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ENRICHMENT AND IMPROVING THE NUTRITIONAL VALUE OF THIS CABBAGE WASTE USING *Beauveria sp*

Hari Sadu[1], Manpal Sridhar[2], Jhansi Lakshmi Bai[3] and D Ravindra Kumar Reddy[3]
[1]Research Associate (Fisheries), Krishi Vigyan Kendra, ANGRAU, Nellore-524004
[2]Manpal Sridhar, Principal Scientist, National Institute of Animal Nutrition and Physiology, Adugodi, Bangalore-560030
[3]Department of Aquaculture, College of Fishery Science Nellore, Andhra Pradesh

Abstract: The present study was conducted to find out enrichment of cabbage waste which is fermented with *Beauveria sp.* Feeds incorporated with 5, 10 and 15% Cabbage waste fermented with *Beauveria sp.* shown uniformity in pellet shape and compactness. The moisture content ranged with higher moisture levels ranging between 7.5 to 8.9 %. The protein contents were higher in feeds having 15 % cabbage waste than in unfermented feeds. Fat content in fermented feeds were also lower than in control feeds. Feeds incorporating 5 to 15% of Cabbage waste fermented with *Beauveria sp.* recorded carbohydrate contents of 23.81, 23.44 and 23.20% respectively.
Keywords: Cabbage Waste, Beauveria sp, proximate composition, Solid State of Fermentation, Feed stability.

Introduction

In aquaculture more than half the investment comprising 50 to 70 % of the total operating costs goes into feeds as they contribute an essential factor for enhancing fish production. With the intensification of culture activities more emphasis is diverted to fish nutrition and compounded feeds in particular

Commercial aquaculture feeds for shrimp generally contain 25-45% of crude protein because shrimp require such high dietary levels. Consequently only high protein oil seed residues have been used for compounding shrimp feeds (New, 1976). Feedstuffs of vegetable origin as a whole are lower in protein when compared to those of animal origin. Nevertheless among all plant protein sources tested for most crustaceans, soyabean meal has been found to be the most superior on account of its high protein content and essential amino acid profile (Kanazawa, 1995; Akiyama, 1988). In order to reduce the escalating cost of aqua feed and make aquaculture sustainable in the long run intensive research is being focused on alternative and more sustainable protein sources for use within compounded aquafeeds (Tacon, 1993). The utility of plant protein as partial replacement for the more expensive animal protein fractions has been examined but results show great variations in the degree of success, which inordinately depend on the species and types of ingredients used

Non conventional ingredients are ingredients that are capable of partially or completely substituting fishmeal. These have been in use since traditional aquaculture in Asia. It has been found that these feed stuffs can be used as substitute for fishmeal as they are no more abundant than fishmeal but are least expensive. Fishmeal has a well-balanced amino acid profile along with essential fatty acids. All foodstuffs need not have the same amounts of amino acids in fishmeal but they usually exceed the levels found in some amino acids.

The advantages of fermentation have been known over the ages as a means of bioconversion and protein enrichment of food and feed ingredients. It is also increasingly evident that the development of low cost, high quality protein foodstuffs is crucial for the future success of the aquaculture industry. Solid state fermentation is a novel technology by means of which cheap ingredients of lesser nutritive value can effectively be converted into nutritionally rich and easily digestible aquafeeds (Nigam and Singh, 1996).

Of the wide variety of feed ingredients available in India for production of aqua feeds (New *et al.,* 1993) most are reported to be of too poor quality to produce high quality aquafeeds, especially for shrimp substrates, cell substances of the microbes and externalized metabolites (Nigam and Singh, 1996).

Solid State fermentation (SSF) has gained importance in the recent past due to its several advantages over submerged fermentation especially for enrichment of protein of agricultural wastes and sub products. The SSF technology has the advantage of direct utilization of none or very few pretreated solid substrates under aerobic conditions to produce microbial Biomass products (MBP), which contain a mixture of unused substrates, cell substances of the microbes and externalized metabolites (Nigam and Singh, 1996).

Cabbage (*Brassica olaracea var. capitata*) is available in the local vegetable markets throughout the year. The outer green leaves are usually discarded as waste and only the inner compact head is utilized. The waste is available in bulk in most market and its incorporation, as a non-conventional ingredient in shrimp feeds would therefore be a lucrative proposition. It would be most pertinent to carry out further enrichment of these substrates as well along with cabbage waste in order to increase their nutritive value and digestibility.

Material Methods

Non-conventional feed ingredients viz. cabbage waste, procured from the local market were analyzed for their proximate composition. Feeds were compounded with varying concentrations of cabbage waste and their physical and physico-chemical characteristics determined. These ingredients were subjected to Solid State Fermentation (SSF) using a fungus, *Beauveria sp.* and the changes in chemical composition were determined. Feeds of varying concentrations were again formulated with the fermented cabbage waste and the physical and physico-chemical characteristics compared to those of the unfermented material.

Feed formulation:

The feed ingredients and other additives like oil, binder, vitamin premix, mineral mix were weighed according to the standard feed formulation given by New, 1987 and Solid State Fermentation of ingredients was performed by following the method of Ramesh and Lonsane (1987). Necessary changes were however, made in the methodology and medium composition after optimization of process parameters.

Preparation of experimental cabbage waste feeds:

Fish and shrimp meal were equally replaced with 5%, 10% and 15% dried cabbage waste and these feeds were designated as CW7, CW 8and CW9 respectively.

Table 1: Percentage composition of control and experimental feeds prepared using varying concentrations of dried powdered cabbage waste.

INGREDIENTS %		EXPERIMENTAL FEEDS		
	Control	**CW 7**	**CW 8**	**CW 9**
Cabbage waste	0.0	5.0	10.0	15.0
Fish meal	15.0	12.5	10.0	7.5
Soya bean meal	36.0	36.0	36.0	36.0
Shrimp Meal	10.0	7.5	5.0	2.5
Wheat flour	18.0	18.0	18.0	18.0
Ground Nut Oil Cake	10.0	10.0	10.0	10.0
Cod liver Oil	2.0	2.0	2.0	2.0
Vegetable oil	2.0	2.0	2.0	2.0
Gelatin	4.0	4.0	4.0	4.0
Vitamin mixture	0.5	0.5	0.5	0.5
Coated vitamin C	0.5	0.5	0.5	0.5
Mineral Mixture*	1.0	1.0	1.0	1.0
Di-Calcium	1.0			
Phosphate		1.0	1.0	1.0
Total	**100**	**100.0**	**100.0**	**100.0**

*Standard USP XVII mixture procured from SISCO laboratories

Proximate composition of experimental feeds compounded with cabbage waste fermented using *Beauvaria sp.*

Three feeds were formulated incorporating 5, 10 and 15% of cabbage waste fermented using *Beauvaria sp.* fungi in the same manner as adopted for compounding unfermented cabbage waste feeds. These were dried to less than 10% moisture after compounding and analysed for their physico-chemical characteristics

Preparation of fermented cabbage waste feeds:

A set of three feeds were compounded by incorporating 5, 10 and 15% of cabbage waste fermented using Beauveria sp. and designated as CW7, CW8and CW9 respectively. The diet devoid of fermented material designated as C served as control. The percentage incorporation of other feed ingredients used in the feed base was as given in Table.1. The prepared were dried to less than ten- percent moisture content and stored in airtight plastic containers at room temperature till further analysis.

Physical evaluation of the feeds:

The physical appearance of the feeds viz. color, shape, size and pellet diameter were recorded.

Hydrostability tests of the feeds:

Water stability of the control feed was determined by the method of Jayaram and Shetty (1981) with minor modifications. Approximately 5 g of diet was weighed, in triplicate and transferred 4" X4" pouches made of bolting silk. These were immersed in 25 liters of sea water (28ppt salinity and 28 ± 2^o C water temperature) in plastic tubs. Pouches were removed from water at 0.5,1,2,3and 4 hours respectively and rinsed with distilled water to remove adhering salts. The contents were transferred to pre-weighed petridishes and dried in an oven at 80 ± 5^o C and the resultant loss in dry matter was calculated.

SOLID STATE FERMENTATION:

Solid State Fermentation of ingredients was preformed by following the method of Ramesh and Lonsane (1987). Necessary changes were however, made in the methodology and medium composition after optimization of process parameters.

Micro-organisms:

Pure cultures of fungi, *Beauveria sp.* were procured from the microbiology section of the Department of Biotechnology, Cochin University of Science and Technology. They were maintained as pure cultures by sub-culturing every week on nutrient agar slants for bacteria and mycological agar containing streptomycin for fungi.

Analytical Methods:

Moisture, ash, crude protein, crude fat and fiber in feed ingredients, fermented substrates and feeds were determined by standard procedures (AOAC 1990) .

Results

Proximate composition of feeds compounded using cabbage waste fermented with *Beauveria sp.* Fungi

The proximate composition of the three feeds compounded using 5, 10 and 15% of cabbage waste fermented using *Beauveria sp.* and designated as CW7, CW8 and CW9 are given in Table 2.

Table 2: Proximate chemical composition of the control and experimental feeds compounded utilizing cabbage waste fermented using *Beauveria sp.*

Nutrient	Control	Feeds*		
		CW7	CW8	CW9
Dry matter	97.05	91.13	92.46	92.07
Moisture	2.95	8.87	7.54	7.93
Crude Protein	43.39	45.24	45.39	45.54
Ether Extract	6.18	5.46	5.3	5.28
Crude Fiber	2.05	2.16	2.42	2.61
Ash	10.77	14.46	15.91	15.44
Acid Insoluble Ash (AIA)	0.62	0.23	0.22	0.2
NFE**	34.65	23.81	23.44	23.2

- Values expressed on dry matter basis.
- ** Nitrogen Free Extractives calculated as (100- % crude protein+crude fat+ash+crude fiber +moisture)

These feeds recorded higher moisture levels ranging between 7.5 to 8.9 %. Protein contents of these feeds was higher than the corresponding feeds prepared from unfermented cabbage waste and ranged from 45.24 for feed CW7 incorporated with 5% of the fermented waste to 45.54 for feed CW9 incorporated with 15% of the cabbage waste fermented with *Beauveria sp.* . Fat content of these feeds was lower than that of the control feed and ranged from 5.28 for CW9 (15% incorporation) to 5.46 in the case of feed CW7(5% incorporation). Crude fiber values of 2.15, 2.42 and 2.61 % were obtained for feeds CW7, CW8 and CW 9 respectively and though these were higher than the crude fiber content of the control feed wherein value of

2.05 was recorded, they were comparatively lower to those recorded in the case of feds prepared incorporating 5,10 and 15% of unfermented cabbage waste. The reverse was the case with regard to ash content as values ranging between 14.46% in the case of feed CW7 to 15.91% in the case of CW8 were recorded which were higher than 10.77% ash of the control feed and also of the three feeds prepared incorporating 5, 10 and 15% of the dried unfermented cabbage waste. Concomitantly, the carbohydrate contents of these three feeds compounded incorporating 5, 10 and 15% of cabbage waste decreased with values of 23.81, 23.44 and 23.20% respectively being recorded. The acid insoluble ash did not vary significantly among these feeds as compared to the feeds prepared utilizing the unfermented cabbage waste but were significantly lower than that of the control feed where in a value of 0.62% was recorded for the AIA.

Physical characteristics of the feeds compounded using cabbage waste fermented with *Beauveria sp.*

The physical characteristics of the three feeds compounded by incorporating 5,10 and 15% of cabbage waste fermented using *Beauveria sp*. fungi and designated as CW7, CW8 and CW9 are elaborated in Table 3. The three feeds had a pale brown color with a whitish tinge on account of the fungus, in comparison to the control feed which had a brown color and an uneven texture. These feeds had a strong fungal odor.

Table 3. Physical characteristics of the control and experimental feeds compounded utilizing cabbage waste fermented with *Beauveria sp.* Fungi

FEED	PELLET SIZE	APPEARANCE (Unfermented)	APPEARANCE (Fermented)
Control	2.0x 3.0 mm	Dark brown uneven texture	Dark brown uneven texture
CW7	2.0x 3.0 mm	pale brown fine and compact	Brown compact
CW8	2.0x 3.0 mm	dark brown greenish tinge fine and compact	Dark brown compact fine texture
CW9	2.0x 3.0 mm	dark brown greenish tinge fine and compact	Dark brown compact fine texture

Changes in percentage of some biochemical parameters upon solid state fermentation of cabbage waste, cotton seed cake and gingelly oilcake are represented in Table 4.

Table 4. Changes in percentage of some biochemical parameters upon solid state fermentation of cabbage waste

Substrate	BIOCHEMICAL PARAMETERS*					
	Dry matter	Protein	Fat	Fibre	Ash	NFE
Cabbage waste (CW)	- 0.464	5.044	-0.473	-6.397	3.018	-1.193

Hydro stability of the feeds compounded using cabbage waste fermented with *Beauveria sp.*

The hydrostabilty of the three feeds prepared incorporating 5, 10 and 15% of cabbage waste fermented using *Beauveria sp.indicated* three feeds viz. CW7, CW8 and CW9 prepared incorporating 5,10 and 15% of cabbage waste fermented using *Beauveria sp.* were also quite stable in seawater with 81 to 85% dry matter remaining at the end of one hour. After that there was an additional 11-13% loss in dry matter upto 4 hours in seawater. However, the feeds prove to be stable exhibiting a profile very similar to that of the control feeds.

Discussion

The present study was designed with the purpose of testing the suitability of cabbage waste (*Brassica olaracea var. capitata)* in shrimp feeds as a non-conventional feed ingredient before and after subjecting to solid sate fermentation. The role of Solid State Fermentation in microbial enrichment and improving the nutritional value of this cabbage waste

Aquatic feeds have so far solely depended upon the highly nutritious but at the same time expensive animal proteins, of which the contributions of fishmeal has been most outstanding (New, 1976; Tacon, 1993). However, it is now evident that the future success of the aquaculture industry depends on the development of low-cost nutritious feeds, utilizing more of plant proteins. Commercial aquaculture feeds for shrimp generally contain 25-45% crude protein and consequently high protein oil seed residues have been used of compounding shrimp feeds (New, 1976).

Further, non-conventional ingredients of plant origin have low protein and high carbohydrate and fiber contents in addition to anti-nutritional factors and toxins. Also variability in nutritional composition (New *et al*, 1996) has been attributed to factors like method of handling and processing, the nutritional status of the environment in which they were grown as well as variations in analytical methodologies.

Gopalan *et al*., (1985) showed the protein content of leafy vegetables to range between 1% to 27% in the dried leaves of rape (*Brassica napus*). In the present study the outer leaves of cabbage discarded as waste recorded a moisture of 4.71% and a moderate protein content of 15.43% making it a non-conventional ingredient worth incorporation in aquafeeds owing to availability round the year. Leafy vegetables have low fat content ranging between 0.1 to 4.8 with low to moderately high carbohydrate contents ranging between 2 to 60.9% in the case of tender tamarind leaves (Gopalan *et al*., 1985). Cabbage waste recorded a low fat content of 1.5% and a relatively high carbohydrate content of 50%. Though certain leafy vegetables have been reported to contain high ash and fiber contents, cabbage waste recorded ash content of 11.46% and fiber content of 14.57%. There are no reports of the presence of any anti-nutritional factors in cabbage and thus cabbage waste was deemed to be suitable ingredient for incorporation into shrimp feeds.

Recommended protein levels vary from 30 to 57% in various species and sizes of marine shrimp While recommended lipid levels for commercial feeds range between 6% to 7.5% and should not exceed 10% (Akiyama *et al*., 1991). Carbohydrates are not a dietary essential for shrimp feeds, however their usefulness and cost effectiveness are undeniable. In the present study the three feeds compounded incorporating 5, 10 and 15% cabbage waste were very much within the nutritional standards prescribed for shrimp nutrition.

The use of micro-organisms to convert carbohydrate, ligno-celluloses and other industrial wastes into protein rich food and feed stuffs has been well documented. Cabbage waste on account of its protein content offers great potential in aquafeed formulation especially in the case of freshwater prawns and herbivorous fish species.

Among the processes that can be used to supply proteins, the most important and promising are those based on microbial growth and production of microbial biomass employing solid state fermentation (SSF). SSF technology has the advantage of direct utilization of none or very few pretreated solid substrates under aerobic conditions to produce Microbial Biomass Products (MBP), consisting of a mixture of unused substrates, cell substances of the micro-organisms and externalized metabolites. The reduced reactor volume per unit substrate converted and the direct applicability of the fermented product for feeding purpose makes SSF a very attractive technology (Nigam and Singh, 1996). With the demand for cheaper plant protein sources to supplement the more expensive fishmeal component as well as bring down the cost of aquafeed the use of non-conventional ingredients like cabbage waste and further improvement of nutritive value of conventional ingredients becomes pertinent. In this investigation SSF technology was employed for the production of microbial protein as well as protein enrichment of cabbage waste and one strain of fungi (*Beauveria sp.*) in order to evaluate their suitability for aquafeed formulation.

In the present study, bacterial fermentations recorded mild color change unlike the fungal fermentations, wherein the pronounced spore formation leading to a visible change in color, with a strong moldy odour is in agreement with the results of Sridhar and Chandrasekhar (1996). These workers reported a 61.08% increase in protein content of wheat bran and 51.08% increase in protein content in groundnut oilcake upon fermentations with *B.licheniformes.* Fermentation with *Beauveria sp.* of both these substrates led to a protein increase of 30% in wheat bran and 31% in groundnut oilcake. However, the reduction observed by them in the dry matter content of all substrates is in agreement with this study.

In this study protein content increased in all three substrates viz. cabbage waste with *Beauveria sp.,* but the increase was higher in the case of fungal fermentations, as compared to the bacterial fermentations, a view contradictory to that observed by Sridhar and Chandrasekhar (1996).

SSF resulted in bioconversion and protein enrichment of all substrates. Thus SSF is a novel technology by means of which cheap ingredients of lesser nutritive value can effectively be converted into nutritionally rich and easily digestible aqua feeds. Studies are preliminary and offer immense scope for further research, to prove beyond doubt that SSF, which is simple and economic, is the appropriate technology for the futuristic aqua feed industry.

The results obtained are very encouraging and clearly indicate that nutritionally inferior substrates can be converted to protein enriched digestible ones for incorporation into aqua feeds

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