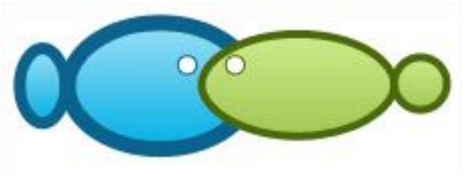

The potential use of yam tuber with probiotic for gonad development of tiger grouper

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Abstract. The effect of yam tuber (YT) with dietary probiotics on the growth performances, intestinal microbiota and reproductive hormones of tiger grouper (*Epinephelus fuscoguttatus*) were assesed after 60 days feeding on supplement diets. The diet contains 30 mg of the ingredients, namely 10 mg of YT, 10 mg of probiotic and 10 mg of skimmed milk as filler. Molecular analysis using PCR-DGGE (denaturing gradient gel electrophoresis) approach revealed that the probiotic was able to populate the gastrointestinal tract and modulate the microbial communities. After exposition, females fed on supplemented diet with isolated probiotic from digestive tract of tiger grouper presented an over-expression on gonad maturation comparing to the control group. It showed gonad weight and gonadosomatic index (GSI) bigger than control. The numbers of goblet cells were significantly higher on YT + probiotic compared to other treatment group. This suggest that probiotics with YT has potential as supplement in tiger grouper maturation diet for improvement of gonad development since probiotic supplementation with YT could affect female reproductive performance.
Key Words: *Epinephelus fuscoguttatus, Dioscorea hispida,* probiotic, reproductive hormone, DGGE.

Introduction. Herbaceous plants are dietary source of hundreds of non-nutritional phytochemicals. Many phytochemicals possess a number of beneficial activities, including antioxidant, anti-tumoral, anti-inflammatory, and estrogenic-like properties, as demonstrated by numerous epidemiologic, clinical, and experimental studies (Pandey & Rizvi 2009; Chong et al 2018). Yams (*Dioscorea* species) are nutritionally rich. Generally, they will have important role to attain food nutrition security in the country. From studied showed, yams have highest nutritional content with respect to protein, fibre, calcium and iron content but comparatively ash, fat, phosporous and zinc contents were low (Bekele & Bekele 2018).

Nowadays yam tuber (YT), or local name as ubi gadong (*Dioscorea hispida*) when processed, is widely used as an important ingredient in pharmaceutical industry as dietary supplement and cosmetic. It also stimulates the sex steroid production and may potentially exert some positive actions on reproductive success of tiger grouper (*Epinephelus fuscoguttatus*) brood stock management (Om et al 2017). Besides, YT has antioxidant properties, prostaglandins (PGE1) (Om et al 2016), the major pyhtoconstituents found in ethanol extraction, which have positive effect on gonads development. Prostaglandins are extremely potent in causing various biological responses, particularly in maturation of the fish gonad (Sorensen et al 1988).

Meanwhile, the indigenous gut microbiota act as a defensive barrier against pathogenic bacteria species, contributing towards digestive function and is implicated in development or maturation of the gut and immune system (Gómez & Balcàzar 2008). Therefore, the use of bacteria as probiotics in aquaculture has an important influence on health and disease.

The alimentary tract of fishes represents an interface between the external environment and the body. It is favored with wide range of microbes with an increase in population, density, types and complexity of interaction. The intestine is a complex multifunctional organ for digesting and absorbing feedstuff. By adding probiotics, with wide range of microbes, the digestion processes of aquatics animals can be enhanced by addition of some microorganism that may participate in the digestions processes. This can be done through production of extra-cellular nutritive enzymes, that have intended abilities for supplying necessary growth factors as fatty acids, vitamins and others (Vine et al 2006).

Using probiotic microorganisms to promote animal health is now backed by strong scientific evidence for some clearly defined and well characterized strains. In aquaculture, probiotics have been proposed as a major nutritional factor influencing gastrointestinal physiology and function (Balcazar et al 2006). This development introduces many challenges, but also creates new opportunities for food and nutrition scientists to improve food quality and develop new products with specific health benefits for different hosts. The administration of probiotics appears to be a very promising research area for nutrition, biological control and disease prevention in aquaculture.

Therefore, this study evaluated the effects of dietary YT supplemented with probiotics on growth performance, intestinal microbiota and hormone reproductive influence of tiger grouper brood stock reared in tanks under controlled conditions.

Material and Method

Raw materials and extraction methods. The yam tubers were procured from local market in Pasir Mas, Kelantan. Tuber was dried in tray dryer and was pulverized to mesh size 40. Fresh Tuber paste was treated as described by Wall et al (1952) with slight modification. The coarse powder was subjected to continuous hot extraction in Soxhlet apparatus using ethanol (80% dilution). The ethanol extract was concentrated under reduced pressure to produce a dark sticky residue. Then the extract obtain was taken with separator funnel. The tuber was concentrated to dryness by evaporating the solvent at 45^{o}c in a rotary evaporator and was maintained for another week in oven. Freeze Dryer was used to remove the remain moisture in raw YT.

Microorganism and culture media. Bacteria were isolated from the gastrointestinal tract of tiger grouper (2.8 kg body weight (BW)) obtained from FRI Tanjong Demong, Besut, Terengganu, Malaysia. MRS agar medium, 50% artificial seawater (ASW) 1,000 mL, pH 6.0-6.5 was used for the maintenance and enumeration of cultural Lab. MRS broth was used to subculture gut microbial samples. A famous brand probiotic (21st Century Probiotics) was used for the enumeration of cultural commercial probiotics, MRS agar medium containing 0.5% sodium alginate and MRS broth was used to subculture the bacteria. Excess water from both probiotics was removed using freeze dryer and kept at -20^{o}C prior used.

Experimental design. An experiment was conducted from July to September, 2017. The tiger grouper averaging 3.3±0.7 kg body weigth (BW) and 52±4.5 cm total length (TL) were obtained from FRI Tanjong Demong facilities. Twelve fish were divided into four type of treatments (three fish per treatment). The fish were fed for 60 days with the four treatments. The first treatment was the commercial probiotic plus yam tuber (C1; commercial probiotics + YT). The second treatment was the positive control without probiotic but only yam tuber (C2; YT only); the third treatment was the probiotic taken from digestive tract (DT) of tiger grouper (C3; probiotic DT + YT), and the fourth treatment acted as negative control without probiotic and without yam tuber (C4; no YT).

For every 30 mg of capsule weight, the amount of the ingredients was 10 mg of YT plus 10 mg of probiotic and plus 10 mg of skimmed milk as filler. The capsule of probiotic with or without YT and filler were placed in the mouth of trash fish and given to the fish thrice a week.

Blood were taken every 15 days' interval for plasma analysis of Alkaline Phosphate (AFP) and Estradiol (E2) hormone with ELISA. All fish were anesthetized in water with a sub lethal dose of tricaine methanesulphonate (MS222; Argent Chemical Laboratories, Redmond, WA, USA). Fish were individually weighed for body weight (kg) and total length (cm). The digestive tract length, gonad weight, and liver weight were sampled for growth performance analysis. Thereafter, the guts of individual fish were aseptically excised and gut homogenates were prepared. The homogenates were kept on ice and analyzed in the laboratory within 2 h of collection for bacterial count and isolation, or moved to frozen storage at -30^{o}C for 16s rRNA gene clone library.

ELISA analysis of serum. The serums collected were analyzed with ELISA according to the manufacture instruction. First, the serums were diluted with carbonate buffer (pH 9.5-9.7) with the ration of 1:1 prior to ELISA assessment. Fifty (50) µL from each serum-diluted sample was added in the ELISA wells with replicates of three. Fifty (50) µL of Horseradish peroxide-conjugated (HRP-conjugated) and 50 µL of antibody (for Estradiol and AFP) were then added to each well. The color of the mixture should be blue. Then they were incubated for 1 hour at 37^{o}C. After incubation, each well was washed with 200-µL wash buffer three times. The plate was shaken lightly for 10 seconds and removed for each time. Removing the liquid is an essential step as it gives better performances for the kit. The excess remaining liquid was removed by aspiration or decanting by inverting the plate and blots it against clean paper towels. After that, 50 µL of substrate A and 50 µL of substrate B were added to each well on ELISA plate and incubated for 15 minutes at 37^{o}C. During this step the plate and the substrates A and B were kept in dark and any temperature fluctuation were avoided. After the incubation, 50 µL of stop solution were added to each well. The plate was gently tapped or shaken to ensure the solution was thoroughly mixed. The optical density (OD) of each well was determined using a microplate set to the wavelength of 450 nm.

Bacterial genomic DNA extraction and PCR amplification of 16S rDNA for DGGE fingerprinting technique. Homogenate tiger grouper gut samples were extracted for their DNA extract using Promega Wizard Genomic DNA purification kit (Promega, Madison) based on manufacturer protocol. A universal primer set (8F and 1492R) was used same in the first PCR. A total of 30 µL PCR reaction mixture was prepared contained the followings: sterile double distilled water, master mix (2 x MyTaq Mix; Bioline) primers (each 0.2 mM) and 1 µL of DNA extract. The PCR was started with 4 minutes of initial denaturation at 95°C followed by 35 cycles of the following conditions: 95°C for 30 seconds, 56°C for 30 seconds and 72°C for 1.5 minutes and ended at 72°C for 7 minutes. Thereafter, Primers (968F with GC clamp and 1401R) which targeted the V6-V8 region of the bacterial 16S rDNA were used for the second PCR (Ziembińska et al 2007). A total of 40 µL PCR reaction mixture contained the followings: sterile double distilled water, PCR-buffer (10 x Ex Taq Buffer; Takara), dNTP (each 2.5 mM), primers (each 0.25 mM), 5 U Ex Taq polymerase (Takara) and 1 µL of first PCR product. The reaction mixture run were started with 3 minutes of initial denaturation at 95°C followed by the following condition: 95°C for 30 seconds, 57°C for 1.5 minutes and 70°C for 30 seconds and ended at 70°C for 6 minutes.

DGGE fingerprinting analysis and sequencing analysis. DGGE was performed in 6% polyacrylamide gels with denaturing gradient of 20-45% of urea and formamide in 1.0 x TAE buffer. A total of 20 µL of each second PCR products were loaded into each well. DGGE was initiated by pre-running for 20 minutes at 60V and later ran at constant voltage of 150 V at 60°C for 6 hours. After the completion, the gel was stained with a 1: 10,000 dilutions of SYBR® Safe DNA gel stain for an hour before photographing using gel and western imaging (Omega Lum^{TM} G). Selected bands were excised and eluted in 20 µL of sterile double distilled water overnight at 4°C. A total of 30 µL PCR reaction mixture contained the followings: sterile double distilled water, master mix (2 x MyTaq Mix, Bioline), same primers but without GC clamp (each 0.2 mM) and 1 µL of eluted DNA. Then, the PCR was run based on first PCR protocol. All PCR products were purified and

sequenced at the external laboratory (1st BASE Laboratory Sdn. Bhd., Selangor). The resulting chromatograms of DNA sequences were examined using Chromas version 2.6.2 software. All sequences were aligned for similarity searches in the GenBank database through National Center for Biotechnology Information (NCBI) using BLAST program (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Histology. To determine the effect of probiotics diets and YT on intestine, liver and gonad, tiger grouper brood stock in each treatment were sampled for histological sections at the end of feeding experiment period. Fish tissue sample were stored in formalin solution (10%) and then eviscerated to remove their digestive tract. Paraffin blocks of fish intestine, liver and gonad were prepared and then sliced by microtome to give sections of 4 to 5 µ. Sections were stained by the coloration methods of hematoxylin and eosin (HE) and studied under microscope (Mumford 2004).

Statistical analysis. Data are expressed as the mean±S.D. Results were statistically analyzed using one-way ANOVA (SAS Institute, Cary. NC). Duncan's multiple range test was used to compare differences among the treatment groups. A p-value of less than 0.05 was considered statistically significant.

Results. The growth performance of culturable bacteria isolated and culturable commercial probiotic sample was compared with that of non-probiotic sample with and without UG (Table 1). There was significant difference ($p > 0.05$) between the digestive tract length with YT plus probiotic compared to negatife and positive control treatment. The length with YT and without probiotic were longer compared to UG with probiotic. Similarly, the gonad weight of the treatment was significantly diferent ($p > 0.05$) with digestive tract length ratio. Probiotic in both treatment (isolated, C1 and commercial, C3) gave better impact on gonad and liver weight development. The gonadosomatic index was 40 percent bigger than positive control and 60 percent than negative control, respectively.

Table 1

Growth performance of cultured figer grouper after 60 days feeding trial

	Body weight (kg)	*Total length (cm)*	*Digestive tract length (cm)*	*Digestive tract / total length ratio (%)*	*Gonad weight (g)*	*Liver weight (g)*	*Gonadosomatic index (GSI) (%)*
C1	3.3	55	88.5±13.4^{a}	162	213±11.3^{a}	62.5±20.5^{a}	55.7±4.3^{a}
C2	3.5	56	109.5±4.9^{b}	197	113±123.0^{b}	48±8.5^{a}	32.8±28.5^{b}
C3	3.3	53	89±1.41^{a}	168	214±23.3^{a}	53±31.8^{a}	56.2±4.2^{a}
C4	2.9	54	49.5± 38.9^{c}	91	33±4.2^{c}	43±12.7^{a}	21.8±6.9^{b}

C1, C2, C3 and C4 represent treatment YT with commercial probiotic and trash fish (C1), treatment YT only (positive control) and trash fish (C2), treatment YT with DT tiger grouper and trash fish (C3), trash fish only, (C4) (negative control). Superscript indicates a significant difference ($p < 0.05$); two-sided paired t-test between treatment.

Reproductive hormonal response. The results on effect of YT with and without probiotic on Estradiol (E2) and Alkaline Phosphate hormone (AFP) are shown in Figures 1 and 2. Estradiol in serum of matured brood stock fish with probiotic isolate (treatment C3) was higher compared to YT plus commercial probiotic (C1) and positive (C3) and negative control (C4). Estradiol hormone–showed highest peak (2450 pg mL^{-1}) in E2 treated fish with yam tuber (probiotic + YT) in the 45 days feeding experiment (3rd sampling). The Alkaline Phosphate hormone was 286 pg mL^{-1} in probiotic plus YT (treatment C3) (Figure 2) after 45 days feeding experiment (3rd sampling), whereas the control group value was low ranging between 143 to 88.5 pg mL^{-1}.

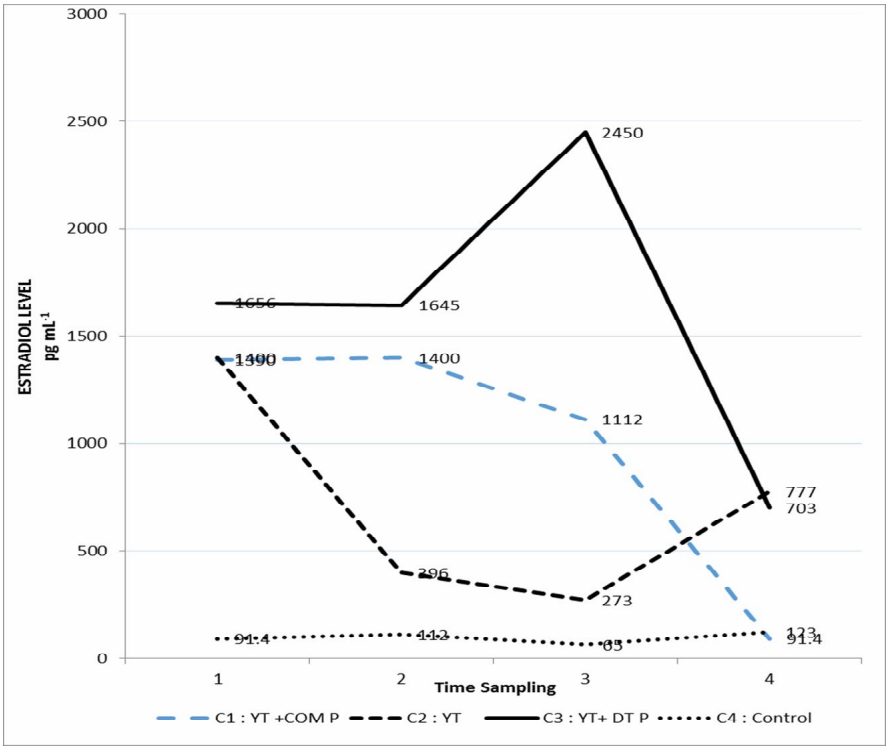

Figure 1. Effect of yam tuber on estradiol (E2) hormone in serum of tiger grouper.

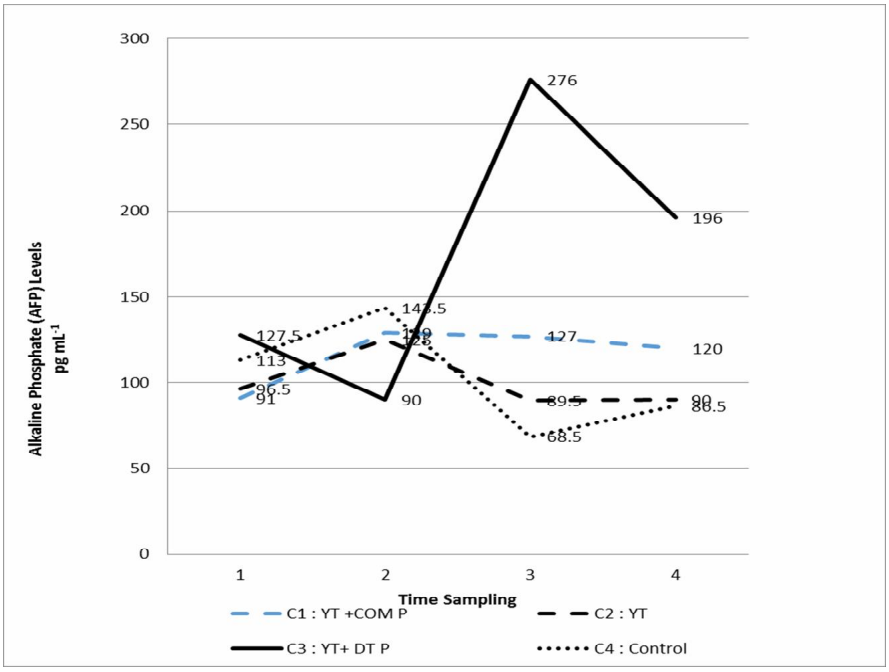

Figure 2. Effect of yam tuber on alkaline phosphate hormone in serum of tiger grouper.

DGGE fingerprinting profiles. To compare the structure of bacteria community of tiger grouper digestive tract in different treatment with or without influence from YT and probiotic, PCR-DGGE fingerprinting technique was developed. Figure 3 shows the DGGE profiles of PCR amplified 16S rDNA obtained from DNA extracted directly from digestive tract sample. Selected dominant and intense bands were subsequently sequenced to describe the phylogenetic diversity of -303 bp partial 16S rDNA sequence (Table 2).

Table 2

16s rRNA sequence identified in the representative isolates from the gut microbes of tiger grouper

DGGE band	*Length (bp)*	*Closest relatives in GenBank database*	*Phylum/Class*	*Similarity*	*C1*	*C2*	*C3*	*C4*
B1	350	*Vibrio harveyi* strain SDMN-GY2 (KY003121)	Proteobacteria; γ-proteobacteria	99	•	•		•
B2	352	*Photobacterium damselae* strain 752 (KU666982)	Proteobacteria; γ-proteobacteria	98	•	•	•	•
B3	356	*Staphylococcus warneri* strain NIOER192 (MG205915)	Firmicutes; Bacilli	99			•	
B4	354	*Staphylococcus pasteuri* strain NIOER120 (MG205859)	Firmicutes; Bacilli	99	•	•	•	•
B5	311	*Macrococcus caseolyticus* strain CAU7860 (MF429580)	Firmicutes; Bacilli	98		•		•
B6	354	*Staphylococcus warneri* strain 40A (KC787352)	Firmicutes; Bacilli	99	•	•	•	•
B7	339	*Epulopiscium* sp. N.I._1_clone_68 (AY844979)	Firmicutes; Clostridia	96				•
B8	345	*Epulopiscium* sp. N.I._1_clone_16 (AY844975)	Firmicutes; Clostridia	96				•
B9	316	Bacterium enrichment culture clone KWE30-49 (JQ670721)	Bacterial; environmental samples	90			•	•
B10	303	*Wohlfahrtiimonas chitiniclastica* strain 122 (MF399392)	Proteobacteria; γ-proteobacteria	94		•		
B11	350	*Koukoulia aurantiaca* strain RCB485 (KT260697)	Proteobacteria; γ-proteobacteria	100				•
B12	353	*Vibrio ponticus* strain HAINUV03 (KY295940)	Proteobacteria; γ-proteobacteria	99	•	•	•	•
B13	354	*Vibrio parahaemolyticus* strain AC7 (MG190872)	Proteobacteria; γ-proteobacteria	100		•		
B14	353	*Photobacterium damselae* subsp. *damselae* strain KC-Na-2 (MF973083)	Proteobacteria; γ-proteobacteria	100	•	•	•	•
B15	353	*Vibrio japonicus* strain Bio7-2 (NR_149201)	Proteobacteria; γ-proteobacteria	100	•	•		•
B16	341	*Exiguobacterium profundum* strain K2 (KY928089)	Firmicutes; Bacilli	100			•	

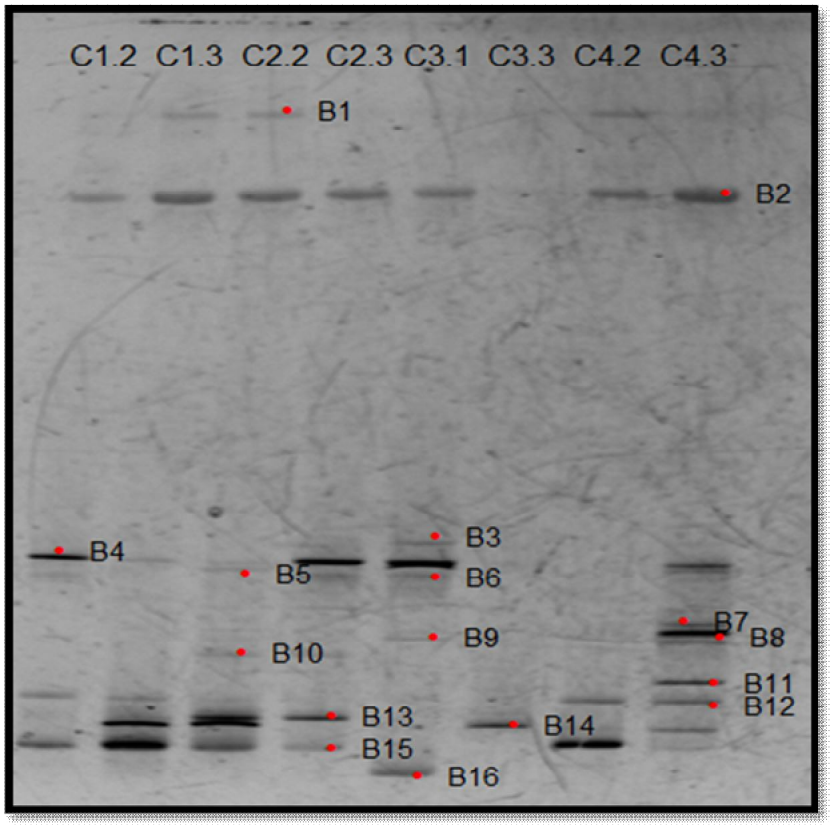

Figure 3. Denaturing gradient gel electrophoresis (DGGE) band profile of microbiota in tiger grouper digestive tract. Each lane shows the bacterial composition of one individual. C1, C2, C3 and C4 represent treatment with commercial probiotic and trash fish (C1), treatment YT only (positive control) and trash fish (C2), treatment YT with DT tiger grouper and trash fish (C3), trash fish only, (C4) (negative control) each treatment duplicates.

The DGGE fingerprinting analysis revealed that host derived probiotics treatment (C3) modified the bacterial composition by reducing some pathogenic bacteria such as *Vibrio harveyi* (B1) and *Vibrio japonicus* (B15) in the gut of cultured tiger grouper. Moreover, some beneficial bacteria like *Exiguobacterium profundum* (B16), which is known as lactic acid producer, were found. It is possibility that these beneficial bacteria improve gut environment and host growth/immune response. In addition, *Epulopiscium* sp. (B7 and B8) and *Koukoulia aurantica* (B11) were absent in gut of tiger grouper fed with feed mixed YT extract. The bacterial pathogenic community growth such as *Vibrio harveyi* (B1) and *Vibrio parahaemolyticus* (B13) was inhibited with YT.

Histological changes in anterior intestine, liver and gonad of fish following bacterial and/or dietary intake were assessed by light microscopy (LM) (Table 3 and Figure 4). Histologically, the intestine villus height and width was bigger than positive and negative control but not significantly different. However, the number of goblet cell in treatment commercial with probiotic (C1) and digestive tract with probiotic (C3) were significantly different. Both of probiotic treatment showed an intact epithelial barrier with abundant goblet cells.

Table 3

Effect of C1, C2, C3 and C4 represent treatment UG with commercial probiotic and trash fish (C1), treatment YT only (positive control) and trash fish (C2), treatment YT with DT tiger grouper and trash fish (C3), trash fish only, (C4) (negative control).

	Villus height (µm)	*Villus width (µm)*	*Number goblet cell*	*Liver lipid size (µm)*	*Gonad size (µm)*
C1	677±167[a]	161±34[a]	325±4.9[a]	11.23±3.7[a]	321±67[a]
C2	538±244[a]	108±25[b]	226±14.5[b]	12.47±3.4[a]	376±57[a]
C3	454±149[a]	138±24[a]	352±43.8[a]	11.56±2.9[a]	366±60[a]
C4	541±204[a]	79±12[c]	257±42.0[b]	15.25±5.1[a]	338±44[a]

Superscript indicates a significant difference ($p < 0.05$); two-sided paired t-test between treatment.

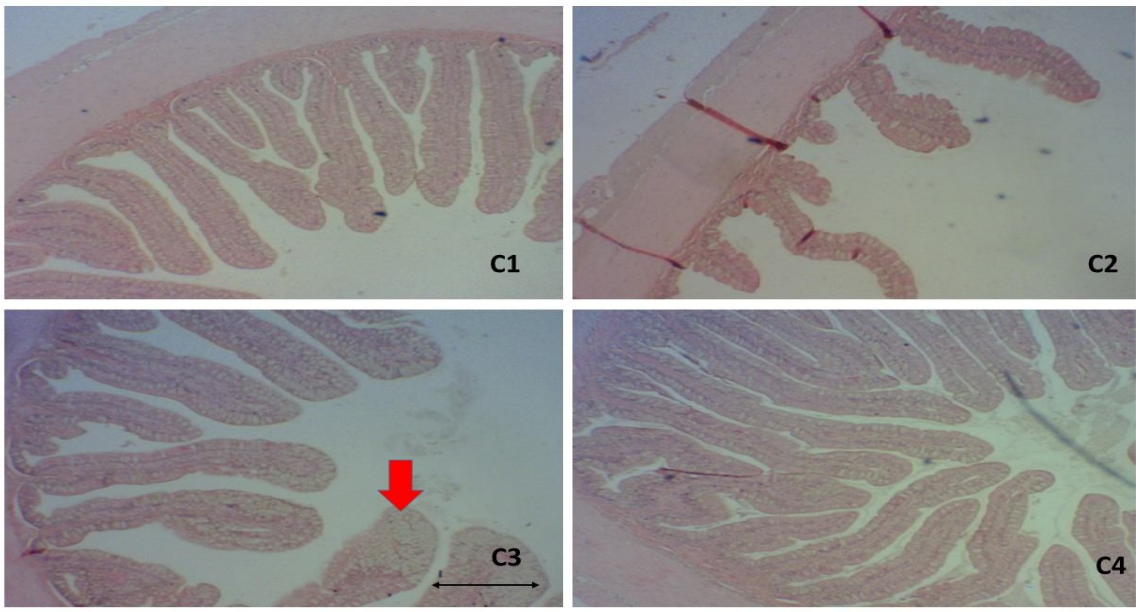

Figure 4. Comparative histology (HE staining) between treatment: C1 - commercial probiotic with *YT*; C2 - positive control (*YT* only); C3 - digestive tract probiotic with *YT*; and C4 - negative control (without *YT* and probiotic). Arrow indicates goblet cells in villus. Bar (insets) 500 µm.

Discussion. This is the first report showing the effect of addition of yam tuber (YT-rhizom) with probiotic on the performance and hormone response of tiger grouper broodstock. However, previous studies have shown the effects of medicinal herbs on performance, eggs quality characteristic and immunity response of laying hens (Reddy et al 1991; Navid et al 2013), but the synergistic effects study was not done in fishes.

In the present study, the herbal YT showed effects on growth perfomance of tiger grouper gonad development. Application of the phytochemicals in diet supplementation is relatively new venture and the potential of herbals with multifunctional active principles are promising. The increased gonadosomatic index and the other parameters in the hormone administered treatment were already reported. Babu (1999) showed that the methanolic extract of the herb significantly influence over the various production parameters in shrimp hatchery industry than its crude powder. The herbal extracts have rapid positive influence over the reproductive performance and biochemical parameters in the spawners as well as offspring quality in the tiger shrimp *Penaeus monodon* during the successive spawning (Babu et al 2008).

A significant finding in the present study was that the gonadsomatic index increased in YT with probiotic groups compared to that of normal control groups. Total length of digestive tract ratio was significantly affected with YT plus probiotic and may influence reproductive output. The study revealed that, YT and probiotic improved the reproductive performance and efficiency nutrient absorption from digestive tract. The herbs (YT) contained several bioactive compounds, including phytosterols, phytoestrogen, polyphenols and fatty acid. The YT, which has been extensively cultivated

as food, contained diosgenin, a chemical used in the laboratory to make various steroids, such as estrogen and progesterone-like compound (Stohs & El-Olemy 1972). Diosgenin is often promoted as a natural alternative to estrogen therapy (Datta et al 1984).

Many reactions that transform naturally occuring phytochemicals into bioactive molecules require the activity of different components of the calonic microbiota. Probiotic are living microorganisms that affect the host in a beneficial manner by modulating mucosal and systemic immunity, as well as improving nutritional and microbial balance in the digestive tract. Therefore, probiotic can affect the kinetics transformation of these precursors, thus improving the bioavalability and/or biological activity of natural phytochemicals (Rossi et al 2013).

It has been well documented that bioactive plant compounds and probiotic bacteria can interact with host cells (Penneret et al 2005), subsequently altering intercellular signal transduction pathways. Furthermore, both secreted bacterial products (e.g. peptides, short-chain fatty acids, bacteriocins, nitric aoxide) and non-viable structural components (e.g. DNA, proteins) of bacteria can mediate specific host responses.

Studied reported that Chinese yam polysacahride (CYPs) (*Dioscorea opposita*) enhanced beneficial gut microbiota, but suppressed bacterial pathogens. Diversity of gut microflora was increased in CYP enriched beneficial gut microbiota, but suppressed bacterial pathogens in rat ceaceum, indicating that CYP is a good source of carbon and energy, and may improve bacterial community diversity and modulate short-chain fatty acid production in hindgut of rats (Kong et al 2009). In another study on pharmacological effects of Chinese yam (*Dioscorea batatas*) on gastrointestinal of Sprague-Dawley rats by Jeon et al (2006) showed that Chinese yam has no effect on growth of normal intestine bacteria. This indicates that Chinese yam extract not only contributes to improvements in digestive capability, but also alters the intestinal floral mix toward helpful bacteria. It acts as useful digestive-aid agent that serves to increase gastrointestinal motility. The present study showed that all the pathogenic bacteria growth were inhibited by YT and that synergetic effect coud happen when it is used together. Probiotic can inhibit pathogens by competition for colonization sites or nutritional source and production of toxic compounds, or stimulation of the immune system.

Thus the symbiotics, as a combination of probiotics and prebiotics, have been studied to expect the synergetistic effects. Prebiotics oligosacharides have strong effect on the survival, growth performance and feed cost-benefit to the European lobster (*Homarus gammarus*) larvae (Daniels et al 2013). YT contained 1.55% bioactive compound which are water-soluble oligosaccharides or 28.80 mg/100 g diosgenin and 7.08% bioactive oligosacharides water unsoluble (Sumunar 2014). Certain indigestible oligosaccharides may benefit gastrointestinal tract health via fermentation and proliferation of desirable bacterial species. Type of oligosaccharides influenced short chain fatty acid production (Campbell et al 1997). Physicochemical properties of dietary fibers may influence their fermentation characteristics (Henningsson et al 2002).

The results of our present study showed that the assayed diets did not improve villus height and villus width or the absorption surface significantly, but the number of goblet cells were affected by probiotics. However, the study of Dimitroglou et al (2010) has demonstrated that prebiotics (mannanologosacharides) are able to increase villus height in different fish species. The goblet cells, present along the entire villi, are responsible for the synthesis and secretion of the protective mucus layer that covers the epithelium surface. This mucus layer acts as a medium for protection, lubrication and transport between the luminal contents and epihelial lining.

Conclusions. In summary, we demonstrated that yam tuber had the strongest bioactivity. Treatment YT with DT tiger grouper and trash fish (C3), enriched beneficial gut microbiota, suppressed bacterial pathogens in the tiger grouper digestive gut. It is clear that herbs and probotics have beneficial effects. These findings suggest that YT may serve as a useful dietary supplement for improving tiger grouper maturation. This study offers promising possibilities of using this herb in sustainable development of tiger

grouper gonad. Further study is needed to evaluate the gastrointestinal mode of action of herbal formulation in response to maturation purpose.

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Received: 23 February 2019. Accepted: 29 May 2019. Published online: 30 August 2019.
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How to cite this article:
Om A. D., Nik Yusoff N. H., Iehata S., Beng Chu K., Jamari Z., 2019 The potential use of yam tuber with probiotic for gonad development of tiger grouper. AACL Bioflux 12(4):1431-1441.