PROTEIN TO ENERGY RATIOS IN GOLDFISH (Carassius auratus) DIETS

Relação proteína/energia em dietas para o kinguio (Carassius auratus)

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ABSTRACT

The optimal dietary digestible protein to energy ratio is critical for the success of fish farming. Considering that it provides the proper development of the animals, this study was undertaken to evaluate the effects of different dietary digestible protein to energy ratio in diets for goldfish fingerlings. Diets were formulated to contain 28.0, 35.0, 42.0 and 49.0% DP and 3,600 and 3,200 kcal/kg. The growth parameters evaluated were daily weight gain (DWG), feed intake (FI), feed conversion ratio (FCR), protein efficiency ratio (PER), protein productive value (PPV) and energy retention ratio (ERR) and specific growth rate (SGR). Regardless FI, all the parameters were affected by protein and energy levels and interaction between both factors was observed. The second-order polynomial equation best fitted to the DWG, SGR, FCR, PER and ERR with estimated requirement values of 38.81, 38.85, 41.05, 41.80 and 39.39% DP, respectively, at the 3,200 kcal/kg. A second-order polynomial model was significant only for the ERR data at the 3,600 kcal/kg with an estimated requirement value of 36.45% DP. Generally, fish fed diets containing 3,200 kcal/kg had better growth performance than fish fed 3,600 kcal/kg (P<0.01), indicating a higher feed efficiency. A 3,200 kcal/kg diet seems to be the proper DE level for meeting goldfish requirement for highest weight gain. Based on this experimental condition, we recommend the use of DP levels between 38.0-40.0% in diets for goldfish with 3,200 kcal/kg or a dietary protein to energy ratio between 119-125 mg/ kcal DE.

Index terms: Energy requirement, feed intake, growth protein requirement, ornamental fish.

RESUMO

A ótima relação energia/proteína é importante para o sucesso da piscicultura. Considerando que a mesma fornece o adequado desenvolvimento dos animais, este estudo foi realizado para avaliar a relação energia/proteína digestível ótima para alevinos de kinguio. Dietas foram formuladas para conter 28,0; 35,0; 42,0 e 49,0% PD e 3600 e 3200 kcal/kg. Foram avaliados: ganho de peso diário (GPD), consumo de ração aparente (CRA), conversão alimentar aparente (CAA), taxa de eficiência protéica (TEP), valor produtivo protéico (VPP), taxa de retenção de energia (TRE) e taxa de crescimento especifico (TCE). Com exceção do CRA, todos os parâmetros foram afetados pelos níveis de proteína e energia e interação entre os dois fatores foram observados. O modelo que melhor se ajustou aos parâmetros GPD, TCE, CAA, TEP e TRE foi o polinomial de segunda ordem com valores de exigência de 38,81; 38,85; 41,05; 41,80 e 39,39% DP, respectivamente, para o nível de 3200 kcal ED/kg. O modelo polinomial de segunda ordem foi significativo apenas para os dados de TRE no nível de 3600 kcal ED/kg, com valor de exigência 36,45% PD. Os peixes alimentados com dietas contendo 3200 kcal/kg apresentaram melhor desempenho de crescimento do que os alimentados com 3600 kcal/kg (P<0,01), indicando uma maior eficiência alimentar. 3200 kcal/kg ED parece ser o nível adequado encontrado para kinguio para maior ganho de peso. Com base nas condições experimentais deste estudo, recomenda-se a utilização de níveis entre 38,0-40,0% PD em rações para kinguios com 3200 kcal/kg ou uma relação proteína/energia entre 119-125 mg/kcal ED.

Termos para indexação: Requerimento energético, consumo de ração, exigência protéica, peixe ornamental.

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INTRODUCTION

Ornamental fish culture is an increasingly growing aquaculture activity in developing countries. This market has been increasing since the 80's with annual profits around U\$ 900 million with fish marketing and three billion dollars with related equipments and feed (FAO, 1999). The Asian countries are responsible for more than half of worldwide production in this activity while the main consumers are the USA, Japan and Europe particularly Germany, France and United Kingdom (FAO, 1999).

Although the economical importance of ornamental fish production, few information are available on nutrient requirements for the species usually kept in aquaria (BLOM; DABROWSKI, 2000). According to Tamaru, Ako and Paguirigan (1997), before elucidating the nutrient requirements of ornamental fish species, studies should

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be performed to test if the diets developed for farmed fish are adequate for ornamental fish.

The nutrient requirements of ornamental fish are not well established. Commercial diets are based on extrapolation of results obtained with farmed fish (YANONG, 1999; SALES; JANSSENS, 2003). A great diversity of ornamental fish species with different feeding plasticity are fed with the same diets. Additionally, diets exceeding the nutrient requirements may be responsible to reduced water quality inducing the appearance of disease outbreaks.

Optimum dietary protein to energy ratio must be used to formulate fish diets in order to promote adequate growth rates. High energy diets concur for a reduced protein and essential nutrients intake (CHOU; SHIAU, 1996; PEZZATO et al., 2001) and an increase on fat deposition in several fish species (MACGOOGAN; REIGH, 1996; MUKHOPADHYAY; RAY, 1997). On the other hand, diets with low energy content increase the use of protein as an energy source, increasing the feed efficiency ratio and the cost of production. Additionally, the increased nitrogen excretion due to deamination of amino acids in fish metabolism impose a serious problems to environmental sustainability of fish culture, reducing water quality and promoting the eutrophication of the environment.

Although the importance of goldfish on ornamental fish market scenario, until now, just two studies have reported the protein and amino acid requirement for this species (LOCHMANN; PHILLIPS, 1994; FIOGBÉ; KESTEMONT, 1995). However, none have studied the influence of digestible energy level on protein requirement. Thus, this study aimed to determine the digestible protein and energy requirement, and proper dietary protein to energy ratio for goldfish fingerlings based on growth performance and nutrient utilization.

MATERIAL AND METHODS

Experimental diet and diet preparation

Diets were manufactured using conventional feed ingredients in different proportions aiming to obtain different digestible protein and energy levels (Table 1). Thus, four digestible protein levels (28.0, 35.0, 42.0, and 49.0% DP) at two digestible energy levels (3,200 and 3,600 kcal/kg) were tested comprising eight diets. Digestible protein and energy content of feedstuffs were calculated based on apparent digestibility coefficients reported for tilapia (PEZZATO et al., 2002; GUIMARÃES et al., 2008, 2012). All ingredients were ground until sieve in a mesh diameter of 500 mm. Diets were mechanically mixed with water (25% of dry weight) and the moist mixture was extruded in a 4.0 mm die of a meat grinder. Diets were oven dried until present moisture <100 g/kg, and stored at -18 °C until further use. At the beginning of the experiment diets were ground and sieved in a mesh diameter according to fish size.

Experimental procedure

Three hundred goldfish with 90 days-old were purchased from a commercial fish farm and acclimatized to the laboratory conditions in two 500 L-aquaria. These fish were fed twice daily with a commercial diet to satiation for two weeks. The feeding trial was conducted in a recirculating system; accumulated feces were removed by siphoning. A homogenous group of 120 goldfish was selected by weight $(1.78 \pm 0.11 \text{ g})$ and randomly stocked into 24 3L-aquaria.

Each diet was fed to triplicate groups of fish for 45 days. Fish were fed until apparent satiation. The animals were fed at 07:00, 12:00, and 17:00 h. During the feeding trial water quality parameters were maintained in the optimum range for fish rearing (pH 6.8±0.3; dissolved oxygen 5.8 ± 0.7 mg/L and ammonia (NH₃) 124.0 µg/L). Water temperature was heater-controlled and kept at 26.0 \pm 0.7 °C. All tanks were maintained under natural photoperiod.

During the experiment, fish mortality was recorded. At the beginning and at the end of the feeding experiment, fish were starved for 24 h, and then weighed by group.

Analysis and measurement

A group of 20 fish at the beginning of the experiment and five fish per aquaria at the end were collected and killed with high benzocaine concentration (193 mg/L). Fish were ground and samples stored frozen (-18 °C) to determine the whole body protein and energy content. Proximate composition analysis on feed ingredients, experimental diets and fish were performed by the standard methods of ASSOCIATION OF OFFICIAL ANALYTICAL CHEMIST - AOAC (1995). Due to the reduced amount of fish samples, only protein and energy content were measured. Samples of diets were dried to a constant weight at 105 °C to determine moisture. Protein was determined by measuring nitrogen (N x 6.25) using the Kjeldahl method; lipid by ether extraction using Soxhlet; ash by combustion at 550 °C, crude fiber by fritted glass crucible method after treated with H2SO4 and NaOH, and energy by an adiabatic bomb calorimeter (PARR1281, USA).

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Ingredients	3,600/28.0	3,600/35.0	3,600/42.0	3,600/49.0	3,200/28.0	3,200/35.0	3,200/42.0	3,200/49.0
Soybean meal	10.80				16.00	5.00		
CSM-28	14.16	14.16	ı	ı	14.80	16.35	ı	ı
CSM-40	ı	ı	ı	ı	ı	ı	7.00	ı
Corn gluten meal	I	7.62	14.16	15.40	ı	7.62	14.16	15.46
Fish meal	5.30	5.30	ı	ı	5.30	5.30	ı	ı
Feather meal	25.83	30.64	30.64	ı	21.23	25.64	26.64	ı
Wheat middlings	ı	5.70	20.42	50.46		5.70	20.42	49.96
Broken rice	I	ı	ı	ı	·	4.00	ı	ı
Cellulose	I	ı	5.00	5.00	ı	ı	5.00	5.00
Lysine HCI	1.57	3.24	3.46	5.60	1.53	2.55	3.58	5.60
DL - Methionine	0.40	0.52	0.75	1.12	0.40	0.52	0.77	1.12
Triptophan	0.04	ı	0.05	0.06		ı	0.04	0.05
Threonine	0.63	0.80	0.80	0.87	0.63	0.80	0.82	0.87
Soybean oil	6.70	5.50	4.20	9.84	1.00	1.00	1.00	2.00
Dicalcium phosphate	1.80	1.80	1.80	1.98	1.80	1.80	1.80	1.98
Limestone	1.11	1.11	1.06	1.11	1.11	1.11	1.11	1.56
Vit C	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
NaCl	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin/mineral mix ^a	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
BHT^{b}	0.02	00.2	0.02	0.02	0.02	0.02	0.02	0.02
Total	100	100	100	100	100	100	100	100
Digestible energy*	3,637.34	3,608.28	3,623.90	3,599.46	3,269.46	3,258.31	3,173.36	3,210.88
Digestible protein*	28.26	35.22	42.25	49.02	28.60	35.64	41.76	49.02
Crude protein	32.82	42.44	49.06	55.47	34.30	42.68	49.39	55.67
Crude fiber	5.08	5.31	5.62	4.87	5.32	5.11	4.50	4.74
Lipid	12.63	12.83	10.86	15.09	6.15	6.12	6.34	7.19

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Calculations and statistical analysis

The following variables were calculated: Specific growth rate (SGR) = (Ln FW – Ln IW)×100/t; Daily weight gain (DWG) = (FW – IW)/t; Feed intake (FI) = Feed consumption (g)/((FW +IW)/2)×t) (WANG et al., 2012); Feed conversion ratio (FCR) = Dry feed fed in g/wet weight gain in g; Protein efficiency ratio (PER) = Wet weight gain in g/protein intake in g; Protein productive value (PPV) = $(FW \times P1 - IW \times P2)/(Id \times P)$; Energy retention ratio (ERR) = $(FW \times E1 - IW \times E2)/(Id \times E)$

where FW is final body weight, IW is initial body weight, t is experimental duration in days, Id is feed intake of dry matter. P, P1 and P2 represent protein contents in diet, final fish body and initial fish body, respectively. E, E1 and E2 represent energy content in diet, final fish body and initial fish body, respectively.

The experiment followed a completely randomized design arranged in a 2 x 4 factorial scheme with three replicates. The data were verified for normality (Kolmogorov–Smirnov test) and homogeneity of variances (Levene's *F* test). Data from each treatment were subjected to one-way analysis of variance (ANOVA), two-way ANOVA and correlation analysis where appropriate. When overall differences were significant (P<0.05), Schefe multiple range test was used to compare the mean values between individual treatment. Additionally, polynomial regression was evaluated to determine the protein requirement in each dietary digestible energy level. Statistical analysis was performed using SAS software (STATISTICAL ANALYSIS SYSTEM, 1999), version 9.1.

RESULTS AND DISCUSSION

Growth performance of goldfish fed diets containing different levels of digestible protein and energy over 45-day feeding trial is presented in table 2. Regardless the feed intake, all growth parameters evaluated were influenced (P<0.05) by dietary protein and energy levels, and their interaction.

Protein is one of the most studied macronutrient in fish nutrition. However, several factors may influence the quantitative protein requirement for different fish species. Factors including fish size, the use of purified ingredients, the lipid content of diets, the amino acid composition of diets, among others have been described to affect the determination of protein requirement (ELANGOVAN; SHIM, 1997; BUREAU; ENCARNAÇÃO, 2006; VELASCO-SANTAMARÍA; CORREDOR-SANTAMARÍA, 2011; NRC, 2011). In general, most of the studies conducted with fish have been using purified or semipurified diets to determine the quantitative protein requirement; however, the use of these types of ingredients/diets reduces the palatability of the diets. Thus, we decided to use conventional ingredients to formulate the experimental diets in this study to reduce the effect of the low nutrient intake on estimated requirement.

Although there are reports in the literature regarding the quantitative protein and amino acid requirement for Goldfish, no reports on the effect of different energy concentrations on protein requirement are available. Thus, we observed in this study that the protein requirement of goldfish is greatly affected by the energy content of the diets, thus corroborating with several reports with different fish species (MCGOOGAN; GATLIN, 2000; SATOH et al., 2004; MOLLA and AMIRKOLAIE, 2011). Until we could reference, there is just two studies in the literature reporting the protein and energy requirement for Goldfish, however none have used different energy content.

No effect of increasing levels of protein and/or energy on feed intake of goldfish was observed in this study. This result was unexpected since there is evidence to suggest that fish control feed intake primarily to meet metabolic energy demands, and that control is closer related to the digestible and metabolisable energy content of the diet (MORALES et al., 1994; BOUJARD; MÉDALE, 1994). Although there are reports in the literature regarding the effects of nutrients on food intake of fish, a multitude of factors could affect the control of food intake in fish (DE LA HIGUERA, 2001), for example, the great variability on ingredients composition of experimental diets may have affected the palatability in some treatments. The high inclusion of fats and animal proteins could have increased the feed intake in high energy diets while the presence of high carbohydrate and vegetable proteins content in low energy diets may have not be sufficient to decrease the palatability of the diets. All these factors may have acted in synergism for the non evident effect of energy on feed intake.

Second order polynomial regression model best fitted to daily weight gain (DWG), specific growth rate (SGR), protein efficiency ratio (PER), feed conversion ratio (FCR) and energy retention ratio (ERR) at 3,200 kcal/kg, while only ERR fitted at 3,600 kcal/kg (Figure 1). Whereas no mathematical models were able to fit the protein productive value (PPV) at both digestible energy levels.

DP (%) DE							
(kcal/kg)	FI (%)	DWG (g)	FCR	SGR (%)	ERR (%)	PPV (%)	PER (%)
28.0	0.019 ± 0.001	4.94 ± 0.85^{ab}	7.43±0.99°	0.25 ± 0.04^{ab}	11.24 ± 0.66^{cd}	14.66±0.31 ^{cd}	0.32 ± 0.04^{cd}
35.0 2,200	0.016 ± 0.001	$8.64{\pm}1.54^{a}$	3.74 ± 0.53^{a}	0.43 ± 0.08^{a}	24.05 ± 1.86^{a}	$19.69{\pm}1.01^{ m ab}$	$0.55\pm0.08^{\mathrm{bc}}$
42.0 5,200	0.015 ± 0.001	7.65 ± 0.85^{a}	3.99 ± 0.40^{a}	$0.38\pm0.05^{\mathrm{ab}}$	21.33 ± 0.58^{a}	$13.81 \pm 0.81^{\rm d}$	$0.90{\pm}0.09^{a}$
49.0	0.013 ± 0.002	$5.68{\pm}1.54^{\mathrm{ab}}$	$4.68\pm0.65^{\mathrm{ab}}$	0.29 ± 0.06^{ab}	15.98 ± 2.72^{b}	$18.69{\pm}1.32^{\rm abc}$	$0.61{\pm}0.08^{\rm b}$
28.0	0.018 ± 0.003	$4.69{\pm}1.13^{ab}$	$7.38{\pm}1.20^{\rm bc}$	$0.25\pm0.07^{\mathrm{ab}}$	9.36 ± 1.24^{d}	22.46 ± 2.49^{a}	$0.49{\pm}0.08^{\rm bc}$
35.0 2,000	0.017 ± 0.001	7.65 ± 1.54^{a}	4.19 ± 0.61^{a}	0.42 ± 0.08^{a}	$14.87\pm0.24^{\rm bc}$	22.43 ± 1.30^{a}	$0.69\pm0.11^{\mathrm{ab}}$
42.0 5,000	0.020 ± 0.005	2.72 ± 0.85^{b}	13.06 ± 0.38^{d}	0.15 ± 0.04^{b}	$9.98{\pm}0.92^{d}$	16.03 ± 1.74^{bcd}	$0.18{\pm}0.01^{ m d}$
49.0	0.017 ± 0.003	$4.69{\pm}1.13^{ab}$	6.71±0.42 ^{bc}	$0.26\pm0.07^{\mathrm{ab}}$	6.71 ± 0.20^{d}	21.88 ± 0.83^{a}	0.31 ± 0.02^{cd}
DE	0.1293	0.0024	<0.0001	0.0052	<0.0001	< 0.0001	<0.0001
DP	0.3643	0.0024	<0.0001	0.0227	<0.0001	< 0.0001	<0.0001
DE*DP	0.5868	0.0035	<0.0001	0.0055	0.0024	0.0014	0.0002

At the 3,200 kcal/kg, the linear increase on dietary protein level improved feed efficiency and growth performance of goldfish reaching a peak, and thus reducing significantly. Estimated protein requirement at 3,200 kcal/ kg based on DWG, SGR, PER, FCR and ERR were 38.81, 38.85, 41.80, 41.05, and 39.39%, respectively. However, the estimated protein requirement based on energy retention at the 3,600 kcal/kg level was lower (36.45%) than the 3,200 kcal/kg level.

The highest PPV was observed in fish fed the 28.0/ 3,600 and 35.0/3,600 diets, while the lowest PPV was observed for fish fed the 28.0/3,200 and 42.0/3,200 diets. Generally, fish fed diets containing 3,600 kcal/kg retained more protein in their bodies compared to the 3,200 kcal/kg level.

At the 3,600 kcal/kg level, the highest growth rates were observed in fish fed diets with 35.0% DP, while the lowest values were observed in fish fed 42.0% DP. The results of feed efficiency showed similar trend to the growth data.

The estimated protein requirement for Goldfish based on growth response seems to be around 39.0% DP at the lowest DE level (3,200 kcal/kg), while Goldfish seemed to grow better when fed a diet with 35.0% at the highest DE level (3,600). These results indicated a possible protein-sparing effect of energy (mainly provided by lipid) in Goldfish. Additionally, the highest PPV values observed in Goldfish fed the highest DE level supports our hypothesis, indicating that fish efficiently used protein when a decrease in DP:DE ratio was attained. This strongly supports the findings of several authors with numerous farmed fish species that the reduction of DP levels with or without concomitant increase in the dietary non protein DE supply lead to improved protein utilization (NRC, 2011).

Closer protein requirement values (42.53% CP) to this study were observed for Goldfish with 1.66 g live weight fed diets with different crude protein concentration formulated with several locally available agro-products (BANDYOPADHYAY, SWAIN; MISHRA, 2005). Conversely, Lochman and Phillips (1994) reported a lower protein requirement (29.0% CP) when fed Goldfish with semipurified diets containing increasing levels of crude protein. Differences on estimated nutrient requirement may be attributable to several factors, including: the type of experimental diet, the statistical model used to determine the requirement and fish size (BUREAU; ENCARNAÇÃO, 2006; SHEARER, 2000). The low protein requirement reported by Lochman and Phillips (1994) may be due to the use of a multiple range test to determine the requirement.

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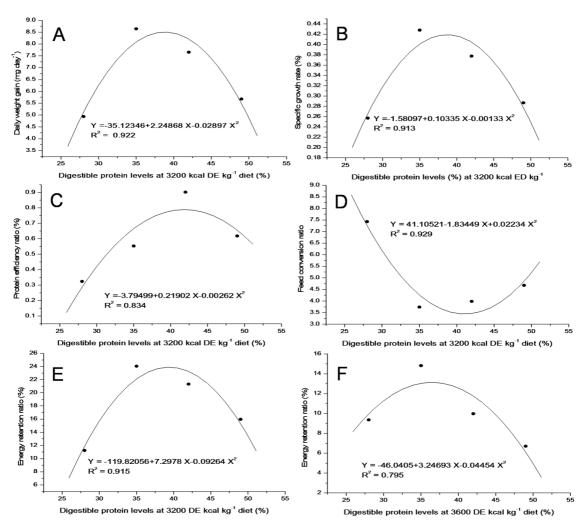


Figure 1 – Effect of dietary protein level on the DWG (a), SGR (b), PER (c), FCR (d), ERR (e) of goldfish fed diets containing 3,200 kcal/kg and the effect of the dietary protein level on the ERR (f) of goldfish fed diets containing 3,600 kcal/kg.

If a regression analysis is fitted to those data, a linear ascending regression would probably fit better, indicating that the protein requirement is higher than that recommended by Lochman and Phillips (1994) for goldfish.

There is strong evidence in several reports that the requirement for maximizing protein gain is higher than that required to maximize weight gain (RODEHUTSCORD et al., 1997; SUSENBETH et al., 1999; HAULER; CARTER, 2001; BUREAU; ENCARNAÇÃO, 2006). Similarly, we observed in our study that Goldfish required a higher amount or DP (between 41.0 and 42.0) to sustain maximum feed utilization which supports previous studies with other farmed fish species.

Contrary to the results of protein retention, the estimated DP requirement for maximum energy retention reduced with the increasing DE level in this study. There is a large variation among the results of the studies with other fish species (BANDYOPADHYAY et al., 2005; DENG et al., 2011; LIU et al., 2011; MOHANTA et al., 2008). Differences on efficiency of energy retention are related to the ability of the species to utilize the non-protein energy (lipid and carbohydrate), since the great variability on the results of the studies may be attributable to this assumption. Based on our results of energy and protein retention for goldfish, we could assume that this species is able to utilize lipid and carbohydrate to spare protein since the diets with the highest DE levels had a higher lipid content while the low DE diet presented higher starch content. However, it seems that lipid has a more pronounced effect on protein-sparing for goldfish since higher PPV and lower ER were, in general, observed for the diets containing higher lipid content (3,600 kcal/kg). Additionally, it seems that goldfish have the ability to convert energy derived from carbohydrate to lipid synthesis since a higher energy retention was observed in fish fed the low energy diet which is in one hand with data on other fish species that energetic efficiency for protein retention in fish is lower than that for fat deposition (SCHWARZ; KIRCHGESSNER, 1995; RODEHUTSCORD; PFEFFER, 1999; LUPTATSCH et al., 2003). However, we did not evaluate the composition of fish growth due to lack of samples to determine the lipid content.

CONCLUSIONS

A 3,200 kcal/kg diet seems to be the proper DE level for meeting goldfish requirement for highest weight gain. Based on this experimental condition, we recommend the use of DP levels between 38.0-40.0% in diets for goldfish with 3,200 kcal/kg diet or a dietary protein to energy ratio between 119-125 mg/kcal DE. A protein sparing effect by dietary lipid seems to occur in goldfish; however it was not clear if the dietary carbohydrate has the same effect. Furthermore, goldfish seems to be able to use lipid more efficiently than carbohydrates, but more studies are needed in this area since low nitrogen excretion is a quality that ornamental fish feed producers and hobbyists search when choosing a diet.

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