THE NUTRITIVE VALUE OF RED SEAWEED, *Kappaphycus alvarezii* MEAL IN FORMULATED FEED OF JUVENILE ASIAN SEABASS, *Lates calcarifer*

NIK SITI ZAIMAH BINTI SAFIIN

BORNEO MARINE RESEARCH INSTITUTE
UNIVERSITI MALAYSIA SABAH
2014

THE NUTRITIVE VALUE OF RED SEAWEED, *Kappaphycus alvarezii* MEAL IN FORMULATED FEED OF JUVENILE ASIAN SEABASS, *Lates calcarifer*

NIK SITI ZAIMAH BINTI SAFIIN

THESIS SUBMITTED IN FULFILLMENT FOR THE DEGREE OF MASTER OF SCIENCE

**BORNEO MARINE RESEARCH INSTITUTE
UNIVERSITI MALAYSIA SABAH**

2014

DECLARATION

I hereby declare that the material in this thesis is original except for quotations, excerpts, summaries and references, which have been duly acknowledged.

29 August 2014

____

NIK SITI ZAIMAH BINTI SAFIIN

PO2011-8328

CERTIFICATION

NAME	: **NIK SITI ZAIMAH BINTI SAFIIN**
MATRIC NO.	: **PO2011-8328**
TITLE	: **THE NUTRITIVE VALUE OF RED SEAWEED, *Kappaphycus alvarezii* MEAL AS AN INGREDIENT IN FORMULATED FEED OF JUVENILE ASIAN SEABASS, *Lates calcarifer***
DEGREE	: **MASTERS OF SCIENCE (AQUACULTURE)**
VIVA DATE	: **16 July 2014**

DECLARED BY

1. SUPERVISOR
(Prof. Madya Dr. Rossita Shapawi) Signature

____

2. CO-SUPERVISOR
(Prof. Dr. Shigeharu Senoo) ______________________

ACKNOWLEDGEMENTS

Alhamdulillah, I am really grateful to Allah S.W.T. for blessing me in my journey to complete my dissertation. A token of appreciation to Director of Borneo Marine Research Institute, Prof. Dr. Saleem Mustafa; Acting Dean of Centre for Postgraduate Studies, Prof. Dr. Mariam binti Abd Latip and also Vice-chancellor, Universiti Malaysia Sabah, Prof. Dr. Harun Abdullah for the encouragement and continuous support to this study.

My supervisors are backbone for this study. I am sincerely thankful to my main supervisor, Prof. Madya Dr. Rossita Shapawi for her continuous advice, support and guidance throughout the study. Having a great co-supervisor, Prof. Dr. Shigeharu Senoo gave me supportive idea and outstanding advice make this study become more valuable.

With this opportunity, I would like to acknowledge Dr. Faihanna Ching Abdullah for her valuable advice and encouragement. Appreciation also goes to the hatchery supervisor, Mr. Norazmi Osman and all UMS hatchery staffs, Borneo Marine Research Institute for the outstanding assistance and technical support during the study. I would like to say thank you to all my fellow friends who have been very supportive and lend me generous ideas, knowledge and love especially during difficult time of this study. Last but not the least, I would like to acknowledge my beloved parents for giving me an opportunity to pursue my dream, unconditional love, encouragement and understanding throughout these years. Support from my beloved siblings and other relatives are also greatly appreciated.

Nik Siti Zaimah Binti Safiin
25 August 2014

ABSTRACT

Two separate trials were conducted to evaluate the potential of seaweed meal (*Kappaphycus alvarezii*) as an ingredient in the formulated diets of Asian seabass, *Lates calcarifer*. Experiment 1 aimed to evaluate the possibility of replacing commercial feed binder, carboxymethyl cellulose (CMC) with different inclusion levels of *K. alvarezii* meal at 2, 4, 6, 8 or 10% (SW2, SW4, SW6, SW8 or SW10). A diet without seaweed inclusion (SW0) served as the control treatment. Triplicate groups of 15 fish were randomly stocked in cylindrical cages placed in a 10-tonne high density polyethylene (HDPE) tank and fed experimental diets for eight weeks. Water stability indices (after 60 min) of SW6, SW8 and SW10 were not significantly different (P>0.05) from the control diet (SW0). The highest weight gain and specific growth rate (SGR) were observed in fish fed SW6 (382.85±58.38% (P<0.05) and 2.81±0.21%/d, respectively) and the poorest was in SW0 (301.95±46.31% and 2.48±0.20%/d, respectively). No significant difference on feed conversion ratio (FCR) (1.54±0.20-1.83±0.05) was detected in all dietary treatments. Survival rate was above 96% and not affected by the inclusion level of seaweed meal. Insignificant (P>0.05) level of whole-body moisture, protein, lipid and ash were shown in all treatments. In Experiment 2, a 10-week feeding trial was carried out to determine if cooking process can improve the utilization of seaweed meal. Experimental diets were formulated with cooked seaweed meal at 6, 10, 14, 18 and 22% inclusion levels (SW6, SW10, SW14, SW18 and SW22, respectively). Diets with raw seaweed at 6% (SW6R) and without seaweed (SW0) inclusion served as the control treatments. Triplicate groups of 20 fish were stocked in a 150 L fibreglass tank and fed until satiation twice daily. Faeces were collected from week 6 until week 10 for the measurement of diet's apparent digestibility coefficient (ADC). Cooked seaweed, SW6 (97.48±0.37), SW10 (97.99±0.32), SW14 (98.35±0.29), SW18 (98.36±0.16) and SW22 (98.31±0.12) performed significantly (P<0.05) better in terms of water stability than the uncooked seaweed, SW6R (95.25±0.50) and comparable (P<0.05) with control diet, SW0 (98.26±0.25). In this experiment, fish fed 6% cooked *K. alvarezii* meal (SW6) showed significantly (P<0.05) higher weight gain and SGR (785.02±30.70% and 3.11±0.05%/d, respectively) and resulted the best FCR (1.18±0.06) compared to other treatment. Survival was affected by the inclusion of seaweed meal in the diets, where the highest inclusion level (SW22) resulted in the poorest survival (83.00±5.77). Dry matter ADC ranged from 59.03±7.88% to 73.65±1.19%. Protein ADC of the diets decreased with increasing seaweed level and ranged from 69.06±0.49% to 92.05±0.09%. Lipid ADC were not significantly difference (P>0.05) among treatments. In conclusion, the best incorporation of seaweed meal as binder was at 6% replacement level either raw or cooked seaweed which consistently giving the best growth performance, feed efficiency, survival, diet's digestibility and cost effective. However, replacement level from 6% to 10% can be considered to be include as it improve diet's stability and suitability used as feed binder to replace CMC and eventually improve the fish performance.

ABSTRAK

Nilai Nutrisi Serbuk Rumpai Laut Merah, Kappaphycus alvarezii Dalam Formulasi Makanan Ikan Juvenil Siakap Asia, Lates calcarifer

Dua percubaan pemakanan telah dijalankan untuk menguji potensi serbuk rumpai laut (Kappaphycus alvarezii) sebagai ramuan dalam formulasi diet ikan siakap juvenil, Lates calcarifer. Eksperimen 1 bertujuan mengenalpasti kemungkinan untuk menggantikan bahan pengikat makanan komersil, carboxymethyl cellulose (CMC) dengan serbuk K. alvarezii pada kadar 2, 4, 6, 8 atau 10% (SW2, SW4, SW6, SW8 atau SW10). Diet tanpa penambahan rumpai laut (SW0) bertindak sebagai rawatan kawalan. Tiga kumpulan ikan sebanyak 15 ekor dimasukkan secara rawak ke dalam sangkar silinder yang distok dalam tangki HDPE 10-ton dan diberi makan selama lapan minggu. Kadar kestabilan air (selepas 60 min) untuk SW6, SW8 dan SW10 menunjukkan tiada perbezaan bererti dengan diet kawalan. Pertambahan berat dan kadar pertumbuhan spesifik (SGR) tertinggi dicatatkan pada SW6 (382.85±58.38% (P<0.05) dan 2.81±0.21%/d) dan yang terendah pada SW0 (301.95±46.31% dan 2.48±0.20%/d). Tiada perbezaan bererti pada kadar penukaran makanan (FCR), (1.54±0.20-1.83±0.05) dalam kesemua rawatan makanan. Kadar kemandirian ikan tidak dipengaruhi oleh kandungan rumpai laut iaitu 96% ke atas. Kandungan proksimat dalam badan ikan menunjukkan tiada perbezaan bererti (P>0.05). Dalam Eksperimen 2, satu percubaan pemakanan selama 10 minggu dijalankan untuk mengenalpasti samada proses memasak boleh meningkatkan kadar penggunaan rumpai laut. Diet dengan rumpai laut yang dimasak dirumuskan dengan kadar kandungan 6, 10, 14, 18 dan 22% (SW6, SW10, SW14, SW18 dan SW22). Diet mengandungi 6% rumpai laut mentah (SW6R) dan diet tanpa rumpai laut (SW0) bertindak sebagai rawatan kawalan. Tiga kumpulan 20 ekor siakap juvenil dimasukkan ke dalam 150 L tangki kaca gentian dan diberi makan sehingga mencapai tahap kepuasan nyata. Najis ikan dikumpulkan dari minggu 6 hingga 10 untuk mengenalpasti koefisien pencernaan nyata (ADC). Rumpai laut yang dimasak, SW6 (97.48±0.37), SW10 (97.99±0.32), SW14 (98.35±0.29), SW18 (98.36±0.16) and SW22 (98.31±0.12) menunjukkan kadar kestabilan air yang lebih baik dan bererti (P<0.05) berbanding rawatan kawalan, SW6R (95.25±0.50) dan setanding (P>0.05) dengan diet kawalan, SW0 (98.26±0.25). Keputusan menunjukkan kadar kestabilan air yang bererti (P<0.05) meningkat (selepas 60 min) dengan peningkatan kandungan rumpai laut dalam diet. Dalam ekperimen ini, rawatan SW6 yang dimasak menunjukkan pertambahan berat dan SGR yang lebih tinggi (P<0.05), (785.02±30.70% and 3.11±0.05%/d) dan nilai FCR terbaik (1.18±0.06) berbanding rawatan lain. Kadar kemandirian dipengaruhi oleh kandungan tahap rumpai laut di mana SW22 menunjukkan kadar kemandirian yang terendah (83.00±5.77). ADC bahan kering di antara 59.03±7.88% ke 73.65±1.19%. ADC untuk protein menurun dengan pertambahan kandungan rumpai laut iaitu di antara 69.06±0.49% to 92.05±0.09%. ADC untuk lipid tidak menunjukkan perbezaan bererti (P>0.05) untuk kesemua rawatan. Keseluruhannya, penggantian serbuk rumpai laut pada 6% sama ada mentah atau dimasak menunjukkan kesan terbaik ke atas kadar pertumbuhan, kecekapan pemakanan, kemandirian, penghadaman makanan dan keberkesanan kos. Walaubagaimanapun, penggantian pada 6% hingga 10% boleh diambilkira kerana peningkatan kestabilan diet dan kesesuaian sebagai pengikat menggantikan CMC dalam makanan dan seterusnya meningkatkan keseluruhan persembahan ikan.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

µm	micrometre
ADCs	Apparent digestibility coefficients
ANOVA	analysis of variance
AOAC	*Association of Official Analytical Chemists*
Bhd.	*Berhad*
BMRI	Borneo Marine Research Institute
BW	Body weight
cm	centimetre
CMC	carboxymethylcellulose
CP	crude protein
CL	crude lipid
DFM	Department of Fisheries Malaysia
DO	dissolve oxygen
et al.	and others, and the rest
ed.	edited, edition, editor
FAO	*Food and Agriculture Organization*
FCR	feed conversion ratio
FM	fish meal
g	gram
h	hours
HDPE	High density polyethylene
HSI	hepatosomatic index
Kg	Kilogram
L	Litre
m	Metre
m^3	cubic meter
mg	Milligram
min	Minute
ml	Milliliter
mm	Millimeter
NFA	National Fisheries Authority

NPU	net protein utilization
NRC	National Research Council
PER	protein efficiency ratio
pH	potential hydrogen
ppt	part per thousand
QPIF	Queensland Primary Industries and Fisheries
RM	Ringgit Malaysia
SD	standard deviation
SGR	Specific growth rate
spp.	Species
SPSS	*Statistical Package for Social Science*
UMS	Universiti Malaysia Sabah
USA	United States of America
VSI	Viscerosomatic index

CHAPTER 1

INTRODUCTION

Feed is one of the most critical inputs in aquaculture farm because it constitutes more than 50% of the production cost and indispensable in intensive culture system. Today the demand for aquaculture feed is increasing as the result of the rapid expansion of the industry throughout the world. As a result, the cost of aquaculture feed and ingredients have been rising and are likely to continue to rise. This will hinder the sustainability of the industry. In marine fish farming industry, the sustainability was threatened by the over-dependence on fish meal and fish oil as the primary ingredients in the feeds (Sugiyama *et al.*, 2004). Malaysia is not a dedicated producer of fish meal and most of the time depends on expensive feed ingredients that must be imported.

Recently, increasing efforts have been made to find the potential alternative ingredients to be included in formulated feed in order to produce cost-effective fish feeds. However, finding alternative ingredients that are produced in sufficient quantities to become standard components of fish feeds requires a thorough evaluation including the understanding of an ingredient's nutritional value, ability to blend with other ingredients, effects on pellet stability and its appeal to fish. Several agriculture by-products of animal and plant origin have been evaluated for possible inclusion in fish feeds with promising findings (El-Sayed, 1994; Williams *et al.*, 2003; Lanari and D' Agaro, 2005; Shapawi *et al.*, 2007; Shapawi *et al.*, 2008; Zhou *et al.*, 2011; Plaipetch and Yakupitiyage, 2012; Shapawi *et al.*, 2013). More attempts are needed considering the rapid growth of aquaculture industry and increasing demand of marine protein. Particular emphasis should be focused on maximizing the use of locally available feed ingredients, nutritionally sound and safe feed ingredient sources whose production and growth can pace with the 8 to 10 percent average annual growth of the fed finfish and crustacean aquaculture sector (Tacon *et al.*, 2006).

Of all the plant protein tested for inclusion in aquaculture feeds, one of the promising natural food resources is the seaweed. Seaweed is well-known for its nutritional value, efficient productivity and has been used as food source for decades. Recently, seaweed is recognized as a novel food source with elevated high nutritional benefits such as proteins, minerals, vitamins and non-starch polysaccharides (Mabeau and Fleurence, 1993, Wong and Cheung, 2000; Kumar and Kaladharan, 2007). In Malaysia, seaweed has played an important role in the development of aquaculture sector and economically important resources especially in the East Coast of Sabah (Sade *et al.*, 2006). They are available commercially either from the wild or cultivated with *Kappaphycus alvarezii* being the most abundantly cultured species with sun-dried product sold in local markets at reasonable prices. Abundantly available dried seaweed contains polysaccharides known as carrageenan has been exported to other countries as hydrocolloid thickening agents and stabilizer especially in food industry. To date, carrageenan extraction has been locally operated by producing semi-refined carrageenan (SRC) powder and used for exporting purpose (Sade *et al.*, 2006). Realizing the fact that seaweed polysaccharides are important tools in providing the food formulators to produce product with increased acceptability, it should be diversified to expand the industry. In related to aquaculture perspective, producing formulated feed based on available and cheaper resources to increase aquaculture production should be introduced. However, the use of seaweed powder/ meal and their extract are still relatively new with regards to their uses as feed ingredients thus require considerable research to ascertain the true potential and value (Tacon *et al.*, 2006). A thorough investigation on the potential of seaweed inclusion in formulated fish feeds will generate new knowledge on alternative aquafeed ingredients to support the rapid development of local and global aquaculture sector.

In general, development of a practical diet would require the combination of several ingredients to meet the nutritional requirement of the target species. In this process, addition of feed binder is necessary to bind all these ingredients so that the nutrients will be delivered to the cultured organisms. Without proper binding, feed are prone to leaching due to poor stability and early disintegration. Seaweed meal may serve as a feed binder and also source of essential nutrients in fish feeds. However, little information are available on binding agents required for fish feed stability. Inclusion of seaweed meal instead of pure binder can reduce the diet costs and reduce the dependency on fishery-based ingredients.

Seaweeds being rich in polyunsaturated fatty acids, minerals, vitamins, as well as phycocolloids (Mabeau and Fleurence, 1993; Jimenez-Escrig and Sanchez-Muniz, 2000; Fleurence, 1999; Wong and Cheung, 2000; Sanchez-Machado, 2004; Dawczynski *et al.*, 2007; Lourenço *et al.*, 2002; Ruperez, 2002; Kumar *et al.*, 2011; Matanjun *et al.*, 2009). Partial substitution of costly protein sources in animal feeds with seaweed protein may improve feed quality (Kumar and Kaladharan, 2007. Specifically, *K. alvarezii* is used in current study due to its functional components such as kappa-carrageenan, good profile of essential fatty acids compared to green and brown seaweed and comparable level of vitamin, essential trace minerals and antioxidants (Santoso *et al.*, 2006; Matanjun *et al.*, 2008; Matanjun *et al.*, 2009; Fayaz *et al.*, 2005; Rajasulochana *et al.*, 2010; Rajasulochana *et al.*, 2011). Due to its nutritive value, *K. alvarezii* and its extracts has successfully used as food supplement, source of natural antioxidants used for health food or neutraceutical supplement, ingredients in functional foods, thickeners and gelling agent in food industry, possess the biological effects such as antimicrobial, anti-inflammatory and immunostimulant (Fayaz *et al.*, 2005; Cheng *et al.*, 2007; Rajasulochana *et al.*, 2010; Kumar *et al.*, 2011; Rajasulochana *et al.*, 2012).

Limited study was conducted to investigate the potential of seaweed meal in the formulated diets of cultured fish (Nakagawa and Montgomery, 2007). The reported use of seaweed meal as a feed binder is also limited to a few aquatic species only such as snakehead, *Channa striatus* (Hashim and Mat Saat, 1992) and tiger prawn, *Penaeus monodon* (Peñaflorida and Golez, 1996) with promising findings.

Asian seabass, *Lates calcarifer* is one of the most cultured fish species in the Asia Pacific region with established culture technique, stable demand and good market price (Catacutan and Coloso, 1995; Boonyaratpalin *et al.*, 1998; Ravisankar and Thirunavukkarasu, 2010). Like other marine fish species, the growth of Asian seabass farming is also challenged by the increase price of feed, dependency on imported feed ingredients and unacceptability of plant-based product. Development of feeds with sustainable resources and preferably using locally available ingredients will be able to ease these problems and eventually improve feed utilization and acceptability. In the present study, two experiments were conducted to evaluate the nutritive value of seaweed meal from *K. alvarezii* and possibility as an ingredient in the practical diets of juvenile Asian seabass. Specifically, seaweed meal from *K. alvarezii* was used as nutritive binder to replace the commercial feed binder, carboxymethyl cellulose (CMC).

1.1 Objectives of Study

Experiment 1 aims to determine the effectiveness of seaweed meal, *K. alvarezii* as binding agents which incorporated in the practical diets of juvenile Asian seabass with the objectives:

(i) to identify the nutritive value and effects as feed ingredient
(ii) to replace commercial feed binder with different inclusion level
(iii) to evaluate the diet's stability through consistency in water
(iv) to evaluate the effects on growth performance, survival, feed utilization and body composition of juvenile Asian seabass.
(v) to evaluate the cost effectiveness of different seaweed inclusion level in fish feed.

Meanwhile, Experiment 2 was conducted to determine the effects of different level of cooked-seaweed meal with the objectives:

(i) to improve feed quality and acceptability of Asian seabass
(ii) to determine the optimum inclusion level in formulated feed
(iii) to evaluate the effects on diet's stability
(iv) to evaluate the growth performance, survival, feed utilization, body composition
(v) to evaluate the diet's digestibility on Asian seabass performance
(vi) to evaluate the cost effectiveness of different seaweed inclusion level in fish feed.

CHAPTER 2

LITERATURE REVIEW

2.1 Aquaculture and Aquafeed Production

Aquaculture contributed 42.2% to the world total fish production by producing around 66.6 million tonnes in 2013 (Table 2.1). Malaysia fish food supply has grown dramatically in the last few decades. The aquaculture production in Malaysia requires at least 690,000 tonnes of formulated feed yearly to sustain aquaculture production. Unfortunately only about 100,000 tonnes were produced locally and the remaining must be imported (Musa and Nuruddin, 2005).

In any aquaculture farms, feed constitutes the highest proportion in operating cost (Boonyaratpalin *et al.*, 1998). This is particularly evident in the farming of carnivorous marine finfish species and shrimp (Tacon *et al.*, 2006). Fish meal has traditionally used as major ingredient in fish feeds due to its protein quality and palatability (Lovell, 1984). However increasing demand, high cost and uncertain availability of fish meal (Barlow, 1989) have resulted in finding new alternative protein sources of plant and animal origins to replace fish meal in the diet formulation. In this chapter, the review will be focusing on the alternative ingredient of plant origins.

Table 2.1: World production trend of aquaculture contribution (million tonnes)

Contribution	2007	2008	2009	2010	2011	2012
Aquaculture						
Million tonnes	49.9	52.9	55.7	59.0	62.0	66.6
Percentage (%)	38.2%	39.9%	38.2%	39.9%	40.1%	42.2%
Capture						
Million tonnes	90.8	90.1	90.1	89.1	93.7	91.3
Percentage (%)	64.5%	63.0%	61.8%	60.1%	59.9%	57.8%
Total Fish Production (Million tonnes)	140.7	143.1	145.8	148.1	155.7	158.0

Source: FAO, 2014

2.2 Alternative Plant By-Product in Fish Feed

Alternative feed ingredients and additives have been actively searched in the development of cost-effective aquaculture feeds. The suitability of plant by-product which is incorporated in fish feed must fulfill the requirement of being widely available, cost-effective and nutritious to produce high quality fish feed which give less environmental impact and favourable fish flesh for human consumption and health (Gatlin *et al.*, 2007). Plant-derived feed ingredients is required for substitution and supplements in aquafeeds to support growth, reduce the usage of expensive ingredient and economically produced of high quality farmed fish. The plant by-products which have been successfully used in aquafeeds to support expanding aquaculture industry are soybean, wheat, cottonseed, barley, canola, oat, peas, rice and sunflower as reviewed by Gatlin *et al.* (2007).

Nowadays, nutritionist has found new high potential source for feed ingredients: the algae-based product which has been actively used in aquaculture feed industry. In spite of the potential used within aquafeed, seaweed meal and the extracts are relatively new in market ingredient and required considerable research to ascertain the benefits and fulfilled the world demand (Tacon *et al.*, 2006).

2.3 Practical Used of Binder in Formulated Feed

Due to scarcity and high cost of fish feed ingredient, several studies have been carried out to evaluate the different types of natural, synthetic or modified substance used as binding agents for aquatic feed (Hung, 1989; Heinen, 1981) and reviewed by Lim and Cuzon (1994) in Table 2.2. Among the binder used, wheat flour, tuber or cereal starches and combination are commonly used for fish feeds. The organic binders such as CMC, alginates and gums have successfully done in laboratory-prepared diets but not commercially produced in large scale production due to high cost and limited adaptability. Meanwhile, the synthetic binders like BASFIN (formaldehyde-based product) have been used in shrimp diet with 0.5-1.0% level but the growth of shrimp was depressed due to decreased on stability and toxicity (Dominy and Lim, 1991).

Starch and guar gum work as botanic glues and classified as plants product with binding properties as described by Hung (1989). Starch is practically used as binder in fish feed and combined with appropriate processing technology in order to form high durable pellet. Starch also serves as energy sources (carbohydrates) in the feed. Ali *et al.* (2005), investigated five different sources of starch, namely corn, maida, rice, tapioca and wheat flour as binder at 15.3% combined with guar gum at levels of 1.0 ,2.0 and 3.0%. Feed pellets containing wheat flour with 2.0% guar gum and processed by using a commercial scale ring-die pellet mill with conditioners and steam injection facility, performed good water stability up to 4 hours (79.5% stability). The starch was gelatinized by adequate moisture, steam cooking and extrusion with steam injection works to complete gelatinization of starch and produce high binding effect.

Study by Ali (1988) stated that 2.0% level of agar-agar, polyvinyl alcohol and sodium alginate gave comparable result with 9.0% level of tapioca starch in prawn feed. Effiong *et al*. (2009) evaluated the duckweed meal as potential binder compared to corn starch and cassava meal in fish feed by incorporating 2.0% in fish feed. The best stable feed was achieved by feeds containing duckweed meal. Falayi (2000) reported that cassava starch gave better binding effects. Other study was reported by Moond *et al*. (2004) compared the various natural and synthetic binding agents in terms of water stability such as colocasia, ladies finger, ripe banana, wheat flour, egg, agar-agar, guar gum, carrageenan and CMC. The highest stability (80%) was observed in 50% wheat flour. Other starch source such as millet starch was combined with yeast (*Saccahromyces cerevisae*) to produce pellets that can float up to 30 minutes in water. While cassava and corn starch can be utilized to obtain pellets that are 50 minutes durable in water. Some binders exert the chemical action after undergoing heat processing, moisture and pressure treatments during feed processing, thus improve the pellet durability (Cuzon *et al*., 1994). However, starch used for expansion can weak the pellet texture (Aarseth *et al*., 2006). Hygroscopic ingredients weaken the pellet structure in water and separating the components and resulted in reduced acceptance (Saalah *et al*., 2010).

Table 2.2: Example of binders used in aquafeed

Binder	Inclusion level	Comment
Natural substances		
Casein	4.0	Fair, expensive
Wheat gluten	3.0-5.0	Good, moderate cost
Starch (rice, sorghum, Corn, cassava, etc.)	>20	Fair, inexpensive
Xanthan and locust bean gum	0.4	Good, expensive
Carrageenan mix	0.5	Good, moderate cost
Modified substances		
CMC	2.0-6.0	Fair, expensive
Alginate (1-3%)	2.0-4.0	Good, expensive
Lignin sulfonate	2.0-4.0	Poor, inexpensive
Synthetic substances		
Polyvinyl alcohol	2.0	Good, expensive
BASFIN	0.5-1.0	Very good, inexpensive but toxic
Aquabind	2.0-4.0	Good, expensive

Source: Lim and Cuzon, 1994