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RESEARCH ARTICLE

Formulation of Feed Pellet with MD2 Pineapple Peel Inclusion as Potential Prebiotic for Local Red Hybrid Tilapia Fish, *Oreochromis Niloticus*

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ABSTRACT - Fruit waste such as pineapple peel is cost-effective, halal and can be applied as feed additives for the value-added feed. Unrestrained use of antibiotics to maintain the health of aquaculture has implicated resistance to pathogenic aquaculture bacteria towards the antibiotics and somehow leaking into the food chain. Therefore, incorporating prebiotics in aquaculture as feed additives could be a harmless and better option to promote aquaculture well-being. The aim of this study is to formulate fish feed for Red Hybrid tilapia, with the inclusion of 5% MD2 pineapple peel (PAP) as a prebiotic supplement and to evaluate the nutritional diet and physical characteristics of the pellet. The tilapia fish diet contains soybean meal, fish meal, corn, corn gluten meal, casava tuber meal, palm oil, mineral premix, vitamins, and MD2 pineapple peel. The formulation comprises calculated values of digestible energy;14MJ/kg and 32%digestible protein. The results for moisture content, bulk density, floatability, expansion ratio and hardness were 25.06%, 385.28kg/m^3, 97%, 1.09 and 3.65 respectively. It can be concluded that the formulated pellet was comparable to the commercial feed, Starfeed brand.

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1.0 INTRODUCTION

Malaysia has an equatorial climate commonly referred to as a tropical rainforest climate which is hot, humid and rainy with a climate year centered on the northeast and southwest monsoons making it appropriate for agricultural practice [1]. The agricultural sector indeed contributes greatly in the overall economic growth in the world including Malaysia, for example, it produced 8.1% or RM89.5 billion to the Gross Domestic Product (GDP) in 2018 according to statistics of the Department of Statistics; Malaysia [2]. Regrettably, due to this fact, 1.2 million tonnes of agricultural waste is disposed of into in landfills every year [3-4]. The production of Malaysia's agricultural waste was 0.122 (kg/cap/day) in 2009 and is predicted to increase to 0.210 (kg/cap/day) by the year 2025 [4]. Managing agriculture waste has been a global issue, Malaysia, for example, have been reported in 2012 had generated solid waste daily at 33,000 tonnes and this figure exceeded the estimated production which is 30,000 tonnes by 2020 [5]. Malaysia, accounts for 80% landfilling for current waste disposal, but in 2020, this method is anticipated to drop to 65%. Therefore, converting agriculture waste into animal feed, as a resource appears to be a good alternative in dealing with the mounting waste in the country.

The increasing cost of fish feed has influenced the farming attainability and profitability. Conventional feed ingredients account for a considerable production cost, elevating the aquaculture feed cost [6]. The demanding and low accessibility to essential ingredients, such as soybean and fish meal progressively causing the increasing price [7]. Malaysia is dependable towards protein from seafood sources this was evidenced by 59 kg per capita of fish consumption in 2016 [8]. Aquaculture accounts for about 20% of the total seafood production in Malaysia and production since 1980 in Malaysia has increased to 1.69 million tonnes in 2017 [9]. The production value of tilapia, *Orechromis* spp. account at 73.79 million USD with the amount at 31,766 tonnes [10]. Malaysia is a halal hub and practices halal compliance hence the products and services marketed toward Muslim populations are compulsory to meet the requirements of Islamic law and the sector accords about 10% of its gross domestic product (GDP) [11]. However, there are reports that some local aquaculture fish were identified fed with animal protein and animal by-products such as blood, tissue, or bone that may be developed from pig and waste [12-13]. In the halal food supply chain, animal feed is an important subject that has undeviating significance for the safety, quality, and halal integrity of animal-based food products. Extensive surveillance and management in the preparation, production, storage, and dispersion of animal feed are compulsory to guarantee acceptable quality standards [14].

Plant by-products such as pineapple waste have been mentioned as a potential prebiotic by few studies and have been supplemented in the diet of animals [15-17]. Prebiotics are types of indigestible food fibers and natural sugars that promote the growth of beneficial bacteria in the gut. Study by [18] had discovered that pineapple peel composed the

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highest amounts of indigestible polysaccharides and oligosaccharides as compared to banana and watermelon peel. Prebiotics is an essential supplement for animals and human and is the food for probiotics, the microbe which inhabits the gut of the host. Prebiotics play a significant role for the growth and sustainability of the probiotics in the gut, while probiotics give many important benefits to the host such as improving host health [19]. This symbiosis act could be a preventive measure for fish diseases substituting the high usage of antibiotics in fish farming that is questionable and raises issues in food safety. Thus, this paper focuses on formulation of fish pellet with the inclusion of MD2 pineapple peel as potential prebiotic supplement in tilapia diet and evaluation of the nutritional and physicochemical properties of the formulated pellets in comparison to commercial feed pellets.

2.0 METHODS AND MATERIAL

2.1 Materials

The raw materials (soybean meal, fish meal, corn, corn gluten meal, tapioca starch DCM), feed additives and fish vitamin for pellet formulation were purchased from local supplier, My Ternak Trading Sdn. Bhd. and Nutrivest Sdn. Bhd. The pineapple peel was collected from a pineapple plantation farm, Saudagar Nenas at Kuala Merap, Selangor, Malaysia. The pineapple peels were pressed, and the liquid was discarded. Pressed peels were dried using oven until the moisture was static at 5%. The raw materials were ground into uniform size, at 0.5µm with mill ZM 300, Retsch, Germany. The size of the grounded materials should be in the same size due to assurance that all ingredients were mixed well and homogenized.

2.1.1 Probiotic Strains

Probiotic strains *Lactobacillus acidophilus* DDS1 NRRL B-3208 and *Bifidobacterium bifidum* BB12 probiotic strains were obtained from UAS Laboratories, The Probiotic Company (Wasau, Wisconsin, USA). The stock cultures were kept in 20% glycerol and stored at -20 °C. The strains were cultured in de Mann Rogosa Sharp (MRS) broth (Oxoid, Basingstoke, UK) and incubated for 24 to 48 h at 37°C prior to use,

2.2. Assessment of the Pineapple Peel Prebiotic Effect

2.2.1 Fermentation

The inoculum of 48 h incubation were harvested by centrifugation (10,000×*g*, 10 mins, 10°C), washed twice with phosphate-buffered saline PBS pH 7.2 (0.1 M Na_2HPO_4, 0.1 M NaH_2PO_4) and suspended in the same buffer. Approximately 1% (w/v) of the homogenised solution was added to 150 mL basal medium that comprised of (2 g/L) peptone, yeast extract (2 g/L), NaCl (0.1 g/L), K_2HPO_4 (0.04 g/L), KH_2PO_4 (0.04 g/L), $MgSO_4.7H_2O$ (0.01 g/L), $CaCl_2$ (0.01 g/L), $NaHCO_3$ (2 g/L) and Tween 80. The pH was adjusted to pH 7.2 using the phosphate buffer mentioned earlier. This basal medium was modified as proposed by [20-21]. The peel was added in 3% (w/v) into the basal medium and prepared in triplicate. The probiotic strains were inoculated aseptically and incubated for 96 h. The growth of the strains and pH of the media were determined.

2.2.2 Formulation of Feed

The nutrient compositions of the diets are formulated by means using the National Research Council (NRC) nutrient requirements of fish and shrimp [22] as a guide, the formulation diets were formulated to meet the minimum specifications for tilapia. The fish pellet was developed with the following ingredients: soybean meal, fish meal, cornmeal, corn gluten meal, casava tuber meal, pineapple peel, vitamin premix, mineral premix and water, about 20 to 30%. The mixing process for feed started with the least amount of ingredients, for example, the fish vitamins then followed by the next least ingredient until all the ingredients were added and mixed, meanwhile, water was usually added for 20% to 25%. The formulation and ingredients of the pellet are as shown in Table 1. The premix formulation was then extruded through a twin screwed pellet extruder (SIMA, Brand, China). The barrel temperature profile was set at 78 ℃ (feeding zone), 112 ℃ (compression zone) and 144 ℃ (forming zone). The main motor speed was set up at 16 rpm, feed motor speed was 26.9 rpm and the cutting speed was 13.3 rpm; while the pressure was at 10.0 MPa. The extrusion process took about 1 h to produce 10 to 15 kg pellets and after that, the pellets were oven-dried at 50 ℃ for overnight. Finally, palm oil was added to condition the pellets. The dried, extruded and conditioned pellets were kept in an airtight container at room temperature for further analysis.

2.3 Physical Properties of Extruded Pellets

2.3.1 Moisture Content

The moisture content was determined using a moisture analyzer, A&D MX-50, Japan. The principle of the apparatus is based on thermogravimetric analysis. The sample was dried using a halogen lamp and the moisture content was determined in percentage. Five grams (5 g) of the ground pineapple peel were placed on the weighing pan inside the halogen moisture analyzer at 105 ℃ and time ranges between 14 to 17 min [23].

2.3.2 Expansion Ratio

The extrusion pelleting needs the premix formulation is extruded by subjecting to heat, moisture, and shear forces/pressure in an extruder barrel just preceding to pelleting. Expansion ratio of the 10 pellets (Df) was measured by using digital calliper and calculated with Equation 1 [24].

$$SEI = \left(\frac{D_E}{D_D} \right)^2 \tag{1}$$

where, *SEI*: Sectional Expansion Index, D_E: Extruded pellet diameter and D_D: Die hole diameter.

2.3.3 Bulk Density

Bulk density is a measurement of the space a feed mass occupies per unit volume (g/mm^3) [25]. The bulk density was measured by pouring each replicate of pellets into a 1000 ml cylinder to measure the weight of 1000 ml pellet. Then the poured density was calculated from the mass and volume of the pellets. The measurements were taken five times.

2.3.4 Floatation Test

Ten replicate pellets were dropped into a 100 ml beaker and observed for 20 min. The amount of floating pellet (F_f) was measured using Equation 2 [26]. Data were reported as the average of five measurements per sample.

$$F = \left(\frac{F_f}{F_i} \right) \times 100\% \tag{2}$$

where, F is Floatability, F_f is the final number of floating pellet and F_i is the initial number of floating pellets.

2.3.5 Hardness

The extruded product was tested for hardness properties by using a texture analyzer (Stable Micro System, TA.XT plus, United Kingdom. The texture analyzer, model is equipped with a 5 kg load cell and 30 kg load cell and compressed with a 5 mm stainless steel cylinder probe at a test speed of 2.00 mm/sec and post test speed was at 10.00 mm/sec cell [27]. The compression test was set up at 75% of extruded product deformation. The hardness unit was given in force (N) at the breakage point. The measurements were done using five randomly selected pellets and the hardness value was calculated based on the average hardness measurement of the feed pellets.

3.0 RESULTS AND DISCUSSION

The probiotic strains *L. acidophilus* DDS1 NRRL B-3208 and *Bifidobacterium bifidum BB12* were examined for in vitro fermentation of pineapple peel. The basal media were supplemented with 3% of pineapple peel and were inoculated with the probiotic strains obtained from the pellet of 24 to 48 hrs full-grown strains under aseptic conditions. The growth kinetics of the strains are depicted in Table 1 while the pH is shown in Figure 1. It was determined that both strains grew effectively in the medium containing pineapple peel suggesting that pineapple peel is a potential prebiotic. The number of bacterial cells was increasing during incubation period. The proliferation values in log10 CFU/mL of the probiotic's strains growing in peel medium are presented in Table 1. It is confirmed that the probiotic strains could utilize pineapple waste as a growth medium for propagation. Study by Akter [18] also on pineapple peel extract showed a significant effect on the bacterial growth because of a significantly higher ($P < 0.05$) viable count in comparison with inulin, watermelon peel and banana peel within 24 h. The pH of the media was also decrease through the fermentation period (Figure 1) indicating that the probiotics break down carbohydrate substrates for propagation and vitality and the major fermentation products are mainly lactic acid, acetic acids, and short-chain fatty acids [28]. The pineapple peel in this study as sole of carbon source supported the growth of the probiotic strains due to the high dietary fibre content, which other studies have reported to be mostly cellulose, hemicellulose, lignin, fructans, pectin, and pectic substances [29]. In addition, some studies have reported pectin-derived oligosaccharides [30]. The prebiotic strains also lowered the pH in the medium to a final value that ranged from pH 6.63 to 3.57.

The feed formulation composition of the feed was depicted in Table 2. The feed formulation was selected based on the study by [31-32]. All of the common ingredients consist in the feed formulation except for the pineapple peel that was added as a prebiotic source in the feed. The inclusion of pineapple peels that contain high in sugar seem to be caramelized and homogenized well with other ingredients. Besides the main ingredients, amino acids; such as methionine and threonine, vitamin concentrate and mineral were among the important supplements for tilapia fish. Formulation compositions, digestible protein and energy of the feed do meet the requirement diets for local tilapia fish. In general, the diet of tilapia varies with developmental phase. The optimum growth rates for tilapia is depend on a balanced diet consisting of protein, carbohydrates, lipids, vitamins, minerals and fibre. The fingerling needs lower amount of carbohydrates and a higher amount of protein (35%), lipids, vitamins, and minerals for the growth of muscles, internal organs and bones [33]. The diet for sub-adult fish requires a lower content of protein (30-32%) and a higher percentage of fat and carbohydrate for growth and metabolism. For an adult fish, a low percentage of dietary protein is needed, however, the amino acids need to be available in certain ratios and a higher carbohydrate content is also needed [33].

The nutrient level of the formulated pellet and commercial pellet is shown in Table 3. The amount of nutrients and energy of the pellet was compared with the local commercial pellet from Starfeed brand. The digestible protein and energy of the formulated feed were 31.97% and 13.97 MJkg^{-1}, respectively, while the Starfeed brand only mentioned the percentage of protein which was 32%. The formulated pellet and commercial pellet are isonitrogenous, by means the protein content are at a similar amount. The dry matter of the formulated pellet was lower, 74.94% compared to the commercial pellet which is 88%. The amount of crude fibre of both pellet is quite similar at 3% to 3.37%, however, the ether extract content which is referred to the fat content is significantly different, formulated pellet has 7.64% of fat compared to commercial pellet is only 3%. The formulated pellet accounted for more than two times higher in fat than the commercial pellet. The ash amount in the formulated pellet was 7.78% but the ash content in the commercial pellet was not mentioned. The nutrient level of both pellet varied due to the ingredients in the pellet formulation. It was mentioned in the Starfeed packaging that the ingredients in the pellet are fish meal, soybean meal, rice bran broken rice, vitamin and mineral. However, the amount of each ingredient was not revealed on the packaging well as the type of binder used in the formulation.

The result of physical properties such as moisture content, expansion ratio (ER), bulk density, floatability and hardness of both types of pellets was interpreted in Table 4. The moisture content of the formulated pellet and commercial pellet was 4.53% and 7.62%, respectively. As for the formulated pellet, the expansion ratio value was determined at 1.09 while the commercial pellet is 1.04 (Table 4). The expansion of extruded products is due to the formation of the starch matrix that capture water vapour. The captured water vapour inside the starch will create bubbles during cooking extrusion [34]. The expansion ratio of the extruded pellet is usually affected by elastic force and bubble growth force when the extruded pellet reaches at the die exit it will be expanded, while this happens the moisture of the mixture evaporates as steam [35]. Previous studies have shown that the increased moisture of the product is the reason that the expansion ratio increases [36]. In this study, it showed that the pellet was expanded in diameter and floats due to the starch gelatinization and increased the air cell size during the extrusion period. According to [37], gelatinization has a significant impact on feed digestibility, expansion, water solubility and particle binding. Additionally, it should be noted that higher moisture content in the pellet will increase the drag force, which will put more pressure on the die and cause the extruded pellet to expand more at the exit [35].

Table 1. Propagation of probiotics strains *L. paracasei* subsp. *paracasei* NTU101, *L. acidophilus* DDS1 NRRL B-3208 in pineapple peel and pomace at 0 hour to 96 hours

Probiotic strains	Log^{10}CFUml^{-1}				
	0 h	24 h	48 h	72 h	96h
Lactobacillis acidophilus DDS1 NRRL B-3208	0	5.304	7.264	8.217	5.863
Bifidobacterium bifidum BB12	0	4.441	6.696	7.52	4.877

Values are presented as mean±standard deviation (n = 3)

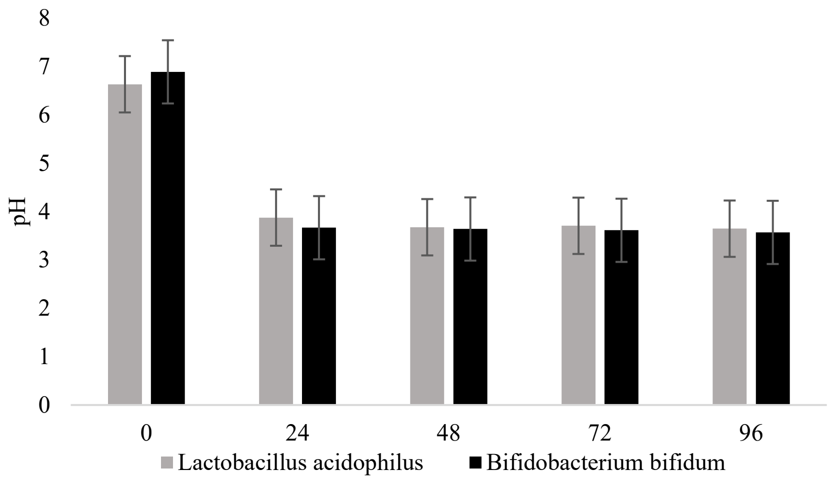

Figure 1. pH of the probiotic strains tested on 0 hr and 72 hrs in pineapple peel
Values are presented as mean±standard deviation (n = 3)

Table 2. The formulation composition of formulated diets (g /100g dry diet), proximate composition of the diet based on analysis and the calculated values of digestible protein and digestible energy

Ingredient	Amount composition (%)
Soybean meal, dehulled	40.00
Fish meal	13.25
Casava tubers meal	13.00
Corn gluten meal	10.25
Palm olein	5.65
Corn, high oil, grain	5.00
Pineapple peel	5.00
Methionine	2.85
DCP	1.95
Threonine	1.95
Mineral premix	0.65
Vitamins concentrate*	0.45
Total	100.00
Nutrient level (as is basis)	%
Dry matter	74.94
Digestible protein	31.97
Crude Fiber	3.37
Ether Extract	7.64
Ash	7.78
Digestible energy (MJ/kg)	13.97

*Vitamin concentrate consisting per kg of ration at 1 kg/tonne inclusion: 20.00 miu/kg vitamin A, 4.00 miu/kg vitamin D3, 52.00 g/kg vitamin E, 4.00 g/kg vitamin K3, 4.00g/kg vitamin B1, 10.00 g/kg vitamin B2, 6.4.00 g/kg vitamin B6, 40.00 mg/kg vitamin B12, 200.00 mg/kg biotin ,3.2.00 g/kg folic acid, 80.00 g/kg niacin, 22.40 g/kg, 22.40 g/kg pantothenic acid and q.s. to 1000.00 g/kg

Table 3. Nutrient level of the formulated pellet and commercial pellet from Starfeed brand

Nutrient level (as is basis) (%)	Formulated pellet (added pineapple peel)	Local commercial pellet Starfeed brand
Dry matter	74.94	88
Digestible protein	31.97	32
Crude Fiber	3.37	3
Ether Extract (Fat)	7.64	3
Ash	7.78	Not mention
Digestible energy (MJ/kg)	13.97	Not mention

Table 4. Determination of physical properties of fish feed pellet

Physical properties	Formulated pellet (added pineapple peel)	Local commercial pellet (Starfeed brand)
Moisture content (%)	4.53±0.22	7.62±0.31
Expansion ratio (ER)	1.09±0.05	1.04±0.03
Bulk density (BD) (kgm^{-3})	385.28±2.17	432.42±0.54
Floatability (F) (%)	98%	99%
Hardness (N)	3.65±0.17	2.92±0.3

Values are presented as mean±standard deviation (n = 3)

Bulk density is an important property for aquaculture feed as it will determine the floatability of the pellet. The pellet will float if its density is lower than that of the water, otherwise it will sink. Consequently, freshwater pellets with densities higher than 1000 kgm^{-3} will typically sink [38]. The bulk density value of the formulated pellets and commercial pellets in this study showed below than 1000 kgm^{-3}, which is 385.28 kgm^{-3} and 432.42 kgm^{-3}, respectively. A lower bulk density and high buoyancy are desired in this study since the tilapia is a surface feeder. The different ratio of starch and different pellet size also could effect the value of bulk density. Starch actually in the formulation has helps the expansion of the pellet, through gelatinization. Torrejon [39] reported the bigger the expansion of the pellets the less the bulk density of the pellet. This is due to the expansion of pellets, that their volumes gain disproportionately with their masses that result in less in bulk density.

The floatability of the formulated pellet and commercial pellet were measured for 30 min and was found to be at 98% to 99%. Both pellets are equally good in floatability this is probably due to the chemical properties of the ingredients used in the formulation, for example, the formulated pellets in this study use casava tubers meal, a type of starch that actually aids in swelling and gelatinization process during extruding [40]. The process definitely increases the buoyancy of the pellets significantly. The hardness value of formulated pellet is 3.65 N and the commercial pellet is 2.92 N. The hardness test showed significance difference between the formulated pellets and commercial pellets, Starfeed brand. The hardness index of a pellet indicates the degree of gelatinization of the raw starch during feed production. The feed pellet diameter and grinding screen size of hammer mill determined the hardness of the feed pellets [41]. The largest pellet diameter yielded the hardest pellets, while the smallest pellet diameter resulted in the lowest hardness. This is agreeable, because the diameter of the formulated pellet is 4 mm compared to the commercial pellet is only 2mm, Other than that, raw starch must be fully gelatinized to ensure strong bonds between particles to improve the hardness of the feed pellet [42]. In view of these factors, the hardness of the feed pellet may be guaranteed [43].

4.0 CONCLUSION

The findings in this study work, it can be concluded that the selected fruit peel extracts can be used to promote the growth of *L. acidophilus DDS1 NRRL B-3208* and *B. bifidum BB12* strains respectively. The physical characteristics of the floating grower tilapia feed pellets, which contain 5% pineapple peel, are comparable to those of commercial feed pellets under the Starfeed brand in terms of moisture content, expansion ratio, bulk density, floatability, and hardness. In conclusion, it was discovered that pineapple peel has the ability to replace some common elements in the formulation of commercial feed. Lower technical fish feed quality is known to impact fish farming uptake ratios and contributes to freshwater and marine pollution. This can be the situation if the pellet sinks faster than expected, disperses when immersed in water or disintegrates during transport. Therefore, it is important to ensure the technical quality of formulated fish pellets is up to the standard of local commercial pellets. This study would be further to the next step of feeding tilapia fish with the formulated fish feed to study the effect on the growth performance of the fish prior to consuming the fish pellet with the inclusion of pineapple peel.

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6.0 CONFLICT OF INTEREST

The authors declare no conflicts of interest.

7.0 AUTHORS CONTRIBUTION

S.A. Rahman (Conceptualization; Visualization; Writing-original draft)

M.Y.A. Shukor (Supervision; Review; Funding acquisition)

S. Mustafa (Supervision; Review)

M. S. Kamarudin (Supervision)

Y. S. Ting (Supervision; Software)

F. I. Jamaludin (Supervision)

N. Samad (Supervision)

S. P. Koh (Supervision; Review; Funding acquisition)

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