



Effects of varying dietary levels of *Carica papaya* seed meal powder (PSM) on growth and histology of gonads in *Oreochromis niloticus* larvae

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ABSTRACT

Sex control in tilapia should provide an invaluable benefit to aquaculture. The effects of different dietary levels of papaya carica seed meal powder on growth and gonad histology in *Oreochromis niloticus* larvae were studied to prevent early maturity and uncontrolled spawning. Four (4) dietary level of seed meal powder, varying from 0 g.Kg⁻¹ as a control; 1 g.Kg⁻¹; 2 g.Kg⁻¹ to 3 g.Kg⁻¹ were each tested in triplicate. The diet were isonitrogenous and were fed to twelve (12) experimental groups of 110 larvae weighing 0.019 g at a feeding rate of 4% body weight, three times a day for 30 days with the experimental diet and then after fed with the basal diet for other 60 days of the experiment. At the end of the experiment, 24 fish from all replicates were randomly sampled, slaughtered and dissected to remove testicles and ovaries. Testicular and ovarian samples were fixed in 10% buffered formalin for 24 h before the histological process. After 90 days of experiment, fish weight increased from 0.019 g ± 0.09 to 32.47g ± 0.02; 30.40g ± 0.05; 34.68 g ± 0.07 and 33.83 g ± 0.09 respectively for *C. papaya* dosage of 0; 1; 2 and 3, respectively. In fish treated with 0 and 1 g.Kg⁻¹ of PSM, the testes showed different stages of spermatogenesis with germ cells moving up to the spermatozoa. Similarly, the ovaries showed follicles at different stages of folliculogenesis up to vitellogenesis. The main lesional changes observed concerned the testicles of fish fed with 2 and 3 g.Kg⁻¹ PSM with in particular the scarcity of germ cells and spermatozoa. For females, the only observations concern vitellogenesis which are important for treatment doses of 0 and 1 g.Kg⁻¹, while they decrease in the group treated with 3 g.Kg⁻¹.

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Keywords: Papaya seed meal powder, Performances, Gonads histology, *Oreochromis niloticus*.

INTRODUCTION

World aquaculture is developing fast enough to overcome the increase in fish demand for human consumption (Nyadjieu et al. 2018). Aquaculture, probably the fastest growing food-producing sector, now accounts for nearly 50 percent of the world's food fish (FAO, 2018). Tilapia (*Oreochromis niloticus*) is the second most important group of farmed

fish after *Cyprinus sp.* and the most widely grown of any farmed fish on the world. It is farmed in at least 85 countries of the world (Burden, 2014). Global production of Tilapia is reported to be 4, 200, 000 metric tons (FAO, 2018). Tilapias are known for their ability to sexually mature at a small size, around 8-10 cm in body length, and at a young age (sometimes when 2-3 months old)

(Mansour, 2001; Chapman, 2012). Adult fish are known to live six to eight years, while some have been reported to reach eleven to twelve years of age. In tropical regions, the spawning season of tilapia usually begins during the raining season when water temperatures begin to rise, and spawning continues throughout the year as long as water temperatures are above 22 °C (Chapman, 2012). Tilapia are suitable for culture in most developing countries where they are most often grown in ponds, cages, rice field, raceways, and concrete tanks (Fagbenro, 2002).

Tilapias spawn and produce offspring with ease and these make them good fish to farm. However, this trait also creates problems, due to the precocious maturity and uncontrolled spawning (Ekanem and Okoronkwo, 2003). Survival of the young is high and grow-out ponds can become overcrowded, fish become stunted as the supply of natural food organisms in the pond is depleted (Fagbenro, 2002). Sex control in tilapia is expected to bring about an inestimable benefit for aquaculture. Several methods have been developed to control reproduction for cost effective production of farmed tilapia (Fagbenro, 2002; Toguyeni et al., 2002; Olufeagba and Okomoda, 2015). Such as monosex culture, tank culture (grading, hybridization, manual sexing), sex reversal by androgenic hormones, cage culture tank culture, the use of predators, high density stocking (stocking manipulation), sterilization (through the use of irradiation, chemosterilants and other reproduction inhibitors), intermittent/selective harvesting, the use of slow-maturing tilapia species, among others have been used. All these control methods, however, have their inadequacies and limitations. The need arises, therefore, to examine less expensive and appropriate technology in addressing the problem of uncontrolled breeding in tilapia using suitable natural alternatives that are biodegradable and biological inhibitory agents. Many plants such as *Carica Papaya* have successfully been used to induce sterility in laboratory animals (Abbas and Abass,

2011) and as medicinal plants in rural communities to cure many human and animal diseases (Dakpogan et al., 2018). *Carica papaya* (Linn) is one of the economically important fruit trees in the *Caricaceae* family (Nwiloh et al., 2009).

Several auteurs reported on the effect of Pawpaw (*C. papaya*) seed meal (PSM) on the sexual determination and growth of fed fish (Ekanem and Okoronkwo, 2003; Ayotunde and Ofem, 2008; Jegede and Fagbenro, 2008; Kobayashi et al., 2008; Omeje, 2016). However, there is paucity of information about the health status of fish fed varying levels of inclusion of this natural sex reversing agent.

Senegalese aquaculturists have faced many inbreeding and stunts population of tilapia in their ponds. Most of them are claiming no profitability of this activity. In addition, hormones are expensive and difficult to obtain, particularly in Senegal. Therefore, the solution is to find local and inexpensive vegetable reproductive inhibitor.

The effect of *C. papaya* PSM as a potential reproductive inhibitor in this species could provide a natural and accessible solution for large number of aquaculture producers to control the reproduction and proliferation of Tilapia individuals in breeding and allow better management of aquaculture production. The objective of this study was to study the effects of various dietary levels of *C. papaya* seed meal powder (PSM) on growth and gonad histology in larval aquaculture of *Oreochromis niloticus* in Senegal in order to prevent inbreeding and stunts population observed in tilapia aquaculture.

MATERIALS AND METHODS

Fish larvae were reared in 12 aquariums of 27.3 liter useful volume, manufactured by the aquaculture department of the University Gaston Berger, Senegal.

Source of plant material

Carica papaya seeds, removed from a papaya fruit bought in local market in Saint-Louis (Senegal), were cleaned, shaded and

dried at 50 °C during 72 hours before being ground to a fine powder. The dried seeds are ground to a fine particle size (< 250 µm) and stored in a clear, dry plastic container, which served as the crude extract.

Experimental design

Four (4) isonitrogenous (30%) diets were prepared from the following raw materials: fish meal, fish oil, peanut cake and corn flour (table 1). Within these diets, the papaya seed meal is gradually incorporated at a rate of 0, 1, 2 and 3 g.Kg⁻¹. The raw ingredients are finely ground and screened using a 250-micron sieve. For each food the ingredients were weighed and mixed until a homogeneous powder to which was added vegetable oil, aqua binder and mineral-vitamin complex. Water was then added at a rate of 20% dry matter, so as to obtain a malleable paste which, passed through the die of a meat grinder, gives filaments 2 mm in diameter (spaghetti). These filaments are then dried in the shade, fragmented to the desired size, bagged and stored frozen until distribution. These foods were tested on *O. niloticus* larvae, with an initial average weight of 0.019 ± 0.09 g (mean weight ± SE), obtained from the Richard-Toll National Aquaculture Agency Station.

Larvae were randomly distributed at 110 per aquarium, thus forming four triplicate treatments each corresponding to a food. Fish were fed 4% of their body weight, three times a day for 30 days with the experimental diet and then after fed with the basal one for the other 60 days of the experiment.

Dechlorinated municipal water was used in this experiment. Temperature (°C), dissolved oxygen (O₂; mg.L⁻¹) and pH were measured using a hand-held multi-probe water meter (YSI 556 MPS; Xylem Inc., Yellow Springs, Ohio) and water transparency (NTU) was measured using a U-53 turbidity meter (Horiba, Osaka, Japan). These measurements

were taken two times a day (8:30 and 16:30) during the treatment phase with *C. papaya* seed meal powder.

At the end of each two experimental weeks, all the fishes in each treatment were weighted until the end of the experiment and their specific growth rate (SGR; g.d⁻¹), were calculated as follows:

$$SGR(\%) = \frac{[\log(WF) - \log(W0)]}{t} \times 100$$

where ln = natural logarithm, WF = final body weight (g fish⁻¹), W0 = initial body weight (g fish⁻¹), and t = time (days).

Histological study of gonads

At the end of the experiment (after 90 days), 24 fish from all replicates were randomly sampled. The fish were weighed to the nearest 1 g, then stored in ice, slaughtered and dissected to remove the testes and ovaries (Figure. 1 A and B). The testes and ovaries samples were fixed in a 10% buffered formalin for 24 h prior to histological process. Formalin fixed tissues were embedded and 5 µm slices were stained with haematoxylin & eosin (Carson et Hladik, 2009) in Animal Histopathology Laboratory of Veterinary Faculty (EISMV) of Dakar.

A semi-qualitative histological assessment protocol was used to appreciate normal gonad tissues and their eventual alterations .

Statistical analysis

Data on water quality and fish growth variables were tested for normality and homogeneity of variance. The data met all the assumptions of one-way analysis of variance (ANOVA), which was used to determine the significant differences between means for water quality and growth variables. Significance was tested at 5% level and all statistical analyses were carried out using the Statistical computing R (R Core Team, 2013).

Table 1: Formulation of the experimental diets of Nile tilapia, *O. niloticus* (CP = crude protein).

Ingredient	Quantity (g.Kg ⁻¹)
Fish meal (58% CP)	250
Peanut meal (47% CP)	250
Maize meal (8.3% CP)	470
Fish oil	20
Premix (mineral, vitamin, binder)	10



Figure 1: Illustration of the testes (A) and ovaries (B) in Nile tilapia (*O. niloticus*).

RESULTS

Environmental conditions during the experimentation

The water temperatures recorded in different treatments varied from 26.9 to 27.4 °C. The result of the ANOVA test showed that the temperature was not significantly different between the treatments ($p < 0.05$). It is the same for the pH and the turbidity ($p > 0.05$) (Table 2).

Growth performances

After 90 days of experiment, fish weight increased from $0.019 \text{ g} \pm 0.09$ to $32.47 \text{ g} \pm 0.02$; $30.40 \text{ g} \pm 0.05$; $34.68 \text{ g} \pm 0.07$ and $33.83 \text{ g} \pm 0.09$ respectively for *C. papaya* dosage of 0; 1; 2 and 3, respectively. The specific growth rate was 4.92%; 4.88%; 4.97% and 4.95% for *C. papaya* dosage of 0; 1; 2 and 3, respectively. No significant differences were found for SGR ($p > 0.05$).

Histological study of the gonads

Several histological sections of thirty-two gonads were examined. Histological

morphology revealed testes and ovaries tissues at different stages of evolution. In fish treated with 0 and 1 g.Kg⁻¹ of PSM, the testes showed different stages of spermatogenesis with germ cells moving up to the spermatozoa (Figure 2). Similarly, the ovaries showed follicles at different stages of folliculogenesis up to vitellogenesis (Figure 3). The main lesional modifications observed were on the testes at fish fed with 2 and 3 g.Kg⁻¹ of PSM with notably the rarefaction of the germinal cells and the spermatozoa (Figure 4), the presence of the cysts and a hyaline substance (Figure 5) sometimes associated with that of the interstitial cells. These modifications were sometimes observed in the controls and in the batches treated, especially with 1g.Kg⁻¹. For females, the only observations concern the folliculogenesis and vitellogenesis which are important for the control and 1 g.Kg⁻¹ treatment dosage, whereas they decrease in the 3g.Kg⁻¹ treated group.

Table 2: Physico-chemical variables (mean ± SE) of four *C. papaya* treatments applied.

Parameter	<i>C. papaya</i> dosage (g.Kg ⁻¹)			
	0	1	2	3
Temperature (°C)	27.12 ± 2.22	27.11 ± 2.22	27.31±2.22	27.21±2.22
pH	6.51±0.31	6.45±0.34	6.46±0.32	6.46±0.33
Turbidity (µS/cm)	136.23±10.57	137.80±10.75	137.02±11.11	136.24±10.71

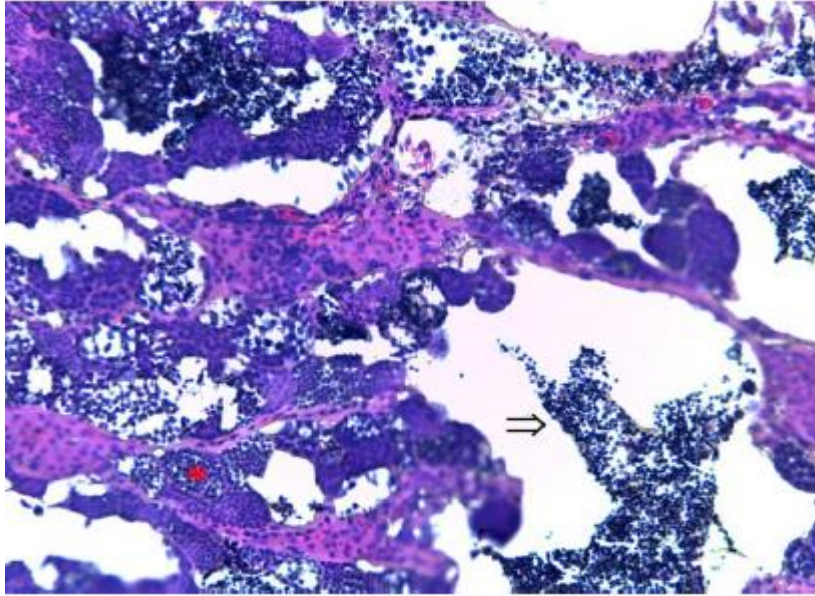


Figure 2: Testis histological appearance of Nile tilapia (*O. niloticus*), with germ cells (*) and a cluster of spermatozoa (\Rightarrow) (H & E, x40).

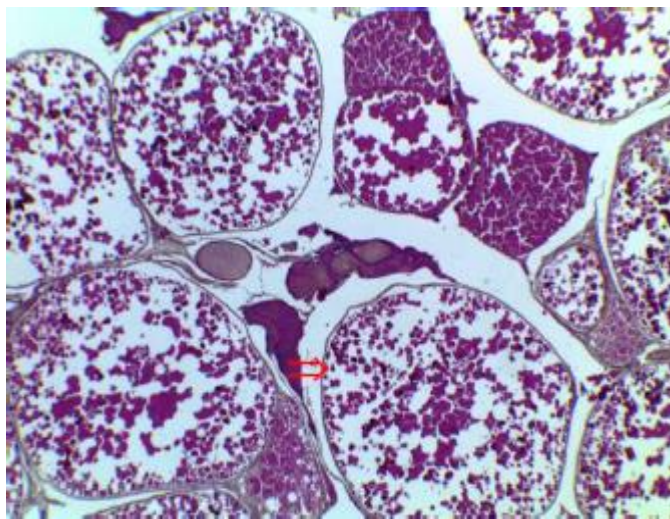


Figure 3: Histologic appearance of Nile tilapia ovary (*Oreochomis niloticus*) with follicles of different stages with follicles vitellogenesis (\Leftrightarrow) (H&E, x40).

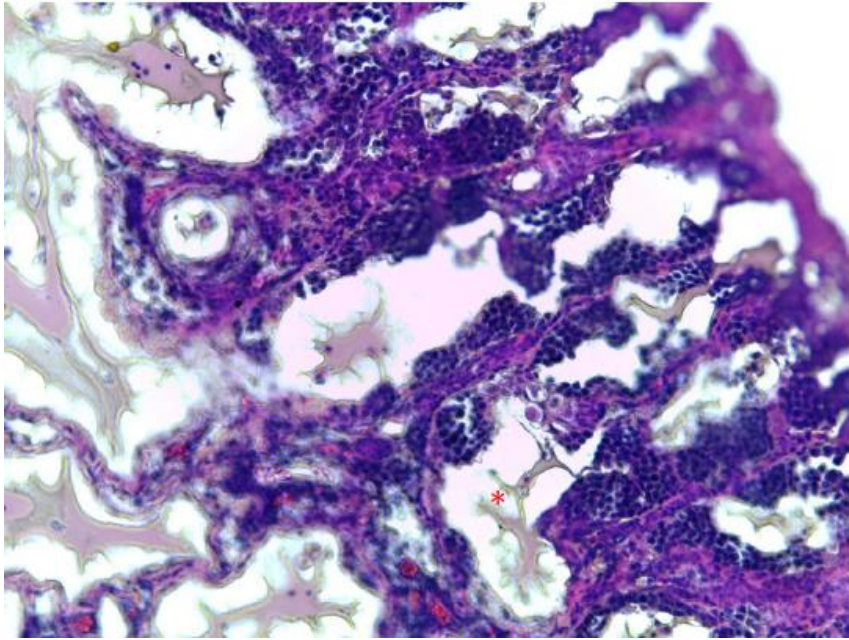


Figure 4: Histological appearance of Nile tilapia testis (*Oreochomis niloticus*) with rarefaction of germ cells and cystic tubes (*) (H & E, x40).

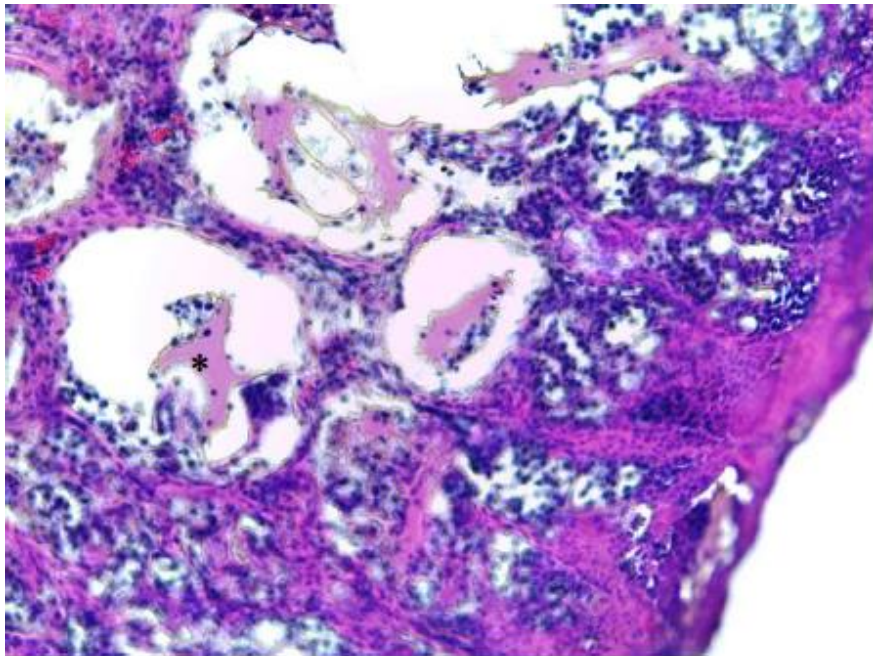


Figure 5: Histological appearance of Nile tilapia testis (*Oreochomis niloticus*) with cystic tubes with hyaline contents (*) (H & E, x40).

DISCUSSION

The results of our study showed that the physico-chemical parameters (temperature, pH and turbidity) have not changed according to the treatments. This can be explained by the fact that the treatments were subject to the same rearing conditions as the aquariums have of the same size with the same amount of water, the frequency of feedings and the amount of food given by treatment are the same and also the frequency of water renewal was the same.

The water temperature is among the major factors that influence tilapia growth during his different stages. The water temperatures recorded in different treatment varied from 26.9 to 27.4 °C. These findings are in accordance with the findings of those reported by other authors (Sarr et al., 2015). Temperature between 20 and 36 °C have been reported by various researchers as being suitable for tilapia culture (Azaza and Kraiem, 2008; Lacroix, 2014). These authors reported that the best growth performances can be obtained for temperatures varying between 24 and 28 °C. According to Lazard (2009), the preferred temperature ranges for optimum *O. niloticus* growth is between 28 and 32 °C.

Crane (2006) reported that highly acidic water with pH less than 5.5 limited fish growth and reproduction. This author noted that the ideal pH range for freshwater aquaculture should range between 6.5 and 7.0, though a pH range of 6.1 to 8.0 is also considered satisfactory for the survival and reproduction of fish. Makori et al. 2017 reported a pH range of between 6.5 and 9.0 as optimum for growth of tilapia. For Heydarnejad (2012), the optimum pH for better survival and growth should vary between 7 and 8. These results are in accordance with our findings.

Consistent with the current study findings, Russell et al. (2011) noted that water turbidity of between 150 and 500 µS/cm is ideal for fish culture. Stone et al. (2013), however, put the desirable range of turbidity for fish ponds at between 100 and 2000 µS/cm. The turbidity values obtained in this study were included in the value ranges indicated by these authors.

Growth of fish is dependent on a wide range of positive or negative impacting

factors. Studies show that growth of fish in aquaculture mainly depends on feed consumption and quality, stocking density, biotic and abiotic factors (Slawski et al., 2011; Ma et al., 2006; Imsland et al., 2007). Currently, feed costs are the major portion of fish rearing costs, commonly 50 to 70% of the total. Vegetable ingredients proposed as alternative must match the growth and survival demonstrated by fish fed animal ingredients. Diets must meet several physical and chemical criteria to insure that the diet is ingested and assimilated. The first chemical characteristic required in formulated feeds is attractiveness. Feed must draw the animals to the particle using traits such as smell and color.

Abbas and Abbas (2011) reported that the action of the active ingredients of pawpaw seeds may critically influence the growth rate and the quality of fish meat. Fish weight increased during this study, but there was no significant difference of weight between treatments. In this study the inclusion of papaya seed powder in tilapia diet did not have a differential effect between the treatments on the growth. This result is different from that of Fathy et al. (2014) who reported that 6 g.kg⁻¹ of PGP in the food given for 45 days after yolk sac uptake of Nile tilapia larvae can be used as a growth promoter by improving most of the performance parameters of Nile tilapia larvae.

The difference between our results and that of Fathy et al. (2014) could be explained by the fact that the doses of PGP used in this study vary between 1 and 3 g against 6 g per kg of food used by Fathy et al. (2014). The specific growth rates of individuals (SGR) also remain the same in the different treatments. The SGR values obtained in our study are similar to those obtained by Bamba et al. (2008) in tilapia fed for 21 days with food formulated from different local by-products.

The histological results revealed that the samples are composed of male and female gonads. The observed changes in gonads have been reported by some authors (Jegade, and Fagbenro (2008). However, such changes have been noted in both the control and the treatment, but they were more marked in groups treated in particular with 2 and 3 g.K⁻¹

de PSM. Nevertheless, the fact that these modifications are more marked in the treatises could justify the effect of treatment on the testicular parenchyma of tilapias, which could corroborate the sterilizing effect of papaya seeds in tilapias as Khalil et al. (2014) and Jegede and Fagbenro (2008) pointed out. In addition, it is reported that a combination of enzymes, alkaloids and other substances in *C. papaya* might themselves inhibit testosterone production and ultimately oestrogen production (Khalil et al., 2014).

Our results did not reveal any lesional changes in the ovaries, contrary to what was reported by Jegede and Fagbenro (2008). This difference could be due to the protocols used but also to the limits of studies undertaken including the conditions of the study, the amount of papaya seed distributed, the duration of treatment. In this study 3g.Kg⁻¹ was the highest inclusion of papaya seed meal powder. Farrag et al. (2013) reported that the dietary Papaya seed powder (PSP) at level 6 g/ kg diet for 45 day after absorbing the yolk sac of Nile tilapia fry may be used as a growth promoter for tilapia fish, which improved the most of growth performance parameters, survival, FCR and fish body composition. In addition, Fathy et al. (2014) reported that the dietary PSP at 6 and 8 g /kg diet for 45 and 60 days after hatching had positive effect in reproductive process control for *O. niloticus*. The PSP seems to decrease the sex hormone (testosterone and progesterone) and to cause several histological alternations in testes and ovaries, which reduces fertility in both males and females Nile tilapia.

Conclusion

This study determined the effects of various dietary levels of *C. papaya* seed meal powder (PSM) on growth and gonads histology in larval aquaculture of *Oreochromis niloticus* in Senegal. The results show that the inclusion of papaya seed meal powder in feed at doses up to 3 g/kg reduces vitellogenesis in female and leads to several histological alternations of the testis in male. Therefore, papaya seed meal powder appears to be a reproductive inhibitor for *O. niloticus*. This information is useful for the proper use of this plant-reproductive inhibitor in the formulation of fish feed. Papaya seed meal

has the advantage of being locally available and accessible to aquaculturists, unlike industrial hormones.

COMPETING INTERESTS

The authors declare that there is no competing interests regarding the publication of this paper.

AUTHOR'S CONTRIBUTIONS

LDM and MAL was the investigator of the work. JK and RF provided technical advices. YK contributed to the supervision of the work. The five authors contributed to the manuscript preparation, writing and corrections.

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