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Original Article

Effect of tamarind seed supplementation on growth performance and immunity in sex-reversed red tilapia's diet (*Oreochromis niloticus* x *O. mossambicus*)♣

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Abstract

Tamarind seed, a by-product of tamarind paste production, contains usable nutrients in the kernel that could induce immunity in rats. This study aimed to determine the possibility of using tamarind kernel powder in red tilapia feed observing its effects on fish growth and immunity. A feeding trial was conducted using tilapia fingerlings fed with 1, 2.5 and 5% tamarind kernel powder (TKP) inclusion in feed for eight weeks. A control group were fed with diet without TKP. The average initial weights of the red tilapia ranged from 4.73 ± 0.10 to 4.87 ± 0.23 g/fish (P>0.05). Growth parameters were monitored during the feed trial. At the end of the trial, lysozyme activity was determined, and fish were subjected to a pathogen challenge by intraperitoneally injecting them with 1.6 x 10^8 CFU *Aeromonas hydrophila*. Results showed that the fish reached 29.07 ± 2.96 to 30.77 ± 2.05 g/fish sizes. The growth parameters (weight gain, ADG, FCR), blood counts, and hematocrit were not significantly different among the treatments (P>0.05). The lysozyme activity also showed no significant difference among the treatments (21-28 units/milliliter). Fish mortality rate post-challenge with pathogen was not significantly different between control and treatments (P>0.05). The results showed that 5% tamarind kernel powder could be included as a binding agent in feed without affecting growth of tilapia during the nursery stage.

Keywords: tilapia, *Oreochromis niloticus x O. mossambicus*, tamarind seed kernel, aquafeed, immunity

1. Introduction

Disease in aquatic animals can result in crop losses due to mortality. Causative factors associated with fish diseases include poor water quality, pathogenic agents, and weak fish health. Treating the fish with chemicals and antibiotics cannot guarantee success, especially when pathogens are fully spreading. Prophylactic treatments with chemicals and antibiotics are also not the solution. Antibiotics and their derivative residues in fish flesh can have large impact on consumer's health, for example by inducing drug resistance. Since aquaculture will play an important role as food support in the future, it is crucial to generate green and clean products for consumers. This practice will not only be beneficial to consumers, but will also be friendly to the environment, which are key aspects of aquaculture sustainability.

An alternative to using antibiotics is to improve the immunity of fish through natural compounds from plants or herbs (Chitmanat, 2017; Rattanachaikulsobhon, & Poom kajorn, 2010). Farmers usually observe high mortalities in fry after pond stocking. This could be due to the still developing immune defense mechanisms against pathogens, and to poor adaptation to the new environment. Therefore, fish at this age are relatively vulnerable to diseases and could be a model for studying immunostimulation. In commercial scale production of sex-reversed tilapia, the fry is usually fed with feed mixes including testosterone hormones until they reach sexual

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dimorphism. They will then be sold to grow-out farmers to be raised in either cages or in earthen ponds. Smaller fry are usually bought by earthen pond farmers to either nurse to sell to other grow-out farmers, or for culturing them until marketable size. Cage farmers would buy larger fingerlings (50-100 grams/fish) from pond farmers to stock in their facilities for a shorter period of only 3-4 months. The fry ranging from 0.5 to 50 grams in body weight are at the most critical age, and were used in this study.

Feed with immunostimulant is a natural delivery method to enhance the immunity of fish. It was suggested that polysaccharide in feed could enhance immunity through either blood cell activities or humoral immunity (Malowsky & Mackay, 2011). The polysaccharide comes in many forms. One of the most extensively reported form of polysaccharide is glucan, and glucan from yeast cells had been shown to enhance immunity in finfish. However, aquafeed mills need to consider the cost of obtaining yeast. Another source of glucan would be helpful. Glucan from plants or xyloglucan could be found in seed kernels in dicotyledon plants (Sinchaikit & Suttijit, 2011). Xyloglucan is an important component stored in seeds that decreases with germination (Do Rosário, Kangussu-Marcolino, Amaral, Noleto, & Petkowicz, 2011). High xyloglucan content was reported for tamarind seeds, which are a by-product from the tamarind processing industry after the pulp has been removed for use in cooking to add acidity to dishes. A seed of Tamarind is also called pericarp or cotyledon. A whole seed is composed of the husk, which is the outermost layer with brownish in color, and the inner part which is the kernel. Tamarind is a plant that stores polysaccharides as xyloglucan for growth of the plantlet. Xyloglucan is responsible for germination of the tamarind plant.

Xyloglucans stored in seeds have cellulosic b (1,4)-glucan backbone, branched at some points with a-(1,6)-xylopyranosyl (forming with the glucose of the backbone as a disaccharide assigned X) or b-(1,2)-D-galactopyranosyl-a(1,6)-D-xylopyranosyl (forming a trisaccharide assigned L) (Ravachol *et al.*, 2016). Except for the absence of terminal fucosyl units a-(1,2) linked to the b-D-galactopyranosyl groups, there is a remarkable similarity between seed-stored xyloglucans and structural xyloglucan from primary walls of dicotyledons, with differences depending on strains. Xyloglucan in tamarind seed kernel has been shown to stimulate macrophage activities (Do Rosário *et al.*, 2011) and to protect skin against cancer stimulated by UV radiation in rats (Strickland, Kuchel, & Halliday, 2004). To digest polysaccharide in tilapia's intestine, gut microbiomes play important roles (Moreno-Indias, Cardona, Tinahones, & Queipo-Ortuno, 2014). Examples of bacteria working on polysaccharide digestion are *Bacillus* sp., *Actinobacteria*, *Firmicutes*, Proteobacteria and *Fusobacterium* (He *et al.*, 2010). Therefore, polysaccharides are used by gut microbes, enhancing the growth of these bacteria and increasing fatty acids together with lactic acid in the gut, as energy sources to the host (Pié *et al.*, 2007). Later, research by Malowsky and Mackay (2011) suggested that gut microbes could digest polysaccharides into short chain fatty acids (SCFA), which would be absorbed through the epithelial on intestine, coupling with receptors on macrophages and hence, controlling inflammation and enhancing phagocytosis. Thus, in order to enhance fish health, immunostimulation via feed could be a potential solution.

Tamarind seed kernel has been widely utilized in various industries, including in animal feed and aquafeed production. Thus, using the seed which is widely available in the sub-northern region of Thailand could be a way to reduce underutilization of this by-product and to provide added value as an ingredient in aquafeeds, while also reducing the feed production costs. In addition, xyloglucan in the seed kernel could be beneficial to fish health.

2. Materials and Methods

Tamarind seed (*Tamarindus indicus*) was obtained from Krok Pra district, Nakorn Sawan province, after the pulp was removed. The seeds were washed and dried at room temperature before toasting at 40^{o}C to remove the coat. The resulting kernels were ground and boiled before use as a feed ingredient. Feed formulations consisted of fish meal, soybean meal, rice bran, coconut meal, vitamins, minerals, and varying concentrations of tamarind kernel powder (TKP). Total protein concentration in all formulations was 37% (Plaipetch, 2016). The ingredients were mixed, and 3 mm feed pellets were produced using a pelletizer. Proximate analysis was done for all four diets.

Red tilapia fry (*Oreochromis niloticus* x *O. mossambicus*) was purchased from Boonrak farm, Prompiram district, Phisanulok province. Initial weight of fry was 0.5 gram/fry with a total length 2.5-3.0 cm). These fries were bathed in 25 ppm formalin to eliminate ectoparasites before stocking in 500-liter circular plastic tanks at a density of 1 fish/liter. The fish were acclimated in this facility for 1 week before starting the experiment.

The fish were then stocked in sixteen of 0.5 m^2 concrete circular tanks, supplied with oxygenation, at a density of 86 fish/m^2. Fish were fed with the experimental diets at 4% of body weight for 60 days. Weight and length of individual fish were monitored once a week.

Blood sample was taken biweekly from the caudal blood vessel of three fish/ tank. A total of 100 microliters was withdrawn from the fish and transferred to EDTA coated tubes. The red blood cells were diluted with Gower's solution before placing in the hemocytometer. The white blood cells were diluted with 3% acetic acid before applying to the hemocytometer. Hematocrit was determined using a coated capillary tube and centrifuging at 12,500 rpm before the packed red cell volume was read.

After 60 days of the experiment, a total of 1 milliliter blood was withdrawn from an individual fish. The blood was left to clot for 60 minutes at room temperature. The serum was separated by centrifugation at 2,500 rpm, and 10 microliters of the serum was loaded to a 96-well microplate. A total of 250 microliters of *Micrococcus lysodeikticus* suspended in buffer was added to each well, and the optical density at 540 nanometers was monitored for 0.5-6 minutes using a microplate reader (Biotek, U.S.A.). Then the serum was further analyzed for lysozyme activity.

A total of ten fish remaining from each tank was transferred for the challenge test in 180-liter glass aquarium. Individual fish received 1.6 x 10^8 CFU *Aeromonas hydrophila.* Mortalities were monitored for 14 days. Food,

oxygenation, and overall good water quality were maintained during the challenge test.

3. Results and Discussion

Formulation of feed in this experiment was done using TKP as a binder. The experimental diets were analyzed for their nutritional value. Results show that the total protein did not significantly differ between the feeds, which on average had 35% protein. Only the control diet had significantly lower fat content (17.44%), which was significantly different from the other diets having 1, 2.5 and 5% TKP (Table 1). However, no significant difference was found in any growth parameters, as shown in Table 2. It was observed that the fish in the control group had slightly lower weight gain and ADG (average daily growth) from the actual experimental groups, but there was no statistically significant difference (P>0.05) among the groups.

Red blood cell counts showed a trend of increasing blood count with increasing levels of TKP in week 6th and week 8th (not statistically significant, P >0.05), as shown in Figure 1. The red blood count agreed with % packed red cells in Figure 2. White blood cells showed similar averages with a lower count for 2.5% TKP inclusion (Figure 3).

Survival rate after pathogen challenge was observed for 14 days. All the fish that received 10^8 CFU of *Aeromonas hydrophila* were dead by the end of the test period. Fish responded to pathogen differently, as shown in Figure 4. On day 9, the mortality rate of fish from 2.5% TKP treatment was 100%. Mortality rates for the other treatments reached 100% soon after: control group (0%TKP) on day 10, 0.5%TKP on day 11 and 5%TKP on day 12. In this experiment, there were no significant differences in WBC and lysozyme activities among the treatments, and in addition, the quantities of white blood cells and lysozyme activity were not in agreement. The 5%TKP fed fish had lower WBC than that of 1%TKP, while they surprisingly exhibited higher lysozyme activity (Figure 5). However, no significant differences between the treatments were observed.

The results of this experiment suggest that sex-reversed red tilapia fed with 0-5% TKP inclusion for eight weeks did not have differences in their growth performance. This is likely due to similar protein content in the feeds (37% protein). Although, there was a significant difference in terms of fat content of the feeds. Control diet (0%TKP) total fat was lower than those of the actual experimental diets. However, the difference in fat content in the diets was minimal and it did not affect growth performance of the fish during nursing, as observed in this experiment. A similar study conducted by Tongsanitkan, Siriratananant, and Worapassu (2017) suggested that 5%TKP inclusion in feed as binder would result in better stability of feed in water. Further experiments could be done with a higher proportion of TKP so that the maximum levels of TKP could be determined.

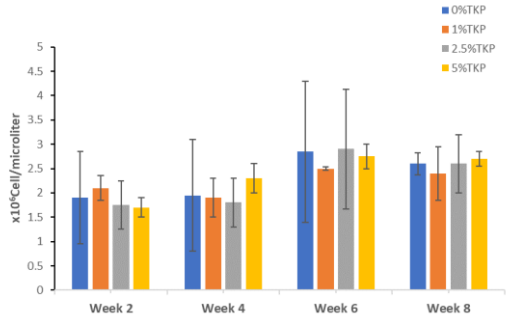

Figure 1. Red blood cell count (cell/microliter) from fish fed with different levels of TKP. T1; 0%TKP or control group, T2; 1%TKP, T3; 2.5%TKP, and T4; 5%TKP

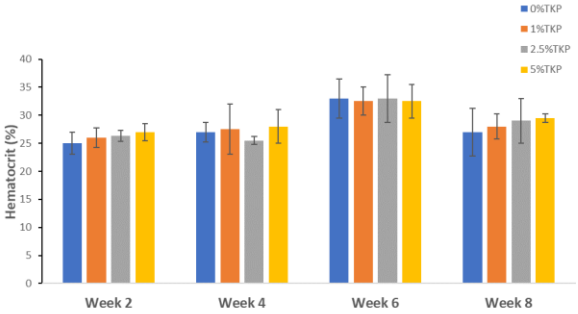

Figure 2. Hematocrit (%packed red cells) of fish fed with different levels of TKP. T1; 0%TKP or control group, T2; 1%TKP, T3; 2.5%TKP, and T4; 5%TKP

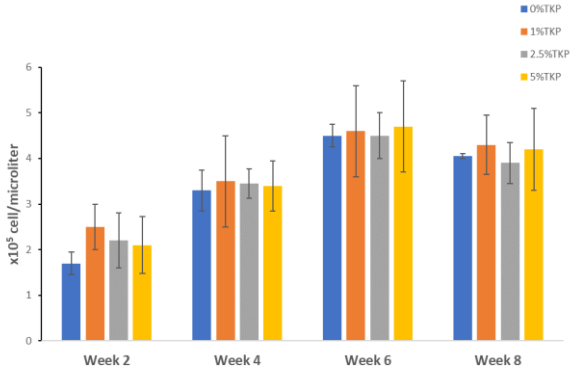

Figure 3. White blood cell count (cells/microliter) for fish fed with different levels of TKP. T1; 0%TKP or control group, T2; 1%TKP, T3; 2.5%TKP, and T4; 5%TKP

Table 1. Proximate analysis of nutritional values (%) in the experimental feeds with alternative levels of tamarind kernel powder (TKP). T1; control diet, T2; 1.0%TKP, T3; 2.5%TKP, and T4 5%TKP

Diet	Protein	Fat	Moisture	Fiber	Ash
T1 (0%TKP)	34.89±0.36	17.44±0.096b	1.28±0.06	3.72±0.43	8.69±0.29
T2 (1.0%TKP)	35.22±0.49	18.01±0.45a	1.04±0.20	3.91±0.35	8.63±0.29
T3 (2.5%TKP)	35.15±0.26	18.02±0.085a	1.38±0.09	3.94±0.99	8.57±0.01
T4 (5.0%TKP)	35.35±0.53	18.17±0.14a	1.19±0.04	3.98±0.43	8.42±0.004

Note: Different letters in the same column indicate significant differences at 95% confidence level (P <0.05).

Table 2. Growth performance of red tilapia fed with the experimental diets

Diet	Parameter				
	Average weight (gram/fish)	Weight gain (%)	Specific growth rate (%/fish/day)	Feed conversion ratio	Survival rate (%)
T1 (0%TKP)	29.07±2.96	496.35±30.55	2.97±0.08	1.06±0.11	93.02±2.33
T2 (1.0%TKP)	30.40±0.70	539.25±77.52	3.10±0.19	1.02±0.02	93.80±4.84
T3 (2.5%TKP)	30.77±2.05	545.96±43.66	3.11±0.11	1.02±0.06	92.25±3.55
T4 (5.0%TKP)	30.57±1.00	545.77±36.26	3.11±0.10	0.99±0.05	93.02±4.03

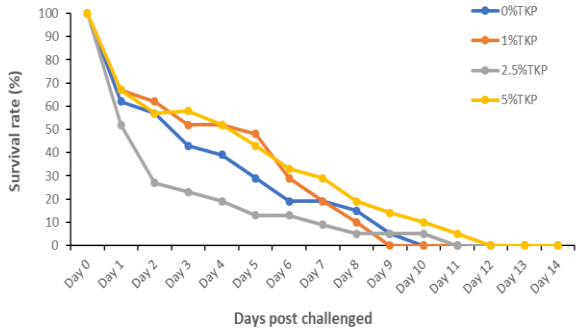

Figure 4. Challenge test results. Survival rates of fish injected with $3.8x10^8$ CFU of *A. hydrophila*. Normal saline was injected in a group of fish for negative control. T1; 0%TKP or control group, T2; 1%TKP, T3; 2.5%TKP, and T4; 5%TKP.

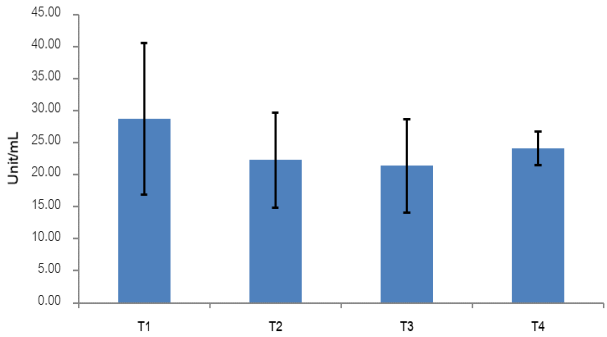

Figure 5. Lysozyme activity (unit/milliliter) in fish fed with different levels of TKP. T1; 0%TKP or control group, T2; 1%TKP, T3; 2.5%TKP, and T4; 5%TKP

Blood parameters in the experimental fish showed an increasing trend with fish growth. The red blood cells count increased from week 2-6, then slightly decreased in the final week. The red blood cell count was comparable to hematocrit, both showing a slight drop at the 8th week. Blood cell counts normally increases with age (Baga & Promkhuntong, 2012; Hrubec, Cardinale, & Smith, 2000). Alteration of red blood cell or erythrocyte count could be caused by environmental stress or indicate pathogenic symptoms. Sayed, Mahmoud, and Mekkawi (2015) demonstrated that hormonal feeding in tilapia might result in morphological changes in erythrocytes, including swollen cells, tear drop cells, and sickle cells. This could indicate an overdose of the male hormone. In this experiment, we did not see morphological alterations of erythrocytes during RBC counting.

Anticoagulants such as EDTA, heparin and sodium citrate could affect the blood counts. It was suggested that EDTA is a reliable anticoagulant for certain kinds of fish, such as *Mugil cephalus* (Fagio, Arfuso, Piccione, Zumbo, & Fazio, 2014). In this experiment, EDTA was used as anticoagulant.

Lysozyme is part of the innate immunity of teleost found in mucus and leucocytes specifically monocytes, macrophage, and neutrophils (Dalmo, Ingebrigtsen, & Bogwald, 1997). These cells are important as first line of defense when a fish is infected with a pathogen. Wongwai pairote, Srisapoome, Unajak, and Areechon (2015) reported that lysozyme activity would be activated by pathogen invasion. In this experiment, there were no significant differences in either lysozyme or white blood cell counts among the treatments. For fish fed with 5% TKP inclusion, the lysozyme activity was lower than that of the control, but higher than with the other TKP inclusion diets. It is possible that TKP inclusion in the feed might not affect immunity within this life stage or with only 60 days feeding trial and the tested inclusion levels.

Fruit of tamarind contains about 55% pulp, 34% seed and 11% shell, with fiber in a pod. The seed comprises 20-30% seed coat and 70-80% endosperm or kernel (Thombare, Chowdhury, & Srivastava, 2014). The seed is considered an underutilized by-product from the tamarind pulp industry. There is a need to utilize by-products from industries in order to minimize biowaste, and they are rich sources of nutrients that could be used for human consumption (Nwanna, Fagbenro, & Olanipekun, 2004) and would be suitable for animal feed. Price of tamarind kernel is around 2-3 THB/kg (US$ 0.07-0.10/kg) as plantlets for propagating in Thailand. In this study, 5% TKP was included to the feed replacing the binding agent and for inducing lysozyme activity. Therefore, utilization of the binding agent available domestically, such as TKP, instead of using imported products for binding agent, will benefit local aquafeed producers.

To examine if TKP would induce immuno stimulation, fish were subjected to a challenge test with a pathogen, *Aeromonas hydrophila*, after feeding with the experimental diets. Intraperitoneally injected 1.6 x 10^8 CFU fish fed with the different experimental diets (0 to 5%TKP) were observed for 14 days in the laboratory. The results showed that, at this pathogen dose, the fish could not survive to day 14. Fish fed with the 5%TKP diet could withstand the pathogen for 12 days, longer than the control group (0%TKP), despite a lower lysozyme activity. The mean number of leukocytes in 5%TKP fed fish was higher than that of the control group (P>0.05). A follow-up study to observe the effects of various profiles of leukocytes may be beneficial.

4. Conclusions

Inclusion of up to 5% tamarind seed kernel powder in tilapia diets is possible during the nursing stage of 4-5 gram tilapia, without affecting their growth performance. We concluded that 5%TKP in the feed gave it improved stability. However, at this level of inclusion and with the feeding strategies of the experiment, immune responses were not significantly different by treatment, as shown in lysozyme activity and in the challenge test. The lack of effects on the former attribute could be due to the size of the fish, while the latter could be determined by the intensity of pathogens.

This experiment corroborates the feasibility of including tamarind kernel powder in fish feed, and its potential to improve fish health during the nursing period. However, extending the duration of the feeding trial might better induce lysozyme activity.

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References

Baga, A. & Promkhuntong, W. (2012). Effects of *Enteromorpha* sp. in diets on growth performance, feed utilization and immune response of red tilapia (*Oreochromis niloticus* x *O. mossambicus*). *50th Kasetsart university Annual conference, January 31st – February 2nd, 2012*. Kasetsart university. Bangkok. pp. 515-524.

Chitmanat, C. (2017). Herbal products effects on fish immunity. *Khon Khaen University Research Journal*, *18*(2), 257-269. Retrieved from http: //www.resjournal.kku.ac.th

Dalmo, R. A., K. Ingebrigtsen, K. & Bogwald, J. (1997). Non-specific defence mechanisms in fish, with particular reference to the reticuloendothelial system (RES). *Journal of Fish Diseases*, *20*, 241–273. doi:10. 1046/j.1365-2761.1997.00302.x

Do Rosário, M. M. T., Kangussu-Marcolino, M. M., Do Amaral, A. E., Noleto, G. R., & Petkowicz, C. L. D. O. (2011). Storage xyloglucans: Potent macro phages activators. Chemico-Biological Interactions, 189(1-2), 127-133. doi:10.1016/j.cbi.2010.09.024.

Fagio, C., Arfuso, F., Piccoine, G., Zumbo, A. & Fazio, F. (2014). Effect of three different anticoagulants and storage time on haematological parameters of *Mugil cephalus* (Linneaus, 1758). *Turkish Journal of Fisheries and Aquatic Sciences 14*, 615-621. doi: 10.4194/1303-2712-v14_3_03

He, S., Zhou, Z., Liu, Y., Cao, Y., Meng, K. & Shi, P. (2010). Effects of the antibiotic growth promoter flavomycin and florfenicol on the autochthonous intestinal microbiota of hybrid tilapia (*Oreochromis niloticus* ♀ × *O. aureus* ♂). *Archives of Microbiology*, *12*(192), 985-994. doi:10.1007/s00 203-010-0627-z.

Hrubec T. C, Cardinale J. L., & Smith S. A. (2000). Hematology and plasma chemistry reference intervals for cultured tilapia (*Oreochromis* hybrid) intestinal microbiota of hybrid tilapia. *Veterinary Clinical Pathology*, *29*(1), 7-12. doi:10.1111/j.1939-165x.2000.tb00389.x.

Komkuntod, R., Krintanyakij, P., Suwittawas, K., Tanongjit, K., Pengchang, P. & Wanichkul, K. (2014). Comparison of growth and productivity of 19 strains of tamarind aged 4 years old at Pakchong Research Station, Nakhon Ratchasima province. *Kaen Kaset, 42*(3), 227-232.

Maslowski, K. M., & Mackay, C. R. (2011). Diet gut microbiota and immune responses. *Nature Immunology*, *12*(1), 5-9. doi:10.1038/ni0111-5.

Moreno-Indias, I., Cardona, F., Tinahones, F. & Queipo-Ortuno, M. I. (2014). Impact of the gut microbiota on the development of obesity and type 2 diabetes mellitus. *Frontiers in Microbiology*, *5*(190), 1-10. doi:10.3389/fmicb.2014.00190.

Nwanna, L., Fagbenro, O. & Olanipekun, S. (2004). Evaluation of Tamarind (*Tamarindus indica*) Seed meal as a dietary carbohydrate for the production of Nile Tilapia *Oreochromis niloticus* (L). *Animal Research International, 1*(3), 164-168. doi:10.4314/ ari.v1i3.40831.

Pié, S., Awati, A., Vida, S., Falluel, I., Williams, B. A., & Oswald, I. P. (2007). Effects of added fermentable carbohydrates in the diet on intestinal proinflammatory cytokine-specific mRNA content in weaning piglets. *Journal of Animal Science*, *85*(3), 673-683. doi:10.2527/jas.2006-535.

Plaipetch, P. (2016). Nutrition management for tilapia aquaculture. *Journal of Science and Technology*, *24*(1), 12-39.

Rattanachaikulsobhon, P. & Poomkajon, P. (2010). Utilization of herbs for preventing and curing fish diseases. *Ubonrachathani University Journal of Science and Technology*, *12*(4), 63-67.

Ravachol, J., Philip, P., Borne, R., Mansuelle, P., Mate, M. J., Perret, S. & Fierobe, H. P. (2016). Mechanisms involved in xyloglucan catabolism by the cellulosome-producing Bacterium *Ruminiclostri dium cellulolyticum*. *Scientific Reports, 6.* 1-17. doi:10.1038/srep22770.

Sayed, A. E.-D. H., Mahmoud, U. M. & Mekkawi, I. A. (2016). Erythrocytes alterations of monosex tilapia (*Oreochromis niloticus*, Linnaeus, 1758) produced using methyltestosterone. *Egyptian Journal of Aquatic Research 42*, 83-90. doi:10.1016/j.ejar. 2015.10.004.

Sinchaikit, P. & Suttijit, M. (2011). Biochemical properties and utilization of tamarind seed kernel. *Naresuan Phayao Journal*, *4*(2), 8-12. Retrieved from https:// www.researchgate.net/publication/304465953.

Strickland F. M., Kuchel J. M. & Halliday G. M. (2004). Natural products as aids for protecting the skin's immune system against UV damage. *Cutis*, *74*, 24-28. Retrieved from http://www.scopus.com.

Thombare, N., Srivastava, S. & Chowdhury, A. R. (2014). Multipurpose application of tamarind seed and kernel powder. *Science Reporter*, 32-33. Retrieved

from https://www.researchgate.net/publication/306038753.

Thongsanitkan, P., Siriratananant, N. & Worapassu, T. (2017). Supplementation of tamarind seed in Nile tilapia diet. *Proceeding of 10th Rajamangala university of Technology Tawan-ok Research conference, 29-31 May 2017*. Retrieved from http://research.pcru.ac.th/rdb/published/datafilescreate/477.

Wongwaipairote, T., Srisapoome, P. Unajak, S. & Areechon, N. (2015). Efficacy of *Streptococcus agalactiae* vaccine prepared by different methods on immunity of Nile tilapia (*Oreochromis niloticus* Linn.). *53th Kasetsart university Annual conference, February 3rd – 6th, 2015*. Kasetsart university, Bangkok. Retrieved from http://www.annualconference.ku.ac.th/cd53/04_003_O24.pdf