Journal of Animal and Feed Sciences, 20, 2011, 131–142

Evaluation of microalgae as sources of digestible nutrients for monogastric animals*

A. Skrede[1,5], L.T. Mydland[1], Ø. Ahlstrøm[2], K.I. Reitan[4], H.R. Gislerød[3] and M. Øverland[1]

Norwegian University of Life Sciences,
[1]Aquaculture Protein Centre, CoE, Department of Animal and Aquacultural Sciences,
[2]Department of Animal and Aquacultural Sciences,
[3]Department of Plant and Environmental Sciences
P.O. Box 5003, N-1432 Ås
[4]SINTEF Fisheries and Aquaculture
N-7465 Trondheim, Norway

(Received 26 January 2011; revised version 3 March 2011; accepted 12 March 2011)

ABSTRACT

The study was carried out to evaluate three microalgae as potential nutrient sources in diets for monogastric animals. In a digestibility experiment with adult mink (*Mustela vison*), the microalgae *Nannochloropsis oceanica*, *Phaeodactylum tricornutum* and *Isochrysis galbana* were fed at 60, 120 and 240 g kg^{-1} as is, replacing fish meal. The *N. oceanica* and *P. tricornutum* had similar crude protein (CP) content (47.7 and 49.0% of DM, respectively), amino acid composition and lipid content (8.4 and 7.4%, respectively), whereas *I. galbana* contained 20.1% CP and 16.2% lipids. There was a significant linear reduction in CP digestibility with increasing dietary inclusion of all algae products. The apparent CP digestibility determined by linear regression for *N. oceanica*, *P. tricornutum* and *I. galbana* was 35.5, 79.9 and 18.8%, respectively. The individual amino acid digestibilities showed acceptable values for *P. tricornutum*, but low and highly variable values for *N. oceanica* and *I. galbana*. Although the algae contributed a minor proportion of dietary lipids, lipid digestibility declined with increasing inclusion of all algae and especially with the highest level of *N. oceanica*. It was concluded from the mink study that among the investigated algae, *P. tricornutum* was the preferable source of digestible nutrients.

KEY WORDS: algae, protein, amino acids, lipid, digestibility, mink

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* Supported by the Research Council of Norway, Grant No. 172580/S40 "Potential of using microalgae to partially replace fish oil and fish meal in aquaculture fish feeds"

[5] Corresponding author: e-mail: anders.skrede@umb.no

INTRODUCTION

The increasing awareness of limitations in cultivatable land areas and the fact that the catches of pelagic fisheries are limited, emphasizes the need to explore the potential of untraditional and sustainable animal feed ingredients. In the search for alternative nutrient sources, microalgae have been considered promising sources of protein and lipids (Spolaore et al., 2006; Becker, 2007). Microalgae may have a protein content of 20-60% of dry matter and variable contents of lipids, depending on species and growth conditions (Reitan et al., 1994; Brown et al., 1997). Production of microalgae may therefore have a potential to be a suitable source for partial replacement of protein and lipids in animal feed. At present, several species of microalgae are mainly used as live feed in aquaculture (Brown et al., 1997; Brown, 2002), whereas about 30% of the current algal production is used in feed for poultry and other terrestrial animals (Becker, 2007).

The application of microalgae as a future nutrient source for the animal feed industry depends on detailed information on parameters such as chemical composition and digestibility. The microalgae species *Nannochloropsis oceanica*, *Phaeodactylum tricornutum* and *Isochrysis galbana* are all used for bivalve molluscs, for the larval and early juvenile stages of crustaceans and certain fish species, and for zooplankton used in aquaculture food chains (Yamaguchi, 1997; Brown, 2002), and it seems interesting to study whether these algae are applicable also in compound feed for monogastric animals. The objective of the present study was to investigate the effect of increasing dietary inclusion of *N. oceanica*, *P. tricornutum* and *I. galbana* on digestibility of crude protein, amino acids and lipids in mink (*Mustela vison*). The authors are unaware of previous studies in which digestibility of microalgae in mink has been studied. The carnivorous mink was used as model animal due to the documented relationship with digestibility in salmonid fish and other monogastric species (Skrede et al., 1998).

MATERIAL AND METHODS

Algae products tested

The *N. oceanica* was isolated from an operational hatchery in western Norway and cultivated in three tubular photobioreactor biofence systems, each of 200 l volume, at Norwegian University of Life Sciences, Ås (Sandnes et al., 2005). The *P. tricornutum* was obtained from Fitoplankton Marino (Spain), and *I. galbana* from Reed Mariculture, Campbell, California (USA). The biomass of all three microalgae was freeze-dried prior to analysis and feed manufacturing.

Animals and diets

The digestibility experiment was carried out at the Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, Ås.

Adult male mink of the standard dark genotype were randomly allotted to 10 groups of four animals each. The mink were housed individually in cages equipped for controlled feeding and quantitative faecal collection, avoiding contamination with urine, in a ventilated room with controlled temperature and light conditions. The animals had continuous access to water. All mink were cared for according to laws and regulations controlling experiments with live animals in Norway (i.e. the Animal Protection Act of December 20, 1974, and the Animal Protection Ordinance concerning experiments with animals of January 15, 1996).

Ten different diets were used in the experiment. In the control diet (diet 1) high quality fish meal was used as the sole source of protein (Table 1). In diets 2, 3 and 4, *N. oceanica* was added at levels of 60, 120 and 240 g kg^{-1} as is, respectively, replacing fish meal. Correspondingly in diets 5, 6 and 7, *P. tricornutum* was added at 60, 120 and 240 g kg^{-1}, respectively. In diets 8, 9 and 10, *I. galbana* was added at 60, 120 and 240 g kg^{-1}, respectively, replacing fish meal.

Table 1. Ingredient composition of control diet

Ingredients	g kg^{-1} as fed
Fish meal [1]	491.8
Maize starch (precooked)	234.2
Soyabean oil [2]	234.2
Cellulose powder	37.5
Vitamin and mineral premix [3]	2.3
Water [4]	

[1] Norse LT-94, low-temperature dried fish meal, Norsildmel, Bergen, Norway; [2] Denofa AS, Fredrikstad, Norway; [3] Norsk Mineralnæring, Hønefoss, Norway. Ingredients per kg: IU: vit. A 2 000 000, vit. D_3 200 000; mg: vit. E 50 000, vit. B_1 15 000, vit. B_2 3 000, vit. B_6 3 000, vit. B_{12} 20, pantothenic acid 3 000, niacin 5 000, biotin 30, folic acid 300, Fe (amino acid-chelated) 20 000, Zn oxide 7 500, Mn oxide 15 000 and Cu sulphate 1 250; [4] added to suitable diet concistency

Proximate composition of diets and calculated percentage contribution of the algae to the total dietary content of CP and lipids are shown in Table 2. The control diet and diets containing *N. oceanica* and *P. tricornutum* were nearly isonitrogenous on a DM basis, whereas the crude protein content of the diets containing *I. galbana* declined slightly with increasing algae inclusion due to the low protein content of this alga. All diets had similar total lipid contents.

All diets were prepared prior to start of the experiment, weighed into plastic cups in individual daily rations, and stored frozen at -22ºC until thawing in a refrigerator starting about 20 h before feeding. The diets were fed for 7 days 3 days of adaptation followed by 4 days of total faecal collection (Skrede, 1979;

Table 2. Analysed proximate composition of freeze-dried diets (g kg^{-1}) and dietary proportion of crude protein and lipids from microalgae, %

Item	Control	*N. oceanica*			*P. tricornutum*			*I. galbana*		
Diet	1	2	3	4	5	6	7	8	9	10
Dry matter, g kg^{-1}	976	971	980	979	974	981	979	972	963	957
In dry matter, g kg^{-1}										
crude protein	378	359	347	313	359	347	317	347	301	241
lipid	311	307	306	301	306	305	299	314	303	311
starch	229	232	236	237	227	229	231	231	236	229
ash	79	72	62	60	84	83	77	84	105	120
organic matter	897	899	918	919	890	898	902	888	858	837
Part of nutrient from algae, %										
crude protein	0	7.7	16.1	35.2	8.1	16.7	36.4	3.4	7.0	17.9
lipid	0	1.61	3.24	6.40	1.45	2.91	5.86	2.87	5.68	11.5

Szymeczko and Skrede, 1990). The individual daily feed intake was recorded; as feed offered minus feed rejected. Feeding, collection of feed residues and collection of faeces were carried out once daily. Pooled faeces from each animal were freeze-dried, ground, and sifted for removal of hair pending analyses.

Chemical analysis

For chemical analysis, samples of all experimental diets and individually pooled faeces were freeze-dried and ground through a 0.5 mm screen. Diets and faecal samples were analysed using standard methods for the European Community: dry matter (71/393/EEC), ash (71/250/EEC), crude protein (Kjeldahl-N x 6.25; 93/28/EEC), amino acids (98/64/EC) and tryptophan (2000/45/EC). Crude lipid was determined by extraction with petroleum ether in an Accelerated Solvent Extractor (ASE200) from Dionex (Sunnyvale, CA, USA). Starch was determined as glucose after hydrolysis (McCleary et al., 1984). Organic matter was calculated as: DM - ash. All ingredients, diets, and faecal samples were analysed in triplicates.

Calculations and statistics

Apparent total tract digestibility (%) was calculated for each individual animal as:

$$((a-b)/a) \times 100$$

where: a - nutrient intake and b - amount of nutrient in faeces.

Statistical analysis was performed by the GLM procedure of SAS (1990) with each mink as the experimental unit. Results are presented as least squares mean (LSmeans) for each diet, and variance is expressed as standard error of the mean

(SEM) for each series of algae inclusion or standard deviation (SD) for treatment groups. Duncan's multiple range test was used to test differences in digestibility between diets. The significance level was set to $P<0.05$.

RESULTS AND DISCUSSION

Proximate composition and amino acid profile

The chemical composition of microalgal biomass is affected by species and cultivation methods, and may be manipulated readily by changing growth conditions (Brown et al., 1997; Fàbregas et al., 1998; Rebolloso-Fuentes et al., 2001). The proximate composition and amino acid profiles of the algae products used in our study in a comparison with LT fish meal are shown in Table 3. The

Table 3. Proximate composition and amino acid contents of fish meal and the microalgae *Nannochloropsis oceanica*, *Phaeodactylum tricornutum* and *Isochrysis galbana*

Algae	Fish meal	*N. oceanica*	*P. tricornutum*	*I .galbana*
Proximate composition				
dry matter, g kg^{-1}	927	949	970	888
crude protein, g kg^{-1} DM	747	477	490	201
fat, g kg^{-1} DM	96.6	84.1	73.7	162
starch, g kg^{-1} DM	0.0	0.3	0.3	0.1
ash, g kg^{-1} DM	147	74.8	158	310
organic matter, g kg^{-1} DM	780	874	812	578
Essential amino acids, g 16 g^{-1} N *				
lysine	6.8	4.8	4.2	3.1
threonine	3.5	3.6	3.7	4.6
methionine	2.5	1.8	2.0	2.5
tryptophan	0.7	1.7	1.3	2.5
valine	4.0	4.6	4.6	6.1
isoleucine	3.7	3.5	3.8	5.1
leucine	6.2	6.7	6.2	9.2
phenylalanine	3.3	3.9	4.2	5.7
histidine	1.7	1.5	1.2	1.7
arginine	5.4	4.9	4.4	4.1
Non-essential amino acids, g 16 g^{-1} N *				
aspartic acid + asparagine	8.1	7.0	8.1	9.7
serine	3.7	3.3	3.5	4.2
glutamic acid + glutamine	12.2	9.7	10.2	11.3
proline	3.3	9.8	4.5	4.9
glycine	4.7	3.8	3.7	5.2
alanine	4.8	5.2	5.6	6.6
tyrosine	2.5	2.4	2.5	0.3
cysteine + cystine	0.8	0.7	0.8	1.0

* determined using water-corrected molecular weights

N. oceanica and *P. tricornutum* had a lower content of CP than fish meal, but comparable contents of lipids. The CP content of *P. tricornutum* was similar to results obtained by Miròn et al. (2003) but higher than reported by Brown (1991), whereas the lipid content was lower than observed by Brown (1991) and Alonso et al. (2000). The *I. galbana* had lower CP content compared with the other algae, about twice as high lipid content, and very high ash content. The relatively low protein content and high lipid content in *I. galbana* is in line with other studies (Sànchez et al., 2000; Pettersen et al., 2010).

According to Brown (1991), the amino acid composition of different microalgae species is similar, and relatively unaffected by conditions of cultivation. In the present study, all species had a profile of essential amino acids similar to fish meal, except that content of lysine was lower and tryptophan content higher in the algae. The *P. tricornutum* used in our study had similar amino acid profile as reported by Brown (1991). The contents of amino acids in *I. galbana* agreed mostly with results obtained by Epifanio (1979), Brown (1991) and Brown et al. (1993), but our data revealed lower contents of lysine, arginine and tyrosine compared with the latter studies.

Digestibility

Inclusion of the different algae in mink diets did not appear to affect diet palatability, as all diets were well accepted by the mink. Average feed intake is shown in Table 4. One animal fed the lowest level of *N. oceanica* was omitted from the experiment due to low feed intake. The average feed intake in different treatment groups amounted to 96-99% of feed offered and was not correlated to inclusion level of any of the algae. Increasing inclusion of all algae products caused a slightly softer faecal consistency.

Substitution of LT fish meal with increasing amounts of *N. oceanica* and *I. galbana*, and to a lesser extent *P. tricornutum*, significantly lowered digestibility of CP and lipids in mink (Table 4). Addition of *I. galbana* and the lowest level of *P. tricornutum* significantly increased digestibility of starch (Table 4). However, the starch was generally very well digested, as might be expected from the use of precooked maize starch as almost the sole source of starch in all diets.

There was a significant adverse effect on the apparent CP digestibility even at the lowest level of *N. oceanica* and *I. galbana* (60 g kg^{-1}), but a significant negative effect of *P. tricornutum* only appeared at the two highest levels. Regression analyses showed linear relationships between algae inclusion and apparent CP digestibility (Figure 1). The apparent CP digestibility in algae estimated by linear regression and extrapolation to 100 % of CP from algae, was 35.5 % for *N. oceanica*, 79.9 % for *P. tricornutum* and 18.8 % for *I. galbana*. The

Table 4. Average feed intake (AFI) and apparent digestibility (%) of crude protein (CP; N x 6.25), lipid, starch and organic matter (OM) in mink fed diets containing the microalgae *Nannochloropsis oceanica*, *Phaeodactylum tricornutum* and *Isochrysis galbana*

Item	*Nannochloropsis oceanica*				P	*Phaeodactylum tricornutum*				P	*Isochrysis galbana*				P	Pooled
Diet	1	2	3	4		1	5	6	7		1	8	9	10		SEM
AFI[1]	49.7	49.6	48.3	49.4		49.7	50.3	50.2	49.3		49.7	49.5	51.9	51.1		
Digestibility																
crude protein	87.2^{a}	81.3^{b}	78.8^{bc}	72.4^{c}	<0.002	87.2^{a}	86.8^{ab}	86.1^{bc}	84.9^{c}	<0.02	87.2^{a}	85.0^{b}	83.6^{b}	75.3^{c}	<0.0001	1.17
lipid	98.1^{a}	96.0^{a}	93.0^{a}	79.8^{b}	<0.002	98.1^{a}	97.4^{a}	95.9^{a}	90.7^{b}	<0.001	98.1^{a}	97.3^{a}	94.6^{a}	87.0^{b}	<0.013	1.85
starch	98.9	99.4	99.3	99.2	<0.13	98.9^{b}	99.4^{a}	99.1^{ab}	98.9^{b}	<0.03	98.9^{c}	99.4^{ab}	99.6^{a}	99.2^{b}	<0.0001	0.11
organic matter	87.0^{a}	85.4^{a}	81.3^{b}	71.7^{c}	<0.001	87.0^{a}	86.3^{a}	85.2^{b}	82.8^{c}	<0.001	87.0^{a}	86.1^{a}	84.9^{a}	79.4^{b}	<0.001	0.86

^{a,b,c} within row and the same alga species, means without a common superscript are significantly different at P<0.05

[1] g DM/day in the faecal collection period

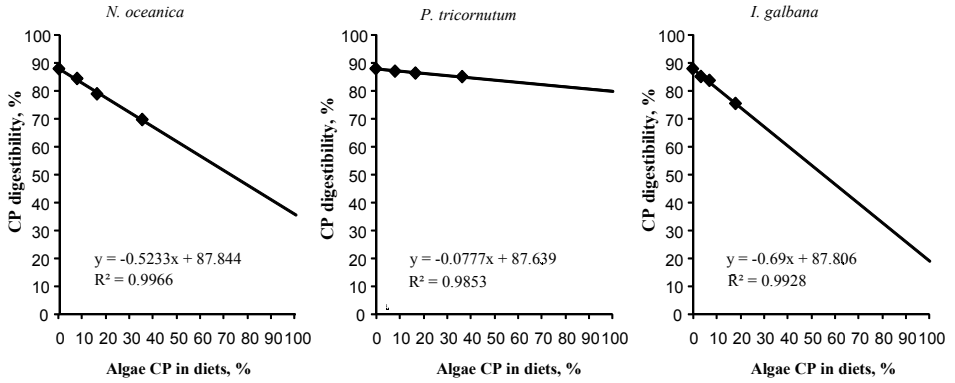

Figure 1. Regression lines for apparent digestibility of CP in the three microalgae (y = apparent digestibility of a diet with x% of CP from the algae)

apparent CP digestibility of *P. tricornutum* was lower than for high quality fish meal, but comparable with the CP digestibilities of common plant protein sources such as soyabean meal and rapeseed meal. The estimated CP digestibilities of *N. oceanica* and *I. galbana* must be considered as very low and probably unacceptable for feed ingredients intended for monogastric animals. Especially for *I. galbana*, it should be pointed out that the rather low contribution to total dietary CP, reduces the accuracy of extrapolation when assuming linearity in digestibility up to 100% of dietary CP. The poor CP digestibility of *N. oceanica* and *I. galbana* may be caused by the resistance of the thick and rigid algal cell wall to disruption by digestive processes (Becker, 2007; Marshall et al., 2010). Although there are no previous digestibility studies with mink, studies with other animals indicate that the digestibility of several algae species is dependent on disrupting the algal cell wall by proper processing (Saleh et al., 1985; Janczyk et al., 2005). Even after cell wall disruption, inherent proteins in the cell wall matrix may be protected against attack by digestive proteolytic enzymes, and advanced technological treatment increases the cost of the algae product. The freeze-drying of algae used in our study probably maintained the cell structure of the *N. oceanica* and *I. galbana* without any cell wall disruption. The cell wall of diatoms as *P. tricornutum* consist of silicon and may be more easily broken during digestion. However, some studies indicate that preserving of microalgae such as *P. tricornutum* by freezing or freeze-drying may lower digestibility due to altering the cell wall or compacting the algal cell (Albentosa et al., 1997).

The apparent digestibilities of amino acids in the algae species, using linear regression and the individual contribution of amino acids from algae to total dietary amino acid intake, are shown in Table 5. The *P. tricornutum* revealed

substantially higher digestibility of potentially limiting essential amino acids such as methionine, lysine and tryptophan compared with *N. oceanica* and *I. galbana*, thus confirming the CP digestibility data indicating that *P. tricornutum* was a better source of digestible amino acids than the other algae. The calculated amino acid digestibilities of *I. galbana* were rather variable, and especially lysine and cysteine (negative value) were poorly digested. However, *I. galbana* contributed minor proportions of total dietary amino acids; hence reducing the precision in the regression calculations of individual amino acid digestibilities.

Table 5. Average apparent amino acid digestibility (%) of LT fish meal and the algae *Nannochloropsis oceanica*, *Phaeodactylum tricornutum* and *Isochrysis galbana* [1]

Amino acids	Fish meal	*N. oceanica*	*P. tricornutum*	*I. galbana*
Essential amino acids				
lysine	86.8	38.1	84.5	12.6
threonine	85.0	50.1	83.0	55.0
methionine	93.5	35.6	83.4	64.8
tryptophan	85.6	38.3	81.7	69.0
valine	91.4	31.6	82.2	62.5
isoleucine	92.4	30.3	75.9	63.5
leucine	93.0	30.9	81.6	68.6
phenylalanine	90.3	31.9	83.2	69.2
histidine	88.9	17.2	76.6	37.1
arginine	93.6	41.2	87.4	56.8
Non-essential amino acids				
aspartic acid + asparagine	82.3	37.7	81.4	60.0
serine	88.3	35.0	76.4	49.4
glutamic acid+ glutamine	92.1	42.8	84.1	52.4
proline	88.7	78.9	88.1	60.5
glycine	88.3	39.7	88.8	54.7
alanine	91.8	30.3	78.6	61.8
tyrosine	80.9	22.7	58.7	77.0
cysteine + cystine	71.7	58.8	54.7	-13.1

[1] determined directly (fish meal) or by linear regression (microalgae)

Lipids are important storage components of microalgae, and several species are considered as promising sources of long-chain PUFAs such as EPA and DHA (Yamaguchi, 1997; Spolaore et al., 2006). The data presented in Table 4 show that increasing inclusion of all algae caused a decline in lipid digestibility. The highest inclusion level resulted in significantly lower lipid digestibility than any other diets within the same alga (Table 4). The lowest lipid digestibility was observed for the diet containing the highest level of *N. oceanica*. Characteristically, lipid digestibility was more variable for diets containing *N. oceanica* and *I. galbana* than for diets with *P. tricornutum*, and the variation increased greatly with increasing levels of algae products. Thus, the standard deviations for percentage lipid digestibility were 0.14 for the fish meal control diet, and 8.46, 2.49 and

6.46 for diets containing the highest levels of *N. oceanica*, *P. tricornutum* and *I. galbana*, respectively.

As shown in Table 2, all algae represented a rather minor proportion of total dietary lipids. Soyabean oil was the major source of lipids in all diets, and fish meal also provided larger proportional levels of lipids than the algae, except for the diet containing the highest level of *I. galbana*. Still all algae appeared to cause a progressively declining lipid digestibility with increasing inclusion of algae lipids (Table 4; Figure 2). Although *P. tricornutum* could be fed at 24% as

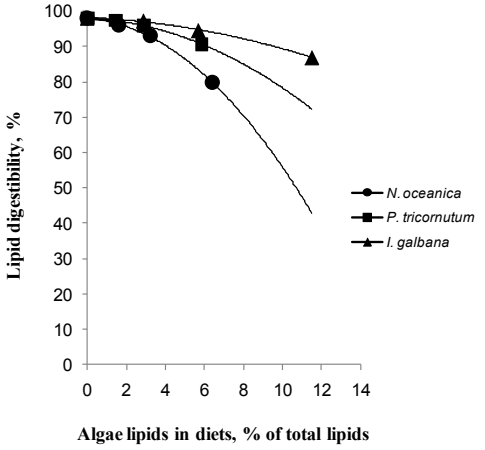

Figure 2. Regression lines for apparent digestibility of lipids in the three microalgae (y = apparent digestibility of a diet with x% of lipids from the algae). *Nannochloropsis oceanica* (–●–); $y = -0.3841x^2 – 0.3724x + 97.962$ ($R^2 = 0.9989$). *Phaeodactylum tricornutum* (–■–); $y = -0.1732x^2 – 0.2497x + 98.107$ ($R^2 = 1.0$). *Isochrysis galbana* (–■–); $y = -0.0653x^2 – 0.2266x + 98.205$ ($R^2 = 0.9982$)

is with moderate negative effects on digestibility of dietary lipids, a regression analysis of lipid digestibility of all algae resulted in negative values. This curvilinear relationship was surprising and can only be explained by negative effects of components in the algae on lipid digestibility. These components are unlikely to be lipids; hence the lipid digestibilities in Table 4 may give no information about digestibility of the inherent algae lipids. Noteworthy, Bitou et al. (1999) reported lipase inhibitor activity in a number of marine algae and identified the terpene caulerpenyene as an active inhibitor interacting directly with the lipase protein. The resistance of the complex algal cell wall structure to digestive enzymes may inhibit lipid digestion, and cell wall constituents may also affect digestion of other lipids in the diets than those derived from algae. It

is concluded that the lipid digestibility as well as protein digestibility might have been improved by using an adequate processing technique to rupture the cell walls prior to inclusion of the algae in the compound feed. The microalgae had very different digestibility of protein and amino acids, and different effects on lipid digestibility. Among the investigated freeze-dried algae, *P. tricornutum* should be the preferred feed ingredient for monogastric animals including salmonid fish, due to the high digestibility of crude protein and individual amino acids, and only minor negative effect on lipid digestibility if fed at moderate levels.

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