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MASS PRODUCTION OF *CHLORELLA VULGARIS* BEYERINK AND ITS SUITABILITY TO BE USED FOR FEEDING SILVER CARP FISH (*HYPOPHTHALMICHTHYS MOLITRIX*)

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Abstract

A traial was carried out to sustain the most suitable conditioins for using *Chlorella vulgaris* cells in feeding silver carp fish. *Chlorella vulgaris* cells were found to be rich in amino acids content Leucine, isoleucine, lysine, threonine, treptophan, phenylalanine + tyrosine and valine contents were higher than those of the FAO pattern of fish diet The sulphur containing amino acids (methionine + cystine) were lower. Fish yield , specific growth rate and protein content reached their maximum level at 20% of dried algal biomass supplemented to artificial fish, but further supplementations were of negative effect. The study of the biochemical composition of fish reared on the different variance of artificial feed (AF) with different dry algal cells supplements showed an increase of protein content of fish up to 20% supplementation, which decreased again on further substitution of AF. The level of ammonia content in water fish tank showed certain success when fish fed contain 20% of dried algal biomass supplemented artificial fish food compared with other treatments. Their content of water ammonia fish tanks increased with the increase of nitrogen content of the tested diet variance.

Key words: *Chlorella Vulgaris, Hypophthalmichthys molitrix*

Introduction

Microalgae are indisepensable as feed nowadays in intensive fish rearing. They are of great importance to the rearing larvae, juveniles and adults of bivalves, Horstmann (1985) and De Pauw *et al.* (1986*).* Silver carp larvae rearing depends on phytoplankton, Zarnecki (1968). Additionally, algae can be kept at the exponential growth phase, through long cultivation periods and trials for increasing their yield per unit volume (Palmer *et al.*, 1975 and Trotta, 1981). Most industrial production of microalgae is largely unialgal semi-continuous or batch culture which are successively cultivated. This was described by Oceantic Institute's (OI), and the shrimp hatchery algae upscaling protocool which illustrates some important principles (Treece and Yates, 1988 and Hoff and Snell, 1989).

Algal species commonly used to feed bivalve larvae were reported by (Guillard, 1975) and De Pauw *et al.*, 1984). Species are usually selected on the basis of size of cells, nutritional value and ease of culturing, it must also be non – toxic. Moreover mixed diet of several species of microalgae has been reported to give better results, (Laing and Millican, 1986 and Hu, 1990). Some strains of fresh water algae (*C. vulgaris, C. ellipsoidea, C. reguralis, etc.*) are heterotrophic or mixotrophic. For this reason, they can be easily massively produced in aseptic, light and dark conditions, and the production coasts are comparatively low (Maruyama *et al.* 1990).

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Among the problems associated with the use of algal cells as fish feed is that the low digestibility of the algal cells makes the algal biomass unsuitable for rearing fishes such as silver carp, common carp and milk fish. The rigid cell walls of the green algae make them even more difficult for the fishes to digest, Soeder (1976). Segner *et al.* (1987) even reported that the larvae of milk fish *(Chanos chanos)* was found to suffer 100% mortality when reared in *Chlorella* sp. Soeder (1976) suggested the use of thermal shock to increase the digestibility of algal biomass for fish cultivation. Edwards (1980) also, reported that the heat – dried algal biomass had a nutrient efficiency level similar to other vegetable proteins, such as soybean. In this study, heat drying was used to increase the digestibility of the algal biomass, thus, the dried algal biomass was used as a fish food supplement for silver carp, which is an economically important fish.

Growth and production in fish culture are generally dependent on stock density, daily feed consumption rate and feeding frequency (Chua and Teng, 1978 and Kayana *et al.*, 1990). In addition, slight variation in frequency size of fed groups was attributed to food intake, Omar and Gunther (1987) and Andrews and Page (1975), they showed that optimum weight gain and feed efficiency in catfish were obtained by two feedings daily. According to Omar and Gunther (1987), increasing feeding frequency from four to six times daily significantly improved body weight gain and feed utilization in minor carp. Kayana *et al.* (1990) found that between two and four satiated feedings daily were the optimum frequencies for fish weighing 0.5g, however in other research Kayana *et al.* (1993) found that the highest muscle mass and the highest levels of muscle protein were obtained at feeding frequency of six times daily.

Microalgal biomass production is economically feasible only when product values are relatively high, such as special chemicals and pigments, or when the microalgae play a critical role in aquaculture production (Spektorova *et al.*, 1997).

The present investigation is a trial to conclude the most suitable conditions for using *Chlorella* cells in feeding silver carp.

Materials and Methods

Chlorella vulgaris was isolated using the technique of Guillard (1973), and identified according to Pascher (1915). Stock cultures of *Chlorella* were prepared in two litres capacity flasks in the laboratory for 5-6 days, then inoculated in carboy cultures at a density of 1 x 10^5 cells ml^{-1}. The carboy cultures are used as inocula for two different phases of production in indoor and outdoor in glass aquaria. Stock cultures of *Chlorella vulgaris* were prepared in tanks with volume ranging from 0.5 m^3 to 20 m^3, kept in open air or in covered sheds with aeration from air compressor. The tanks were connected together by polyethylene tubes. The harvesting of the algal cells was achieved at a density of 5 x 10^6 cells ml^{-1} by switching the aeration system to allow cells to sediment at the base of the tank, where they were collected as thick slurry. Cells were separated by centrifugation and dried at 110°C till constant weight, then dried algal pellets can be stored in adesicator.

Crude protein, crude fat and fiber contents of the dried algal biomass was estimated according to American Puplic Health Association (APHA 1985). Total carbohydrate content was estimated as glucose according to Dubois *et al.* (1956). The DNA content of *Chlorella vulgaris* was estimated by diphenyl amine procedure described by Burton (1956) while RNA content was estimated colorimetrically by the orcinol procedure described by Dische and Schwarz (1937). The amino acid content was determined using automatic amino acid analyzer Beckman, Model 126 AA, (Mondino, 1969).

Feeding fish experiments were carried out in indoor fish glass aquaria, (75 x 40 x 50 cm.) filled with fresh water to a depth of 0.3 m (approximately 90 L.). Aeration was achieved using sterile air by using air pumps with tubes connected to air stones submerged at the bottom of the tanks. The fish in all treatments were kept at temperatures ranging from 25.5 to 27.5°C, pH was determined daily using pH meter (Orian model 420 A). Dissolved oxygen concentration was measured using a YSI oxygen meter. The experimental fish silver carp fry (*Hypophthalmichthys molitrix*) with initial weight of about 3.5 – 4 g was used. Each aquarium contained 10 fish fry and treatments were carried in triplicates. Each group of fish was fed by 2% of fresh fish body weight (w/w) four times daily, with one of the following different diet variants : Artificial fish food (AF) as a control, to which algal cells were substituted in different dry weight 5%, 10%, 20%, 50%, 75% and 100%. Also another treatment was carried out using fresh harvested living algal cells (A), air dried.

The composition of artificial fish food (contains 25% protein) was : fish meal 9%, meat meal 9%, cotton seed 10%, soybean meal 10%, wheat bran 15%, yellow corn 30%, rice bran 15%, and mollase 2%, as recommended in Oceantic Institute "OI".

Growth of the experimented fish was monitored for 32 days. The specific growth rate (SGR) was calculated according to Degani and Viola (1987). Food conversion ratio (FCR) was determined according to Berger and Halver, (1987). Protein, fat and ash content of fish were measured after 32 days following APHA (1985). Ammonia concentration in water tank was determined on day 14 and 21. Samples were taken at 10.0 am and analyzed immediately after collection following APHA (1985). Also survival rate of treatments was observed daily. Statistical analysis of the experimental results were carried out following Statistical Analysis Systems SAS Program (SAS, 2000).

Results and Discussion

The results indicated that the *Chlorella vulgaris* has 18 amino acids (Table 1). They were well – balanced except that the level of the sulfur – containing amino acids (methionine and cystine) was low compared with the FAO pattern. On the other hand, phenylalanine + tyrosine recorded higher values compared with the FAO pattern. Glycine, aspartic and alanine recorded higher values and were not recorded in the FAO pattern. The other amino acids presented were recorded in larger amounts than that of FAO pattern. These data are in agreement with Fowden (1951) and Combs (1952) who obtained similar results and found that *Chlorella* is deficient in cysteine and methionine. It might be also noted that *Chlorella* contains higher levels of lysine and threonine than those detected in bread wheat.

Chlorella vulgaris and *Scenedesmus bijuga* algae served as rich sources of vitamins, minerals and essential amino acids (Dawah *et al.*, 2002). In accordance to the present resent results, both *Skeletonema costatum* and *Chaetoceras calcitrans* showed aspartic acid as the dominant amino acid (more than 10%). The second main amino acid was serine for *C. vulgaris* and glutamic acid for *S. bijuga* (Derrien *et al.*, 1998).

The results in Table (2) show that the total carbohydrate content of oven dry tissue of *C. vulgaris* was 10.10 mg/g dry wt. The ash content and the crude fiber of the *C. vulgaris* was 18.87% and 8.2%. This result was in support the data obtained by Millamena *et al.* (1990).

Table (1): Individual amino acid content of *Chlorella vulgaris*

Amino acids	g/100 gm dry weight	% of total	FAO pattern*
Alanine	8.1	9.29	
Arginine	5.3	6.08	
Aspartic acid	8.5	9.75	
Glutamic acid	5.1	5.85	
Glycine	12.1	13.88	
Histidine	1.5	1.72	
Isoleucine	4.3	4.93	4.2
Leucine	7.4	8.49	4.8
Lysine	5.0	5.73	4.2
Methionine + Cystine**	1.8	2.06	6.4
Phenylalanine + tyrosine**	7.0	8.03	5.8
Proline	4.8	5.50	
Serine	4.3	4.93	
Threonine	4.2	4.82	2.8
Trypyophan	2.3	2.64	1.4
Valine	5.5	6.31	4.2
Total of amino acids	87.2		

* Recommended essential amino – acid content for an ideal protein for animal consumption (WHO/FAO) (Bhumiratana, 1976).

** Essential amino acids in vulgaris.

Table (2): Chemical composition of oven dry *Chlorella vulgaris*.

Component	g/100 gm dry weight
Protein	45.60 ± 1.4
Fact	17.90 ± 2.5
Total carbohydrate	10.10 ± 1.0
ASH	18.87 ± 2.15
Fiber	8.20 ± 1.1
RNA	2.10 ± 0.3
DNA	0.90 ± 0.1

The total nucleic acid content was quite low compared with that reported for algal cells by Bhumiratana (1976). The low level of nucleic acids in the algal cells is an advantage for the use of algal biomass as an animal feed supplement, since high levels of nucleic acid induce harmful effects on animals (Edozein *et al.,* 1970).

Badour *et al.* (1970) reported that algae can be used as direct or indirect material feed for fish. Algae can be incorporated into a food chain which eventually feeds to fish production Nikolsky, 1963).

The results in Table (3) showed the average body weight (± SE) of silver carp fish fed with diferent diets containing artificial fish food (AF) alone, 5%, 10%, 20%, 50%, 75%, 100% of dried algal biomass supplemented artificial fish food and on live algae. The data showed that there were no significant differences between 100% of dried algal biomass supplemented artificial fish food and on live algae ($P < 0.05$). The average body weight of fish fed artificial fish food supplemented with 20% dried algal cells was significantly the highest as compared with the fish fed with other ratios. The average body weight of silver carp fed with the living algal cells (A) was significantly lower than the average body weight of silver carp fed with (A_{20}) diet. It could be noticed that there were significant differences between AF and A_{100} diets. The daily gain in weight for fry fed with A_{20} diet gave gains which were significantly highest than the other diets. Feeding with A_5, A_{10}, A_{50} and A_{100} diets gave daily weight gains which were showed no significantly differences inbetween, ($P < 0.05$).

The survival rate of fish in all treatments was 100. The means of specific growth rates showed significant differences at $P > 0.05$ among treatments. The highest values (1.0) was observed following artificial fish food supplementation provided by 20% dried algal cells where the lowest values (0.54) was recorded for the living algal cells as fish died (A).

The average of specific growth rate (SGR) of silver carp fed on the diet of 20% which recorded 0.997 was higher than those that fed on the control (artificial fish food) and other diets. The average total of SGR of the whole silver carp fish tested ranged from 0.535 to 0.997. The present results are in harmony with those of Viola (1975). Kiessling and Askbrandt (1993) found SGR ranged from 0.34 to 0.65 for carp fish fed standard feed (15% fish meal). Viola and Zohar (1984) in tilapia ranged from 1.06 to 1.18 which fed on different levels of protein. Degani and Viola (1987) found that the averages of 0.56, 0.80 and 0.67 for eel fed diets contained 50, 40 and 30% of protein indoors.

Table (3): Mean individual weight and net daily growth of the silver carp *(Hypophthalmichthys molitrix)* fed on different diets of dried algal biomass supplemented artificial fish food and on live growth algal cells (A) for 32 days growth.

Diet	Stock group	* Individual mean wt(g)			Net daily gain (g)	Fish Survival%
		Initial ± StD.	Final ± StD	** gain (g)		
AF	10	3.69 ± 0.16	4.47 ± 0.13 ^{CD}	0.78 ^{E}	0.0244 ^{C}	100
A5	10	3.52 ± 0.19	4.49 ± 0.04 ^{C}	0.79 ^{D}	0.0304 ^{B}	100
A10	10	3.38 ± 0.14	4.65 ± 0.17 ^{B}	1.27 ^{A}	0.0397 ^{A}	100
A20	10	3.75 ± 0.16	4.77 ± 0.20 ^{A}	1.02 ^{BC}	0.0318 ^{B}	100
A50	10	3.39 ± 0.16	4.42 ± 0.05 ^{ECD}	1.03 ^{B}	0.0321 ^{B}	100
A75	10	3.33 ± 0.09	4.40 ± 0.12 ^{ED}	1.01 ^{C}	0.0315 ^{B}	100
A100	10	3.57 ± 0.34	4.36 ± 0.27 ^{EF}	0.79 ^{E}	0.0247 ^{C}	100
A	10	3.64 ± 0.17	4.32 ± 0.15 ^{F}	0.68 ^{F}	0.0213 ^{C}	100

* Means in the same column with the same letter are not significant.

** Gain = Final mean weight – Initial mean weight.

Probability (P) from t-test for the value was significant a: $P < 0.05$.

The data presented in Table (4) showed clearly that artificial fish food supplemented with 20% dried algal cells (A_{20}) was the best treatment for sliver carp grown in mass culture, it supported much better growth and lower food conversion ratio (FCR – 1.70) than that of artificial fish food alone 3.04. The living algal cells as fish died (A) was not suitable since it recorded the lowest growth and higher FCR of all treatments.

The data showed that FCR followed as opposite trend to those of feed uptake. This might indicate that the presence of dried algal biomass supplemented artificial fish food improved the feed conversion ratio. Similar results of FCR (2.0 to 3.54) were obtained by Viola *et al.* (1981) and Wang and Chan (1990) for carp; Viola and Arieli (1983) for tilapia AUREA (2.1 TO 3.45); Degani and Viola (1987) for eel (1.61 to 2.97) and Conrad et al. (1988) for catfish (2.4 to 2.9). In contrary, Viola and Zohar (1984) found that FCR ranged from 1.49 to 2.15 for tilapia fed on different protein levels while Shiau and Huang (1989) reported that feed conversion ratio had ranged from 1.23 to 5.64 for hybrid tilapia.

Fry fed with live grown algal cells (A) and 100% of dried algal biomass recorded significantly lower yields than other treatment groups ($P < 0.05$). There were no significant differences between the yield of fish fed on artificial fish food (AF), 5%, 50% and 75% of dried algal biomass supplemented artificial fish food. Fish fed on 10% and 20% showed significantly higher yields than thos fish fed on other treatments.

The protein percentage in various diets were significantly different. The artificial fish food and algal contain 25% and 46% protein respectively. The highest protein content was recorded in (A_{100}) and (A) 46.0%.

Table (4): Specific growth rate, food conversion and yield of the silver carp (*Hypophthalmichthys molitrix)* fed on different diets for 32 days growth.

Diet	Growth rate mg day^{-1}	Specific Growth Rate (SGR)	* Food Conversion Ratio (FCR)	Total yield (g/10 fish)
AF	24.4^{C}	0.60^{E}	3.04^{B}	44.7
A5	30.4^{B}	0.76^{BC}	2.37^{D}	44.9
A10	31.8^{B}	0.75^{C}	2.38^{D}	46.5
A20	39.7^{A}	1.00^{A}	1.70^{G}	47.7
A50	32.1^{B}	0.83^{BC}	2.03^{F}	44.2
A75	31.5^{B}	0.87^{B}	2.21^{E}	44.0
A100	24.7^{C}	0.63^{D}	2.93^{C}	43.6
A	21.3^{C}	0.54^{F}	3.42^{A}	43.2

*** Food conversion ratio (FCR) = Total feed (g) / Total gain (g).**

Table (5): Total feed (g) and composition of diets consumed by silver crap *(Hypophthalmichthys molitrix)* after 32 days growth.

Type of Diet	Stock group	Total feed (g)/ all fish/32 days	Feed (g)/ one fish/ 32 days	Feed (g) fish/ day	Diet composition (g) / fish /32 day	
					Artificial	algae
AF	10	236.8	23.86	0.74	23.68	00
A5	10	224.0	22.4	0.70	21.28	1.12
A10	10	240.00	24.000	0.75	20.0	2.40
A20	10	217.6	21.76	0.68	17.41	4.35
A50	10	208.0	20.8	0.65	10.4	10.4
A75	10	208.0	20.8	0.65	5.2	15.6
A100	10	227.2	22.72	0.71	00	22.72
A	10	233.6	23.36	0.73	00	23.36

Data presented in Table (6) showed the biochemical composition of experimental fishes. The protein content of fish grown in artificial fish food supplemented with 20% dried algal cells represented the highest value (64.88%), but the lowest protein value of silver carp fish was observed in artificial food and living algal cell (A) treatments. These results indicated the suitability diet A_{20} as a source rich in crude protein instead of traditional diet (AF).

However the percentage of protein in fish tissues fed on live algal cells (A), was significant lower than other treatment ($P < 0.06$). So, the present study, could be seen that crude protein percentage (CP%) in whole fish bodies received dried algal biomass diets (62.42 to 64.88%) were higher than that of fish fed the control (artificial fish food) diet (61.08) or living algal cells diets (61.82) Table (6) the obtained results agreed with those of Wee and Shu (1989) who found that the CP% in whole fish ranged from 61.42 to 64.23% in Nile tilapia fed diets contained boiled felled – fat soybean. Also, Shiau and Huang (1989) obtained the similar values of crude protein (61.2, 62.7 and 63.5%) in hybrid tilpaia.

The lowest fat content of the silver carp was observed in A20 ratio (16.56%). The highest fat percentage was observed in (A) treatment with living cells (19.42%). This result agreed with those of Shiau and Huang (1989), who found that the percentage of fat ranged from 19.81 to 26.13 in tilapia.

The lowest ash content was observed in A_{20}, A_{50} and A_{100} treatments while the highest content was recorded in artificial food (AF) treatment followed by A_5 treatment.

Table (6): Biochemical composition of fish tissues fed on different diets after 32 days growth.

Diet	% Ash	% Fat	% Protein
Af	17.66	18.09	61.08
A5	17.32	17.48	62.42
A10	16.33	17.04	63.72
A20	15.46	16.56	64.88
A50	15.36	17.44	64.08
A75	16.46	18.86	63.48
A100	15.58	18.98	63.06
A	17.23	19.42	61.82

The results in Table (7) showed that the ammonia level in the water was affected by the type of feed given. On day 14 significant differences ($P < 0.05$) between treatment means were recorded. Significantly higher ammonia levels were observed when fish were feed with 100% or 75% dried algal biomass supplemented with artificial fish food compared with other treatment. The ammonia levels in fish diet with AF and A_5 of dried algal biomass supplemented artificial fish were significantly lower compared with the other treatments. On day 21, the ammonia level in water containing fish feed with 20% of dried algal biomass supplemented to artificial fish food (A_{20}) was significantly lower compared with the other treatment ($P < 0.05$). The mean concentration of ammonia during the experiment showed that the water in tanks contained fish fed on 20% of dried algal biomass supplemented artificial fish food had lower concentration of ammonia 11.13 μg L-1 compared with other treatments. The highest concentration of ammonia was recorded in water aquaria of fish fed on 100% dried algal biomass ($p > 0.05$). The increase in ammonia can be attributed to the level of nitrogen excretion which depends primarily on the nitrogen content of the diet considering all other factors equal Meade (1985).

The availabilty of low cost algal concentrates would also allow the blending of these concentrates to produce nutritionally superior feeds (Borowitzka, 1997).

Then this study incare that *Chlorella vulgaris* could be used as a high source of protein to improve the quality of artificial fish food affected the daily silver carp growth, the protein content in flush and ammonia concentration of water, especially when artificial fish food substituted 20% dried algal biomass.

Table (7) :Water quality measurements temperature (°C), pH, dissolved oxygen (mg L^{-1}) and ammonia content (μg L^{-1}) during feeding experiment (32 days).

Diet	Temperature (°C)	pH	Disolved O_2 (mg L^{-1})	Ammonia (μg L^{-1})		% Nitrogen
				Day 14	Day 21	
AF	26.31	7.59	7.28^{AB}	11.3^{G}	15.27^{D}	0.945
A5	26.40	7.61	7.23^{CB}	11.7^{F}	15.62^{C}	0.939
A10	26.37	7.58	7.22^{A}	11.75^{F}	15.21^{D}	1.041
A20	26.22	7.61	7.15^{CD}	12.3^{E}	9.96^{G}	1.009
A50	26.25	7.60	7.0^{D}	15.57^{C}	14.3^{F}	1.174
A75	26.51	7.58	7.24^{B}	37.9^{B}	16.45^{B}	1.363
A100	26.33	7.62	6.94^{E}	45.4^{A}	22.54^{A}	1.68
A	26.35	7.60	6.85^{F}	13.1^{D}	14.7^{E}	1.713

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الأنتاج الكمى لطحلب الكلوريلا فولجارس وملائمة استخدامه لتغذية أسماك المبروك الفضى المرباه فى أحواض زجاجية.

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فى هذه الدراسة أشارت التحاليل المختلفة إلى أن خلايا طحلب الكلوريلا فولجارس غنية بثمانية عشرة حامض أمينى والمحتوى البروتينى ٤٦,٥ % وكانت نسب بعض الأحماض الأمينية أعلى من النسب الموصى بها من منظمة الأغذية والزراعة لتغذية الأسماك. ومن ثم تم النظر إلى استخدام خلايا الطحلب كعلف للأسماك. وقذ تم تنمية خلايا طحلب الكلوريلا فولجارس كمياً. وكان الهدف الرئيسى من هذه الدراسة هو أستخدام معدلات احلال مختلفة من خلايا طحلب الكلوريلا فولجارس فى تغذية أسماك المبروك الفضى المرباه فى أحواض زجاجية.

أشارت النتائج أن معدل النمو اليومى، معدل النمو النوعى، الإنتاج السمكى كانت الأعلى عند تغذية الأسماك باستخدام العليقة الصناعية مع نسبة إحلال ٢٠% خلايا طحالب جافة، ومن جهة أخرى أشارت النتائج إلى أن الزيادة من الأحلال من خلايا طحلب الكلوريلا فولجارس الجافة أكثر من ٢٠% أدت إلى نتائج سلبية ووجدت الدراسة ان تغذية أسماك المبروك الفضى على عليقة صناعية تحتوى على معدلات مختلفة من الإحلال حتى ٢٠% من خلايا الطحلب الجافة يتزامن معها الزيادة فى محتوى البروتين فى الأسماك. وبزيادة نسبة الحلال عن ٢١% تنخفض هذه النسبة. وقد وجد أن محتوى الأمونيا فى ماء الأحواض فى المدى الملائم لنمو أسماك وذلك أثناء إستخدام العليقة الصناعية مع نسبة احلال ٢٠% من خلايا الطحلب الجافة.