### EFFICACY OF FERMENTED VEGETABLE DISCARDS AS A DIETARY INGREDIENT FOR SHRIMP FEED

THESIS SUBMITTED
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF

DOCTOR OF PHILOSOPHY (MARICULTURE)

OF THE

#### CENTRAL MARINE FISHERIES RESEARCH INSTITUTE

Indian Council of Agricultural Research
COCHIN - 682 018
INDIA

THE LIBRARY
CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
KOCHI - 682 018

Ву

SAJITHA, S., M. F. Sc. (MC- 226)



CENTRAL INSTITUTE OF FISHERIES EDUCATION (DEEMED UNIVERSITY)

MUMBAI – 400061 MAY 2010



Dedicated to my family



#### केन्द्रीय समुद्री मात्स्यिकी अनुसंधान संस्थान

(भारतीय कृषि अनुसंधान परिषद)

पोस्ट बॉक्स सं 1603, एरणाकुलम उत्तर डा.घ., कोचिन - 682 018 CENTRAL MARINE FISHERIES RESEARCH INSTITUTE

Telegram Telex

Phone:(Off)

: 2394867/.....Ext. 2391407 CADALMIN, EKM 0885-6435 MFRI IN 0091-0484- 2394909/2396685 mdcmfri@md2.vsnl.net.in

Fax E-mail

(Indian Council of Agricultural Research) Post Box No.1603, Ernakulam North P.O. Cochin - 682 018, Kerala State, India.

Dated: 31 May, 2010

#### CERTIFICATE

Certified that the entitled "EFFICACY OF FERMENTED thesis VEGETABLE DISCARD AS A DIETARY INGREDIENT FOR SHRIMP FEED" is a record of independent bonafide research work carried out by Mrs. Sajitha, S (MC-226), during the period of study from October 2004 to May 2010 under our supervision and guidance for the degree of Doctor of Philosophy (Mariculture) at Central Marine Fisheries Research Institute, Cochin, and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or any other similar title.

Major Advisor/Chairperson

Dr. Imelda Joseph, Senior Scientist

Mariculture Division CMFRI, Cochin-18

**Advisory Committee** 

Dr. R. Paul Raj, Member secretary Coastal Aquaculture Authority

Govt. of India, Chennai

Co-chairman

Dr. L. Krishnan, Senior consultant

IGNOU Cochin

Member

Dr. D. Prema, Senior scientist FEMD, Member

Dr. Somy Kuriakose, Senior scientist

FRAD, Member

#### **DECLARATION**

I hereby declare that the thesis entitled "EFFICACY OF FERMENTED VEGETABLE DISCARDS AS A DIETARY INGREDIENT FOR SHRIMP FEED " is an authentic record of the work done by me and that no part thereof has been presented for the award of any degree, diploma, associateship, fellowship or any other similar title.

31<sup>st</sup> May 2010 Cochin.

(Mrs. SAJITHA, S)

Ph.D. Scholar

Post Graduate Programme in Mariculture Central Marine Fisheries Research Institute



ACKNOWLEDGEMENTS

#### **ACKNOWLEDGEMENTS**

"Thanks be to God in the highest."

It is my privilege to work under the guidance of Dr. (Mrs.) Imelda Joseph, Senior scientist, Mariculture Division. I wish to express my sincere gratitude and indebtedness to her for suggesting the problem, valuable advice and for her generous support that she provided in her capacity as my major advisor during my study.

I submit my sincere thanks to **Dr. R. Paulraj**, presently working as Member secretary, Coastal aquaculture authority, co-chairman for giving me proper guidance and support. His valuable suggestion has helped me a lot throughout the course of my experiment.

I sincerely thank **Dr. L. Krishnan**, currently working as senior consultant, IGNOU, Kochi, member of my advisory committee for providing me all the facilities and support to carry out the feeding trial in the hatchery which is a major fraction of my work.

I wish to acknowledge my deep indebtedness to Dr. D. Prema, Senior scientist, FEMD, member of my advisory committee for her valuable advice and timely help in doing the mineral analysis.

I am also thankful to **Dr. Somy Kuriakose**, Senior scientist, FRAD, member of my advisory committee for her kind suggestions and valuable help in doing statistical analysis of the results that had supported my study.

I extremely thank **Prof. Dr. Mohan Joseph Modayil**, Former Director Central Marine Fisheries research Institute Cochin, for providing all necessary facilities and help for my work during his tenure

I also thank **Dr. G. Syda Rao**, Director Central Marine Fisheries research Institute Cochin, for providing all necessary facilities and help for my work.

I wish to thank **Dr. P.C. Thomas**, Scientist in-charge, HRD cell for their constant support and valuable advice which helped me in the timely submission of the thesis.

I express my sincere and heartiest thanks to Dr. Boby Ignacious and Dr. K, S. Sobhana for their timely help without which I would never be able to complete my work.

I am incredibly thankful to Mrs. G. S. Shailaja, Technical Officer, Sri. S. Nandakumar Rao, Technical Assistant, MBTD and K, K, Surendran Technical Assistant, PNPD, Mrs. Uma, staff, Hindi cell for their valuable suggestions and timely help and support that helped me in successfully completing my work.

I would also like to express my sincere thanks to Mr. Venugopalan, K. M., Mr. C.. S. Sasidharan, Binu annan, Mr. Suresh, Mr. Kuttan, Mr. Surendran and Mr. Santhosh for providing me necessary help and support throughout my work in hatchery.

I owe the deepest sense of gratitude to Chandrasehkaran sir, Subhash sir, Devaki madom, Savithri chichi, Prasanna chechy and Binni chechy for providing the necessary help and support for my work.

I would also like to express my sincere thanks to Mrs. Annie Selva Sonia, Mrs. Hena Vijayan, Mrs. Remya sivanandan, Mrs. Jeeja P. K and Mr. N Rajesh for their timely help and support during my work.

I sincerely thank Mrs. Susmitha for her timely help without which I would never be able to submit the thesis in time.

I am indebted to the entire staff members of CMFRI library, for helping me in collection of reference for my work.

I pay tribute to all my teachers both past and present who taught me all that I know today.

Last but not the least, I express my profound gratitude and credit to my family whose everlasting and unlimited care and sacrifice have kept me in good spirits throughout my life. I owe my success to them.

Finally, I express my deep sense of gratitude to ICAR for providing me monetary assistance for my study.

Date 31th May 2010

Place Cochin

Sajitha S)

### सारांश

स्थानीय बाज़ारों से छोडे गए मिश्रित तरकारियों को जीवाणु, बासिलस लिचेनिफोर्मिस एम टी सी ो 6824 और बासिलस कोयागुलन्स एम टी सी सी 2449 और कवक, आस्पेरगिल्लस ओरीज़िए एन ी आइ एम 2010 अलग अलग प्रकार उपयुक्त करके किण्वन किया गया। जीवाण् किण्वन के लिए 5 वस और कवक किण्वन के लिए 15 दिवस लग गए और प्रोक्सिमेट मिश्रण और अमिनो आसिड ोफाइल के परिवर्तन का अध्ययन किया गया। बी. लिचेनिफोर्मिस और ए. ओरीज़िए द्वारा क्रमश: 4 गौर 12 दिनों में किण्वित उत्पादों (बी एल एफ पी और ए ओ एफ पी) को उनके एमिनो आसिड ोफाइल, प्रोक्सिमेट मिश्रण, खनिज (Cu, Zn, Mg. Mn और Fe) फाइटिक अम्ल स्तर के आधार पर वंगट खाद्य के संघटक के रूप में उपयुक्त किया गया। दोनों बी एल एफ पी और ए ओ एफ पी का रीक्षण I और परीक्षण II में क्रमशः *पी. मोनोडोन* के आहार में पश्च डिम्भक के लिए 10, 15 और :0% की दर में और किशोरों के लिए 5, 9 और 12% की दर में समावेशन हुआ। सभी आहारों का फ्रष्मीय मान 3240 cal g <sup>-1</sup> से ऊपर था। लगभग 45 दिनों के लिए परीक्षणात्मक आहार से खिलाए गए चंगटों की प्रतिक्रिया की तुलना >50% पशु प्रोटीन और अकिण्वित मिश्रित तरकारी चूर्ण से बनाए गए ग्राणिज्यिक विंगट खाद्य (यू एफ पी) से खिलाए गए चिंगटों से की गयी। बढ़ती, खाद्य परिवर्तन अनुपात एफ सी आर) और प्रोटीन क्षमता अनुपात (पी ई आर) के आधार पर 9-12% ए ओ एफ पी और 10-5% बी एल एफ पी समाविशत आहार की क्षमता बेहत्तर देखी गयी। सामान्यतः किण्वित तरकारी उत्पादों युक्त खाद्य से खिलाए गए चिंगटों की बढ़ती और खाद्य उपयोगिता क्षमता यू एफ पी खाद्य से खलाए गए चिंगटों की अपेक्षा उच्चतम देखी गयी। पी. मोनोडोन पश्च डिंभक में किए गए परीक्षण I में 10% ए ओ एफ पी युक्त खाद्य वज़न बढ़ाव (375%), एस जी आर (0.67%), पी ई आर (0.43) और रफ सी आर (3.93) की दृष्टि से बेहत्तर देखा गया। 20% बी एल एफ पी3 (1439%) युक्त खाद्य में एपारेन्ट नेट प्रोटीन यूटिलाइसेशन उच्चतम था। *पी. मोनोडोन* के किशोरों में किए गए परीक्षण II में, 9-12% ए ओ एफ पी युक्त खाद्य वज़न बढ़ाव और एस जी आर की दृष्टि से बेहत्तर देखा गया। 9% और 12% ए ओ एफ पी (0.26) युक्त खाद्य में प्रोटीन क्षमता अनुपात उच्चतम दिखायी पडी। 12% ए ओ एफ पी युक्त खाद्य में खाद्य परिवर्तन क्षमता कम देखी गयी। 9% बी एल एफ पी (79.24%) युक्त खाद्य में ए एन पी यू अधिकतम था। परीक्षण I में 15% बी एल एफ पी (34.26%) युक्त खाद्य ने उच्चतम प्रोटीन पाच्यता और 10% बी एल एफ पी (74.23%) युक्त खाद्य ने उच्चतम वसा पाच्यता दिखायी। दूसरे परीक्षण में, 9% ए ओ एफ पी युक्त खाद्य ने उच्चतम प्रोटीन (68.93%) और वसा (77.24%) पाच्यता दिखायी। परीक्षण I और II में क्रमशः 20% बी एल एफ पी (75%) और 9% बी एल एफ पी (68%) युक्त खाद्य से खिलाए गए चिंगटों में कारकास प्रोटीन का उच्चतम संचयन देखा गया। परीक्षण I और II में क्रमश: 15% बी एल एफ पी (7.93%) और 12% ए ओ एफ पी (8.09%) युक्त खाद्य सं खेलाए गए चिंगटों में ऊतक लिपिड का स्तर उच्चतम देखा गया। परीक्षण I और II में क्रमशः ए ओ एफ पी1 (21.66%) और ए ओ एफ पी 2 (23.86%) के लिए सूखी चीज़ों की प्रतिशतता देखी गयी। त्रर्तमान अध्ययन से यह मालूम पडा कि पी. मोनोडोन के खाद्य में 9-12% ए ओ एफ पी और 10-15% बी एल एफ पी समावेशित करने से बढ़ती, अतिजीवितता और शारीरिक संघटन में कोई प्रतिकृत प्रभाव

#### **ABSTRACT**

Mixed vegetables discards from the local market were fermented using bacteria, Bacillus licheniformis MTCC 6824 and Bacillus coagulans MTCC-2449, and fungus, Aspergillus oryzae NCIM 2010 individually. The bacterial fermentation was carried out for 5 days and fungal fermentation for 15 days and changes in the proximate composition and amino acids profile etc. were studied. B. licheniformis and A.oryzae fermented products (BLFP and AOFP) derived on days 4 and 12 respectively were used as a shrimp feed ingredient based on their amino acids profile, proximate composition, mineral (Cu, Zn, Mg, Mn and Fe) and phytic acid levels. Both BLFP and AOFP were incorporated in P. monodon diet at the rate of 10, 15, 20 % and 5, 9, 12 % in trial I and trial II respectively. The calorific value of all the diets was above 3240 cal g<sup>-1</sup>. The response of shrimp fed on the experimental diets for 45 days was compared with a commercial shrimp feed (CF), a feed prepared with >50% protein of animal origin (NCF) and an unfermented mixed vegetable discard powder incorporated feed (UFP). On the basis of growth, food conversion ratio (FCR) and protein efficiency ratio (PER), 9-12% AOFP and 10-15 % BLFP incorporated diets were found to exhibit improved performance. In general, growth and feed utilization efficiencies of shrimp fed diets containing fermented vegetable product were superior to those fed diets containing UFP. In trial I with P. monodon postlarvae, diet with 10% AOFP recorded better performance in terms of weight gain (375%), SGR (0.67%), PER (0.43) and FCR (3.93). ANPU was the highest for diet with 20% BLFP (1439%). In trial II P. monodon juveniles fed diets containing 9-12 % AOFP performed better in terms of weight gain and SGR. PER was the highest for diet with 9% and 12% AOFP (0.26). Lower FCR (5.73) was observed in diet 12% AOFP. ANPU was the maximum for diet with 9% BLFP (79.24%). Diet with 15% BLFP showed the highest protein digestibility (34.26%), and diet with 10% BLFP showed highest fat digestibility (74.23%) in trial I. In the second trial, diet with 9% AOFP showed the highest protein (68.93%) and fat (77.24%) digestibility. The highest accumulation in carcass protein was recorded in shrimp reared on diet with 20% BLFP (75%) and 9 % BLFP (68%) in trial I and II respectively. The highest tissue lipid level occurred in shrimp fed with the diet containing 15% BLFP (7.93%) and 12% AOFP (8.09%) in trial I and II respectively. The dry matter percentage was obtained for AOFP1 (21.66%) and AOFP2 (23.86%) in trial I and II respectively. From the present study, it is concluded that an inclusion level of 9-12 % AOFP or 10-15 % BLFP in the diet of P. monodon do not have any adverse effect on growth, survival, and body composition.

# CONTENTS

SI. No.	Particulars	Page No
1.	INTRODUCTION	1
2.	REVIEW OF LITERATURE	4
2.1	Solid state fermentation (SSF)	5
2. 2.	Microorganisms	6
2. 3.	Parameters influencing SSF.	9
2.4.	Applications of SSF	10
2.5.	Aquaculture and shrimp nutrition	13
3.	MATERIALS AND METHODS	18
3.1	Substrate	18
3. 2.	Microorganisms	19
3. 2.1.	Bacteria	19
3. 2.1.1.	Bacillus licheniformis MTCC 6824	19
3. 2.2.2.	Bacillus coagulans MTCC-2449	20
3.2.2.	Fungus	20
3. 3.	Chemical analysis	21
3. 3.1.	Proximate composition	21
3. 3.1.1.	Moisture	21
3.3.1.2.	Crude protein	21
3.3.1.3.	Crude fat	22
3.3.1.4.	Crude fibre	22
3.3.1.5.	Crude ash	22

3.3.1.6.	Nitrogen free extract (NFE)	23
3.3.2.	Minerals	23
3.3.3.	Phytic acid	24
3.3.4.	Amino acids analysis	25
3.3.5.	Gross Energy of diets	26
3.3.6.	Hydrostability of test diets	27
3.3.7.	Chromic Oxide	27
3.4.	Feeding trials using Penaeus monodon	28
3.4.1.	Trial I	28
3.4.2.	Trial II	30
3.5.	Diet performance	31
3. 6.	Statistical Analysis	32
4.	RESULTS	40
<b>4.</b> 4.1.	Nutrient analysis	<b>40</b> 40
4.1.	Nutrient analysis  Bacillus licheniformis MTCC 6824 fermented	40
4.1. 4. 1. 1.	Nutrient analysis  Bacillus licheniformis MTCC 6824 fermented product (BLFP)  Proximate composition	40 40
<ul><li>4.1.</li><li>4.1.1.</li><li>4.1.1.1.</li><li>4.1.1.1.</li></ul>	Nutrient analysis  Bacillus licheniformis MTCC 6824 fermented product (BLFP)  Proximate composition	40 40 40
4.1. 4. 1. 1. 4.1.1.1. 4.1.1.1.1. 4.1.1.1.2.	Nutrient analysis  Bacillus licheniformis MTCC 6824 fermented product (BLFP)  Proximate composition  Moisture	40 40 40 40
4.1. 4. 1. 1. 4.1.1.1. 4.1.1.1.1. 4.1.1.1.2. 4.1.1.1.3.	Nutrient analysis  Bacillus licheniformis MTCC 6824 fermented product (BLFP)  Proximate composition  Moisture  Crude Protein	40 40 40 40
4.1. 4.1.1. 4.1.1.1. 4.1.1.1.2. 4.1.1.1.3. 4.1.1.1.4.	Nutrient analysis  Bacillus licheniformis MTCC 6824 fermented product (BLFP)  Proximate composition  Moisture  Crude Protein  Crude Fat	40 40 40 40 40
4.1. 4.1.1. 4.1.1.1. 4.1.1.1.2. 4.1.1.1.3. 4.1.1.1.4. 4.1.1.5.	Nutrient analysis  Bacillus licheniformis MTCC 6824 fermented product (BLFP)  Proximate composition  Moisture  Crude Protein  Crude Fat  Crude Fibre	40 40 40 40 40 40
4.1. 4.1.1. 4.1.1.1. 4.1.1.1.2. 4.1.1.1.3. 4.1.1.1.4. 4.1.1.5.	Nutrient analysis  Bacillus licheniformis MTCC 6824 fermented product (BLFP)  Proximate composition  Moisture  Crude Protein  Crude Fat  Crude Fibre  Crude Ash	40 40 40 40 40 41

4.1.1.4.	Amino acids profile	41
4. 1. 2.	Bacillus coagulans MTCC-2449 fermented product (BCFP)	42
4. 1. 2. 1.	Proximate composition	42
4.1.2.1.1.	Moisture	42
4.1.2.1.2.	Crude Protein	42
4.1.2.1.3.	Crude Fat	42
4.1.2.1.4.	Crude Fibre	42
4.1.2.1.5.	Crude Ash	42
4.1.2.1.6.	Nitrogen Free Extract (NFE)	43
4. 1. 2. 2.	Minerals	43
4. 1. 2. 3.	Phytic acid	43
4. 1. 2. 4.	Amino acids profile	43
4. 1. 3.	Aspergillus oryzae NCIM 2010 fermented product (AOFP)	44
4. 1. 3. 1.	Proximate composition	44
4.1.3.1.1.	Moisture	44
4.1.3.1.2.	Crude Protein	44
4.1.3.1.3.	Crude Fat	44
4.1.3.1.4.	Crude Fibre	44
4.1.3.1.5.	Crude Ash	44
4.1.3.1.6.	Nitrogen Free Extract (NFE)	45
4. 1. 3. 2.	Minerals	45
	Phytic acid	45
4. 1. 3. 4.	Amino acids profile	15

4. 2.	Test diets incorporated with the fermented product	
4.2.1.	Gross energy	64
4.2.2.	Hydrostability	64
4.2.3.	Efficacy of test diets in Trial I	64
4.2.3.1.	Growth parameters	64
4.2.3.2.	Digestibility	65
4.2.3.3.	Body composition	65
4.2.4.	Efficacy of test diets in Trial II	66
4.2.4.1.	Growth parameters	66
4.2.4.2.	Digestibility	66
4.2.4.3.	Body composition	66
5.	DISCUSSION	79
5. 1.	Fermentation using B. coagulans and B. licheniformis	81
5. 2.	Fermentation using A. oryzae	84
5. 3.	Utilization of fermented product in shrimp feed	88
5. 4.	Growth Performance of <i>P. monodon</i> fed diets formulated with fermented products	90
6.	SUMMARY	95
7	PEEEDENCES	99

# LIST OF TABLES

Table No.	Particulars	Page no.
Table 1.	Proximate composition (% dry weight) of feed ingredients	34
Table 2.	Ingredient composition (percentage) of the test diets in Trial I	35
Table 3.	Ingredient composition (percentage) of the test diets in trial II	36
Table 4.	Composition of commercial vitamin/mineral mixture (Supplevite–M)	37
Table 5.	Proximate composition (% dry weight) of test diets in trial I	38
Table 6.	Proximate composition (% dry weight) of test diets in trial II	39
Table 7.	Proximate composition of Bacillus licheniformes fermented product (BLFP)	47
Table 8.	Proximate composition of <i>Bacillus coagulans</i> fermented product (BCFP)	47
Table 9.	Proximate composition of <i>A. oryzae</i> fermented product (AOFP)	48
Table 10.	Amino acid profile of <i>B. licheniformes</i> fermented product (g100g <sup>-1</sup> sample)	49
Table 11.	Amino acid profile of <i>Bacillus coagulans</i> fermented product (expressed in g/100g sample)	50
Table 12.	Amino acid profile of Aspergillus oryzae fermented product (expressed in g/100g sample)	5 <b>0</b> A
Table 13.	Gross energy of the test diets used in trial I	68

Table 14.	Gross energy of the test diets used in trial II	68
Table 15.	Hydrostability of test diets in trial I (expressed in %)	69
Table 16.	Hydrostability of test diets in trial II (expressed in %)	69
Table 17.	Growth performance and feed utilization efficiencies in <i>P .monodon</i> post larvae after 45 days feeding trial	70
Table 18.	Growth performance and feed utilization efficiencies in <i>P. monodon</i> juveniles after 45 days feeding trial	71
Table 19.	Comparison between percentage inclusion of the fermented product in different treatments with feed digestibility and weight gain (Trial I)	72
Table 20.	Comparison between percentage inclusion of the fermented product in different treatments with feed digestibility and weight gain (Trial II)	72
Table 21.	Body composition of shrimp PL after 45 days of experiment (% dry matter basis)	73
Table 22.	Body composition of shrimp juveniles after 45 days of experiment (% dry matter basis)	73

# LIST OF FIGURES

Figure No.	Particulars	Page No.
Fig. 1.	Mineral profile of B. licheniformes fermented product	51
Fig. 2.	Mineral profile of B. coagulans fermented product	
Fig. 3.	Iron, Magnesium and Phosphorus profile of <i>A. oryzae</i> fermented product	
Fig. 4.	Copper, Zinc and Manganese profile of A. oryzae fermented product	54
Fig. 5.	Phytic acid profile of <i>B. licheniformes</i> fermented product	55
Fig. 6.	Phytic acid profile of B. coagulans fermented product	56
Fig. 7.	Phytic acid profile of A. oryzae fermented product	57
Fig. 8.	Essential amino acid profile of BLFP during fermentation (g/100g sample)	58
Fig. 9.	Non-essential amino acid profile of BLFP during fermentation (g/100g sample)	59
Fig. 10.	Essential amino acid profile of BCFP during fermentation (g/100g sample).	60
Fig. 11	Non essential amino acid profile of BCFP during fermentation (g/100g sample)	61
Fig. 12	Essential amino acid profile of AOFP during fermentation (g/100g sample)	62

Fig. 13	Non-essential amino acids profile of AOFP during fermentation (g/100g sample).	63
Fig. 14	Growth (weight gain) of <i>P. monodon</i> fed with BLFP in trial I	75
Fig. 15	Growth (weight gain) of <i>P. monodon</i> fed with AOFP in trial I	76
Fig. 16	Growth (weight gain) of <i>P. monodon</i> fed with BLFP in trial II	77
Fig. 17	Growth (weight gain) of <i>P. monodon</i> fed with AOFP in trial II	78

# LIST OF PLATES

Plate No.	Particulars	Page No.
Plate 1.	Experimental set up	33

INTRODUCTION