MINI-REVIEW

THE USE OF ALGAE CONCENTRATES, DRIED ALGAE AND ALGAL SUBSTITUTES TO FEED BIVALVES

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Abstract

Microalgae has high nutritional value and are used to feed adult and larval stages of bivalves, the larvae of some fish and crustaceans and zooplankton. However, microalgae production for aquaculture animal is very expensive. To overcome this, the use of preserved microalgae such as algae concentrate and dried algae, or algal substitutes has been developed. There are both advantages and disadvantages to this alternative food. For example, even though the cost production for algal substitute yeast-based diet is cheaper, their nutritional value is much lower compared to fresh microalgae. Moreover, there is no significant difference in nutritional value between preserved (concentrated or dried) and fresh microalgae; however, preserving microalgae for long periods will affect their nutritional value. In spite of this problem, preserved microalgae such as algal concentrate and dried algae seem to be more effective to feed bivalves than algal substitutes yeast based diet due to their availability and relatively high nutritional value. Furthermore, algae concentrates are more suitable to replace fresh algae than dried algae.

Keywords: algae concentrate, bivalves, dried algae, microalgae, yeast

1. Introduction

Microalgae has potential uses as a food source in aquaculture because of their nutritional value [1-4]. Microalgae are required for the nutrition of larvae and adult bivalves, the larvae of other molluscs, crustacean, fish, or live prey used in culturing such as rotifers and brine shrimp (*Artemia*) [2,5-7]. The content of lipids, proteins (amino acids), carbohydrates and vitamins of various microalgae species is one of the main reasons for considering these organisms as feed source for aquaculture animal [8]. Furthermore, their content of highly unsaturated fatty acids (HUFAs) especially eicosapentaenoic acid (20: n-3) (EPA), arachiodonic acid (20: n-6) (ARA), docosahexaenoic acid (22: n-3) (DHA) and linolenic acid (18: n-3) (LA) provides the most prominent determinant of the nutritional composition of microalgae (Table 1) [2,9-10].

Bivalves (clams, oysters, mussels, scallops) obtain their nutrition by filter feeding on fine particles such as phytoplankton throughout their lives. Aquaculture of bivalves consumes the highest production of microalgae diets compared to other aquaculture organisms (gastropods, crustacean and fish) [11-12]. The requirement of microalgal diets for bivalves varies depending on their developmental stage (broodstock, larval or post larval rearing). The larval stage needs a small amount but require a high quality of microalgae. On the other hand, broodstock preparation needs lots of microalgae quality [2]. Nowadays, bivalve aquaculture industries collect their larvae or spat from hatchery production to reduce the need to collect them from the wild. It occurs because the uncontrollable environmental conditions (salinity, food availability, temperature, etc) in the wild provide unreliable stock [13]. Therefore, it is important to provide the mass production of microalgae to feed larvae of in bivalve hatchery production's field.

The main difficulty associated with microalgae supply is the high operational cost which can run at almost half of the hatchery operating cost [8]. The price of microalgae inoculums is expensive, around AUD $120 and AUD $190 for 20 mL test tube and 250 mL tissue-culture flask, respectively [14]. Moreover, the introduction of diseases is possible and if the microalgae culture becomes infected with pathogenic organisms or if the systems fail, a shortage of food for aquaculture animals may occur [15]. Thus, some specialized facilities and equipment are necessary to scale-up the

Table 1. The n-3 Fatty Acid Composition (% total fatty acids) of Selected Species of Microalgae Used in Aquaculture [8]

Species	Fatty Acid		
	18:3n-3 (LA)	20:5n-3 (EPA)	22:6n-3 (DHA)
Golden-brown flagellates			
Isocrhysis clone T-ISO	3.6	0.2	8.3
Pavlova lutheri	1.8	19.7	9.4
Diatoms			
Chaetoceros gracilis	-	5.7	0.4
Chaetoceros calcitrans	Trace	11.1	0.8
Thalassiosira pseudonana	0.1	19.3	3.9
Green flagellates			
Tetraselmis suecica	11.1	4.3	trace
Dunaliella tertiolecta	43.5	-	-
Nannochloris atomus	21.7	3.2	trace

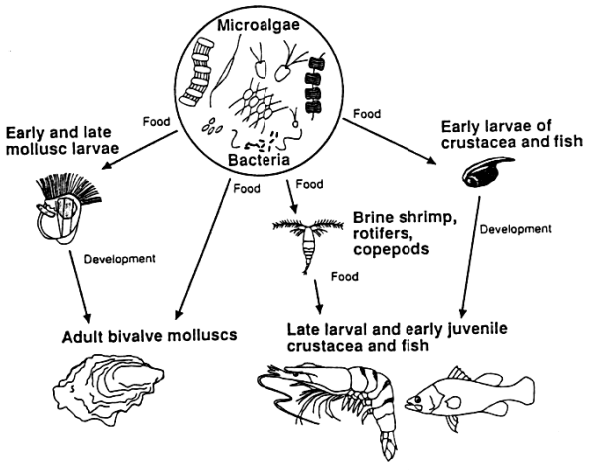

Figure 1. The Central Role of Microalgae as Feed for Aquaculture Animals [11]

culture and to prevent damage of the microalgae cultures and therefore loss of the food source. These include very expensive laminar cabinets which are placed in a special room with high illumination for inoculation and sterilization using either physical (filtration, autoclaving, pasteurization, UV irradiation) or chemical methods (chlorination, acidification, ozonisation) [12,15-16]. In addition, microalgae culture requires dedicated personnel on a daily basis and a large availability of space [8].

Although microalgae could be cultured using a simple and inexpensive method known as the Wells-Glancy method through the "bloom" of a local species of phytoplankton in a pond or tank, production using this method is unreliable due to contamination and fluctuating climatological conditions [8-9]. To deal with the challenges of microalgae culture, aquaculturists have examined an alternative method using artificial or alternative diets [17]. The application of alternative diets must meet several requirements. They must be easily digested, have adequate size to allow ingestion, have adequate nutritional composition and must be non toxic. Moreover, diets must be stable in seawater (not break apart easily) and should not trigger bacterial contamination [18]. So far, there are many studies on the use of alternative or artificial diets to feed bivalves such as the use of microalgae concentrate, dried microalgae, microcapsules diet, microbound diets [18], yeasts or yeast-based diets [19-20] and bacteria [21].

Concentrated algae have been successfully used as part of a mixed or complete diet for bivalves [22-24], however time appears to be a key factor in its success. The use of microalgae concentrates for longer than 4 weeks resulted in significantly lower growth of *C. gigas* juveniles compared to fresh microalgae [25]. This may occur because the longer storage period could decrease the quality of the stored concentrates [23,25]. The evidence available suggests that algae concentrates can be used to fully replace fresh microalgae to feed bivalves. However, further research is needed to determine more precisely the appropiate storage time to avoid the reduction of nutritional value of the algae concentrate.

Therefore, this review will examine on algae concentrate, dried algae and algal substitute like yeast as an alternative diets. In particular this review will discuss advantages and disadvantages of each diet and suggest which the best alternative diet to replace fresh microalgae to feed bivalves.

2. Algae Concentrates

One potential replacement of fresh microalgae as a food for bivalves is concentrated algae paste which can be formed by concentrating algae from mass cultures and preserving the resultant paste through refrigeration, freezing or drying [17,24,26]. The harvesting procedures to concentrate microalgae can be obtained by centrifugation [27], flocculation [28], filtration [29], foam fractionation [30] or photobioreactors [24]. Nowadays, the most commonly used techniques to produce concentrate algae are centrifugation and flocculation [28,31].

Algae paste production. Centrifugation has been reported as an effective method to harvest microalgae with a solid external structure including *Tetraselmis suecica*, *Skeletonema costatum* and *Chaetoceros* spp [25,32,33]. Centrifugation can also reduce the number of bacteria present in the microalgae culture, hence, improving the storage period of the concentrate [34]. However, this method has some drawbacks. The process

involves exposing cells to high gravitational forces which may damage the cell structure of some microalgae species [27]. Furthermore, the processing of large volumes of culture can be time-consuming and require costly equipment [23,27].

Flocculation is a procedure to remove suspended solids in solution by chemical reaction and can be applied with success to microalgae in suspension. Flocculation method has advantages in its simplicity and low cost [35]. Moreover, there is evidence of better growth when using flocculation instead of centrifugation. Knuckey *et al.* (2006) reported that juveniles of *Crassostrea gigas* showed better growth when fed *T. Pseudonana* concentrates from pH-induced flocculation than those from laboratory or cream separator centrifugation [28]. However, the main limitation of flocculation is that the residual liquid volume after concentration is low and would be problematic for storage in hatcheries [25]. Moreover, after flocculation, most of the cells lose motility and it would be difficult to disaggregate them back to a single cells which is a requirement condition for feeding them to bivalves [28].

Preservation of algae pastes. Once the microalgae concentrate is obtained, several methods have been used to preserve its quality during storage, such as adding additives or preservatives [36], freezing [27], and refrigeration [31]. The microalgae concentrate may be stored for several days [37], several weeks [25] or several months [38].

Generally, the maximum time the paste can be kept and still retain its nutritional value equivalent to fresh algae ranges from about 1 week to 4 weeks, depending upon the species of alga [27]. For instance, microalgae *Chaetoceros* sp., there are dramatic decreases in protein and carbohydrate content of microalgae concentrates

Table 2. Growth Trials with Bivalves Fed Microalgae Concentrates

Author	Species	Feed replacement	Result
Nell and O'Connor [41]	Larvae of *Saccostrea glomerata*	Mixed algae concentrated of *P. lutheri+Isochrysis galbana* and *P. lutheri+Chaetoceros calcitrans*	Equivalent and even better growth rate than larvae fed same diets of live microalgae
Knuckey [35]	Juvenile of *C. gigas*	Flocculated concentrates of *T. pseudonana* (1-4 week old)	70% growth rate of juveniles fed live algae
Mc Causland *et al*. [26]	Juvenile of *C. gigas*	Centrifuged concentrates (1-2 week old) of *C. calcitrans* and *Skeletonema costatum*	Similar growth rate to juvenile fed fresh microalgae
Heasman *et al*. [27]	Larvae of *Saccostrea glomerata*	Centrifuged concentrates of *P.lutheri* in combination with *C. calcitrans* or *Skeletonema costatum*	85-90% of the growth of larvae fed mixed diet of live microalgae
Robert *et al*. [32]	Umboned *C. gigas* larvae	Preserved *Tetraselmis suecica* concentrates	No significant differences in growth compared to fresh *T. suecica*
Brown and Robert [33]	Larvae and juvenile of *C. gigas*	Flocculated concentrates of *C. calcitrans*, *C. calcitrans* forma *pumilum*, *Chaetoceros* sp., *Skeletonema costatum* and *I. galbana*	Partial substitution or supplementation of concentrate algae to the diet gave a similar growth rate to the control
Ponis *et al*. [25]	Larvae and juvenile of *C. gigas*	Mixed diet of *P. lutheri*+T-Iso and T-iso+*C. calcitrans* concentrated (1-2 weeks storage)	Better growth for larvae but lower growth rate for juveniles fed live microalgae
Ponis *et al*. [23]	Larvae of *C. gigas*	Replacement of 50% or 80% of live *C. calcitrans* forma *pumilum* with *Pavlova lutheri* concentrated	Not adversely affect survival and growth rate of larvae
Knuckey *et al*. [28]	Juvenile *C. gigas* and the scallop *Pecten fumatus*	*Chaetoceros muelleri* concentrated	Effective as supplementary diets to improve the growth of juveniles under commercial conditions
Ponis *et al*. [24]	Larvae of *C. gigas*	*P. lutheri* concentrated	No significant difference in growth and survival of larvae fed live *P. lutheri*

during the first week of storage [33]. Preservation may cause microalgae lose carbohydrate are probably due to dark respiration by cells [39]. Furthermore, after a 1-month storage period, the algae concentrate of *Chaetoceros calcitrans* forma *pumilum*, *Chaetoceros* sp., *Skeletonema costatum* obtained by flocculation showed a loss in chlorophyll *a* of approximately 25% [33]. In contrast, chlorophyll *a* of *Tetraselmis suecica* centrifuged concentrates shows just less than 10% loss after around 14 weeks storage period at 4 °C [35]. Ponis *et al*. (2003a) found that the chemical composition of concentrated *C. calcitrans* cells remained stable during the first 3 weeks of storage but the organic matter decreased significantly after that period [25].

Temperature and dark condition are important factors that can increase the period of viability of algal concentrates. In terms of shelf life, microalgae concentrates of *C. muelleri*, *C. calcitrans*, and *Skeletonema* sp. remained viable for 6–7 weeks when stored in the dark under refrigeration [27,31]. Moreover, *Nannochloropsis sp*. suspensions at different cell concentrations preserved in darkness at 4 °C, maintained around 85% of their viability after two months storage [40]. Beside temperature and light, oxygen can also be an important factor. For instance, the shelf life of *P. lutheri* concentrate stored at low temperature with air bubbling was significantly longer than those stored at higher temperature and without air bubbling. This occurred because air bubbling supplies oxygen that can be used for respiration [24]. In some algae, storage at low temperature (above 0 °C) with addition for air bubbling will increase viability [24,27].

Although the production of concentrated microalgae is feasible, the issue is whether or not this concentrate is adequate for the culture of bivalve larvae. To date, the nutritional value of these concentrates of different microalgal species has been evaluated with larval and juvenile oysters with some promising results [25-26, 32,41,]. For example, concentrates of *Chaetoceros calcitrans* and *Skeletonema costatum* obtained by centrifugation and stored at 4 °C for a period of 1–2 weeks gave equivalent growth to fresh microalgae when used as part of a mixed diet for *Crassostrea gigas* juveniles [26]. Similarly, cold-stored *Tetraselmis* produced no significant difference in the growth rates or survival to live microalgae when tested as food for *C. gigas* larvae [32].

Nutritional value of algae paste. The nutritional value of some microalgal concentrates for bivalves is outlined in Table 2. Preserved concentrates of microalgae *Pavlova lutheri* have been evaluated on *Saccostrea glomerata* larvae, *Pecten fumatus* [27] and *C. gigas* larvae [23-24] with some good results have been obtained. For instance, a substitution of 50% or 80% of fresh *C. calcitrans* forma *pumilum* with the preserved *P. lutheri* concentrates did not adversely affect growth rate or survival of *C. gigas* larvae [23]. Furthermore, better growth rates than the equivalent fresh diets have been reported for *Saccostrea glomerata* larvae when fed *P. lutheri* + *Chaetoceros calcitrans* concentrate [41] and for *C. gigas* larvae when fed concentrated diets of *P. lutheri* + *T-Iso* stored for 7–14 days [25]. A recent study by Ponis *et al*. (2008) also found that there was no significant difference in survival and growth of *C. gigas* fed preserved or fresh *P. lutheri* after 14 days of rearing [24]. Thus, there is mounting evidence that algal concentrates also known as algal paste can produce the same or even better results than fresh microalgae when used for a short period of time.

3. Dried algae

Another alternative to artificial diets that may overcome the costly and unpredictable production of fresh microalgae is the use of algae that has been preserved by drying [17,42]. Early studies were carried out on the use of freeze dried *Isochrysis galbana* and *Dunaliella euchlora* compared to fresh algae as a food source to observe the growth rate of the hard shell clam larvae *Mercenaria mercenaria* [43]. However, the use of freeze dried algae to feed bivalves is still undeveloped probably due to unpromising results and their low viability. For example, the growth rate of the little-neck clam *Ruditapes decussates* seed fed on a diet of several dried microalgae was significantly lower than seed fed fresh microalgae [44]. Moreover, the substitution of a small proportion of freeze dried algae with fresh algae was enough to give a significant increase in growth rates of the seed culture. In addition, the viability of the algae is also affected by the drying process [44]. Molina-Grima *et al*. (1994) pointed out that *Isochrysis galbana* showed a 2% survival and decrease of viability over time after freeze drying [45]. Similarly, microscopic examination of freeze-dried *Chaetoceros* sp. and *Phaeodactylum tricornutum* after rehydration also showed that only a few cells were apparently viable [42].

Heterotrophic microalgae strain. Another method of producing dried algae is by heterotrophic production. This heterotrophic technique is achieved by growing algae using organic carbon instead of light as an energy source and is grown in fermenters [46,47]. The benefit of the heterotrophic technique is that microalgae production is at much higher densities and more cost effective than the photoautotrophic culture. The costs of intensive fresh microalgae production can reach more than US$200 per kg dry weight [48], while, spray dried microalgae products such as *Spirulina, Schizochytrim* and *Tetraselmis suecica* are approximately US$33, US$45 and US$170 per kg respectively [13,46]. Some species of microalgae are suited to heterotrophic production and some of these have been dried for

commercial production [13,46]. The resulting spray dried microalgae have shown satisfying results for feeding bivalves [20,49].

Nutritional value of spray dried algae. Successful partial replacement of a diet of fresh microalgae has been reported in several studies using spray dried diets (Table 3). For example, spray dried *Spirulina* for bay scallop [50], *Tetraselmis suecica* for juvenile *Tapes philippinarum* and *Crassostrea gigas* [51,52], *Spongiococcum excentricum* for *C. gigas* [49], and *Dunaliella salina* for larvae of *Saccostrea commercialis* [18]. According to Southgate *et al*. [20], spray dried *Tetraselmis* (TET) successfully replaced around 50% of the equal mixture of the three fresh microalgae diets (TAD) without significantly affecting survival and growth rate of the blacklip pearl oyster *Pinctada margaritifera* larvae, and there was a significantly greater survival and growth of larvae fed on a mixture of 1 : 1 TET and TAD than those fed TAD alone. Similarly, spray dried *Tetraselmis suecica* and *Nannochloris* sp. diets supported growth equal to or greater than their fresh algae counterparts but less than a mixture of two high nutritional live microalgae *Chaetoceros calcitrans* and *Isochrysis galbana* (clone T-ISO) [53]. However, *Ostrea edulis* larvae which were fed exclusively dried *Tetraselmis* suffered high mortality and low growth rates [53]. Furthermore, there have been many studies reporting experiments of growth trials with bivalves fed dried microalgae as shown in Table 3.

A growth experiment carried out by Boeing found that spray dried *Schizochytrium* can replace about 40% of mixed fresh *Tetraselmis suecica* and *Chaetoceros* sp. fed to clams without reducing the growth rate [54].

Table 3. Growth Trials with Bivalves Fed Dried Microalgae

Author	Species	Feed replacement	Result
Hidu and Ukeles [43]	Larvae of *Mercenaria mercenaria*	Freeze-dried *Dunaliella* and *Isochrysis.* Heat dried *Scenedesmus*	Early study with successful larvae survival to metamorphoses
Laing *et al*. [53]	Larvae of *Tapes philippinarum*	Spray dried *Nannochloris* and *Tetraselmis*	Growth equal to same fresh algae but less than mixture of fresh algae
Laing and Verdugo [51]	Spat/Juvenile of *Crassostrea gigas, Ostrea edulis, Tapes philippinarum, Tapes decussate, Mecenaria mercenaria*	Spray dried *Tetraselmis*	Growth similar to fresh *Tetraselmis* but less than for *Chaetoceros* or mixed algal diets
Laing and Milican [52]	Juvenile of *Tapes philippinarum*	Mixture of spray dried 30:70 of *Cyclotella cryptica* and *Tetraselmis suecica*	Growth rate was not significantly different from juveniles fed live microalgae
Boeing [54]	Juvenile of *Tapes semidecussata, C. gigas*	Spray dried algal product *Schizochytrium* sp.	Successful as a partial substitute for fresh algae and at 40% replacement not significantly reducing clam growth rate
Albentosa *et al*. [44]	Larvae of *Ruditapes decussatus*	Freeze-dried *Isochrysis galbana*, clone T-ISO; *Tetraselmis suecica*; and *Phaeodactylum tricornutum*	Growth rates of seed fed dried microalgae were significantly lower than fresh algae diets
Southgate *et al*. [20]	Larvae of *Pinctada margaritifera* (L.)	Dried *Tetraselmis*	Growth of larvae fed a 1:1 mixture of fresh algae and dried *Tetraselmis* was significantly greater than that of oyster fed fresh algae
Langdon and Onal [46]	Juvenile of *Mytilus galloprovincialis*	Spray dried algal products *Schizochytrium* sp., *Spirulina Platensis, Hematococcus pluvialis*	Growth rate was significantly greater than that of mussels fed live microalgae

Moreover, both dry organic and wet weight of juveniles *Mytilus galloprovincialis* fed diets composed of spray-dried microalgae products (*Spirulina platensis, Hematococcus pluvialis, Schizochytrium* sp.) were greater than those fed a full fresh microalgae ration (Langdon and Önal 1999). The results suggested that these spray dried products can satisfy all nutritional requirements of mussels. This is further supported by the study of Langdon and Onal, who reported that *Schizochytrium* sp. (Docosa Gold) is rich in DHA which is nutritionally essential (support growth rate) for mussels [46].

In spite of the promising results there are several disadvantages of using heterotrophic techniques, including the limited number of microalgae species that can be grown by this method. To date, just a few spray dried microalgae are grown commercially for use as aquaculture feeds such as *Crypthecodinium* sp. and *Schizochytrium* sp. which are marketed by Aquafauna BioMarine, Inc. CA, USA and Advanced BioNutrition Corp., MD, USA [47]. Another disadvantage is that heterotrophic cultivation has potential to be contaminated by bacteria and growth inhibition when cultured in low organic substrate concentration [47]. Overall, dried algae appear to be a good feed supplement but may not fully replace fresh microalgae due to their lower nutritional value.

4. Yeast Based Diets

Yeast can be used as an algal substitute to feed bivalves because it can be produced much more economically (using inexpensive culture media) and efficiently (shorter generation time) than photosynthetic microalgae [55-56]. There is some evidence of positive results using yeasts. Juvenile *Mytilus edulis, Argopecten irradians* and *Mercenaria mercenaria,* which were fed

Table 4. Growth Trials with Bivalves Fed Yeast Based Diets. Control Animals were Fed Fresh Microalgae

Author	Species	Feed replacement	Result
Epifanio [57]	- Juvenile of *Mytilus edulis, Argopecten irradians, Mercenaria mercenaria* - Juvenile *Crassostrea virginica*	- Diets containing 50% spray dried *Candida utilis* yeast - Yeast as partial algal rations	- Grew faster than juvenile control - Growth rate decreased with the amount of yeast in the diets
Alatalo [63]	Juvenile *C. virginica*	Mixture diet of 1:1 *I. galbana* and yeast	No significant difference in the increase of dry tissue weight than control but reduced growth when fed more than 50% replacement
Urban and Langdon [55]	*Crassostrea virginica*	More than 10.4 mg of yeast/day	Growth was depressed
Nell [41]	Adult *S. commercialis*	*Candida utilis* yeast	Successful as a protein source in fattening diets
Coutteau and Sorgeloss [60]	*Tridacna* clam	100% yeast	Capable of development when fed yeast only
Nell *et al*. [64]	Spat of *Saccostrea commercialis*	Yeast as a supplement to algal diet	Can improve the growth rate
Coutteau *et al*. [56]	Juvenile *M. mercenaria*	50% or 80% *Saccoromyces cerevisiae*	Not adversely affect growth compared to control
Nell *et al*. [64]	Spat of *S. commercialis*	80% substitution of live and dry yeast	Spat grew around 70% than control
Brown *et al*. [19]	Juvenile Sydney rock oyster (*S. commercialis*)	Substitution with 86% live yeast	Weight increases around 70% of those obtained on an algal diet
Southgate *et al*. [20]	Larvae of *Pinctada margaritifera*	Commercial yeast based diet	The growth rate of larvae fed yeast alone was significantly lower but not different in survival than those fed live microalgae.

on diets containing 50% yeast, grew faster than controls [57]. However, there have also been negative results [43,58]. The growth rate of *Crassostrea virginica* declined with the amount of yeast in the diet [57] and their growth was depressed if more than 10.4 mg of yeast per day was added to the partial algal rations [55]. Therefore, the benefit of using yeast in the diets of cultured bivalves remains inconclusive.

Yeast based diets have been shown to be of generally poor nutritional value for bivalves and this has been attributed to the low digestibility of the cell wall and a deficiency or imbalance of essential nutrients [19-20]. The bivalve's stomach and digestive diverticular are well equipped for the digestion of algal carbohydrates by the presence of various enzymes such as aminarinase and chitinase [59]. However, these enzymes may not be necessarily appropriate for efficient digestion of the polysaccharides composition the cell wall of intact yeast cells [60]. In addition, yeast lacks polyunsaturated fatty acids although they have high levels of good quality protein [19]. Therefore, yeast alone cannot be used to feed bivalve but can be used as a supplement with live microalgae rich in HUFAs.

The development of techniques to improve the nutritional composition and digestibility of yeasts has resulted in a modified or manipulated yeast product with great potential to feed bivalves [17]. There are many reports of promising results by using modified yeast diets as an algal substitute (Table 4). For example, replacing 80% of the algal diet for seed of *Crassostrea gigas* and *Tapes philippinarum* yielded an average daily grown rate around 75% of that measured in the algal control treatment [61]. Similarly, the Sydney rock oyster (*Saccostrea commecialis*) had a growth rate of up to 76% of that obtained for an algal control when replacing 80% of the algal ration with "Microfest" yeast. Moreover, about 50% of an algal ration fed to juvenile clams *Mercenaria mercenaria* could be replaced with modified yeast without affecting growth rates significantly [56]. Albentosa *et al*. (1989) also reported that the Manila clams *Tapes semidecussata* achieved 64% to 93% growth rates of the algal control when fed by replacing 80% of the algae with manipulated yeast [62]. Therefore, manipulated yeast is a better food alternative than untreated yeast due to lack of PUFA in yeast [19]. Moreover, technology development will support culturing media to grow manipulated yeast which has high nutritional value for bivalves.

5. Conclusion

Microalgae culture requires high investment and is very costly, so, minimizing production cost is required in bivalve culture as microalgae are very important for both larvae and adult bivalves. Today, this problem may be solved by using algal substitutes such as yeast based diets but research is still needed to make these diets suitable for bivalves. On the other hand, alternative diets or preserved microalgae show more promising results than yeast based diets. Experimental results suggest that the algae concentrate is more suitable to replace fresh microalgae than dried algae. Algae concentrate may be used to fully replace fresh microalgae but better period of storage for algae concentrate need to be study further. Moreover, microalgae concentrate tends to clump when added in to the water as food for bivalves. On the other hand, dried algae may be just useful to partially replace fresh microalgae due to the heterotrophic algae strain's lack of fatty acid although their cost production is cheaper and their density is higher than microalgae concentrate.

Therefore, algae concentrate is the best replacement for fresh microalgae for feeding bivalves. For future development, more research is needed to find the best way to minimise problems with the use of algae concentrate such as storage time and mechanisms to maintain the nutritional value and their availability, and also to prevent clumping in the water.

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