

Dietary combination of pawpaw seed and onion peel powder: Impact on growth, haematology, biochemical and antioxidant status of *Clarias gariepinus*

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Abstract

A feeding trial was conducted to evaluate the effect of pawpaw–onion powder (POP) mixture on the growth, and haemato-biochemical and antioxidant responses of *Clarias gariepinus* ($4.02 \pm 0.01\text{g/fish}$) for 60 days. Five trial diets were formulated as control (without POP or antimicrobial growth promoter (AGP)), AGP (basal diet + 10ml AGP/kg diet), POP 2.5 (basal diet + 2.5g POP/kg diet), POP 5.0 (basal diet + 5.0g POP/kg diet) and POP 10 (basal diet + 10g POP/kg diet). Two hundred and twenty-five fish were equally distributed into five groups in triplicate and fed twice daily. The results indicate that AGP or POP supplementation exerted no effects on the growth and blood profile among the various groups, but a significantly higher lymphocyte count was observed in POP 10g/kg. The highest whole-body protein and lipid contents were noticed in fish fed the control diet ($p < .05$), whereas POP 5.0g/kg group recorded the highest hepatosomatic value. Furthermore, the cholesterol level was found to be lower in the AGP- and POP-fed fish compared with the higher level recorded in the control. The glucose concentration and superoxide dismutase enzyme activity were found to be higher in POP 2.5-fed fish, whereas POP 10-fed fish showed higher catalase activities compared with other groups ($p < .05$). Based on the result obtained, this study showed that dietary POP had no significant impact on the growth performance but has direct effects on the whole-body lipid content, lymphocyte count, cholesterol level, alanine aminotransferase, alkaline phosphatase and antioxidant response of *Clarias gariepinus*.

KEYWORDS

blood profile, *Clarias gariepinus*, onion peel, pawpaw seed, serological indices

1 | INTRODUCTION

The demand for aquaculture products continues to increase as the food-fish contribution from capture fisheries, whose productivity is already stretched, levelled off (FAO, 2018). In 2016, the global aquaculture volume amounted to 80 million tonnes, up from 76.6 million tonnes produced in 2015 (FAO 2017a, 2018). African catfish (*Clarias*

gariepinus) is the most dominant aquaculture fish species in sub-Saharan Africa with a reported production figure of 231, 091 metric tonnes (11% of total African aquaculture output) and value of USD 630, 678 in 2016 (FAO, 2017b). The growth of African catfish (*C. gariepinus*) production can be partly linked to increased intensification of culture system, which has engendered increased incidence of stress-related diseases, high mortality, immune system depression

and growth reduction. Various measures have been introduced over the years to proffer solutions to these negative effects such as the use of antibiotics, anthelmintic agents and hormones (Cabello, 2006; Fawole, Sahu, Pal, & Ravindran, 2016). However, the frequent use of antibiotic has led to the development of drug-resistant pathogens, complications in human health, bioaccumulation and ecosystem pollution (Arciuli et al., 2017; Cabello, 2006; Magrone et al., 2016). Nevertheless, the needs for the production of antibiotic-free animal protein and increased awareness of organic food have led to an upsurge in the use of medicinal plants or phytogetic as feed additive in aquaculture.

Phytogenic feed additives, commonly known as PFAs, are plant origin substances included in the diets of animals at varying levels with the sole aim of improving animal performance (Landy, Ghalamkari, & Toghyani, 2011). Herbs, spices and essential oils are major groups of PFAs which serve as sources of bioactive compound (e.g. alkaloid, terpenoid, quinone, polyphenols and flavonoids) and are known to have antimicrobial, antifungal, antiviral and antioxidative properties (Yang, Chowdhury, Hou, & Gong, 2015). The use of phytoadditives either singly or in combination has been reported to enhance a number of important biological processes in fish. For instance, Manaf et al. (2016) reported that the combination of *Aloe vera* and *Vitex trifolia* significantly improved the growth and reduces mortalities in red hybrid tilapia challenged with *Streptococcus agalactiae*. Other studies have also shown that the combination of different phytogenic additives produces more beneficial effects on the growth performance and health of fish (Yang et al., 2015; Karásková, Suchý, Straková, 2015; Fawole et al., 2016; Lee, Lee, Katya, Park, & Bai, 2017; Wang et al., 2018; Fawole, Sahu, Shamna, & Adeoye, 2018).

Onion, *Allium cepa*, is a multipurpose and commonly used traditional spice in foods. It is richly packed with flavonoids, phenolics, saponins and sulphur compounds (Amrevuawho, Akinyemi, Oyewusi, Bankole, & Ezeri, 2016; Mousavi, Mohammadiarzam, Mousavi, & Ghatrami, 2016). Onion also contains valuable trace minerals such as selenium, a strong antioxidant (Durigon, Kunz, Peixoto, Uczay, & Lazzari, 2019), and quercetin which play a vital role in the reduction in oxidative stress in fish (Pes et al., 2018). It has been reported that the dietary administration of onion powder resulted in growth improvement and enhanced lysozyme activity in fish (Cho & Lee, 2012; Saleh, Michael, & Toutou, 2015).

Pawpaw, *Carica papaya*, is an all-important fruit known for its high level of vitamins C, A and beta-carotene, and are found throughout the tropics (Chukwuka, Iwuagwu, & Uka, 2013; Kadiri, Olawoye, Fawale, & Adalumo, 2016; Vij & Prashar, 2015). Pawpaw (*C. papaya*) is a rich source of iron, magnesium and potassium, and contains several bioactive substances such as alkaloids, flavonoids, glycosides, saponin and tannins (Vij & Prashar, 2015). Several studies have reported various health benefits of pawpaw to include antibacterial, antioxidant, anti-inflammatory and antifungal. These beneficial effects are due to the presence of β -carotene, flavonoid, vitamins, β -sitosterol and polyphenols (Abbas & Abbas, 2011; Ayotunde & Ofem, 2008; Owoye, Adebukola, Funmilayo, &

Soladoye, 2008; Oyoyede, 2005; Starley, Mohammed, Schneider, & Bickler, 1999), thus making *C. papaya* a promising therapeutic plant material for health management of fish. According to Wong and Kong (2014), pawpaw seed is a good source of food antioxidants and was reported to have higher antioxidant activity than *Annona squamosa* seeds (Vijay & Sriram, 2010).

Based on the aforementioned potential of each plant material (onion and pawpaw), their combination may be more beneficial as feed additive for fish but need to be investigated. Therefore, this study was designed to determine the effect of pawpaw seed and onion peel (POP) mixture on the growth, haematology, serum biochemistry and antioxidant response of *Clarias gariepinus* fingerlings.

2 | MATERIALS AND METHODS

2.1 | Collection and identification of plant material

The pawpaw seed and onion peel were collected fresh and air-dried for further processing. Prior to drying, samples were taken to the Herbarium of the Department of Plant Biology, University of Ilorin, for identification, and deposited with voucher number UILH/001/2019/1332 and UILH/002/2019/945 for *Allium cepa* (Onion peel) and *Carica papaya* (pawpaw seed) respectively.

2.2 | Preparation of onion peel and pawpaw seed

The onion skin was manually peeled as well as fresh pawpaw seed and air-dried separately. They were ground with a kitchen blender separately into a fine powder, sieved, and equal portions of pawpaw seed powder and onion peel powder (hereafter referred to as POP) were mixed at a ratio of 1:1 (w/w) and packaged in an airtight bottle and stored at room temperature until required for use.

2.3 | Experimental fish and feeding trial

African catfish, *Clarias gariepinus* (4.02 ± 0.01 g/fish), fingerlings used in the present study were procured from Members of Standard Fish Alliance (MCS), Afon (8.3139° N, 4.5271° E), Asa Local Government, Kwara State, Nigeria, and transported to the research laboratory, Faculty of Agriculture, University of Ilorin (8.4912° N, 4.5950° E), Nigeria, in a plastic containers and were acclimatized for 14 days. The fish were fed 2mm on-farm made feed during the acclimatization period. Prior to the commencement of the feeding trial, the fish were left unfed for 24 hr, after which they were stocked randomly into five experimental groups at fifteen fish per tank ($45.8 \times 32.3 \times 23.7$ cm) in triplicate for each treatment group. During the feeding trial, fish were fed twice a day (8:00 hr and 16:00 hr) to satiation, and each tank was supplied with bore-well water up to 70% capacity throughout the experimental period. Water in each tank was replaced every

three days, and daily syphoning was done to prevent fouling from feed residues and water replaced accordingly. The tanks were well aerated using air pumps (pressure > 0.03Mpa; output: 70L/min). Temperature and hydrogen ion concentration (pH) were measured thrice weekly using HANNA Instruments pH/EC/TDS/Temperature Meter (Model H19813-5, Hanna Instruments, Romania), while dissolved oxygen concentration was measured using D.O meter (Model JPB-607A). The water quality parameters monitored were within the range for African catfish, viz. dissolved oxygen (5.75 ± 0.05 mg/L), temperature ($27.50^\circ\text{C} \pm 0.05$) and pH (7.53 ± 0.01). For conducting this study, no ethical approval was required.

2.4 | Experimental diets

Five isoproteic and isolipidic diets (crude protein—415.9 g/Kg; crude lipid—86.1 g/Kg) were formulated as control (without POP or antimicrobial growth promoter (AGP)), AGP (basal diet + 10ml AGP/kg diet), POP 2.5 (basal diet + 2.5g POP/kg diet), POP 5.0 (basal diet + 5g POP/kg diet) and POP 10 (basal diet + 10g POP/kg diet). The ingredients listed in Table 1 were used for the diet formulation, weighed, ground, measured based on the percentage composition of each diet and mixed thoroughly. Water was added, and the resulting dough was cold-pelleted through a pelletizer with 2-mm die attached to produce a sinking pellet. The pelleted diets were dried, packed in an airtight bag, labelled and stored until use. All the diets were the same except the POP and AGP (enrofloxacin 20%) supplements. The proximate analysis of the basal diet was determined following the AOAC (2005) protocol and is presented in Table 1. Briefly, the moisture of the samples (feed and fish) was determined by drying in an oven set to $103^\circ\text{C} \pm 2$ until a constant weight was achieved. Samples were transferred to desiccator to cool, reweighed and moisture content determined. The nitrogen content of the samples was analysed using the Kjeldahl method, and the crude protein content was determined by multiplying the nitrogen value by the standard factor. For total ash determination, about 20 g samples were weighed into a clean dried crucible and placed in a muffle furnace at 550°C for 6 hr until a light grey ash resulted. After cooling in a desiccator, samples were reweighed, and the ash content was determined. The lipid content of the feed and whole body was analysed using Soxhlet extraction technique.

2.5 | Growth

The fish response to diet was examined fortnightly, changes in weight recorded, and feeding level adjusted accordingly. The fish were weighed using an electronic weighing balance (Virgo™ compact scale). At the end of the 60-day trial, fish were batch-weighed to obtain final mean weight after the evacuation of stomach content by starving for 24 hr prior to sampling. The following biological response (i.e. growth) and nutrient utilization parameters including body indexes were determined using the formulae documented below (Fawole et al., 2020).

TABLE 1 Composition and proximate analysis of the basal diet

Ingredient (g/Kg)	Basal diet
Fishmeal	100
Soya bean meal	380
Feather meal	190
Maize	170
Wheat bran	75
Vegetable oil	45
Vitamin premix	20
Dicalcium phosphate	10
Starch (binder)	10
POP	0
AGP	0
<i>Proximate analysis of basal diet (g/kg)</i>	
Dry matter	927
Crude protein	415.9
Crude lipid	86.1
Crude fibre	87.2
Ash	60.4
NFE	350.2
GE (MJ/kg)*	19.53

Note: POP, pawpaw-onion powder (2.5g/kg, 5g/kg, 10g/kg); AGP, antimicrobial growth promoter (10ml/kg diet)

Fish premix: A = 20,000,000IU, D3 = 4,000,000IU, E = 200,000IU, K = 1200mgr, B1 = 10,000mgr, B2 = 30,000mgr, B6 = 19000mgr, B12 = 1,000mgr, niacin = 200,000mgr, folic acid = 5,000mgr, panth acid = 50,000mgr, biotin = 400mgr, antioxidant = 125gr, vitamin C = 150gr, choline chloride = 400gr, manganese = 30gr, zinc = 40gr, iron = 40gr, copper = 4gr, iodine = 5gr, selenium = 0.2mgr, cobalt = 0.2mgr, calcium = 600gr, lysine = 100g, phosphorus = 4,000gr, methionine = 100gr. (Henan Lvyan Pharmaceutical Co., Ltd).

*Calculated gross energy (GE; MJ/kg) = $[23.9 \times \text{CP (g/kg)} + 39.8 \times \text{CL (g/kg)} + 17.6 \times \text{NFE (g/kg)}]/1000$.

$$\text{Weight gain (WG)} = \text{final body weight} - \text{initial body weight}$$

$$\text{Specific growth rate (SGR)} =$$

$$(\ln \text{Final body weight} - \ln \text{Initial body weight} / \text{trial duration in days}) \times 100$$

$$\text{Daily growth coefficient (DGC)} =$$

$$\left(\text{Final body weight}^{1/3} - \text{Initial body weight}^{1/3} / \text{number of days} \right) \times 100$$

$$\text{Feed conversion ratio (FCR)} = \text{Weight of dry feed (g)} / \text{Weight gain of fish (g)}$$

$$\text{Protein efficiency ratio (PER)} = \text{Gain in weight of fish (g)} / \text{Protein intake (g)}$$

$$\text{Feed intake (FI; g/fish)} = \text{total dry feed given (g)} / \text{number of fish}$$

$$\text{Hepatosomatic index (HSI)} = \text{Liver weight (g)} / \text{Bodyweight (g)} \times 100$$

$$\text{Viscerosomatic index (VSI)} = \text{Visceral weight (g)} / \text{Bodyweight (g)} \times 100$$

2.6 | Haematological analysis

Feeding was stopped 24 hr prior to sampling, and blood was collected after the fish had been anaesthetized with clove oil (100 mg clove oil/L of ethanol). Blood was obtained from 3 fish per tank ($n = 9$ fish per experimental group) via the caudal vein using 2-ml syringe and 23-gauge needles rinsed with 10% ethylene diamine tetraacetic acid (EDTA solution) and transferred to an EDTA-coated tube. The collected blood sample was used for the determination of haematological parameters such as haemoglobin, total erythrocyte count (TEC/RBC), total leucocyte counts (TLC/WBC) and packed cell volume (PCV). Briefly, the haemoglobin concentration was determined using Drabkin's cyanide-ferricyanide solution. Aliquot of 1000 μ l Drabkin's solution was pipetted into a sample tube, and 4 μ l of blood was added and mixed well. Similarly, 4 μ l of standard water and 4 μ l of distilled water (blank) were pipetted into the respective test tubes, mixed thoroughly and incubated for 5 min at room temperature, after which the absorbance of the standard and test samples was read at 540 nm against the blank. The haemoglobin concentration (g dL⁻¹) was calculated using the formula below.

$$\text{Haemoglobin concentration (g dL}^{-1}\text{)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Dilution factor}$$

The WBC counts were determined using Natt Herrick's methods (Campbell, 1996), while total erythrocyte count (RBC) was determined using the haemocytometer method (Schalm, Jain, & Carroll, 1975). The packed cell volume (PCV) was determined using the microhaematocrit method (Coles, 1986). For differential leucocyte counts, blood smear was made on a clean glass slide and air-dried, fixed in methanol for 15 min and then stained with May Grünwald stain. The smears were rinsed in Sorensen's buffer, counterstained with Giemsa stain (diluted 1:9 with Sorensen's buffer, pH 6.8), and the slides were left to dry after a final rinse in buffer. Neutrophil, lymphocyte and monocyte counts were determined following the procedure described by Rowley (1990). The haematimetric indices such as mean corpuscular volume (MCV), mean haemoglobin concentration (MCH) and mean corpuscular

haemoglobin concentration (MCHC) were calculated according to the formulae given below.

$$\text{i. MCV} = \frac{\text{Hct (\%)} \times 10}{\text{RBC (x } 10^{12}/\text{L)}}$$

$$\text{ii. MCHC} = \frac{\text{Hb (g/L)}}{\text{Hct}} \times 100$$

$$\text{iii. MCH} = \frac{\text{Hb (g/dL)} \times 10}{\text{RBC (x } 10^{12}/\text{L)}}$$

2.7 | Serum biochemical analysis

Fresh blood samples were collected from another 3 fish per tank ($n = 9$ fish per experimental group) without anticoagulant into a plain tube. The blood was kept in an ice-cold cooler for 4 hr after which the samples were centrifuged (Centrifuge 80–2 Medfield Equipment, UK) at 3,000 rpm for 10 mins at 4°C to obtain the serum which was stored in the refrigerator at –20°C for later use. The biochemical indices such as total cholesterol, total protein, glucose, aspartate aminotransaminase, alanine aminotransaminase, alkaline phosphatase, superoxide dismutase, catalase and malondialdehyde concentration were analysed using a diagnostic kit in line with the manufacturer's procedure (Randox™, United Kingdom). The analysis was carried out at the Central Research Laboratory, Tanke, Ilorin.

2.8 | Statistical analysis

All data were expressed as means $\pm$ standard error of the mean. Data were checked for normality and homogeneity of variances using the Kolmogorov-Smirnov test and Barlett's test respectively. Where normality assumptions were met, data were subjected to one-way analysis of variance (ANOVA) and the mean separated using Duncan's multiple range test to determine significant differences. The analysis was performed using the IBM SPSS (Statistical Package for Social Sciences) version 20. Significant level was set at $p < .05$.

TABLE 2 Growth, nutrient utilization and survival of *Clarias gariepinus* fed different levels of phytogenic mixture

Inclusion levels						
Parameters	Control	AGP	POP 2.5	POP 5.0	POP 10	<i>p</i> -value
Weight gain (g)	5.75 $\pm$ 1.07	4.49 $\pm$ 0.16	4.30 $\pm$ 0.43	4.48 $\pm$ 0.22	4.18 $\pm$ 0.35	0.328
SGR (% day ⁻¹)	1.46 $\pm$ 0.18	1.25 $\pm$ 0.03	1.20 $\pm$ 0.09	1.25 $\pm$ 0.04	1.19 $\pm$ 0.07	0.319
DGC (% days ⁻¹)	3.19 $\pm$ 0.59	2.49 $\pm$ 0.09	2.39 $\pm$ 0.24	2.49 $\pm$ 0.12	2.32 $\pm$ 0.20	0.328
FCR	3.19 $\pm$ 0.43	3.54 $\pm$ 0.09	3.82 $\pm$ 0.32	3.65 $\pm$ 0.18	3.81 $\pm$ 0.29	0.546
PER	0.79 $\pm$ 0.12	0.68 $\pm$ 0.02	0.64 $\pm$ 0.05	0.66 $\pm$ 0.03	0.64 $\pm$ 0.05	0.487
FI (g/fish)	17.37 $\pm$ 0.63	15.87 $\pm$ 0.19	16.16 $\pm$ 0.37	16.30 $\pm$ 0.19	15.79 $\pm$ 0.43	0.105

Note: Data expressed as mean $\pm$ SE, $n = 3$ (each replicate tank was stocked with 15 fish), and values along row with the same superscript were not significantly different ($p > .05$). POP, phytogenic mixture; SGR, specific growth rate; DGC, daily growth coefficient; FCR, feed conversion ratio; PER, protein efficiency ratio; FI, feed intake.

3 | RESULTS

3.1 | Growth and nutrient utilization

The growth performance indices of *Clarias gariepinus* fed different levels of POP and AGP are shown in Table 2. There was no significant difference in weight gain, SGR, DGC, FCR, PER and feed intake among the fish fed the control-, AGP- and POP-based diets ($p > .05$). The cumulative mortality at the end of the 60-day trial ranged from 17.7% for AGP-fed group to 42% for the control (Figure 1). The lower mortality seen in the AGP- and POP-fed fish could probably be associated with their therapeutic potential and ability to improve survival.

3.2 | Whole-body composition and body indexes

The whole-body proximate composition of *Clarias gariepinus* fingerlings fed different diets is shown in Table 3. The highest crude protein and crude lipid were recorded in fish fed the control diet and differ significantly from other groups ($p < .05$). However, the crude protein among the POP groups was found to be similar. A significantly higher HSI value was noticed in fish fed POP 5g/kg diet, whereas VSI, total ash and moisture content showed no variation ($p > .05$) among the groups.

3.3 | Haematological analysis

The results depicted in Table 4 shows the haematological changes in the blood of *Clarias gariepinus* fed diets containing pawpaw seed and onion peel mixture (POP). No significant difference ($p > .05$) was recorded in Hb, RBC and PCV among the groups. The fish fed POP 10 g/kg registered the highest MCV which is similar to POP 5g/kg and the control but differ significantly from others ($p < .05$). The WBC count was not different among the dietary groups ($p > .05$). A

significantly ($p < .05$) higher lymphocyte count was observed among the fish fed POP-based diets, whereas neutrophil showed a reverse trend with the control group registering the highest value ($p < .05$).

3.4 | Biochemical response and oxidative stress marker

The lowest cholesterol level was seen in AGP- and POP-fed groups compared with the control ($p < .05$) (Figure 2). Serum glucose level was observed to be higher in POP 2.5 while AGP recorded the lowest value ($p < .05$), whereas no differences were observed in the serum total protein among the groups ($p > .05$). A significantly lower ALT activity was noticed in POP 2.5 which is statistically similar to the POP 5.0 and AGP but differed significantly from the higher value recorded in the control ($p < .05$). The serum activities of AST and ALP enzymes were found to be lower in POP 10g/kg fed group ($p < .05$) (Table 5).

Higher superoxide dismutase enzyme activity was noticed in fish exposed to POP 2.5-based diet compared with POP 10 which recorded the lowest value ($p < .05$). The activity of catalase enzyme showed the opposite trend with the highest activity and lowest activity recorded in POP 10 and POP 2.5 respectively. No significant variation ($p > .05$) was noticed in the MDA value among the various experimental groups ($p > .05$) (Table 5).

4 | DISCUSSION

The use of phytogetic additives in aquaculture is of great interest globally as synthetic drugs are being discouraged and banned due to their unwholesome effects on food-fish consumers and the aquatic environment. Earlier studies had shown that the dietary administration of guava and mango leaf mixture significantly improved the growth performance of *Labeo rohita* by 36% compared with the control (Fawole et al., 2018). Similarly, olive flounder (*Paralichthys*

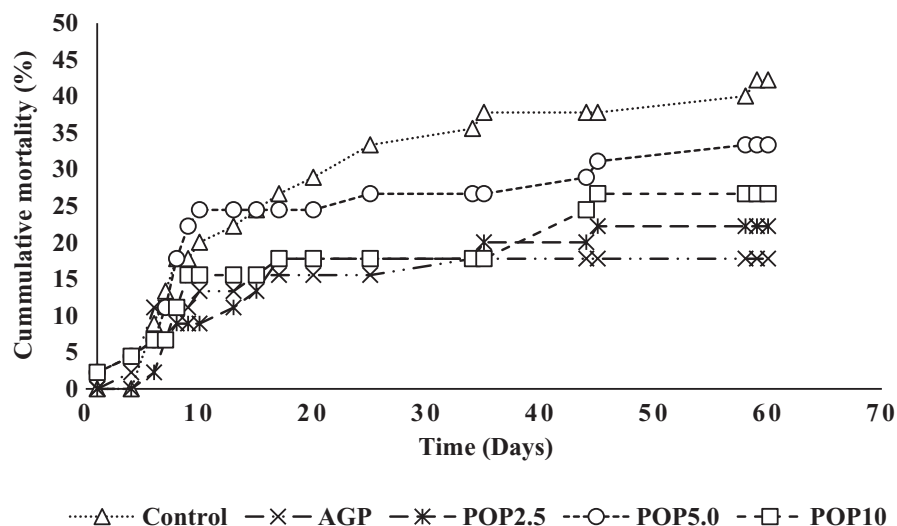


FIGURE 1 Cumulative mortality as a percentage of total fish in each treatment over the period of the trial

TABLE 3 Whole-body composition (wet weight basis) and somatic indices of *Clarias gariepinus* fingerlings fed experimental diets

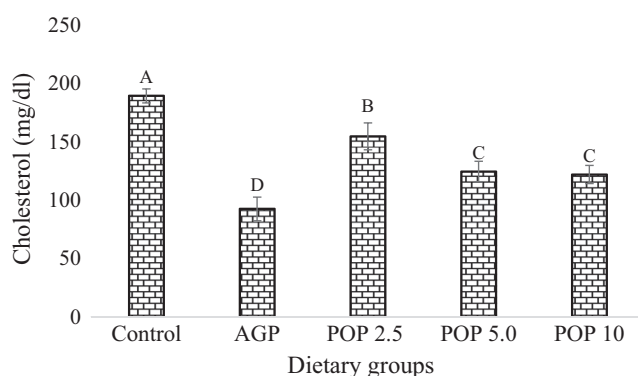
Inclusion levels						
Parameters	Control	AGP	POP 2.5	POP 5.0	POP 10	p-value
Moisture	74.09 ± 0.11	75.98 ± 0.24	75.89 ± 0.73	77.24 ± 0.45	76.00 ± 0.81	0.070
Crude protein	16.74 ^a ± 0.20	15.51 ^b ± 0.02	15.87 ^b ± 0.18	15.62 ^b ± 0.29	15.42 ^b ± 0.08	0.016
Crude lipid	6.48 ^a ± 0.35	4.63 ^b ± 0.28	4.55 ^b ± 0.51	3.01 ^c ± 0.41	4.62 ^b ± 0.01	0.009
Total ash	2.68 ± 0.43	3.96 ± 0.05	3.68 ± 0.05	4.09 ± 0.59	4.00 ± 0.71	0.308
HSI	1.62 ^b ± 0.24	1.74 ^{ab} ± 0.027	2.11 ^a ± 0.11	2.13 ^a ± 0.035	1.52 ^b ± 0.16	0.035
VSI	5.03 ± 0.21	5.76 ± 0.35	6.32 ± 0.67	5.94 ± 0.18	5.02 ± 0.43	0.176

Note: Data expressed as mean ± SE, $n = 3$ (each replicate tank was stocked with 15 fish), and values along the rows with different superscripts differ significantly ($p < .05$). POP, pawpaw-onion powder; AGP, antimicrobial growth promoter; HSI, Hepatosomatic index; VSI, Viscerosomatic index.

TABLE 4 Haematological changes in the blood of *Clarias gariepinus* fingerlings fed different diets

Variables	Control	AGP	POP 2.5	POP 5.0	POP 10	p-value
Hb	8.63 ± 0.15	6.40 ± 0.92	5.80 ± 0.35	7.30 ± 0.86	6.63 ± 0.42	0.069
RBC	4.52 ± 0.14	3.81 ± 0.53	3.22 ± 0.30	3.76 ± 0.37	3.34 ± 0.25	0.141
PCV	28.0 ± 1.53	22.67 ± 4.06	20.0 ± 1.15	25.0 ± 3.06	24.0 ± 2.00	0.334
WBC	5.53 ± 0.85	7.07 ± 1.27	7.97 ± 0.78	6.43 ± 0.91	6.20 ± 0.81	0.470
MCV	65.00 ^{ab} ± 1.00	58.67 ^b ± 2.33	62.33 ^b ± 3.93	66.33 ^{ab} ± 2.60	71.33 ^a ± 0.67	0.039
MCH	19.10 ^a ± 0.32	16.70 ^b ± 0.10	18.17 ^{ab} ± 1.13	19.33 ^a ± 0.72	19.87 ^a ± 0.27	0.037
MCHC	29.40 ± 0.25	28.60 ± 0.98	28.97 ± 0.09	29.20 ± 0.15	27.73 ± 0.64	0.286
Differential counts						
Lymphocyte (%)	59.33 ^b ± 1.76	60.00 ^b ± 2.52	68.67 ^a ± 0.67	67.33 ^a ± 1.45	72.00 ^a ± 3.79	0.011
Monocyte (%)	2.67 ± 0.67	3.00 ± 0.60	1.33 ± 0.67	3.00 ± 0.58	3.33 ± 0.88	0.331
Neutrophil (%)	38.00 ^a ± 2.31	37.00 ^{ab} ± 2.08	30.67 ^{bc} ± 0.67	28.33 ^{cd} ± 1.86	23.33 ^d ± 3.33	0.004

Note: Mean ± SE, $n = 3$, and values along rows with different superscripts are significantly different ($p < .05$) from each other. Hb = haemoglobin (g/dL); PCV = packed cell volume (%); WBC = white blood cell ($\times 10^9$ /L); RBC = red blood cell ($\times 10^{12}$ /L); MCV = mean corpuscular volume (fL); MCH = mean corpuscular haemoglobin (pg); MCHC = mean corpuscular haemoglobin concentration (g/dL); POP, pawpaw-onion powder; AGP, antimicrobial growth promoter.

**FIGURE 2** Cholesterol levels of *Clarias gariepinus* fed the experimental diets for 60 days. Data expressed as mean ± SE. Values with different superscripts are significantly different ($p < .05$) from each other. POP, pawpaw-onion powder; AGP, antimicrobial growth promoter

olivaceus) fed with a herbal mixture of *Massa medicata fermentata*, *Crataegi fructus*, *Artemisia capillaries* and *Cnidium officiale* (2:2:1:1) showed significantly higher weight gain and feed efficiency than the

control (Ji et al., 2007). However, in the current study, POP or antimicrobial growth promoter had no beneficial effect on the growth performance of *C. gariepinus*. Blends of phytochemical compound at times have an indifferent or antagonistic effect on growth response in fish, and these could be due to high concentrations of compounds with unpleasant taste/ smell in the mixture (Aanyu, Betancor, & Monroig, 2020). Ugona, Solomon, Olufegba and Okomoda (2018), and Liping (2019) observed that the inclusion of pawpaw seed meal in the diet of *Oreochromis niloticus* and *Oreochromis anderssonii* resulted in growth depression, and they opined that the reduced growth performance was caused by low feed palatability and/ or the presence of carpasemine. Thus, in the current study, the differences observed compared with the control could be that the quantity of pawpaw seed powder added to the POP mixture was too high as the fish showed a slow response to feeding (visual observation). Nevertheless, no negative impact on growth was noticed and this may be attributed to the synergistic effects of the phytochemical compounds.

The nutritive value of fish depends upon the whole-body composition. In the present study, the dietary POP supplementation

TABLE 5 Biochemical changes and oxidative stress marker of *Clarias gariepinus* fed different diets

Variables	Control	AGP	POP 2.5	POP 5.0	POP 10	p-value
Glucose (mg/dL)	171.09 ^b ± 3.97	132.20 ^c ± 7.26	240.15 ^a ± 16.90	187.80 ^b ± 14.82	201.53 ^b ± 12.72	0.001
Total protein (mg/dL)	11.13 ± 0.82	11.39 ± 0.32	9.92 ± 1.02	11.02 ± 0.23	12.96 ± 0.70	0.106
ALT (U ml ⁻¹)	170.90 ^a ± 13.76	143.59 ^{abc} ± 10.26	115.26 ^c ± 8.27	135.96 ^{bc} ± 5.67	164.36 ^{ab} ± 10.41	0.018
AST (U ml ⁻¹)	248.25 ^b ± 1.98	173.68 ^c ± 11.44	235.79 ^b ± 11.90	281.40 ^a ± 6.99	159.12 ^c ± 14.50	<0.001
ALP (U ml ⁻¹)	103.18 ^{ab} ± 5.40	112.68 ^a ± 5.92	117.76 ^a ± 8.50	107.95 ^{ab} ± 9.81	83.41 ^b ± 10.00	0.003
Superoxide dismutase*	605.42 ^b ± 15.17	586.40 ^b ± 16.64	685.57 ^a ± 16.45	605.44 ^b ± 12.43	517.87 ^c ± 28.59	0.001
Catalase*	231.36 ^c ± 10.48	523.64 ^b ± 38.34	163.44 ^c ± 19.93	470.74 ^b ± 48.93	718.76 ^a ± 26.48	<0.001
Malondialdehyde*	7.48 ± 0.28	7.19 ± 0.16	7.38 ± 0.25	7.31 ± 0.13	7.33 ± 0.02	0.869

Note: Mean ± SE, $n = 3$, and values along rows with different superscripts are significantly different ($p < .05$) from each other. *(U/mg protein). AST, aspartate aminotransferase; ALP, alanine aminotransferase; ALP, alkaline phosphate; POP, pawpaw-onion powder; AGP, antimicrobial growth promoter.

resulted in a decreased whole-body protein and lipid compared with the control. A similar decrease in whole-body protein was observed in Nile tilapia fed pawpaw seed powder (PSP) (Abass & Abass 2011; Farrag, Khalil, Mehrim, & Refaey, 2013). The lower whole-body lipid content observed in fed POP-based group could be attributed to the POP capability in inducing lipid catabolism for optimum utilization by the fish. Onion has been reported to induce endogenous lipogenesis and increase lipid catabolism in rat (Kumari & Augusti, 2007). Also, Waweru, Raburu, and Odhiambo (2019) reported that Nile tilapia fed pawpaw seed powder had a significant decreased whole-body lipid composition. Furthermore, an inverse relationship between carcass lipid and moisture content ($y = -1.087x + 87.093$; $R^2 = 0.99$) noticed in this study agrees well with the submission of Farrag et al. (2013) and Waweru et al. (2019) who stated earlier that whole-body constituent depletion often results in tissue hydration of inverse relationships between muscle lipids and moisture content.

In aquatic vertebrate, haemato-biochemical indices of blood are efficient indicators often used to examine the physiological condition and welfare of cultured fish (Abdel Rahman, Hassanin, & ElHady, 2019; Fawole et al., 2017; Hoseinifar, Mirvaghefi, & Merrifield, 2011). In the current study, the RBC, Hb and PCV values were similar among the group, and this signifies that the POP did not elicit any negative effect on the fish physiological status, and the values were within the normal range reported for *Clarias gariepinus* (Bello, Olaifa, & Emikpe, 2014). On the other hand, Ayotunde et al. (2010) reported a significant decrease in the RBC counts of *Clarias gariepinus* fingerlings fed different levels of pawpaw seed extract. Additionally, the increase in MCV and MCH recorded in fish fed POP mixture in the current study is in line with the results of Saleh et al. (2015) in sea bass fed onion powder.

Leucocytes such as lymphocytes, monocytes and granulocytes participate in the cellular immune response and are used as an indicator of fish health status (Emeish & El-deen, 2016; Fazlollahzadeh, Keramati, Nazifi, Shirian, & Seifi, 2011). In the present study, fish fed POP 10g/kg recorded the highest lymphocyte and monocyte

(numerically) among the dietary group, and this shows that the phyto-genic mixture (i.e. POP) has the potency to enhance the basic elements of the innate immunity in *C. gariepinus*. A similar proliferation of lymphocytes and enhanced leucocyte counts was reported in Nile tilapia fed graded level of Indian lotus (*Nelumbo nucifera*) leaf powder (Abdelrahman et al., 2019). Also, Gultepe, Bilen, Yilmaz, Guroy, and Aydin (2014) showed that the monocyte level was higher in *Oreochromis mossambicus* fed diets supplemented with thyme, rosemary and fenugreek. The immunostimulatory effects observed in the POP groups could be related to the bioactive constituents present in both the onion (flavonoid, quercetin, polyphenols, etc.) and pawpaw seeds (saponins, tannin, flavonoid, etc.) (Akrami, Gharaei, Mansour, & Galeshi, 2015; Vij & Prashar, 2015). The decreasing neutrophil count observed in POP-fed groups may be due to the higher level of other leucocyte cells (i.e. lymphocyte and monocyte respectively), and this shows there is an inverse relationship between lymphocyte and neutrophil ($y = -0.8794x + 93.138$; $R^2 = 0.94$). A similar increase in lymphocytes and a decrease in neutrophil count were also reported in Beluga *Huso huso* and African catfish *Clarias gariepinus* (Akrami et al., 2015; Tiamiyu, Olatoye, & Adedeji, 2019).

Most previous studies have demonstrated the hypocholesterolaemic effect of plant powder/extract in fish (Kim, Bai, Koo, Wang, & Kim, 2002; Akrami et al., 2015; Fawole et al., 2017; Vinodhini, 2010), and this was further supported by the current study with a significant decrease in total cholesterol as the POP level increases. Similarly, Abdel-Tawwab, Adeshina, Jenyo-Oni, Ajani, and Emikpe (2018) reported that serum cholesterol levels decreased significantly with increasing *Ocimum gratissimum* leaf extract in the diet of *Clarias gariepinus*. SOD and CAT are significant antioxidant defence systems whose role in the transformation of superoxide anion and decomposition of hydrogen peroxide to prevent oxidative stress has been reported (Wang et al., 2018; Zhang et al., 2019). The literature indicates that high blood glucose could induce oxidative stress by increasing the formation of ROS (Furukawa et al., 2004). From our result, it was observed that the fish fed POP 2.5g/kg had the highest glucose

concentration and highest activity of the SOD enzyme. This implies that the hyperglycaemic effect noticed in this group resulted in an increased production of ROS which was adequately counteracted by the antioxidant enzyme (SOD) to prevent oxyradical damage. However, the activity of catalase enzyme showed the opposite trend with the highest and lowest activity recorded in POP 10 and POP 2.5 respectively. According to Shamna, Sahu, Fawole, Jain, and Sarvendra (2017), SOD enzyme activity often increases with the intensity of stress, but the activity of CAT and glutathione peroxidase (GPx) could vary depending on the type of stress. Hence, the lower CAT activity noticed in POP 2.5 could be due to the action of GPx which has more affinity for H_2O_2 than CAT. Nevertheless, the highest CAT activity found in POP 10g/kg compared with the control and AGP groups points to the potency of onion-pawpaw (i.e. POP) mixture in enhancing the antioxidant response in *C. gariepinus*. This effect could be explained by the presence of antioxidant compounds such as quercetin, polyphenols and β -sitosterol present in onion and pawpaw, which might have enhanced the antioxidant enzyme function and regulated the ROS reactivity.

High serum levels of ALT, AST and ALP in fish have been related to hepatic tissue damage or inflammation and possible bile duct obstruction (Akrami et al., 2015; Fawole et al., 2017). This enzyme gives a clue on the health of the liver and to examine whether the diets fed causes toxicity and injury to the fish liver. In the current study, the activities of these enzymes were found to be lower at higher inclusion levels of POP (AST and ALP) compared with the control and AGP. This indicates that the inclusion of POP did not cause any hepatic cell alteration which is in line with the observation of Shalaby, Khattab and Abdel-Rahman (2006) who reported that the ALT and AST enzyme activities decreased significantly with increasing level of *Allium sativum* in Nile tilapia. On the other hand, Akrami et al. (2015) and Cho and Lee (2012) recorded that dietary onion powder did not show any significant effect on the activities of ALP, ALT and AST in Beluga *Huso huso* and Olive flounder, *Paralichthys olivaceus* respectively. Therefore, the significant impact noticed in the current study might be due to the combined synergistic and therapeutic effect of the pawpaw and onion mixture.

In conclusion, this study showed that the dietary addition of pawpaw-onion mixture enhanced the lymphocyte count and monocyte count, reduced the cholesterol and liver function enzymes (AST, ALT and ALP) and had a stimulatory impact on the antioxidant enzyme response of *C. gariepinus*. Based on the comparable effect with the antibiotic growth promoter (AGP), POP could be used as a natural feed additive in farmed African catfish (*C. gariepinus*). However, a follow-up study on the individual impact of each plant material and their synergistic effect at much lower inclusion level and or different ratios of POP mixing than those used in the present study on the growth performance of *C. gariepinus* is warranted.

CONFLICT OF INTEREST

There is no conflict of interest to declare.

AUTHOR CONTRIBUTION

Fawole F.J. conceptualized and designed the study, supervised and analysed the primary data, corrected the draft and approved the final version of the manuscript. Adeoye, A.A. and Tiamiyu L.O contributed to reviewing the literature and corrected the manuscript. Samuel F.C., Omosuyi O.M. and Amusa M. T. conducted the feeding trial, sampling and laboratory analysis.

DATA AVAILABILITY STATEMENT

The data that support the findings are available from the corresponding author upon reasonable request.

REFERENCES

- Aanyu, M., Betancor, M. B., & Monroig, O. (2020). The effects of combined phytonutrients on growth and nutritional physiology of Nile tilapia *Oreochromis niloticus*. *Aquaculture*, 519, 734867.
- Abbas, H. H., & Abbas, W. T. (2011). Assessment study on the use of pawpaw; *Carica papaya* seeds to control *Oreochromis niloticus* breeding. *Pakistan Journal of Biological Sciences*, 14(24), 1117–1123. <https://doi.org/10.3923/pjbs.2011.1117.1123>
- Abdel Rahman, A., Hassanin, M., & ElHady, M. (2019). Growth performance, haematology and intestinal histo-morphology of Nile tilapia fed on Indian Lotus (*Nelumbo nucifera* Gaertn.) leaf powder at different concentrations. *Aquaculture Research*, 00, 1–12.
- Abdel-Tawwab, M., Adeshina, I., Jenyo-Oni, A., Ajani, E. K., & Emikpe, B. O. (2018). Growth, physiological, antioxidants, and immune response of African catfish, *Clarias gariepinus* (B.), to dietary clove basil, *Ocimum gratissimum*, leaf extract and its susceptibility to *Listeria monocytogenes* infection. *Fish & Shellfish Immunology*, 78, 346–354.
- Akrami, R., Gharaei, A., Mansour, M. R., & Galeshi, A. (2015). Effects of dietary onion (*Allium cepa*) powder on growth, innate immune response and hemato-biochemical parameters of beluga (*Huso huso* Linnaeus, 1754) juvenile. *Fish & Shellfish Immunology*, 45(2), 828–834. <https://doi.org/10.1016/j.fsi.2015.06.005>
- Amrevuawho, M. O., Akinyemi, A. A., Oyewusi, A. J., Bankole, O. M., & Ezeri, G. O. (2016). Effects of onion (*Allium cepa*) and chloramphenicol on haematological parameters, histopathology and survival of catfish *Clarias gariepinus* (Burchell, 1822) sub-adult infected with *Pseudomonas aeruginosa*. *Vom Journal of Veterinary Science*, 11(1), 1–12.
- AOAC (Association of Official Analytical Chemists), (2005). *Official Methods of Analysis of the Association of Official Analytical Chemists International* (18th ed.). Gaithersburg, USA: AOAC International.
- Arciuli, M., Fiocco, D., Fontana, S., Arena, M. P., Frassanito, M. A., & Gallone, A. (2017). Administration of a polyphenol-enriched feed to farmed sea bass (*Dicentrarchus labrax* L.): Kidney melanomacrophages response. *Fish and Shellfish Immunology*, 68, 404–410. <https://doi.org/10.1016/j.fsi.2017.07.043>
- Ayotunde, E. O., & Ofem, B. O. (2008). Acute and chronic toxicity of pawpaw (*Carica papaya*) seed powder to adult Nile tilapia (*Oreochromis niloticus* Linne 1757). *African Journal of Biotechnology*, 7(13), 2267–2274.
- Ayotunde, E. O., Offem, B. O., Okey, I. B., Ikpi, G. U., Ochang, S. N., Agbam, N. E., & Omini, D. E. (2010). Toxicity of pawpaw (*Carica papaya*) seed powder to sharptooth catfish *Clarias gariepinus* fingerlings and effects on haematological parameters. *International Journal of Fisheries and Aquaculture*, 2(3), 71–78.
- Bello, O. S., Olaifa, F. E., & Emikpe, B. O. (2014). Haematological and blood biochemical changes in African catfish, *Clarias gariepinus* fed walnut (*Tetracarpidium conophorum* Mull Arg) leaf and onion (*Allium cepa* Linn) bulb supplemented diets. *American Journal of Experimental Agriculture*, 4(12), 1593–1603. <https://doi.org/10.9734/ajea/2014/6622>

- Cabello, F. C. (2006). Heavy use of prophylactic antibiotics in aquaculture: A growing problem for human and animal health and for the environment. *Environmental Microbiology*, 8(7), 1137–1144. <https://doi.org/10.1111/j.1462-2920.2006.01054.x>
- Campbell, T. W. (1996). Clinical pathology. In D. R. Mader (Ed.), *Reptile medicine and surgery* (pp. 248–257). Philadelphia, PA: WB Saunders Company.
- Cho, S. H., & Lee, S. M. (2012). Onion powder in the diet of the olive flounder, *Paralichthys olivaceus*: Effects on the growth, body composition, and lysozyme activity. *Journal of the World Aquaculture Society*, 43(1), 30–38. <https://doi.org/10.1111/j.1749-7345.2011.00489.x>
- Chukwuka, K. S., Iwuagwu, M., & Uka, U. N. (2013). Evaluation of Nutritional Components of *Carica papaya* L. at different stages of Ripening. *IOSR Journal of Pharmacy and Biological Sciences*, 6(4), 13–16.
- Coles, E. H. (1986). *Veterinary clinical pathology* (4th ed.). Philadelphia, PA: WB Saunders Company, p. 486.
- Durigon, E., Kunz, D., Peixoto, N., Uczay, J., & Lazzari, R. (2019). Diet selenium improves the antioxidant defense system of juveniles Nile tilapia (*Oreochromis niloticus* L.). *Brazilian Journal of Biology*, 79(3), 527–532.
- Emeish, W. F., & El-deen, A. G. (2016). Immunomodulatory effects of thyme and fenugreek in sharptooth catfish. *Clarias gariepinus*. *Assiut Veterinary Medical Journal*, 62(150), 1–7.
- FAO. (2017a). FAO Aquaculture Newsletter. No. 56 (April). Rome, p. 66. <http://www.fao.org/documents/card/en/c/cbeb55d3-cf9c-428f-9450-3c5f0f3191d3/>
- FAO. (2017b). Food and Agriculture Organization of the United Nations. 2017. FAOstat. <http://www.fao.org/faostat/en/#home>.
- FAO. (2018). *The State of World Fisheries and Aquaculture 2018*. (pp. 2–227). Rome: FAO Fisheries and Aquaculture Department. <http://www.fao.org/state-of-fisheries-aquaculture/en/>
- Farrag, F., Khalil, F., Mehri, A., & Refaey, M. (2013). Pawpaw (*Carica papaya*) seeds powder in Nile tilapia (*Oreochromis niloticus*) diets: Growth performance, survival, feed utilization, carcass composition of fry and fingerlings. *Journal of Animal and Poultry Production, Mansoura University*, 4(6), 363–379.
- Fawole, F. J., Adeoye, A. A., Tihamiyu, L. O., Ajala, K. I., Obadara, S. O., & Oluwaseun, G. I. (2020). Substituting Fishmeal with *Hermetia Illucens* in the Diets of African Catfish (*Clarias gariepinus*): Effects on Growth, Nutrient Utilization, haemato-physiological Response, and Oxidative Stress Biomarker. *Aquaculture*, 734849.
- Fawole, F. J., Sahu, N. P., Jain, K. K., Gupta, S., Rajendran, K. V., Shamna, N., & Poojary, N. (2017). Haemato-biochemical, non-specific immunity, antioxidant capacity and histopathological changes in *Labeo rohita* fingerlings fed rubber protein isolate. *Fish Physiology and Biochemistry*, 43(3), 677–690. <https://doi.org/10.1007/s10695-016-0322-3>
- Fawole, F. J., Sahu, N. P., Pal, A. K., & Ravindran, A. (2016). Haemato-immunological response of *Labeo rohita* (Hamilton) fingerlings fed leaf extracts and challenged by *Aeromonas hydrophila*. *Aquaculture Research*, 4, 3788–3799. <https://doi.org/10.1111/are.12829>
- Fawole, F. J., Sahu, N. P., Shamna, N., & Adeoye, A. A. (2018). Effect of *Psidium guajava* and *Mangifera indica* leaves extracts on growth, antioxidant and metabolic enzymes activities of *Labeo rohita* fingerlings. *Journal of the Association of Nigerian Fisheries Scientists*, 1, 40–49.
- Fazlolahzadeh, F., Keramati, K., Nazifi, S., Shirian, S., & Seifi, S. (2011). Effect of garlic (*Allium sativum*) on hematological parameters and plasma activities of ALT and AST of Rainbow trout in temperature stress. *Australian Journal of Basic and Applied Sciences*, 5(9), 84–90.
- Furukawa, S., Fujita, T., Shimabukuro, M., Iwaki, M., Yamada, Y., Nakajima, Y., ... Shimomura, I. (2004). Increased oxidative stress in obesity and its impact on metabolic syndrome. *The Journal of Clinical Investigation*, 114(12), 1752–1761.
- Gultepe, N., Bilen, S., Yilmaz, S., Guroy, D., & Aydin, S. (2014). Effects of herbs and spice on health status of Tilapia (*Oreochromis mossambicus*) challenged with *Streptococcus iniae*. *Acta Veterinaria Brno*, 83(2), 125–131. <https://doi.org/10.2754/avb201483020125>
- Hoseinifar, S. H., Mirvaghefi, A., & Merrifield, D. L. (2011). The effects of dietary inactive brewer's yeast *Saccharomyces cerevisiae* var. *ellipsoideus* on the growth, physiological responses and gut microbiota of juvenile beluga (*Huso huso*). *Aquaculture*, 318(1–2), 90–94. <https://doi.org/10.1016/j.aquaculture.2011.04.043>
- lipinge, I. N. (2019). *Effect of pawpaw (Carica papaya) seed meal on growth performance, feed utilization, survival and masculinization of sexually undifferentiated three spotted tilapia (Oreochromis andersonii) fry. A thesis submitted in fulfilment of the requirements for the degree of Masters of Science in Fisheries and Aquatic Sciences of the University of Namibia*. (pp. 59–61).
- Ji, S.-C., Jeong, G.-S., Gwang-Soon, I., Lee, S.-W., Yoo, J.-H., & Takii, K. (2007). Dietary medicinal herbs improve growth performance, fatty acid utilization, and stress recovery of Japanese flounder. *Fisheries Science*, 73(1), 70–76. <https://doi.org/10.1111/j.1444-2906.2007.01303.x>
- Kadiri, O., Olawoye, B., Fawale, O. S., & Adalumo, O. A. (2016). Nutraceutical and Antioxidant Properties of the Seeds, Leaves and Fruits of *Carica papaya*: Potential Relevance to Humans Diet, the Food Industry and the Pharmaceutical Industry-A Review. *Turkish Journal of Agriculture-Food Science and Technology*, 4(12), 1039–1052.
- Karásková, K., Suchý, P., & Straková, E. (2015). Current use of phyto-genic feed additives in animal nutrition: A review. *Czech Journal of Animal Science*, 60(12), 521–530. <https://doi.org/10.17221/8594-CJAS>
- Kim, K., Bai, S., Koo, J., Wang, X., & Kim, S. (2002). Effects of dietary *Chlorella ellipsoidea* supplementation on growth, blood characteristics, and whole-body composition in juvenile Japanese flounder *Paralichthys olivaceus*. *Journal of the World Aquaculture Society*, 33, 425–431. <https://doi.org/10.1111/j.1749-7345.2002.tb00021.x>
- Kumari, K., & Augusti, K. T. (2007). Lipid lowering effect of S-methyl cysteine sulfoxide from *Allium cepa* Linn in high cholesterol diet fed rats. *Journal of Ethnopharmacology*, 109, 367–371. <https://doi.org/10.1016/j.jep.2006.07.045>
- Landy, N., Ghalamkari, G. H., & Toghyani, M. (2011). Performance, carcass characteristics and immunity in broiler chicks fed dietary neem (*Azadirachta indica*) as alternative for an antibiotic growth promoter. *Livestock Science*, 142(1), 305–309. <https://doi.org/10.1016/j.livsci.2011.08.017>
- Lee, S. H., Lee, Y. K., Katya, K., Park, J. K., & Bai, S. C. (2017). Natural dietary additive yellow loess as potential antibiotic replacer in Japanese eel, *Anguilla japonica*: Effects on growth, immune responses, serological characteristics and disease resistance against *Edwardsiella tarda*. *Aquaculture Nutrition*, 24(3), 1–7. <https://doi.org/10.1111/anu.12641>
- Magrone, T., Fontana, S., Laforgia, F., Dragone, T., Jirillo, E., & Passantino, L. (2016). Administration of a polyphenol-enriched feed to farmed sea bass (*Dicentrarchus labrax* L.) modulates intestinal and spleen immune responses. *Oxidative Medicine and Cellular Longevity*, 68, 1–11. <https://doi.org/10.1155/2016/2827567>
- Manaf, S. R., Daud, H., Alimon, A., Mustapha, N., Hamdan, R., Muniand, K., ... Hamid, N. (2016). The Effects of *Vitex trifolia*, *Strobilanthes crispus* and *Aloe vera* Herbal-mixed Dietary Supplementation on Growth Performance and Disease Resistance in Red Hybrid Tilapia (*Oreochromis* sp.). *Journal of Aquaculture Research and Development*, 7, 425. <https://doi.org/10.4172/2155-9546.1000425>
- Mousavi, E., Mohammadiazarm, H., Mousavi, S. M., & Ghatrami, E. R. (2016). Effects of inulin, savory and onion powders in diet of juveniles carp *Cyprinus carpio* (Linnaeus 1758) on gut micro flora, immune response and blood biochemical parameters. *Turkish Journal of Fisheries and Aquatic Sciences*, 16(4), 831–838. https://doi.org/10.4194/1303-2712-v16_4_09
- Owoyele, B. V., Adebukola, O. M., Funmilayo, A. A., & Soladoye, A. O. (2008). Anti-inflammatory activities of ethanolic extract of *Carica*

- papaya leaves. *Inflammopharmacology*, 16, 168–173. <https://doi.org/10.1007/s10787-008-7008-0>
- Oyoyede, O. I. (2005). Chemical profile of unripe pulp of *Carica papaya*. *Pakistan Journal of Nutrition*, 4(6), 379–381.
- Pês, T. S., Saccol, E. M. H., Londero, É. P., Bressan, C. A., Ourique, G. M., Rizzetti, T. M., ... Pavanato, M. A. (2018). Protective effect of quercetin against oxidative stress induced by oxytetracycline in muscle of silver catfish. *Aquaculture*, 484, 120–125. <https://doi.org/10.1016/j.aquaculture.2017.10.043>
- Rowley, A. F. (1990). Collection, separation and identification of fish leucocytes. In J. S. Stolen, T. C. Fletcher, D. P. Anderson, S. L. Kaattari, & A. F. Rowley (Eds.), *Techniques in fish immunology, Fish Immunology Technical Communications*, no. 1 (pp. 113–136). Fair Haven, NJ: SOS Publications.
- Saleh, N. E., Michael, F. R., & Toutou, M. M. (2015). Evaluation of garlic and onion powder as phyto-additives in the diet of sea bass (*Dicentrarchus labrax*). *Egyptian Journal of Aquatic Research*, 41(2), 211–217. <https://doi.org/10.1016/j.ejar.2015.03.008>
- Schalm, O. W., Jain, N. C., & Carroll, E. J. (1975). *Veterinary haematology* (3rd ed.). Philadelphia, PA: Lea and Febiger, pp. 125–129.
- Shalaby, A. M., Khattab, Y. A., & Abdel -Rahman, A. M. (2006). Effects of garlic (*Allium sativum*) and chloramphenicol on growth performance, physiological parameters and survival of Nile tilapia. *Journal of Venomous Animal Toxins including Tropical Diseases*, 12(2), 172–201. <https://doi.org/10.1590/S1678-91992006000200003>
- Shamna, N., Sahu, N. P., Fawole, F. J., Jain, K. K., & Sarvendra, K. (2017). Haemato-Biochemical and Hepatic Changes in Labeo rohita Fingerlings Exposed to Multiple Stressors of Crowding and Feed Deprivation. *International Journal of Pure and Applied Biosciences*, 5(5), 617–628. <https://doi.org/10.18782/2320-7051.2694>
- Starley, I. F., Mohammed, P., Schneider, G., & Bickler, S. W. (1999). The treatment of paediatric burns using topical papaya. *Journal of the International Society of Burn Injuries*, 25(7), 636–639. [https://doi.org/10.1016/s0305-4179\(99\)00056-x](https://doi.org/10.1016/s0305-4179(99)00056-x)
- Tiamiyu, A., Olatoye, I., & Adedeji, O. (2019). Blood indices of African catfish (*Clarias gariepinus*) following dietary administration of *Talinum triangulare*. *International Journal of Research - GRANTHAALAYAH*, 7(4), 185–198. <https://doi.org/10.5281/zenodo.2653843>
- Ugonna, B. O., Solomon, S. G., Olufegba, S. O., & Okomoda, V. T. (2018). Effect of Pawpaw *Carica papaya* Seed Meal on Growth and as a Natural Sex-Reversal Agent for Nile Tilapia. *North American Journal of Aquaculture*, 80(3), 278–285. <https://doi.org/10.1002/naaq.10032>
- Vij, T., & Prashar, Y. (2015). A review on medicinal properties of *Carica papaya* Linn. *Asian Pacific Journal of Tropical Disease*, 5(1), 1–6. [https://doi.org/10.1016/S2222-1808\(14\)60617-4](https://doi.org/10.1016/S2222-1808(14)60617-4)
- Vijay, K., & Sriram, S. (2010). Antioxidant activity of seed extracts of *Annona squamosa* and *Carica papaya*. *Nutrition and Food Science*, 40(4), 403–408.
- Vinodhini, R. (2010). Detoxifying effect of *Nelumbo nucifera* and *Aegle marmelos* on hematological parameters of Common Carp (*Cyprinus carpio* L.). *Interdisciplinary Toxicology*, 3(4), 127–131. <https://doi.org/10.2478/v10102-010-0052-9>
- Wang, C. Y., Li, Z. B., Sun, Y. Z., Chen, Q., Li, W. J., Huang, Y. C., & Lu, J. (2018). Effects of chinese herbal medicines mixture on growth performance digestive enzyme activity immune response of juvenile Japanese seabass. *Lateolabrax japonicus*. *Aquaculture Nutrition*, 24(2), 683–693. <https://doi.org/10.1111/anu.12597>
- Waweru, J. N., Raburu, P. O., & Odhiambo, E. A. (2019). Gonad Histology, Proximate Composition and Growth Efficiency of Nile Tilapia Fed with Pawpaw (*Carica papaya*) Seeds Powder. *Asian Journal of Fisheries and Aquatic Research*, 1–9.
- Wong, N. K., & Khor, E. S. (2014). Potential antioxidant and cytotoxic properties of secondary metabolite extracts from *Carica papaya* fruits and seeds. *International Journal of Pharmacy and Pharmaceutical Sciences*, 6(7), 220–224.
- Yang, C., Chowdhury, M. A. K., Hou, Y., & Gong, J. (2015). Phytogetic compounds as alternatives to in-feed antibiotics: Potentials and challenges in application. *Pathogens*, 4(1), 137–156. <https://doi.org/10.3390/pathogens4010137>
- Zhang, B., Li, C., Wang, X., Zhou, H., Mai, K., & He, G. (2019). The effects of dietary *Eucommia ulmoides* Oliver on growth, feed utilization, antioxidant activity and immune responses of turbot (*Scophthalmus maximus* L.). *Aquaculture Nutrition*, 25(2), 367–376. <https://doi.org/10.1111/anu.12862>

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