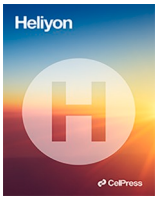
Heliyon 10 (2024) e28224

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Comparative analysis of growth and health of juvenile African catfish (*Clarias gariepinus*) fed with different starch diets

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ARTICLE INFO

Keywords:
Hematology
Digestive enzyme
Antioxidative
Gut microbiota

ABSTRACT

This study evaluated the effects of potato, wheat, rice, and corn starch on growth performance, blood parameters, digestive enzyme activity, antioxidative response, and gut microbiota of African catfish, *Clarias gariepinus*. A control diet (a commercial fish diet) and four different starch (potato, PO; wheat, WH; corn, CO; rice, RC) formulations were fed to African catfish with average weight of 10.5g (n = 30) for eight weeks. The experiment was conducted in triplicates. At the end of the feeding trial, the growth performance of African catfish fed with potato starch (PO) was significantly higher than other treatment groups. Furthermore, this group recorded significant and lowest feed conversion ratio (FCR) compared to other groups. Meanwhile, there were no significant differences in all tested hematological parameters and antioxidative response between the groups. Digestive enzyme activities in the fish intestines, including amylase, lipase, and protease, were significantly higher in African catfish fed with the PO diet. In addition, this group demonstrated substantially lower viscerosomatic index (VSI) and hepatosomatic index (HSI) than other groups, indicating that the fish has more meat on its body. The PO diet group also recorded significantly higher *Akkermansia muciniphila,* a good gut microbiota. Therefore, the PO diet potentially improves African catfish's growth performance and health status.

1. Introduction

African catfish, *Clarias gariepinus*, is a popular freshwater fish in Malaysia and well accepted by the local market due to its desirable characteristics, such as fast-growing, tolerance to high stocking density, short production cycle, and unique taste. [1]. African catfish farming has intensified over the years to fulfill the rising demands, resulting in an increasingly competitive industry. Therefore, aquaculturists seek cost-effective and sustainable African catfish feed formulation to boost farm productivity and investors' income

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https://doi.org/10.1016/j.heliyon.2024.e28224
Received 22 October 2023; Received in revised form 20 February 2024; Accepted 13 March 2024
Available online 19 March 2024

[1]. Furthermore, economically-sustainable feed ingredients are crucial in fish farming as feed cost is estimated to be 60% of the total operational expenditure [2].

Starches, such as various grains (wheat, wheat bran, rice, rice bran) are excellent sources of carbohydrates in aqua feed [3] and act as a binder in the diet formulation [4]. This affordable nutrient source provides energy for aquaculture species [5], but they do not rely solely on carbohydrates to generate energy for metabolic activities. Alternatively, aquatic animals catabolize lipids and protein for energy [6], resulting in protein retention impairment and excessive nitrogen release into the environment [7,8]. Moreover, lipids and proteins such as fish oil, fish meal, and soybean meal are more expensive than carbohydrates as raw material for aqua feed formulation. Thus, carbohydrate inclusion at optimal levels is essential in fish feed formulation to induce the protein-sparing effect and allow aquatic animals to fully utilize protein for growth performance, besides boosting the income for investors.

Generally, starch is incorporated in feed at 15%–20% and 30%–40% for herbivorous and carnivorous aquatic animals, respectively [9,10]. Excessive starch in aqua feed may lead to growth impairment, fatty liver, and visceral lipid accumulation in aquatic animals [11,12]. For instance, high dietary starch resulted in poor growth performance and blood parameters, fatty liver, high hepatosomatic index (HSI) and visceral somatic index (VSI) in Nile tilapia, *Oreochromis niloticus* fed 45% corn starch diet [13]. Dietary corn starch (28%)also adversely impact the growth and health of juvenile golden pompano, *Trachinotus ovatus* fed [14,15]. On the other hand, juvenile cobia, *Rachycentron canadum* received 20% corn starch diet exhibited normal growth performance [16]. Therefore, optimal starch inclusion in aqua feed formulation is crucial to avoid adverse effects in aquatic animals while maintaining their health status and productivity.

Corn, potato, and wheat are the common starches incorporated in aqua feed. Each starch impacts the growth performance and health status of aquatic animals differently due to the varying nutritional profiles. Previous studies have investigated the impacts of starches such as tapioca, sago, rice, potato, wheat, barley, corn, pea, and pregelatinized starch on grouper, *Epinephelus morio* [17], bagrid catfish, *Hemibagrus nemurus* [18], common carp, *Cyprinus carpio* [19], Nile tilapia [20], and largemouth bass, *Micropterus salmoides* [21,22]. Discrepancies were evident between these studies regarding the outcomes of the aquatic species despite having similar dietary habits. For instance, corn was a better starch source for yellow perch, *Perca flavescens,* than wheat [23], while dietary wheat was well accepted by Atlantic salmon, *Salmo salar,* and gilthead seabream, *Sparus aurata* [24,25]. Meanwhile, olive flounder, *Paralichthys olivaceus*, could tolerate corn and wheat in their feed without significant adverse outcomes in growth performance and health status [26].

Corn and wheat are also good starch sources for common carp and blunt snout bream, *Megalobrama amblycephala* [27], while striped catfish only accepted wheat, potato, and sago as starch sources in their feed [28]. Malaysian mahseer, *Tor tambroides* preferred corn and tora as their starch sources instead of tapioca and sago [29]. Carnivorous fish tolerate starches less than herbivorous/omnivorous fish. For instance, dietary tapioca starch at 20–35% have beneficial effects on growth performance of hybrid lemon fin barb [30]. Conversely, a high wheat starch diet (19.93%) caused hepatic inflammation in largemouth bass, *Micropterus salmoides* [31]. These findings indicated the variable outcomes of different species despite all being carnivorous.

Starch source and inclusion level are factors that determine the ability of a fish to digest and metabolize the ingredient, which vary betweenfish species [30]. Overall, omnivorous and herbivorous fish demonstrated better starch utilization than carnivorous fish [32]. As different aquaculture species respond differently to multiple starch sources, this study evaluated the impacts of potato, wheat, rice, and corn on the growth performance, health status, and gut microbiota disparities of juvenile African catfish.

Table 1
Feed formulations used in feeding trial.

Raw materials	Diet formulation in different treatments (%)				
	Control (Commercial feed)	PO	WH	RC	CO
Soybean meal	N/A	30	30	30	30
Fish meal	N/A	39	39	39	39
Potato starch	N/A	20			
Wheat starch			20		
Rice starch				20	
Corn starch					20
Premix	N/A	2	2	2	2
Fish oil	N/A	3	3	3	3
Vegetable oil	N/A	3	3	3	3
Carboxymethyl cellulose (CMC) binder	N/A	3	3	3	3
Total	N/A	100	100	100	100
Nutritional profiles					
Protein	32%	33.1%	32.8%	31.9%	32.4%
Crude fat	3%	5%	6%	6%	5%
Moisture	12%	7%	7%	8%	7%

*N/A = Not available; PO = potato starch diet; WH = wheat starch diet; RC = rice starch diet; CO = corn starch diet.

2. Materials and methods

2.1. Ethics statement

The study has been approved by the animal care and use committee of Universiti Malaysia Kelantan, Jeli, Kelantan, Malaysia (UMK/FIAT/ACUE/PG/02/2023) and conducted according to the ethical protocol and guidelines for experimental animals.

2.2. Fish feed formulation

The fish feed formulation for the current experiment was prepared according to previous studies [1,33] (Table 1). The formulated feeds were subjected to proximate composition analysis using Soxhlet and Kjeldahl extraction methods [34,35]. The range for each nutrient in the experimental feed is as follows: protein = 31.9%–33.1%, crude fat 5%–6%, and moisture 7%–8%. A commercial fish pellet (Star Feed, Malaysia) was used for the control group. The starch sources selected for this study were potato powder, wheat bran powder, rice bran powder, and corn powder. First, the dry raw materials were sieved (<0.4 mm), weighed, and homogenized in a mixer. Subsequently, other raw materials were added to the mixture, followed by water (200 mL/kg) and stirred to form a dough. The dough was then pelleted using a small meat grinder and oven-dried at 50 °C. Finally, the pellets were cooled at room temperature before packing and stored in the refrigerator.

2.3. Feeding trial

Juvenile African catfish were purchased from a commercial farm in Tanah Merah, Kelantan, Malaysia. The fish were acclimatized for two weeks in a 300 L holding tank and provided with commercial starter feed (Star feed, Malaysia). The fish were fed *ad libitum* once daily in the morning and 100% water exchange was carried out in the afternoon. After the acclimatization period, a total of 450 healthy fish with an average weight of 10.5g (swims normally without any lesion or wound on its body) were selected for the feeding trial and distributed into five groups in triplicates. Juvenile African catfish were fed diets containing 20% potato starch (PO), wheat starch (WH), rice starch (RC), or corn starch (CO) once daily for eight weeks. Each aquarium (100 L) was equipped with aeration and contained 30 fish. Feeding was conducted in the morning at a feeding rate of 3% of their body weight, and a 100% water exchange was carried out in the evening by using aged tap water. The water parameters of the aged tap water were monitored weekly by using a multiparameter pH meter (YSI, USA) and maintained at a normal range: temperature = 24–28 °C, dissolved oxygen = 5–6 ppm, ammonia <0.2 ppm, and pH 6–7.

2.4. Growth performance parameters

Experimental fish from each tank were sampled randomly and weighed. At the end of the feeding trial, the growth performance parameters were determined as below.

Total weight gain (WG)	=	Final body weight - initial body weight
Total weight gain rate (WGR)	=	(Total weight gain/initial body weight) X 100
Feed conversion rate (FCR)	=	Total Feed Intake/total weight gain
Viscerosomatic index (VSI)	=	Total viscera weight/total body weight
Specific growth rate (SGR)	=	([log Final body weight – log initial body weight]/experiment duration) X 100
Hepatosomatic index (HSI)	=	Total liver weight/total body weight

2.5. Hematological parameters analysis

Experimental fish (n = 3) were sampled randomly from each group for hematological parameters analysis. First, the fish were anesthetized using clove oil (100 ppm) [36]. Then, the blood was collected and placed in heparinized tubes for further analysis via an automated hematology analyzer (Mythic 18 Vet, USA) and VetTest analyzer (IDEXX, USA) [37].

2.6. Antioxidative activity

The fish sampled in Section 2.5 were also used in the antioxidative assays. First, the fish liver was extracted, homogenized in phosphate-buffered saline (PBS), and centrifuged at 10000 rpm for 10 min. The supernatants were used to detect glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT) activities via colorimetric kits (Elabscience, USA) (Cat. No. E-BC-K096-S; E-BC-K019-S; E-BC-K031-S). The GPx, SOD, and CAT detection were performed according to the manufacturer's instruction and the results were obtained using a microplate reader (BioRad, USA) at 280 nm [1,38].

2.7. Digestive enzyme activity

The fish sampled in Section 2.5 were also used to determine the digestive enzyme activities. First, the fish intestines were harvested,

homogenized in PBS, and centrifuged at 8000 rpm for 10 min. The supernatants were stored at 4 °C until further use. The total protein content in the supernatant was determined as described in the previous study [39] by using bovine serum albumin as a standard. Meanwhile, the amylase and protease activities were detected using iodine solution and the Folin-Ciocalteu phenol reagent. Finally, the lipase activity was evaluated using olive oil as a substrate, following the method described by Ref. [40].

2.8. Gut microbiota analysis

2.8.1. DNA extraction

Experimental fish intestines were sampled from each group (n = 3) and subjected to DNA extraction as described by previous studies [41,42]. The samples were pelleted via centrifugation at 10000×*g*, washed with sorbitol buffer, and resuspended with homogenization buffer. Subsequently, samples were homogenized using a vortex at 4000 rpm for 30 min. After homogenization, the samples were subjected to protein precipitation, where saturated sodium chloride (NaCl) solution was added to the sample and incubated on ice for 5 min. The samples were then centrifuged at 10000×*g* for 10 min. The resulting supernatants were mixed with isopropanol and centrifuged at 10000×*g* for 10 min to precipitate the DNA pellet. Finally, the DNA samples were washed with 75% ethanol and resuspended in Tris-EDTA (TE) buffer.

2.8.2. DNA sequencing assay

Primers 341F: CCTACGGGNGGCWGCAG and 518R: ATTACCGCGGCTGCTGG PCR were used in the PCR assay [43,44]. The PCR was performed using WizBio HotStart PCR mastermix (WizBio, Korea) and the following profile: 95 °C for 3 min, followed by 28 cycles of 95 °C for 30 s, 55 °C for 20 s, and 72 °C for 10 s. The PCR products were visualized on gel, normalized and pooled based on intensity, and purified with 1.5 x vol of solid-phase reversible immobilization (SPRI) beads. The pooled and purified amplicons were processed with the NEB Ultra II Library. The obtained library was quantified with Denovix high-sensitivity assay and sequenced on an iSeq100 (Illumina, San Diego) for 2 x 150 paired-end sequencing.

2.8.3. DNA sequencing analysis

The DNA sequencing results were overlapped using fastp v0.21 [45]. The demultiplexing and primer were removed using cutadapt v1.18, imported to QIIME2 v.2021.4, and denoised with dada2 [46,47]. A total of 254090 bacterial and 4316 archaeal genomes was organized into 45555 bacterial and 2339 archaeal species clusters based on the taxonomic assignment [48,49]. The DNA sequencing readings were subjected to MicrobiomeAnalyst-compatible input that can perform SparCC co-occurrence network construction [50,51]. Statistical analysis was cperformed using the linear discriminant analysis (LDA) effect size (LEfSe) method [52]. The relative abundance was used to generate Krona plots that characterized the relative abundances within the hierarchies of taxonomic classifications. Only clusters with a cumulative relative abundance of >1% and sample prevalence of >20% were used to generate a heatmap [53].

2.9. Statistical analysis

Growth performance, hematological parameters, antioxidative response, and digestive enzyme activities data were compared and analyzed using one-way analysis of variance (ANOVA) followed by Tukey post hoc at $p < 0.05$ by using Statistical Package for the Social Sciences (SPSS) version 16.0 (IBM, USA). The results were expressed as mean ± standard deviation (SD).

3. Results

3.1. Growth performance of experimental fish

The African catfish fed with PO diet exhibited the best growth performance, followed by WH, RC, control, and CO groups (Table 2). The PO-treated fish recorded significant ($p < 0.05$) and highest final weight, SGR and WG. Furthermore, this group demonstrated significant ($p < 0.05$) and lowest FCR. VSI, and HSI.

Table 2
Growth performance parameters of experimental fish fed with different starch diets in an eight-week feeding trial.

Parameters	Control	PO	WH	RC	CO
Initial weight (IW) (g)	10.5 ± 0.06	10.4 ± 0.10	10.5 ± 0.00	10.4 ± 0.15	10.4 ± 0.10
Final weight (FW) (g)	203.8 ± 6.09^{b}	247.3 ± 9.19^{a}	207.6 ± 4.02^{b}	209.0 ± 7.16^{b}	177.7 ± 10.41^{c}
Weight gain (WG) (%)	1847.1 ± 68.89^{b}	2277.9 ± 98.71^{a}	1876.8 ± 38.30^{b}	1903.3 ± 82.44^{b}	1608.2 ± 94.28^{c}
Specific growth rate (SGR) (%)	2.3 ± 0.03^{b}	2.5 ± 0.03^{a}	2.3 ± 0.02^{b}	2.3 ± 0.03^{b}	2.2 ± 0.04^{c}
Visceral somatic index (VSI) (%)	3.4 ± 0.15^{b}	2.4 ± 0.08^{a}	3.4 ± 0.01^{b}	3.4 ± 0.08^{b}	2.5 ± 0.27^{a}
Hepatosomatic index (HSI) (%)	1.6 ± 0.04^{b}	1.2 ± 0.11^{a}	1.5 ± 0.11^{b}	1.6 ± 0.13^{b}	1.2 ± 0.11^{a}
Feed conversion ratio (FCR)	1.3 ± 0.04^{b}	1.1 ± 0.04^{a}	1.3 ± 0.03^{b}	1.3 ± 0.05^{b}	1.5 ± 0.09^{c}

*Data expressed as mean ± SD.
*PO = potato starch diet; WH = wheat starch diet; RC = rice starch diet; CO = corn starch diet.
*Values in the same row with different superscripts showed significant differences at $p < 0.05$.

3.2. Hematological parameters

The PO diet group demonstrated significant ($p < 0.05$) and highest mean value of white blood cell (WBC), hemoglobin (HGB), and mean corpuscular hemoglobin (MCH) (Table 3). Meanwhile, the RC diet group recorded significant ($p < 0.05$) and highest monocytes (MON). There were no significant differences in lymphocytosis (LYM), red blood cell (RBC), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and platelet (PLT) levels between the treatment groups.

3.3. Digestive enzyme activities in experimental fish intestines

Lipase, amylase, and protease activities were significantly ($p < 0.05$) higher in African catfish fed dietary PO than other starch groups and control. In addition, the fish fed with dietary RC, WH, and control exhibited similar digestive enzyme activities, which were significantly ($p < 0.05$) higher than the CO diet group (Fig. 1a–c).

3.4. Antioxidative response of African catfish fed different starch diets

The antioxidant responses, particularly SOD, GPx, and CAT among the groups, were statistically similar in the present study (Fig. 2a–c). Nevertheless, African catfish fed with PO recorded the highest CAT value, the RC group had the highest SOD value, and the control group had the highest GPx level. No specific trend was observed in this experiment.

3.5. Gut microbiota analysis

A total of 15 gut microbiota groups were identified in the present study (Fig. 3). *Bacteroides* were highly abundant in the dietary WH group, followed by control. Meanwhile, the PO, RC, and CO groups exhibited similar *Bacteroides* distribution. A large population of *Proteus mirabilis* was present in the RC group, whereas the PO group is mainly host to *Akkermansia muciniphila*. *Anaerorhabdus furcosa* was highly abundant in the WH and control groups compared to other treatments and absent in the RC group. Furthermore, *Phocaeicola massiliensis* was highly distributed in the intestines of fish fed with the WH diet, followed by CO and RC groups. *Edwardsiella tarda* was the most abundant gut microbiota in the PO-treated group. Meanwhile, *Cetobacterium* was highly abundant in the PO and control groups.

4. Discussion

Starch is an energy source for all livestock, including aquatic animals [4].This compound is a combination of amylose and amylopectin; the former is known for its low digestibility, while animals easily hydrolyze the latter [4]. Therefore, the ratio of amylose and amylopectin in starch will affect its digestibility [54]. A starchrich in amylopectin can be quickly digested and increases glycemia [54]. A study revealed that European sea bass, *Dicentrarchus labrax,* pdemonstrated higher digestibility of waxy maize starch (99% amylopectin) compared to normal starch (72% amylopectin) [55]. Therefore, impacts of different starches vary on the growth performance of aquatic animals [12,56].

In this study, African catfish fed with PO had significantly superior growth performance (WG, SGR, and FCR) than other starch diet groups, indicating the fish's ability to utilize PO better than WH, RC, and CO. Similar findings were observed in striped catfish, *Pangasianodon hypophthalmus*, supplemented with dietary PO [28]. A different study reported no significant differences in the growth performance of yellow perch, *Perca flavescens* [57], tinfoil barb, *Barbonymus schwanenfeldii* [6], or blunt snout bream, *Megalobrama amblycephala* [58] after corn, potato, and wheat diets supplementation. This outcome reflected the ability of the fish species to utilize corn, potato, and wheat effectively. The superior growth performance of African catfish when supplemented with dietary PO can be

Table 3
Hematological parameters of experimental fish fed with different starch diets in an eight-week feeding trial.

Hematological parameters	Control	PO	WH	RC	CO
WBC/μl	119.6 ± 1.85^{b}	138.1 ± 6.90^{a}	124.8 ± 6.08^{b}	124.1 ± 5.35^{b}	120.9 ± 7.01^{b}
LYM (%)	87.0 ± 1.91	88.2 ± 2.9	85.7 ± 4.65	89.4 ± 5.46	88.8 ± 3.35
MON (%)	13.2 ± 0.52^{b}	12.9 ± 0.15^{b}	13.4 ± 0.35^{b}	15.6 ± 1.81^{a}	15.0 ± 1.84^{a}
RBC10^3/μl	2.4 ± 0.35	2.7 ± 0.15	2.5 ± 0.15	2.3 ± 0.21	2.2 ± 0.12
HGB (g/dl)	6.1 ± 0.20^{b}	7.2 ± 1.15^{a}	6.07 ± 0.25^{b}	6.23 ± 0.15^{b}	6.07 ± 0.25^{b}
HCT (%)	25.4 ± 0.55	25.7 ± 1.91	25.4 ± 1.47	23.6 ± 2.36	22.2 ± 0.65
MCV (μm^3)	127.9 ± 5.98	121.1 ± 1.61	124.5 ± 1.31	123.5 ± 0.36	126.1 ± 4.55
MCH (pg)	33.4 ± 2.67^{c}	45.5 ± 2.57^{a}	40.7 ± 1.98^{b}	37.0 ± 1.16^{b}	36.1 ± 3.44^{b}
MCHC (g/dl)	26.3 ± 2.18	29.8 ± 1.42	30.7 ± 1.44	31.0 ± 0.42	30.0 ± 2.39
PLT (10^3/μl)	38.2 ± 0.70	35.1 ± 1.38	34.0 ± 2.42	36.6 ± 1.32	33.4 ± 1.53

*Data expressed as mean ± SD.
*PO = potato starch diet; WH = wheat starch diet; RC = rice starch diet; CO = corn starch diet.
* Values in the same row with different superscripts showed significant differences at $p < 0.05$.
*WBC = White blood cell, LYM = Lymphocytosis, MON = Monocytes, RBC = Red blood cell, HGB = Hemoglobin, HCT = Hematocrit, MCV = Mean corpuscular volume, MCH = Mean corpuscular haemoglobin, MCHC = Mean corpuscular haemoglobin concentration, PLT = Platelet.

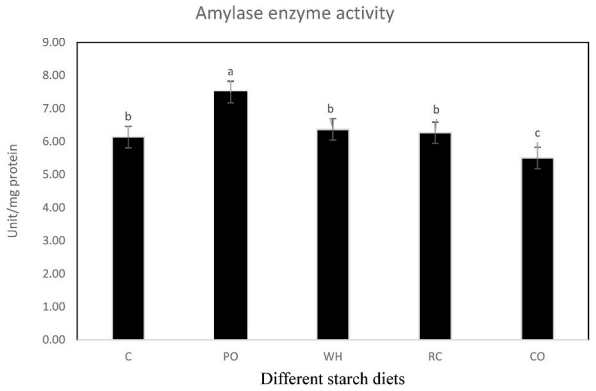

Fig. 1a. Amylase enzyme activity of African catfish fed different starch and control diets after an eight-week feeding trial * PO = potato starch diet; WH = wheat starch diet; RC = rice starch diet; CO = corn starch diet *Values in the same row with different superscripts showed significant differences at $p < 0.05$.

Fig. 1b. Lipase enzyme activity of African catfish fed different starch and control diets after an eight-week feeding trial * PO = potato starch diet; WH = wheat starch diet; RC = rice starch diet; CO = corn starch diet *Values in the same row with different superscripts showed significant differences at $p < 0.05$.

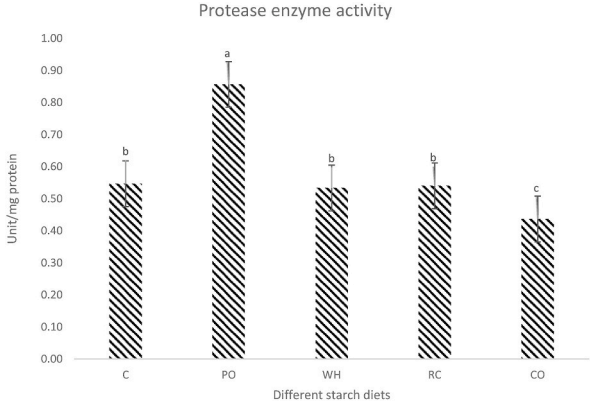

Fig. 1c. Protease enzyme activity of African catfish fed different starch and control diets after an eight-week feeding trial * PO = potato starch diet; WH = wheat starch diet; RC = rice starch diet; CO = corn starch diet *Values in the same row with different superscripts showed significant differences at $p < 0.05$.

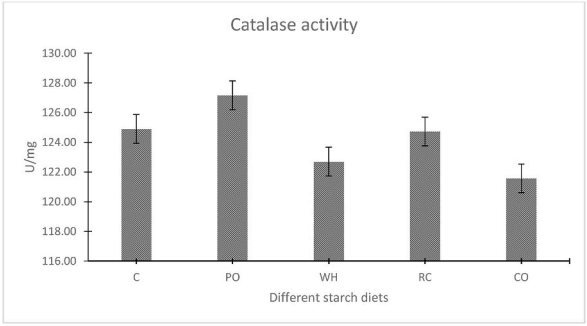

Fig. 2a. Catalase (CAT) activity of African catfish fed different starch and control diets after an eight-week feeding trial. * PO = potato starch diet; WH = wheat starch diet; RC = rice starch diet; CO = corn starch diet *Values in the same row with different superscripts showed significant differences at $p < 0.05$.

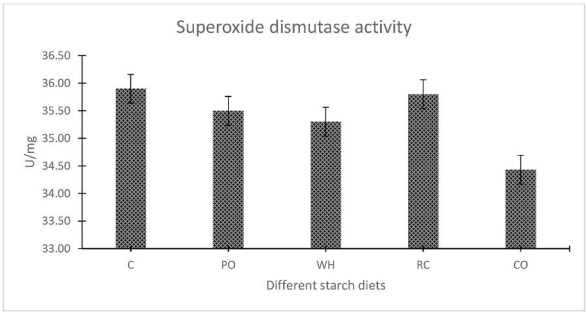

Fig. 2b. Superoxide dismutase (SOD) activity of African catfish fed different starch and control diets after an eight-week feeding trial. * PO = potato starch diet; WH = wheat starch diet; RC = rice starch diet; CO = corn starch diet *Values in the same row with different superscripts showed significant differences at $p < 0.05$.

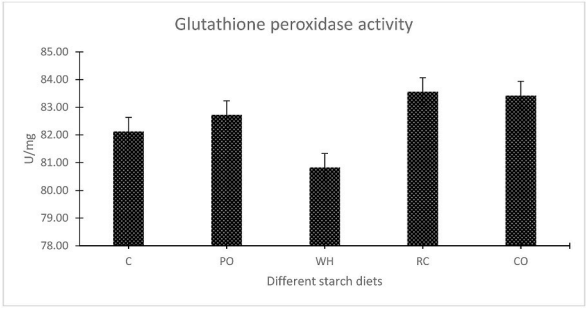

Fig. 2c. Glutathione peroxidase (GPx) activity of African catfish fed different starch and control diets after an eight-week feeding trial. * PO = potato starch diet; WH = wheat starch diet; RC = rice starch diet; CO = corn starch diet *Values in the same row with different superscripts showed significant differences at $p < 0.05$.

explained by the activation of the fish's digestive enzymes, such as lipase, amylase, and protease. Proteinases are vital for digestion and amino acid absorption [59,60]. In summary, the PO diet is more suitable for promoting the growth performance of African catfish than other starch diets in this study.

The current study also revealed that the growth performance of the control, WH, and RC diet groups were relatively similar, suggesting that the commercial diet possibly contains identical ingredients as the WH and RC diets prepared in this study. Furthermore, the growth performance of the CO diet group was significantly lower than other starch diet groups, consistent with an earlier study on juvenile grass carp. This findingindicated the fish's tolerance towards WH and RC diets [61]. Meanwhile, the CO diet is a suitable carbohydrate source for golden pompano, *Trachinotus ovatus* [2], but could not be utilized effectively by gilt head bream which tolerateddietary WH better [24]. This outcome may be attributed to the cuticular coating in corn, resulting in its lower digestibility and utilization by aquatic animals [12,62].

The VSI and HSI were also significantly lower in the PO diet group than other starch diet groups, indicating that the fish had lower

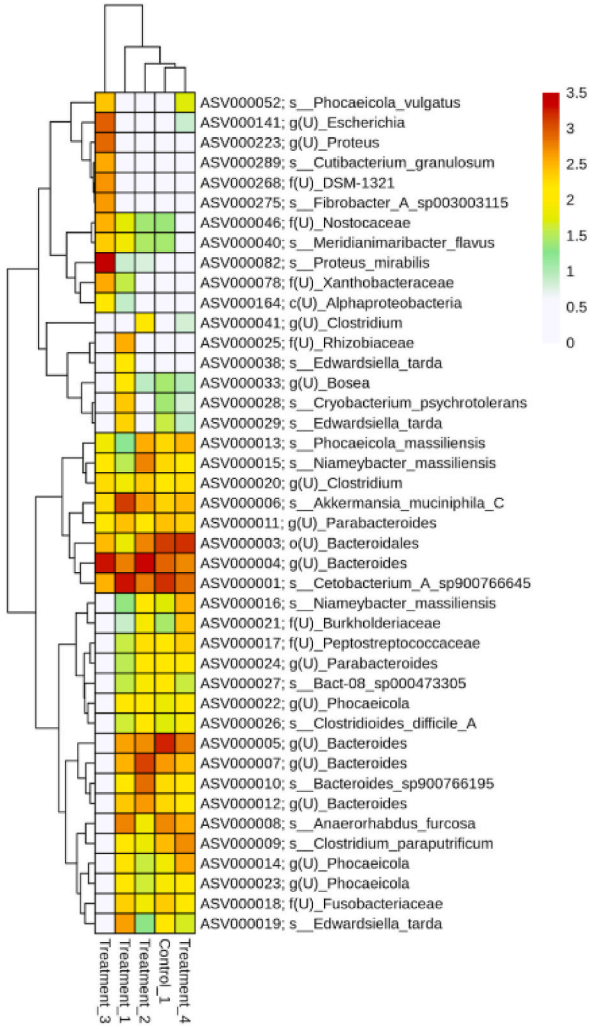

Fig. 3. Heatmap of core microbiota relative abundance across samples of fish fed different starch diets for eight weeks *Control: Experimental fish received commercial pellet Treatment 1: Experimental fish received potato starch, PO Treatment 2: Experimental fish received wheat starch, WH Treatment 3: Experimental fish received rice starch, RC Treatment 4: Experimental fish received corn starch, CO.

fat accumulation within their liver and whole body. These findings align with previous reports in Hybrid catfish, *Clarias gariepinus* ♀ x *Heterobranchus longifilis* ♂, Japanese flounder, *Paralichthys olivaceus*, and Siamese fighting fish, *Betta splendens* [37,63,64]. In addition, WH and CO tend to cause fatty liver and fat deposition in the abdomen of juvenile grass carp, *Ctenopharyngodon idella* compared to other starches [61]. Consuming the CO diet led to fatty liver in European seabass, *Dicentrarchus labrax* [65], while striped catfish and yellow perch fed with the WH diet demonstrated similar outcomes [28]. The current study also discovered that dietary CO, WH, and RC led to higher fat deposition in African catfish compared to the PO diet. Nonetheless, all dietary starches used in this study did not have adverse impacts on the fish's health, indicated by the lack of significant differences in antioxidative response (SOD, GPx, and CAT) and hematological parameters analysis between the treatment groups. This finding is also in agreement with a previous study [57].

Hematological parameters analysis did not reveal any specific trend or effect of the different dietary starches. Despite that, the PO group recorded significantly higher WBC, HGB, and MCH than other starch groups. Higher WBC levels may indicate the good health status of African catfish, which is crucial to combat diseases. Additionally, WBC levels may be influenced by various factors such as season, gender, feeding habits, stress, and the presence of pollutants [66,67]. The MON and LYM levels are also key indicators of a fish's health status [68,69]. Higher HBG and MCH levels reflect the fish's normal respiration and absence of anemia [70].

Cetobacterium washighly abundant in the PO and control groups. This gut microbiota was dominant in various freshwater fish, including *Arapaima gigas* [71], *Cyprinus carpio* [72], *Oreochromis niloticus* [73], *Ictalurus punctatus*, *M. salmoides*, and *Lepomis macrochirus* [74]. An earlier study revealed that tilapia and carp do not require vitamin B-12 [75] as the abundant *Cetobacterium* in the fish intestine was able to producethe nutrient [73]. Furthermore, the presence of *Cetobacterium* would suppress the *Bacteroides* propagation in the fish intestine [76]. TIn the present study, *Bacteroides* was found at low abundance in the PO and control group due to highly distribution of *Cetobacterium* in the African catfish intestine.

The current findings also demonstrated that dietary PO modulated and enhanced the gut microbiota of African catfish, indicated by the highly abundant *Akkermansia muciniphila* in all treatment groups. This gut microbiota is a crucial health biomarker in human and animal intestines [77–79]. The presence of *A. muciniphila* is desirable, suggesting a good health status of a particular organism [53]. Moreover, this gut microbiota is essential for gut health maintenance, immunity, and metabolic activities [80,81]. The absence of this gut microbiota is often linked to diseases [82–85]. Thus, the high abundance of *A. muciniphila* in the gut of African catfish fed with dietary PO reflects their good health status.

5. Conclusion

This study findings revealed that dietary PO could enhance growth performance, feed digestion, and health status of African catfish. Their enhanced growth performance could be attributed to the significant improvements in the digestive enzyme activities. In addition, the fish in PO group had more flesh on their bodies due to the significant and lowest VSI and HSI. However, additional investigations such as proximate body analysis is essential future studies to verify this finding. This study also confirmed that dietary PO promotes the distribution of good gut microbiota, *Akkermansia muciniphila* associated with the fish's good health status. Therefore, dietary PO is a promising carbohydrate source to enhance African catfish production and offers an alternative starch source for aqua feed formulation.

CRediT authorship contribution statement

Lee Seong Wei: Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation. **Mohd Shaiful Azman Abdul Rahim:** Project administration, Methodology, Investigation. **Kon Yeu Hooi:** Methodology, Investigation. **Martina Irwan Khoo:** Visualization, Validation. **Azra Mohamad Nor:** Funding acquisition, Formal analysis, Data curation, Conceptualization. **Wendy Wee:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Lee Seong Wei, Associate Editor of Heliyon. Other authorsdeclare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

This project was funded by Universiti Malaysia Kelantan Matching Grant (R/MTCH/A0700/00387A/009/2023/01161) and the Ministry of Higher Education, Malaysia, under the Niche Research Grant Scheme (NRGS) (R/NRGS/A0.700/00387A/006/2014/00152).

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