

Effect of feeding pineapple waste on growth performance, texture quality and flesh colour of Nile tilapia (*Oreochromis niloticus*) fingerlings

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1. ¹¹ Introduction

Nile tilapia (*Oreochromis niloticus*) is wide culture freshwater species in over 100 countries (Makori et al. 2017). Tilapia is easy to breed, disease-resistant and highly adaptable to a wide range of environmental conditions. To suit local protein needs, tilapia was introduced to undeveloped countries and cultivated on a subsistence level. Tilapia has made its way into mainstream seafood markets as production processes have improved and off-flavors have been reduced. Meanwhile, in highly industrialized countries, only modest markets for live and frozen tilapia were formed and dominated by immigrant groups (FAO, 2020). In Malaysia, *O. niloticus* is the major fish species for freshwater aquaculture where contributes approximately 90% of the total production of tilapia species. Its production skyrocketed from 28,401 (2005) to 38,642 tones (2010), exhibiting a 36% increment with almost USD60 millions of trades (Azhar, 2014).

Aquaculture feeds expenses represent almost 70% of the total operating costs in intensive farming systems. Although the operating costs vary, farmers usually look for new practical alternative feed sources that enhance fish growth and concurrently reduce feed costs (Zulhisyam et al. 2021). Most countries have the main problem with agricultural waste (i.e: pulp, crop residues, leaf litter, fruit, and vegetable by-products) to fulfil the food supply worldwide. To eliminate or recycling such waste is costly hence, a swift resolution and strategies are pertinent to prevent environmental pollution. Several studies have documented the use of various waste materials in the composition of aquaculture feed. The substitution of various waste products in aquaculture feed formulation was reported in several studies. For instance, as reported by Tonsy ³⁴ et al. (2019), the utilization of orange peel waste has given a significant effect to the growth performance of *O. niloticus*. Meanwhile, apple peel waste has been reported as a good potential feed additive for genetically improved farmed tilapia (GIFT, *O. niloticus*) (Qiang et al. 2019). It shows that agricultural waste from agro-based industry may be useful to animal feeding with a comprehensive study implementation.

Malaysia is one of the biggest pineapple producers worldwide besides Thailand, the Philippines, Indonesia, China, Kenya, and India (Lasekan and Hussein, 2018). Currently, there are approximately 16,500 hectares of pineapple plantations in Malaysia. It is worthy of note that about 59.36% of pineapples are considered agro-industrial waste

(Sukruansuwan and Napathorn, 2018). Pineapple waste is used as a fertiliser and animal feed on occasion. However, the trash is frequently burned or dumped on the ground, where it is susceptible to microbial deterioration and poses an environmental threat.. Pineapple waste contains has a high sugars, carbohydrates, protein, and fibre contents (Ketnawa et al. 2012). It also contains bromelain, bioactive and functional compounds and is considered a dietary supplement (Azizan et al. 2020; Pavan et al. 2012). Thus, it reveals that pineapple waste has a significant advantage to be converted as value-added products.

Most of the findings shown agricultural waste has no negative consequences effects on the studied fish. Notwithstanding, the most important characteristics are growth performance and feeding efficiency before considering the substitute by-product as an alternative source of aquaculture feed. The main concern is whether the usage of agricultural waste in feed can affect the seafood texture and colour. Indeed, the texture and colour of fish flesh are crucial in the aquaculture industry to sustain quality control and product development. Therefore, as a result, the goal of this study is to see how pineapple waste affects the growth of *O. niloticus*. Texture quality analysis and colour evaluation of the flesh were also carried out since both contribute to consumer acceptance and marketability.

2. Materials and Methods

2.1 Ethics statements

This study was carried out at laboratory animals and fish hatchery and was approved by the Animal Ethical Committee of Universiti Malaysia Kelantan (Malaysia)

2.2 Experimental diets and design

Pineapples were obtained from a private farm in Jeli, Kelantan, Malaysia. The pineapple waste, including leaves, crowns and peel, was sliced and chopped into smaller pieces and dried for 48 hours at 70°C. Then, the dry waste was then crushed in a heavy-duty blender and filtered through a screen with a mesh size of 250 microns to produce a fine powder. Four experimental diets were prepared based on common requirements of fish nutrition. Table 1 shows the different levels of diets where Diet 1 (0% of pineapple

waste), Diet 2 (10% of pineapple waste), Diet 3 (20% of pineapple waste) and Diet 4 (30% of pineapple waste). To attain 32% of dietary protein, the specified feedstuff and pineapple wastes were mixed together. Feed formulation was carried out using Winfeed Software (Winfeed 2.8), and the chemical analysis and formulation content of the trial diets are shown in Table 1. Chemical analysis and diets were conducted according to AOAC method (2012).

Table 1: Chemical analysis and fish feed formulation diet

Ingredients	Diet 1 (g)	Diet 2 (g)	Diet 3 (g)	Diet 4 (g)
Rice bran	20.75	18.25	15.75	13.25
Fish meal	20.75	18.25	15.75	13.25
Corn meal	20.75	18.25	15.75	13.25
Soybean meal	20.75	18.25	15.75	13.25
Vitamin and mineral premix ²	2	2	2	2
Vegetable oil ¹	5	5	5	5
Binder	10	10	10	10
Pineapple waste	0	10	20	30
Total	100	100	100	100
Proximate analysis (%)				
Crude protein	32	32	32	32
Crude Fat	4.53	4.26	4.43	4.33
Crude fiber	6.54	6.88	7.23	7.33
Moisture	8.18	8.03	7.80	7.94
Ash	10.60	9.53	9.55	9.43
Energy	3200	3200	3200	3200

¹ Visawit vegetable oil ² Vitamin and Mineral premix (g/kg pemix): Vitamin C, KCL, 90; KI, 0.04; CaHPO₄.2H₂O, 500; NaCl, 40; CuSO₄.5H₂O, 3; ZnSO₄. 7H₂O, 4; CoO₄, 0.02; FeSO₄.7H₂O, 20; MnSO₄.H₂O, 3; CaCO₃, 215; MgOH, 124; Na₂SeO₃, 0.03; NaF1

2.3 Fish and culture condition

The healthy farm-raised fingerlings were obtained from the local hatchery in Jeli, Kelantan. All fingerlings were acclimatized for seven days to prevent stress and fed commercial pellets before diet treatments. At the beginning of the experiment, triplicate groups of 30 healthy fingerlings with an approximate weight of 6.0 ± 1.0 g were arbitrarily selected and reared in 12 aquaria. All experimental fingerlings were fed three times daily for eight weeks. Through the experiment, water quality measures such as temperature,

pH, dissolved oxygen, ammonia level, total alkalinity and salinity were recorded. To maintain water quality, detritus, uneaten feed and dead fish were syphoned out of the tank, and half of the water was swapped daily (Soundarapandian et al. 2009; Sukri et al. 2016).

2.4 Growth performance

At the end of feeding trial session, each fingerling was individually weighed. The following formulas were used to compute the Weight Gain (WG), Specific Growth Rate (SGR), Percentage of Survival and Feed Conversion Ratio (FCR):

$$\text{Weight Gain (WG)} = W_f - W_i$$

$$\text{Specific Growth Rate (SGR)} = \frac{(\log W_f - \log W_i)}{T} \times 100$$

where,

W_f = Final fish weight (g),

W_i = Initial fish weight (g),

T = experimental period in days.

$$\text{Survival Rate (SR \%)} = \frac{N_f}{N_i} \times 100$$

where,

N_f = the number of fish stock at the end of experiment,

N_i = the number of fish stock at the beginning of the experiment.

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{Total weight of dry feed given}}{\text{Total wet weight gain}}$$

2.5 Texture analysis

Texture profile studies of tilapia were performed after eight weeks of the trial, including hardness, chewiness, springiness, cohesiveness, and gumminess using Brookfield CT3 Texture Analyzer. Three fillet samples of each replicate of treatments were

evaluated for texture quality. The fillet was cut from head to tail and dorsal to ventral according to Bland et al. (2018) method. Fillet samples were placed on the stage of texture analyser and compressed with a cylinder probe. Samples were compressed twice as has been prescribed in the protocol: a pre-test speed, a test speed, target value and trigger value were set as 1.0 mm/s, 10.00 mm/s, 1.00 cm and 5 g, respectively.

2.6 Colour evaluation

The flesh colour was evaluated according to Cheng et al. (2015) method where L* is indicated as lightness, a* is redness and b* is yellowness using CR-400 Chroma meter (Konica Minolta, Osaka, Japan).

2.7 Statistical analysis

One-way variance analysis (ANOVA) and Duncan's Multiple Range test were used to analyse the data. The statistical analysis was performed using SPSS Version 25 at 0.05 significant level.

3. Results

3.1 Effects of pineapple waste on the growth performance

Table 2 shows the growth performance of Nile tilapia. Diet 1 showed the highest survival rate throughout the study. Meanwhile, in Table 3, Diet 4 recorded the lowest FCR among all treatments. The results, however, show that there was no statistically significant difference in both parameters ($p > 0.05$). Contrariwise, weight gain, percentage of weight gain, and specific growth rate were significantly higher in the pineapple waste diet-fed fish than control treatment ($p < 0.05$). Thus, the present study indicated the addition of pineapple waste, even at a different level, have increased the weight gain of Nile tilapia fingerlings. Of these, Diet 4 gave the best performance as the weight gain, percentage of weight gain, and specific growth rate were significantly superior compared to other diets ($p < 0.05$).

Table 2. Growth performance of Nile tilapia with average mean \pm SD

Treatment	Initial Weight (g)	Final Weight (g)	Weight Gain (g)	Percentage of Weight Gain (%)	Specific Growth Rate (%)
Diet 1	6.67 ±0.19 ^a	14.23 ±0.31 ^a	7.57 ±0.12 ^a	113.54 ±1.59 ^a	0.59 ±0.01 ^a
Diet 2	6.83 ±0.24 ^a	14.87 ±0.41 ^a	8.03 ±0.12 ^{ab}	117.66 ±2.98 ^a	0.60 ±0.01 ^a
Diet 3	6.73 ±0.25 ^a	15.13 ±0.25 ^a	8.40 ±0 ^b	124.93 ±4.69 ^a	0.63 ±0.02 ^a
Diet 4	6.80 ±0.28 ^a	16.47 ±0.68 ^b	9.67 ±0.52 ^c	142.30 ±7.71 ^b	0.69 ±0.03 ^b

Diet 1= contain 0% of pineapple waste, Diet 2= contain 10% of pineapple waste, Diet 3= contain 20% of pineapple waste, Diet 4= contain 30% of pineapple waste

Table 3. Feed Conversion ratio (FCR) and survival rate of Nile tilapia

Treatment	Feed Conversion Ratio (Mean ± SD)	Survival Rate (%) (Mean ± SD)
Diet 1	4.91 ±0.62 ^a	71.11 ±15.48 ^a
Diet 2	4.32 ±0.25 ^a	64.45 ±3.14 ^a
Diet 3	4.03 ±0.46 ^a	61.11 ±11.33 ^a
Diet 4	3.87 ±0.49 ^a	65.56 ±8.31 ^a

Diet 1= contain 0% of pineapple waste, Diet 2= contain 10% of pineapple waste, Diet 3= contain 20% of pineapple waste, Diet 4= contain 30% of pineapple waste

3.2 Texture analysis

Table 4 shows the hardness, cohesiveness, springiness, gumminess and chewiness of tilapia fed different levels of pineapple waste. Although there was no significant difference among treatments, the study found that fish diet with pineapple waste (Diet 2 to 4) showed a high hardness value than control (Diet 1). Of these, Diet 4 recorded the highest value. The study also showed that the means value of chewiness for Diet 2 (37.00 mJ) and Diet 1 (0.40 mJ) was the highest compared to Diet 3 (-0.32 mJ) and Diet 4 (-1.23 mJ). The means value of cohesiveness was decreased from 7.25 for Diet 1 to -0.40 for Diet 3. As for springiness, Diet 1 shows the highest value at 1.02 cm. The gumminess for Diets 2 and 4 showed the highest value at 1001.00±915.03 g and 3474.11±2817.56 g than of the control diet at only 763.45±125.51 g.

Table 4. Hardness, cohesiveness, springiness, gumminess and chewiness values of each sample in different experimental diets

Treatment/Parameter	Hardness (g)	Cohesiveness	Springiness (cm)	Gumminess (g)	Chewiness (mJ)
Diet 1	80.11±3.97 ^a	7.25±2.00 ^a	1.02±0.54 ^a	763.45±125.51 ^a	0.40±31.64 ^a
Diet 2	107.78±46.00 ^a	0.03±5.05 ^a	0.29±0.31 ^a	1001.00±915.03 ^a	37.00±24.19 ^a
Diet 3	81.17±8.26 ^a	-0.40±5.48 ^a	-0.04±0.00 ^a	337.67±39.37 ^a	-0.32±0.13 ^a
Diet 4	149.89±7.86 ^a	2.74±0.77 ^a	-0.02±0.00 ^a	3474.11±2817.56 ^a	-1.27±0.33 ^a

¹Value are mean±SE. Data within the same column with different superscript letters are significantly (p<0.05).

²Diet 1= contain 0% of pineapple waste, Diet 2= contain 10% of pineapple waste, Diet 3= contain 20% of pineapple waste, Diet 4= contain 30% of pineapple waste

3.3 Colour evaluation

The present results in Table 5 showed that although the lightness chromaticity (L*) was not statistically significant, Diet 2 had a higher value than Diet 1 (without pineapple waste). Meanwhile, Diet 2 to 4 showed the lowest value of yellow chromaticity (b*) compared with Diet 1 (p > 0.05). Only red chromaticity (a*) shows a significant difference in all treatments (p < 0.05) as the value of redness of Diet 4 was higher than Diet 1.

Table 5. Lightness (L*), red chromaticity (a*) and yellow chromaticity (b*) values of each sample in different experimental diets

Treatment/Parameters	L*	a*	b*
Diet 1	34.08±0.41 ^a	9.01±0.18 ^b	9.31±0.17 ^a
Diet 2	34.38±1.05 ^a	7.96±0.24 ^c	8.82±0.25 ^{ab}
Diet 3	33.34±0.78 ^a	8.55±0.43 ^{bc}	8.96±0.16 ^{ab}
Diet 4	33.49±0.53 ^a	9.91±0.08 ^a	8.29±0.34 ^b

¹Value are mean±SE. Data within the same column with different superscript letters are significantly (p<0.05).

²Diet 1= contain 0% of pineapple waste, Diet 2= contain 10% of pineapple waste, Diet 3= contain 20% of pineapple waste, Diet 4= contain 30% of pineapple waste

4. Discussion

Our present findings were similar to the recent studies by Van Doan et al. (2021) and Yuangsoi et al. (2018), which reported the optimum growth performance of Nile tilapia. Meanwhile, Deka et al. (2003) have previously reported that the best growth performance of rohu (*Labeo rohita*) fingerlings was by feeding a diet with 25% pineapple waste. Their study also found that other fruits processing waste such as orange and sweet lime at a level of 25% are saved for rohu fingerlings to consume in the diet. Indeed, the agricultural waste addition in animal feed can reduce the environmental issue and

simultaneously increase farmers' economy.

The addition of supplements in fish feed is a common practice to enhance the optimum growth and healthy fish cultivated on the farm. Besides, the livestock industry has also utilised agriculture waste in animal feeding to increase feed conversion. Pineapple skin (peels) contains a high concentration of bromelain followed by core, crown and stem (Misran et al. 2019). Bromelain is an enzyme that helps the texture of flesh become more tender and soft. It also reduces inflammation in the body. The active ingredients in bromelain are the mixture of cysteine proteases. These enzymes break down the proteins in the food, releasing small peptides. It is critical to improve growth performance by increasing protein digestibility and rapid absorption. A previous study has shown that the supplementation of exogenous protease treatment improved the growth performance of gibel carp, *Carassius auratus gibelio* (Shi et al. 2016). Their study also reported the apparent digestibility of dry matter, crude protein, protein and lipid retention. Meanwhile, according to Li et al. (2016) adding of protease in a low fish meal diet can improve white shrimp, *Litopenaeus vannamei* and tilapia, *Oreochromis niloticus* × *O. aureus* growth, where the growth performance was similar to those fed a high fish meal diet.

The present results are also parallel to other animals which fed pineapple waste. For instance, broiler fed pineapple leaf powder has significantly increased the weight gain, growth performance and feed conversion ratio (Rahman and Yang, 2018). Similar results were also observed for ruminants fed pineapple waste (Adekanbi et al. 2017; Costa et al. 2007). It shows that the flavour and smell of pineapple waste are acceptable to be consumed by livestock. Hence, the addition of pineapple waste into fish and livestock feeds do not produce any adverse effects. The pineapple waste also has bioactive compounds that can alter the gut microbiome to enhance digestion and assimilation of nutrients (Rahman and Yang, 2018). In addition, the high fibre content in the pineapple waste plays a significant role in reducing digestion of other dietary components that affect the efficiency of digestibility and absorption of nutrients.

Good aquatic diets are crucial for fish health, texture, and colouration. It also improved the water quality and reproductive potential of farmed fish. The characteristics of raw meat represent the overall quality and acceptability of the fish product. There are

no standard parameters to determine the physical meat quality of most edible species (Komolka et al. 2020). However, texture property assessment could exhibit the quality of fish and fish-based products (Gonçalves et al. 2018; Hultman and Rustad, 2002; Kilinc et al. 2009). Certainly, freshness is the primary aspect in assessing the quality of fish meat as it is directly related to the consumers' perception of the appearance, texture, and taste.

Jun et al. (2014) reported that the dystrophin disappeared quickly after the fish died. Hence this resulted in the detachment of myofibers and myocommata and a reduction in textural resilience. Meanwhile, the actin and desmin collapsed until the muscle tissues exhibited a decayed sensory appearance and texture. Bromelain, which is derived from pineapple peels, has previously been shown to successfully tenderise beef, poultry and squid (Ketnawa and Rawdkuen, 2011). Bromelain is an inexpensive enzyme and has become an effective tendering alternative for meat and has attracted considerable attention from the industry (Arshad et al. 2014). The flesh texture regularly depends on the fish type or species, their size and age, fat content and distribution and muscle density (Hultman and Rustad, 2002). The flesh texture of fish can also be affected by the muscle metabolism post-mortem mechanisms. These mechanisms include the rigour mortis process, proteolysis, microbiological process and storage environments (Hultman and Rustad, 2002).

Chewiness is the energy needed to chew a solid sample into a steady state of swallowing (Zhao et al., 2017). The decrease in chewiness value in the present study indicates the breakdown of peptide and disulphide bonds in myofibrillar proteins structure by bromelain (Feng et al. 2017; Shin et al. 2008). Cohesiveness refers to a material's ability to accept a second deformation in comparison to its resistance to the first (Floury et al. 2009). It shows that the diets that contain pineapple waste have a substantial effect on gumminess. The present study postulates that the bromelain enzyme may probably affect the flesh texture of the Nile tilapia. Thus, our result corresponds with previous studies demonstrating the tenderness of meat increased after applying bromelain (Calkins and Sullivan, 2007).

The colour of the flesh is crucial to determine the consistency of the fish fillets, such as white (lightness), red, and yellow. In general, white fillets are of the best quality, while pink and red represent poor bleeding techniques (Sørensen, 2005). However, the

colour of the flesh is also related to the fish species. Ponsano et al. (2014) reported that besides white fish, the red colour of some fish species is also preferred of which create a trend in the market. Thus, other than freshness, colour qualities are also one of the main criteria in consumers' perception before purchasing meat products. It indicates that consumers' perception is affected by their level of knowledge and sensory appeal (Thongdonphum et al. 2016).

According to Sørensen (2005), the yellow fillet represents the low colour quality caused by the poor water quality in fish farming. Our study cultivates the fish in the glass aquarium, where the water quality is monitored within an optimum range. Hence, we postulate that the low value of yellow chromaticity is probably affected by the compounds in the pineapple waste. Shekarabi et al. (2020) also reported the significant increment of redness of the rainbow trout (*Oncorhynchus mykiss*) fillet fed a diet with black mulberry (*Morus nigra*) juice powder. Their study also found that the carotenoid concentrations in mulberry influence the flesh colourimeter as its high contents increased the redness and yellowness while reducing the lightness of the flesh ($p < 0.05$). Ponsano et al. (2014) reveal that the carotenoids fed to Nile tilapia increased the red ($p < 0.05$) and yellow ($p > 0.05$) values of the fish flesh. Hence, the present finding corresponds with previous studies except for the yellow chromaticity (Brown and Shahidi, 1997; Rahman et al., 2016).

Unlike farmed fish, the flesh colour of wild fish in the natural ecosystem reaches more redness as they consume krill and phytoplankton (Breithaupt, 2007). Most fish on the farm have pale and greyish skin and fillet compared to those obtained in nature (Diler and Dilek, 2002). As to improve this, previous studies have utilised synthetic or natural sources of carotenoid in fish feeding. Hardy and Lee (2010) reveal that the salmonids skin and muscle colour improved after consumed carotenoids pigments such as astaxanthin in the fish feed. We postulate that the feed formulation with pineapple waste used in the present study has the same ability as astaxanthin to improve the redness colour of the tilapia flesh, particularly for farmed fish. It is also suggested that the redness colour of tilapia flesh could garner customers perception towards the improvement of flesh quality by noting the high content of carotenoids.

5. Conclusion

The present study showed the feed formulation with pineapple waste could improve the ²⁵ growth performance of Nile tilapia. There were no significant effects on the texture quality. Diet supplemented with pineapple waste has shown that only red colour was significant and can improve the redness of fillet in farmed fish. The supplementation level of the pineapple waste in the diet was 30% of the total feed formulation for the Nile tilapia fingerlings.

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