffer (Ohsugi *et al.,* 1978), rainbow trout (Adron and Mackie, 1978), red sea bream (Goh and Tamura, 1980), Dover sole (Mackie and Mitchell, 1982), seabass (Mackie, 1982), European eel (Mackie and Mitchell, 1983) and Japanese eel (Takeda *et al.,* 1984). However, *P. monodon* had higher daily feed intake when betaine was supplemented at the rate of 2%. The reduced response of betaine observed in the study by Nunes *et al.* (2006), could be due to to the fact that they used betaine alone, whereas Smith *et al.* (2005) used betaine along with additional amino acids of hydrolysed proteins. Though the nucleosides of inosine and inosine-5-monophosphate could be considered as important feed attractants, generally they are omitted by the nutritionists in aquafeed formulation, since they are relatively higher in cost compared to other chemical stimulants. Nunes *et al.* (2006) evaluated various commercially available chemoattractants, which included vegetable dried biomass, complex amino acids, betaine, biogenic amines, dried fish soluble and squid protein hydrolysate in *P. vannamei.* Their results concluded that, of all the analysed chemoattractants, fish soluble protein, squid protein hydrolysate and complex amino acids have shown higher feeding response in shrimp followed by betaine and dried fish soluble, whereas chemoattractants obtained from vegetable-based materials failed to show positive results, which indicates that chemoattractants from vegetables have no or very poor capability to attract and stimulate aquatic species, in particular shrimp.

Conclusion

There are numerous chemical agents and ingredients from marine sources used as effective feed attractants and growth stimulators in aquafeeds, in particular for crustaceans. Use of chemoattractants helps to develop more suitable aquafeeds by increasing nutrient utilisation of feed, which could be attributed to enhanced attractability and palatability. In addition, it would also help to make environmental friendly feeds by reducing waste production during culture. However, some of them have the ability only in promoting the stimulatory effects on the process of feed intake but not on growth performance. Among the marine sources, krill meal plays an important role in balancing both attractive and stimulatory effects by providing dietary nutrients compared to others. Most of the chemoattractants from marine sources have a vital role in aquafeeds, especially in fishmeal challenged diets, and thereby helps to formulate low cost feeds. But in the case of chemical components used as chemoattractants, it would be more effective when used in combination than individually. No beneficial effects have been documented in aquatic species fed a diet containing chemoattractants sourced from plant origin. Future research needs to address the issue by coordinated efforts in the field and pond conditions rather than under indoor laboratory conditions to predict the actual effects of chemoattractants on aquatic species. This can definitely conserve the aquafeed industry and also provide a sustainable base for the expansion of aquaculture sector to a great extent.

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