RESEARCH MODE

CHARACTERIZATION OF DIFFERENT TYPES OF SPENT MUSHROOM SUBSTRATE (SMS) FOR AFRICAN CATFISH (*Clarias gariepinus*) DIET SUITABILITY

by

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TABLE OF CONTENT

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LIST OF TABLES

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LIST OF FIGURES

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LIST OF ABBREVIATIONS

SMS	Spent Mushroom Substrate
MOA	Ministry of Agriculture
FCR	Feed Conversion Ratio
CAGR	Compound Annual Growth Rate
KMT	Kilo Metric Tones
DAN	Dasar Agromakanan Negara
PVC	Poly (vinyl chloride) pipe
SAMS	The Scottish Association of Marine Science
SAI	Scientific Analytical Institute
FAO	Fisheries and Aquaculture Department
ETP	Economic Transformation Programmed
NKEA	National Key Economic Area
EPPs	Entry-Point Projects
GDP	Gross Domestic Product
UV	Ultraviolet
UV-Vis	Ultraviolet Viscometer
GC	Gas Chromatography
kcal	kilo calories
BSA	Bovine Serum Albumin
HCL	Hydrochloric Acid
Na_2CO_3	Sodium Carbonate
Cu_2SO_4	Copper (II) Sulphate
NaOH	Sodium Hydroxide

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$KNaC_4H_4O_6.4H_2O$	Sodium Potassium Tartrate
rpm	Rotation Per Minutes
SCE	Strong Cation Exchange
H_2SO_4	Sulphuric acid
AAS	Atomic Absorption Spectrometer
kJ	Kilo Joule
AOAC	Official Method of Analysis
ADCP	Aquaculture Development and Coordination Program
NRC	National Research Council
mg	milligram
mL	milliliter
mJ	milijoule
pH	Water properties
OH	Hydroxyl group
RM	Ringgit Malaysia

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LIST OF SYMBOLS

Symbol		Unit
%	Percentage	
DE	Digestibility energy level	kJ g^{-1}
kg	weight	kg
RM	Malaysia currency	RM
Y	Absorption wavelength	A
X	Total leaching protein concentration	mg/ml
m	gradient value	
C	y-intercept	
v	volt	v
Y1	Sum of Overall size catfish left	cm
Z1	Number of catfish left	
X2	Average pH in different types of tank	
Y2	Sum of Overall pH water in tank	
Z2	Number of tank	

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ABSTRAK

Sisa Bongkah Cendawan (SMS) merupakan salah satu daripada sisa pertanian yang dihasilkan dari perkembangan industri cendawan di Malaysia. Secara kimianya, ia merupakan lebihan bahan organik yang berpotensi to digunakan terutamanya di dalam sektor makanan haiwan termasuk akuakultur. Pengunaan SMS sebagai makanan ikan tidak pernah dikendalikan sebelum ini disebabkan maklumat tentang komposisi biokimia SMS yang terhad. Merujuk kepada permasaalahan ini, formulasi baru di cipta dengan memasukkan SMS dan bahan lain seperti sisa kacang soya, makanan ikan, tepung ubi kayu, dan dedak beras. Kebiasaannya, pelbagai jenis SMS dituai sebanyak enam hingga tujuh kali kitaran dikumpul dan dikenal pasti sebagai Tiram Putih (*Pleuratos ostreatus*), Tiram Kelabu (*Pleuratos sajor-caju*), Abalone (*Pleuratos cystidiosus*), Ganoderma (*Ganoderma lucidium*) and Telinga Kera (*Auricularia polytricha*). SMS ini telah diuji komposisi biokimianya termasuk analisa protein mentah, karbohidrat, lemak, lignin, dan abu. Secara keseluruhannya, trend menunjukkan terdapat pertambahan di dalam protein mentah dan jumlah lemak, manakala karbohidrat dan lignin menunjukkan jumlah pengurangannya. Ganoderma mencatatkan jumlah protein tertinggi , 36.6 g, diikuti Telinga Kera , Tiram Putih, Tiram Kelabu dan Abalone. Sebaliknya, pengurangan jumlah karbohidrat yang paling rendah diperhatikan dalam Ganoderma sebanyak 70.42 g and kebayakannya dalam Telinga Kera. Pertambahan lemak dan pengurangan lignin menunjukkan nilai yang serupa bagi setiap SMS. Jumlah protein bagi nilai tertinggi (Ganoderma) , sederhana (Tiram Putih) dan terendah (Abalone) merupakan asas pemilihan SMS untuk di gabungkan di dalam penghasilan formulasi pelet ikan menggunakan cara Pearson Square yang menumpukan protein dan tenaga. Kesemua bahan di campur dan di peletkan dan dikeluarkan selepas 40 minit proses pengewapan pada suhu 80° C. Sejumlah ujikaji fizikal untuk pellet telah dilakukan untuk mengesahkan kesesuaian formulasi pelet sebagai makanan ikan dengan menilai dengan makanan ikan komersial. Eksperimen pemeliharaan ikan dijalankan selama 12 minggu and analisa berat, saiz, kadar survival dan pH air dijalankan bagi setiap jenis pelet yang dihasilkan. Ikan keli yang diberi pelet berasaskan Ganoderma mencatatkan jumlah berat yang tertinggi iaitu purata sebanyak 40.11g/ekor diikuti pelet berasaskan Tiram Putih 38.80g/ikan dan pelet berasaskan abalone 22.86g/ekor berbanding pelet ikan komersial , 45.43g/ikan. Manakala, saiz ikan keli masing-masing menunjukkan saiz yang hampir sama secara keseluruhanya dari pelet komersial, pelet Ganoderma, pelet Tiram Putih iaitu 23.84cm/ekor, 20.75 cm/ekor, 19.62 cm/ekor. Kadar survival bagi ikan keli yang diberi pelet berasaskan SMS menujukkan nilai survival yang lebih tinggi sebanyak 30% lebih dari ikan keli yang diberi makanan pelet komersial. Kesimpulannya adalah SMS mengandungi kandungan nutrisi yang dipercayai untuk digabungkan di dalam pembangunan makan ikan. Pelet ikan berasakan SMS sesuai untuk dimakan, mengekalkan kadar survival ikan keli dan menunjukkan pertambahan pertumbuhan pada saiz yang boleh diterima dan dibandingkan dengan pelet komersial.

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ABSTRACT

Spent Mushroom substrate (SMS) is one of agricultural wastes produced by the ver expending mushroom industries in Malaysia. Chemically, it is rich in organic matter which potentially can be utilized for aquaculture. Utilization of SMS as fish feed has never been conducted before as very limited information on SMS biochemical composition is available. Regarding to this matter, new formulation of fish feed was developed by incorporating SMS and other ingredients namely soybean waste, fish meal, topioca flour and rice bran. Commanly, different types of SMS that being cultivated at six to seven cycles were collected which identified as white oyster (*Pleuratos ostreatus*), grey oyster (*Pleuratos sajor-caju*), abalone (*Pleuratos cystidiosus*), ganoderma (*Ganoderma lucidium*) and black jelly (*Auricularia polytricha*). Those SMS were tested on their biochemical composition which involving the analysis of crude protein, carbohydrate, fat, lignin and ash. Overall trend showed an increment in crude protein and fat content, whereas carbohydrate and lignin showed a reduction in the content. Ganoderma attained the highest protein value, 36.6 g, followed by black jelly, white oyster, grey oyster and abalone. Contradictory, lowest carbohydrate reduction was observed in ganoderma at 70.42 g and the most was in black jelly. Increment in fat and reduction in lignin showed almost similar value for each SMS. The highest (ganoderma), medium (white oyster) and lowest (abalone) protein content were the basis of SMS types selection to be incorporated in pellet formulation using Pearson square method which emphasized on the protein and energy. The ingredients were mixed and a pelletalization was carried out after 40 minutes of steaming process at 80° C. A series of physical properties test of SMS pellets were conducted to confirm the applicability of the formulated pellet for fish consumption by comparing with commercial fish feed. Fish rearing experiment was conducted within 12 weeks and analysis of the weight, size, survival rate and pH of the water were conducted for each pellet type. The catfish fed with ganoderma based pellet obtained the highest weight with an average of 40.11 g/fish followed by white pellet 38.80 g/fish and abalone pellet 22.86 g/fish compared to commercial feed, 45.43 g/fish. By the way, the size showed almost similar for the catfish fed by commercial feed, ganoderma based pellet and white based pellet that are 23.84 cm/fish, 20.75 cm/fish, 19.62 cm/fish respectively. The survival rate of catfish fed with SMS-based pellet showed more resistance and could survived about 30% higher than catfish fed with commercial pellet. The conclusion is the SMS contained a reliable amount of nutrition that can be incorporated in fish feed development. The SMS based pellet also was able to be consumed, retain catfish survival and promoted growth at the acceptable and comparable size by the commercial pellet.

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CHAPTER 1

INTRODUCTION

1.1 Background study

Spent Mushroom Substrate (SMS) is one of the organic wastes produced from mushroom industry. Subsequently, it is refered to the composted material substrates that have been fully utilized after several harvesting cycle of mushroom cultivation which contain high organic matter (Department of Plant Pathology Pennsylvania State University, 2014). Based on increasing production of SMS over the time as 1 kg production of mushroom will generate 5kg of SMS (Sample et al., 2001) , an efficient and practical method is necessary in managing this valuable organic waste so it can be utilize at their optimum varied usage and ultimately ensuring the safe disposal of SMS in environmental management.

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Conventionally, mushroom industry is a new and small industry in Malaysia. However, it is growing fast as one of the highest-valued sources of food and sources of incomes for the farmers. Malaysia government has categorized mushroom as one of the seven high-value crops that will be commercially grown intensively with government support (Mohd Tarmizi et al., 2013). At present, around 320 farmers and companies cultivating mushroom most of whom are small farmers producing about 50 to 500 kg of mushroom per day and total production estimate is about 24,000 kg per day in 2012 and

the demand keep increasing in the population (Department of Agriculture Malaysia, 2012).

Consequently, the world demand for fresh mushroom is projected to grow at the rate of 15% a year and consumption per capita in Malaysia is projected to grow from 1.0 kg in 2008 to 2.4 kg in 2020. As a result, the total demand for mushroom is projected to increase from 23,000 tones/year in 2008 to 72,000 tones/year in 2020 (MOA, 2010). Parallel with the time, production of agricultural waste which is SMS from mushroom industry will multiple 5 times higher in the near future which gives nearly 360,000 tones of SMS in 2020 ahead. Facts that need to overcome before it come to serious environmental problem ahead.

Apparently, the obvious solution to increase in the demand for SMS is through exploration of new application for its utilization. The ever increasing human demand to utilize agro waste for animal feed usage have resulted the exploration of low cost production of animal feed from agro waste source (Mukherjee et al., 2004). By considering of high organic matter in SMS, it has the potentially to be used as animal feed supplement yet at the same time providing additional animal feed resources.

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Another factor that contribute to the exploration of SMS usage which focuses on fish compared another livestock such as cattle, sheep and poultry is because of the feed conversion ratio, (FCR) itself. FCR is being used to measure the amount of feed that being used for every livestock from earlier stage until it can be commercially harvest and sell to the market. Fish indicate the lowest FCR which is 1.5 to 2.0 compared to other livestock FCR such as cattle; 5.0 to 10.0 and sheep 4.0 to 6.0. Therefore, fish is

selected after being considered from economic stand point and time consumer. As fish consume smaller amount of feed, yet will give a huge profit same as other livestock and will showed the result in short time compared others.

Prior to these issues, feed is a major expenditure for fish farmers as it accounts for 40 - 50 per cent of the production cost. By utilizing the potential agro waste from the surrounding, it is capable to minimize the cost of fish feed and has good impact to natural biological cycles and controls environmental safely (Hasan, 2001) (Gold, 1999). The global aqua feeds market is estimated to grow at a compound annual growth rate; (CAGR) of 11.7 per cent from 2013 to 2018, reaching 82,390.5 kilo metric tones; (KMT) by 2018. The global aqua feeds market has grown exponentially in the last few years and similar trend is expected to follow for the next 5 -7 years (Reportlinker, 2013). As the matter of concern, the aquaculture sector is giving priority as it can utilize the product from agricultural waste which is SMS itself.

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1.2 Problem statement

1.2.1 Amount of SMS generated from industry.

A large amount of SMS being discarded after mushroom production will cause environmental problem due to unsystematic disposal system. Due to existing demand of mushroom intake in Malaysia, approximately based on 324 gram intake of mushroom per person will lead the to the production of 8,424 metric tons of mushroom and subsequently generates an approximate value of 42,120 metric tons of SMS monthly (Laupa, 2008); facts that create a great environment challenge in terms of its effective handling solid waste management.

1.2.2 A few studies on characterization of SMS and its application as fish feed compared as other livestock feed.

There were already many studies being conducted on the suitability of agricultural wastes in fish feed production. Unfortunately, for this new agro waste which is SMS, there was very limited scientific information on the characterization of different SMS types in Malaysia was found. Most of the characterization study only focused on popular types of SMS in Malaysia such as white oyster and grey oyster SMS. Furthermore, there was no study on the application of SMS as fish feed in pellet form especially for the African catfish compared to the research conducted by Hanan, et.al, 2010; Zarina, et.al, 2012; Hanan, 2012.

1.2.3 Ability of African Catfish to consume formulated fish feed from SMS waste

Continuing from statement 1.2.2, there was no research on the ability and acceptances of fish especially African catfish to consume the formulated fish feed from SMS waste in term of growth performance and digestibility analysis. Therefore, more information is needed for this kind of waste to be used in aquaculture sector and in the same time will reduce its disposal problem.

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1.3 Hypothesis

It is hyptothesized that SMS contain a tolerable and utilizable amount of biochemical composition that can be potentially be used in the formulation of fish feed. The formulated fish feed in pellet form could also features a realiable physical properties and can be consumed, digested and promoted growth at acceptable rate.

1.4 Objective

This research is meant to follow certain and rigid objectives as listed below:

i. To determine and classify biochemical composition; crude protein, amino acid, total carbohydrate, lignin and fat; heavy metal and ash content of different types of SMS after several cycle of mushroom cultivation.
ii. To evaluate the physical properties of formulated fish feed after formulation using Pearson Square method and compare with commercial ones.
iii. To investigate the growth performance in term of weight and size of the Catfish throughout fish experimental.
iv. To analyze the digestibility of Catfish throughout the feeding trial.

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1.5 Scope and significance of research

This research is a lab scale experiment that focusing on reutilization of agricultural waste which is Mushroom Spent Substrate (SMS) and its suitability to be incorporated in fish feed formulation for African Catfish (*Clarias gariepinus*) in minimizing the environmental pollution issues.The biochemical composition analysis of SMS need to be conducted so that the requirement of nutrient necessary for growth can be readjusted and synchronize according to the availability and amount of sample. The biochemical analysis such as crude protein (%), amino acid; % of lysine and methionine, carbohydrate (%), lignin (%) and fat (%), ash (%) have been conducted to different types of SMS that being cultivated at six to seven cycles such as Ling Zhi, Black Jelly, Abalone and Oyster. After the characterization process, selection of selected SMS such as Ganoderma (highest), Abalone (lowest) and White Oyster (available SMS) will be incorporate into fish feed formulation with ratio of protein category (fish meal and soybean waste) ; 2: 1 whereas energy category (SMS, Tapioca starch and rice bran); 3: 2: 1. Others element such as vitamin mixed and calcium bishydrogen; 2: 1. Next, physical properties characteristic of pellet were conducted such as protein leaching test that investigate protein leach from pellet (mg/ml), hardness test (mJ) that can deform the pellet and lastly floating test to test how long (min/100mL) that pellet floating before eaten by catfish.The fish experimental focuses on growth performance which weight (g/fish), height (cm/fish), survival rate (number fish survive/week) had been analyzed . Also, for digestibility analysis concentrate on % digestible of protein, % digestible fat, % digestible gross energy, and % dry matter of Catfish feces.

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1.6 Organization of Thesis

Chapter 1 emphasizes on overall view and ideas for this project. The key aspects discussed here were study background, problem statement, research objectives, scope and significant of research regarding process of reutilizing this agricultural waste; SMS and incorporate it into fish feed formulation. Chapter 2 reviews on mushroom industry in Malaysia which subsequently explain the relationship between reutilization of this agricultural waste; SMS yet incorporating it into fish feed formulation with aquaculture field in Malaysia. Also, selection of formulation method; Pearson Square method and another contribution factor in fish feed quality such as feeding quality; feeding rate, pH, FCR and other are briefly discussed here altogether with physical properties of fish feed; leaching, floating, hardness and others. Meanwhile, in chapter 3 covers method and experiment flow of biochemical composition of preliminary analysis of SMS such as protein, carbohydrate, fat, heavy metal and ash before proceed with selection process on which types of SMS that will be incorporating with other ingredient such as fish meal, soybean waste, vitamin mixed and others. Process flow on growth and digestibility study also reported in this chapter. In chapter 4, all the finding data starting from biochemical compound SMS study till growth and digestibility performances are presented and discussed clearly in this chapter. Lastly for final chapter which is chapter 5 concludes the research outcomes of the project and some prospect in industry in future. Recommendation also brought forward and highlighted for next study.

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CHAPTER 2

LITERATURE REVIEW

This chapter highlight on reutilization of agricultural waste in the form of SMS that are produced from mushroom industry in Malaysia and the selection criteria of SMS compared to other agriculture waste for fish feed production. This chapter also provides the trend production of SMS which corresponding to the amount of mushroom production and overview of fish feed market forecast throughout the nation. Also, another contribution factor in justifying fish feed quality such as feeding quality, physical properties, digestibility study and growth performance are documented here.

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2.1 Mushroom industry in Malaysia

Mushroom is a new and small industry in Malaysia. However, it is rapidly growing fast as one of the highest-valued sources of food and source of incomes for the farmers. The use of mushroom has evolved into many products such as pharmaceutical, nutraseutical, and cosmetics product. Malaysia government has categorized mushroom as one of the seven high-value crops that will be commercially grown intensively with government support (Mohd Tarmizi et al., 2013).

Malaysia mushroom export grew at a rate of 19% per year from RM12 million in 2000 to RM67 million in 2020 as illustrated in Figure 2.1 (MOA, 2013). Almost 49 % of the mushroom exported is in the fresh form, mostly button mushroom (*Agaricus spp.)*