

# Effects of feed supplemented with steamed pumpkin (*Cucurbita moschata*) on growth performance and immune response in hybrid catfish (*Clarias gariepinus* × *Clarias macrocephalus*)

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**Abstract.** This study researches the effect of diet supplemented with pumpkin (*Cucurbita moschata*) on growth performance and immune response in hybrid catfish (*Clarias macrocephalus* × *Clarias gariepinus*). Fish with an average initial weight of  $72.90 \pm 1.30$  g were selected for each feeding experiment with diet containing steamed pumpkin flesh. The fishes were observed after feeding with steamed pumpkin supplemented diet at 0 % (control), 1%, 5%, and 10%  $\text{kg}^{-1}$ , each, for 8 weeks. Significant increases in growth parameters such as average weight, weight gain, average daily growth, and specific growth rate were apparently gained up to 10.51 - 17.70 %, 21.95 - 38.45 %, 22.43 - 38.32 % and 14.81 - 25.93 %, respectively, across treatments supplemented with steamed pumpkin. Moreover, enhancing the feed conversion rate of the supplementary fed could be obtained with the ratio of 1.41 - 1.59 in comparison to 1.78 of the control group. Concurrently, the survival rate of the fish was not affected in every experiment. Immunological investigation based on phagocyte amount and phagocytic index showed higher values as 15.44 - 33.24 % and 11.27 - 38.73 %, respectively, in the supplemented fed groups, while only 9.31 - 13.82 % and 8.84 - 25.66 % ( $p < 0.05$ ) were gained from the control group. No significant differences were found in the amount of hematocrit index and white blood cell counts that ranged around 44.93 - 58.57 % and  $3.71 - 4.87 \times 10^8$  cells  $\text{mm}^{-3}$  ( $p > 0.05$ ), respectively in all fish groups. A remarkable concomitance by these results has thus indicated an outstanding efficiency of the pumpkin dietary supplementation for hybrid catfish profitable culture.

**Key Words:** local feed plant, hematological, phagocytic cell activity.

**Introduction.** Catfish is one the main freshwater cultured fish species worldwide including Thailand due to its fast growing with high environmental and dense tolerance. At present, the most popular catfish being reared by Thai farmers is the hybrid catfish namely Big Oui catfish which is a crossbreed between a male giant catfish (*Clarias gariepinus*) and a female broadhead catfish (*Clarias macrocephalus*) that yield much better quality in their meat flavor, growth and disease resistance (Na-Nakorn et al 2004). However, the hybrid catfish is often cultured in high-density conditions thus encountering several difficulties in feeding, immune system, disease immergence and subsequent huge loss in production. This is also recognized as having a significant impact on freshwater fisheries around the world (Austin & Austin 1999; Cerezuela et al 2012; Giri et al 2014; Yousefi et al 2016). Fish in such conditions are easily infected especially with bacteria that hasten excessive use of many unnecessary antibiotics and concomitantly producing harmful residues in both the meat and the environment (Carlson et al 2017). Moreover, it also causes an antimicrobial phenomenon which makes the disease difficult to control and treat (Palú et al 2006; Cabello et al 2016; Gasser et al 2019; Okocha et al 2018).

According to the safe food production policy of the government, managing the growth and health of aquatic animals using natural supplementary diet sources such as fruits and vegetables have become the choices with most emphasis on. Fruits and

vegetables that have long been known as a source of healthy nutrients such as carbohydrates, protein, essential oils, vitamins, minerals and dietary fiber which help reduce the risk of heart disease, respiratory disorders, cancer and premature aging etc. Notable high energy and nutritional value fruits, for example mango (*Mangifera indica*) and banana (*Musa* spp.) have been used as dietary supplements for aquatic and terrestrial animals to increase growth and promote health (Awad & Austin 2010; Aderolu et al 2011; Srangyanang 2004). Effectiveness of the local cultivated banana CV. Kluai Namwa ripe fruit has been reported, previously, as suitable for enhancing growth vigor, feed conversion efficiency, and texture quality of the Nile tilapia (*Oreochromis niloticus*) fish (Palintorn et al 2019).

Pumpkin (*Cucurbita moschata*) is another attractive local feed plant because of its high nutritional value and medicinal properties (Ei-Aziz et al 2011). It is also available and inexpensive for daily consumption worldwide, referring to the global pumpkin production that has been rated at least 23 million tons per year (FAO 2019). Among these, China and India lead the output at 7,838,809 and 5,073,678 tons/year, respectively. The local production in Thailand is seasonal and varies from 51,520 to 117,032 tons yearly (Agricultural Production Information System 2020). Pumpkin, in regard to human consumption, is considered a superior healthy food especially for middle-aged, weak, and elderly people. Not only as a rich source of carbohydrates, protein, pectin and dietary fiber but it also contains important minerals such as calcium, iron, potassium, magnesium, phosphorus, copper, manganese, selenium, sodium, iodine, zinc, cobalt, nickel, and chromium that helps against osteoporosis and hypertension (Ahmad and Khan 2019; Hussain et al 2022; Singh & Kumar 2022). Several healthy nutritive substances in particular vitamin A, B1, B2, B6, B9, C and E including oleic and linoleic fatty acids nourishing are strengthening the immune system from infections within the body (Younis et al 2000; Choi et al 2012; Valenzuela et al 2014; Gajewski et al 2008; Blessing et al 2011; Islam et al 2014; Dhok et al 2020).

In addition, pumpkin has been claimed to possess pharmacological effects associated with anti-diabetes mellitus, anti-hyperlipidemia, lowers blood pressure, antioxidants, antimicrobial, anti-intestinal parasites, anticancer, stimulates the immune system and anti-inflammatory properties, owing to its biological active carotenoid, lutein, lycopene, zeaxanthin, phytosterols, polysaccharides, vitamins, minerals and other antioxidants (Sharma et al 2013; Elella & Mourad 2015; Muruganantham et al 2016). The pumpkin extract rich in carotenoid can also prevent disease from various infections, increase the white blood cell production and reduce cell proliferation in chronic lymphocytic leukemia cells in humans (Kim et al 2016; Spencer & Belkaid 2012; Veldhoen & Ferreira 2015; Carr & Maggini 2017; Huijskens et al 2014; Moccia et al 2020). The symptoms of diabetes and oxidative stress levels in cells and tissues of animals and humans have been found to decrease by the action of pumpkin polysaccharides (Fang et al 2021). Biological effects of pumpkin have been intensively studied in various animals (Sojak & Głowacki 2010; Adams et al 2011). However, there are still quite a few reports concerning its use in aquaculture. Thus, this present study has been initiated to investigate the effect of pumpkin supplemented diet on hybrid catfish growth performance and immune response. Information gained from this study would certainly provide subsequent productivity and security data for aquatic animal farming models.

## Material and Method

**Experimental fish.** Hybrid catfish (*Clarias gariepinus* × *Clarias macrocephalus*) with an average weight of 70-80 g, were provided by commercially available Udomphan Farm, Maha Sarakham Province, Thailand. Three hundred sixty fishes were randomly distributed into twelve experimental 1.0 × 1.0 × 1.5 meters floating cages (30 fishes per floating cage), with three replicates for each treatment group. All fish were acclimatized for 2 weeks and fed with a commercial pellet diet at 3% of body weight before being used for experimentation. Experimental fish were fed twice daily at 8:00 h and 16:00 h.

**Preparation of experimental diets.** The pumpkin variety (*Cucurbita moschata*) fruits at maturity from a growing period of 180 days with an average weight of 2.5-3.0 kilograms per fruit were selected from the Night Plaza Market, Buriram Province, Thailand. The fruit peel was removed thoroughly and only their flesh was collected. The pumpkin flesh was cut into small pieces and steamed at 100°C for 20 minutes. These steamed flesh pieces were then grounded with a blender at a speed of 12,000 rpm for 2 minutes until a homogeneous sticky paste could be gathered. The commercial pellet diet CP 9910 containing 30% crude protein was purchased from Charoen Pokphand Food PCL. CPF, Thailand. Ready prepared pumpkin's flesh paste was subsequently mixed with this basal pellet diet at the level of 0% (control), 1%, 5%, and 10% kg<sup>-1</sup> diet. All the made-up diets were dried at room temperature for 1 h and then stored in a plastic bag at 4°C until use. The proximate composition of the experimental diets was determined by standard procedures according to AOAC (1990) method. Experimental diets contained the proximate analysis composition as shown in Table 1. These experimental diets were freshly prepared every 3 days.

Table 1

Proximate analysis composition of the experimental diets supplemented with specified percentage of steamed pumpkin's flesh

Proximate composition	Steamed pumpkin levels (WW <sup>-1</sup> )			
	0 %	1 %	5 %	10 %
Protein (%)	31.80	30.52	29.68	28.74
Fat (%)	7.97	7.60	7.39	7.07
Nitrogen free extract (%)	35.56	37.03	37.92	39.01
Ash (%)	9.85	9.45	9.15	8.55
Moisture (%)	10.28	11.01	11.59	12.49
Fibre (%)	4.54	4.39	4.27	4.14
Digestible energy, DE (kcal/100g)	264.75	260.95	258.53	255.38
Gross energy, GE (kcal/100g)	400.73	396.06	393.00	389.16

**Experimental design.** Hybrid catfish with an average initial weight of 72.90 ± 1.30 g, were divided into 4 groups and fed for 8 weeks with a feeding diet containing the steamed pumpkin at 0 % (control), 1%, 5%, and 10% kg<sup>-1</sup> diet, with three replicates for each treatment group. Experimental fishes were fed twice daily at 8:00 h and 16:00 h. Feeding continued toward apparent satiation based on visual observation. All fish were weighed every 2 weeks to calculate growth performance throughout the experiment of 8 weeks. Growth performance and feed utilization were assessed in terms of average weight, weight gain (Iwama & Tautz 1981), average daily growth (Jantrarotai et al 1994), specific growth rate (Iwama & Tautz 1981), feed conversion ratio (Dupree & Sneed 1966) and survival rate (Nankervis et al 2000). Then, 10 fishes per floating cage (30 fish per experiment group) were randomly collected for blood sampling. To measure the hematocrit index according to the method of Blaxhall and Daisley (1973), 4 fish per floating cage (12 fish per experimental group) were randomly collected. White blood cells were isolated from the liver to calculate % phagocytes and phagocytic index as measured by phagocytosis of zymosan A according to the method of Scott and Klesius (1981).

**Growth performance and feed conversion ratio.** All fish were weighed every 2 weeks to calculate growth performance throughout the experiment of 8 weeks. Growth performance and feed utilization were assessed by evaluating the percentage of average weight, weight gain, average daily growth, specific growth rate, and feed conversion ratio. The parameters were calculated as per the following formulae:

$$\begin{aligned} \text{Average weight (AW) (g)} &= \text{total final body weight/number of fish} \\ \text{Weight gain (WG) (g)} &= \text{FW} - \text{IW} \\ \text{Average daily growth (ADG) (g day}^{-1}\text{)} &= (\text{FW} - \text{IW})/\text{t} \\ \text{Specific growth rate (SGR) (\% day}^{-1}\text{)} &= 100[(\ln\text{FW} - \ln\text{IW})/\text{t}] \end{aligned}$$

Feed conversion ratio (FCR) (g) = total feed supplied (g)/WG

Where FW is final weight (g), IW is initial weight (g) and t is the feeding trial period (days).

**Survival rate.** The fish's survival was expressed in percentage. Mortality for each experimental group was noted daily throughout the 8 weeks experimental period. The calculation was conducted using the following formulae:

Survival rate (%) = 100[number of survived fish/initial number of fish]

**Hematocrit index.** Blood sampling of fish was collected by venipuncture at 1 ml and this blood was transferred into a microcentrifuge tube containing 1% ethylenediamine tetraacetic acid (EDTA) as an anticoagulant to prevent clotting, then blood was taken into capillary tubes, tubes were then centrifuged with a haematocrit centrifuge (Hettich centrifuge, Germany) at a speed of 12,800 rpm for 3 min. The percentage hematocrit value was measured by overlaying the tubes on a sliding scale haematocrit reader.

**White blood cell separation.** Fish's abdomen was opened with an incision made to collect the liver to separate white blood cells. These liver samples were transferred into Petri dishes glass and washed twice to remove any residues with 2 ml of phosphate buffer solution (PBS, pH 7.4). Then the liver tissue was crushed through 100 µl nylon mesh in PBS solution and centrifuged at 2500 rpm for 5 min. The white blood cells were counted using a Neubauer hemocytometer using a Nikon (Tokyo, Japan) light microscope at a magnification of x 200. Their white blood cells from this count number per blood sample 1 mm<sup>3</sup> could be calculated by the following formulae:

White blood cells (WBC) (10<sup>4</sup> cells mm<sup>-3</sup>) = mean white blood cell count per chamber x 10 (chamber of depth) x 200 (dilution)

**Determination of % phagocyte and phagocytic index.** The ability of phagocyte cells to ingest zymosan A (Sigma) was investigated by adding 100 µl of zymosan A to the prepared 100 µl white blood cells. This sample was incubated for 1 h at room temperature. A smear from the incubated suspension was prepared on a glass slide, air-dried, and stained with Wright Giemsa for 20 min. The stained smear was observed under a microscope to count the numbers of white blood cells and phagocytes cells. Determination of phagocytic index (PI) and % phagocytes was performed and the calculation was conducted using the following formulae:

% phagocyte = 100[number of phagocyte/number of white blood cells (200 cells)]

Phagocytic index parameters were determined from the number of zymosan A engulfed in each phagocyte cell (40 cells):

Phagocytic index = number of zymosan A engulfed in phagocyte/number of phagocytes with engulfed zymosan A (40 cells)

**Statistical analysis.** Values were expressed as mean ± standard error (SE). Growth performance, survival rate, hematocrit index, white blood cells, percentage of phagocyte, and phagocytic index parameters were statistically evaluated for their comparative mean values at a 5% level of significance concerning the one-way ANOVA and Duncan's multiple range test by the aid of IBM SPSS Statistics (version 23, 2015, SPSS Inc.) software program.

**Ethics statement.** This research has been approved by the Institute Animal Care and Use Committee, Buriram Rajabhat University; laboratory experiment type document number .002-2567.

## Results

**Growth performance and fish survival.** The effects of a steamed pumpkin supplementing diet on growth performance and survival rate are shown in Table 2. A clear-cut difference value ( $p < 0.05$ ) between the pumpkin supplementing groups and the control group could be seen in growth parameters. Measurements on average weight, weight gain, average daily growth, and the specific growth rate have placed the control's performance to the least, while the highest values of 156.06 %, 83.07 %, 1.48 %, and 1.36 % with the relative increase of 17.70 %, 38.45 %, 38.32 % and 25.93 %, respectively, are from hybrid catfish fed with 10% pumpkin supplementing diet. However, differences among the supplementary level (5 % and 10 %) are not statistically significant, but there is a statistically significant difference with the fish group fed the pumpkin supplement at 1% in the diet. Better value on feed conversion ratio is shown up following the supplemented diet feeding as well. Although the best value at 1.41 from 10% supplementation has apparently been gained, the values are not significantly different from another level. The survival rate of all was 100%.

**Hematological parameters.** The hematological parameters of hybrid catfish showed no significant differences between the hematocrit index and white blood cell count in the experimental groups and control group ( $p > 0.05$ ). The best values at  $58.57 \pm 3.40$ ,  $56.07 \pm 6.67$ ,  $52.77 \pm 3.45$ , and  $51.00 \pm 3.15$ , respectively, were revealed from those of the 10% steamed pumpkin supplemented trial, followed by 5 %, 1 % and control groups, respectively (Table 3). In case of the white blood cell count value, it could be seen that the fish group supplementation of steamed pumpkin in the diet had white blood cell counts ranging from  $3.71 \pm 1.40$  to  $4.87 \pm 0.92 \times 10^8$  cells  $\text{mm}^{-3}$  (Table 4).

**Immunological parameters.** The immunological parameters of hybrid catfish performed in this experiment indicated significant differences between the % phagocyte in the experimental groups and control group ( $p < 0.05$ ). Percentage of the phagocyte value from fish fed pumpkin supplemented diet was significantly higher than the control group ( $p < 0.05$ ). The % phagocytes values were highest in the hybrid catfish group fed steamed pumpkin supplemented with 10 %, followed by 5 %, 1 % groups and in the control group. The values obtained were  $32.71 \pm 2.65$  %,  $29.01 \pm 1.96$  %,  $28.34 \pm 1.49$  % and  $24.55 \pm 1.26$  %, respectively (Table 5). As for phagocytic index, it was found that the fish fed with a 5 % and 10 % of steamed pumpkin supplemented diet held a higher phagocytic index value than the control group and 1% pumpkin supplemented diet ( $p < 0.05$ ). However, there was no statistical difference in the phagocytic index of fish fed with steamed pumpkin at the supplement level of 1% and the control fish group ( $p > 0.05$ ). The highest phagocytic index found were steamed pumpkin supplemented in 10% diet, followed by 5 %, 1 % groups and control fish group were  $1.97 \pm 0.08$ ,  $1.88 \pm 0.10$ ,  $1.58 \pm 0.18$  and  $1.42 \pm 0.08$ , respectively (Table 6). Figure 1 illustrates the morphological characteristics and functional activity of phagocytic cells isolated from the liver of hybrid catfish following the ingestion of Zymosan A at 1-hour post-exposure.

The effect on the immune value of hybrid catfish for 8 weeks feeding with steamed pumpkin compliment diet was measured. It was then clearly revealed that the complementary diet feeding treatments significantly produced higher % phagocytes values than those of the control group ( $p < 0.05$ ).

Table 2

Growth parameters of hybrid catfish fed with diets supplemented at 3 levels of steamed pumpkin compared with the control group after 8 weeks

Growth parameters	Steamed pumpkin level (WW <sup>-1</sup> )			
	0 %	1 %	5 %	10 %
Average weight (g)	132.59 ± 2.12 <sup>C</sup>	146.52 ± 3.12 <sup>B</sup> (10.51)	152.90 ± 2.44 <sup>A</sup> (15.32)	156.06 ± 3.69 <sup>A</sup> (17.70)
Weight gain (g)	60.00 ± 0.98 <sup>C</sup>	73.17 ± 1.67 <sup>B</sup> (21.95)	80.22 ± 1.40 <sup>A</sup> (33.70)	83.07 ± 2.11 <sup>A</sup> (38.45)
Average daily growth (g day <sup>-1</sup> )	1.07 ± 0.02 <sup>C</sup>	1.31 ± 0.03 <sup>B</sup> (22.43)	1.43 ± 0.03 <sup>A</sup> (33.64)	1.48 ± 0.04 <sup>A</sup> (38.32)
Specific growth Rate (% day <sup>-1</sup> )	1.08 ± 0.00 <sup>D</sup>	1.24 ± 0.01 <sup>C</sup> (14.81)	1.34 ± 0.01 <sup>B</sup> (24.07)	1.36 ± 0.01 <sup>A</sup> (25.93)
Feed conversion ratio (FCR) (g)	1.78 ± 0.06 <sup>A</sup>	1.56 ± 0.28 <sup>B</sup>	1.49 ± 0.07 <sup>B</sup>	1.41 ± 0.05 <sup>B</sup>
Survival rate (%)	100.00 ± 0.00 <sup>A</sup>	100.00 ± 0.00 <sup>A</sup>	100.00 ± 0.00 <sup>A</sup>	100.00 ± 0.00 <sup>A</sup>

Note: Values representing means ± SD in the same row with different subscripts are significantly different ( $p < 0.05$ ).

Table 3

Hematocrit index parameters of hybrid catfish fed with diets supplemented at 3 levels of steamed pumpkin compared with the control group for 2, 4, 6, and 8 weeks

Steamed pumpkin level (%) (WW <sup>-1</sup> )	Hematocrit index (%)			
	Week 2	Week 4	Week 6	Week 8
0	43.43 ± 4.35 <sup>A</sup>	47.47 ± 3.97 <sup>A</sup>	48.77 ± 5.53 <sup>A</sup>	51.00 ± 3.15 <sup>A</sup>
1.0	44.93 ± 3.88 <sup>A</sup> (3.45)	47.60 ± 4.16 <sup>A</sup> (0.27)	51.60 ± 3.61 <sup>A</sup> (5.80)	52.77 ± 3.45 <sup>A</sup> (3.47)
5.0	46.60 ± 4.64 <sup>A</sup> (7.30)	50.63 ± 4.07 <sup>A</sup> (6.66)	55.60 ± 4.48 <sup>A</sup> (14.00)	56.07 ± 6.67 <sup>A</sup> (9.94)
10.0	49.40 ± 5.03 <sup>A</sup> (13.75)	52.50 ± 4.92 <sup>A</sup> (10.60)	55.93 ± 5.10 <sup>A</sup> (14.68)	58.57 ± 3.40 <sup>A</sup> (14.84)

Note: Values representing means ± SD in the same row with different subscripts are significantly different ( $p < 0.05$ ).

Table 4

White blood cell parameters of hybrid catfish fed with diets supplemented at 3 levels of steamed pumpkin compared with the control group for 2, 4, 6, and 8 weeks

Steamed pumpkin level (%) (WW <sup>-1</sup> )	White blood cells (cells mm <sup>-3</sup> )			
	Week 2	Week 4	Week 6	Week 8
0	4.37 ± 1.22 <sup>A</sup>	3.71 ± 1.13 <sup>A</sup>	3.11 ± 0.18 <sup>A</sup>	3.63 ± 0.34 <sup>A</sup>
1.0	4.44 ± 1.73 <sup>A</sup> (1.60)	3.87 ± 1.10 <sup>A</sup> (4.31)	3.71 ± 1.40 <sup>A</sup> (19.29)	3.98 ± 0.47 <sup>A</sup> (9.64)
5.0	4.51 ± 1.42 <sup>A</sup> (3.20)	4.53 ± 1.23 <sup>A</sup> (22.10)	4.36 ± 0.36 <sup>A</sup> (40.19)	4.57 ± 0.64 <sup>A</sup> (25.90)
10.0	4.66 ± 0.53 <sup>A</sup> (6.64)	4.87 ± 0.92 <sup>A</sup> (31.27)	4.38 ± 0.51 <sup>A</sup> (40.84)	4.60 ± 1.01 <sup>A</sup> (26.72)

Note: Values representing means ± SD in the same row with different subscripts are significantly different ( $p < 0.05$ ).

Table 5

% phagocyte parameters of hybrid catfish fed with diets supplemented at 3 levels of steamed pumpkin compared with the control group for 2, 4, 6, and 8 weeks

Steamed pumpkin level (%)( $WW^{-1}$ )	phagocyte (%)			
	Week 2	Week 4	Week 6	Week 8
0	21.57 ± 1.91 <sup>A</sup>	23.58 ± 1.28 <sup>B</sup>	26.38 ± 1.15 <sup>C</sup>	24.55 ± 1.26 <sup>C</sup>
1.0	23.13 ± 1.54 <sup>A</sup> (7.23)	26.84 ± 1.49 <sup>AB</sup> (13.83)	27.63 ± 1.42 <sup>C</sup> (4.74)	28.34 ± 1.49 <sup>B</sup> (15.44)
5.0	23.39 ± 1.07 <sup>A</sup> (8.44)	28.75 ± 1.85 <sup>A</sup> (21.93)	32.21 ± 1.33 <sup>B</sup> (22.10)	29.01 ± 1.96 <sup>B</sup> (18.17)
10.0	23.57 ± 1.36 <sup>A</sup> (9.27)	29.67 ± 2.20 <sup>A</sup> (25.83)	34.21 ± 1.26 <sup>A</sup> (29.68)	32.71 ± 2.65 <sup>A</sup> (33.24)

Note: Values representing means ± SD in the same row with different subscripts are significantly different ( $p < 0.05$ ).

Table 6

Phagocytic index parameters of hybrid catfish fed with diets supplemented at 3 levels of steamed pumpkin compared with the control group for 2, 4, 6, and 8 weeks

Steamed pumpkin level (%)( $WW^{-1}$ )	Phagocytic index			
	Week 2	Week 4	Week 6	Week 8
0	1.13 ± 0.06 <sup>B</sup>	1.23 ± 0.05 <sup>C</sup>	1.32 ± 0.03 <sup>C</sup>	1.42 ± 0.08 <sup>B</sup>
1.0	1.15 ± 0.04 <sup>B</sup> (1.77)	1.40 ± 0.06 <sup>B</sup> (13.82)	1.50 ± 0.10 <sup>B</sup> (13.64)	1.58 ± 0.18 <sup>B</sup> (11.27)
5.0	1.24 ± 0.10 <sup>B</sup> (9.73)	1.41 ± 0.06 <sup>B</sup> (14.63)	1.77 ± 0.11 <sup>A</sup> (34.09)	1.88 ± 0.10 <sup>A</sup> (32.39)
10.0	1.50 ± 0.12 <sup>A</sup> (32.74)	1.64 ± 0.09 <sup>A</sup> (33.33)	1.87 ± 0.03 <sup>A</sup> (41.67)	1.97 ± 0.08 <sup>A</sup> (38.73)

Note: Values representing means ± SD in the same row with different subscripts are significantly different ( $p < 0.05$ ).

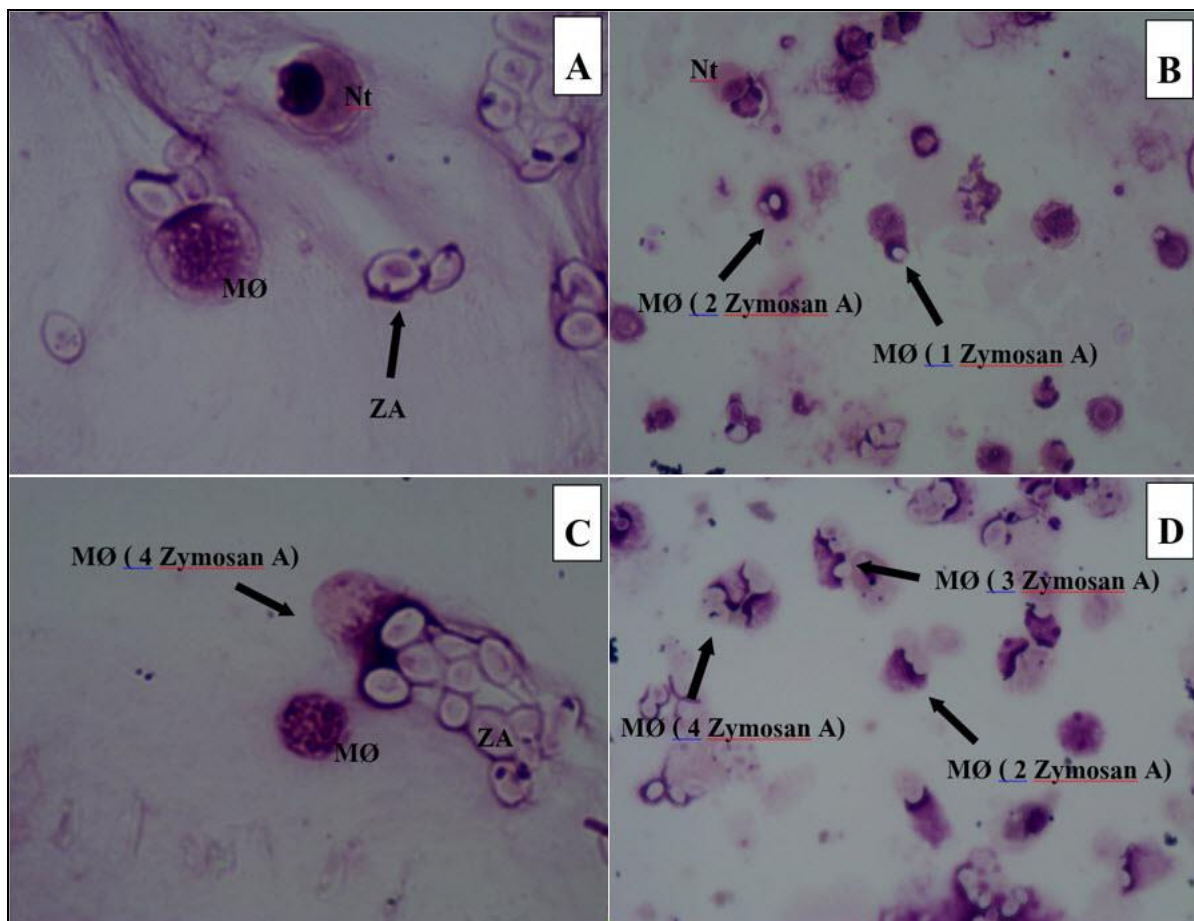


Figure 1. Morphological characteristic and phagocytes activity of hybrid catfish 1 h after testing the ability of phagocytes cells to ingest zymosan A, stained with Wright Giemsa. (A) after 8 weeks feeding with unsupplemented diet (control group) (x100), (B) after 8 weeks feeding with the diet supplemented with steamed pumpkin at 1% (x40), (C) after 8 weeks feeding with the diet supplemented with steamed pumpkin at 5% (x100), (D) after 8 weeks feeding with the diet supplemented with steamed pumpkin at 10% (x40). MØ: macrophage; Nt: neutrophils; ZA: zymosan A.

**Discussion.** Gratifying results have been obtained from the measurement on growth performance, feed conversion and survival rate of the experimental hybrid catfish cultured using steamed pumpkin flesh supplementing diets. The highest growth values were those from the group with 10.0 % supplementation of diet. Although there is not quite different in feeding conversion rate among the 1 % to 10 % supplementing, but their better values than the un-supplement control one is significantly indicated. None of the fishes were seen degenerated through the experimentation. As the pumpkin flesh contains at least 71.28 % of high energy source carbohydrate necessary for their growth supporting (Gajewski et al 2008). This nutrient, especially in the fructo-oligosaccharides form, therefore, favors prebiotic intestinal microorganisms such as lactobacilli and bifidobacteria that fruitfully activate digestion and nutrient absorption (Gibson et al 1995; Gibson 1999). Carbohydrate itself induces an appetite and ensures adequate energy without any need for other sources such as protein content. This circumstance provides a reduction of protein catabolism process and increases the protein reserve promoting better fish health including the death rate prevention (Halver & Hardy 2002). Similar evidence using such locally high energetic fruit has previously been reported in the culture of Nile tilapia (*Oreochromis niloticus*) fish with banana CV. Kluai Namwa ripe fruit complementary diet as well (Palintorn et al 2019).

Even though pumpkin has been used as dietary supplement for raising other animals, for instance the Wister rat groups that were supplemented with dried pumpkin at concentrations of 5 % and 10 % in the diet which showed subsequent growth increasing tendency in terms of their average weight and average daily growth rate

(Koziorzewska et al 2018). But there was only a limit report on the use of pumpkin as a dietary supplement along with the hybrid catfish and other aquacultured species. The only closely reported system was the case report by Klahan et al (2014) that hybrid catfish fed with 5-10 % pumpkin supplemented diet had yielded higher values in weight gain and average daily growth than the control group ( $p < 0.05$ ). However, the effects concerning other details in growth performances and health has yet not been observed. Therefore, the findings of this study provide important evidence supporting the use of pumpkin as a dietary supplement to enhance productivity and promote the health of hybrid catfish, thereby offering practical benefits for aquaculture applications.

Determination of hematocrit index of the hybrid catfish displayed an increasing tendency from 44.93 % to 58.57 % during the 8-week period in groups cultured with steamed pumpkin flesh complementary diets while an average count from 43.43 % to 51.0 % was for the control group. Regardless of their statistical irrelevance, it is certainly indicated vigorous health performance according to a normal standard at 36 % to 54 % for diagnosis of animal abnormalities or hematological symptoms (Mulero et al 1998). These index amounts obtained neither lower nor exceeding the norms that imply either hemolytic anemia or serious stress response (Suckow et al 2012; Clauss et al 2008). Likewise, the white blood cell count from steamed pumpkin diets fed groups exhibited  $3.71$  to  $4.87 \times 10^8$  cells  $\text{mm}^{-3}$  while quite insignificant amount at about  $4.37$  down to  $3.63 \times 10^8$  cells  $\text{mm}^{-3}$  along the cultured period were from the control group. These amounts indicate considerate healthy growth of the fish in agreement with the standard normal amount of white blood cells in fish blood that equal to  $3-15 \times 10^8$  cells  $\text{mm}^{-3}$  (Feldman et al 2000). Several works on the fish white blood cell count also denote similar assumption, for example, the Mekong giant catfish (*Pangasianodon gigas*) with the amount  $5.30 \times 10^4$  cells  $\text{mm}^{-3}$  (Phoonsamran et al 2008) and the Nile tilapia cultured with ginger (*Zingiber officinale*) supplementary diet for 10 days by the amount between  $16.87 - 19.19 \times 10^5$  cells  $\text{mm}^{-3}$  (Pisuttharachai et al 2017).

Phagocytosis process of the phagocyte cells has long been known as nonspecific immune response against pathogens of animals including the fish phylum by activation of accumulating oxygen consumption in respiratory burst to produce more germicidal hydrogen peroxide and superoxide antioxidants (Secombes & Fletcher 1992; Verlhac et al 1996). This study showed prominent increase in % phagocytes and phagocytic index values among the hybrid catfish groups fed with diet containing steamed pumpkin flesh. The highest increasing values of phagocytes by 33.24% and of phagocytic indexing by 38.73% were from the fish group that received steamed pumpkin at 10% level. A nutrient beta-carotene in pumpkin has been principally assumed to be a foundation of effective body defense activation (Zamora et al 1991; Iyama et al 1996). Comparative experiments on rats feeding with pumpkin supplemented diet have demonstrated a significant enhancement of glutathione peroxidase and superoxide dismutase due to higher intake of beta-carotene and carotenoid in the diet (Chang et al 2004). Chang et al (2004) similarly reported the increasing of these enzymes both in the bloodstream and in the liver of the mice feeding with pumpkin extract. Another nourishment as polysaccharides containing in pumpkin is also claimed to be a key factor activating the antioxidant superoxide dismutase need for the body defense system in tumor mice serum (Xu 2000). In consequence, the results obtained in the present observation, an activation of the above defense enzymes mentioned should also be expected. These remarkable features as defined by the increasing % phagocytes and phagocytic index values hence are considerably influenced the immune incentive of the fish as well.

**Conclusions.** This choice for hybrid catfish (*Clarias gariepinus* × *Clarias macrocephalus*) culture using steamed pumpkin flesh as dietary supplementation is the first issue suggested for new efficient, healthful care and ecofriendly rearing. The present study indicates that this steamed pumpkin supplemented diet at 1 %, 5 %, and 10% each, has satisfied desirable increase of the growth performance and survival of hybrid catfish. Investigation on their immunological indices has also certified adequate superior proficiency regarding the phagocytic indexing and white blood cell counts. Thus, besides

the prime feature of pumpkin that is locally available at a reasonable price, it can be concluded that the steamed pumpkin flesh is suitable for hybrid catfish culture feed.

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