HEALTH STATUS, PRODUCTION PERFORMANCE INDEX AND HISTOPATHOLOGICAL CHANGES IN *Clarias gariepinus* JUVENILES FED ONION (*Allium cepa*) BULB AND WALNUT (*Tetracarpidium conophorum*) LEAVES EXTRACTS SUPPLEMENTED DIETS

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ABSTRACT
The study investigated the health status, production performance index and histopathological changes in *Clarias gariepinus* juveniles fed onion bulb (OB) and walnut leaves (WL) supplemented diets. The experimental diet of composed control (0%), OB_2 (0.5%), OB_3 (1.0%), OB_4 (1.5%), OB_5 (2.0%), WL_6 (0.5%), WL_7 (1.0%), WL_8 (1.5%) and WL_9 (2.0%). The fish (6.41±0.01g) were replicated twice with 20 fish per replicate and were fed twice daily at 3% body weight for twelve (12) weeks. Mean weight gain, survival rate, production performance index, condition factor and histology of the fish (gill, intestine, liver, muscle, kidney and testis) were determined using a standard method. Data were analyzed using descriptive statistics and ANOVA at P=0.05. The values of weight gain, survival rate, and production performance index were significantly higher ($P < 0.05$) in the treated groups compared to the control. The well-being of the fish was better in the treated groups compared to the control and there was a significantly different ($P<0.05$) among the dietary groups. No lesion was observed in the testis, kidney, gills, and intestine of the treatments except in control while generalized fatty degeneration and multifocal mild necrosis with mononuclear infiltration were observed in the liver among all the treatments. This study suggests the potential effects of onion bulbs and walnut leaves in the diet of *C. gariepinus* and could positively enhance the growth and health status of *C. gariepinus* juveniles. Keyword: *Clarias gariepinus*; Onion bulb; Walnut leaves; Health status; Histology; Growth

INTRODUCTION
Globally, the consumption of fish and fishery products as a protein source has increased considerably over the years constituting about 20% of total protein (FAO, 2006). The cost of feed is considered to be the highest recurrent cost in aquaculture (De Silva and Anderson, 1995). The reduction in feed costs either through diet development is therefore crucial to the development and well-being of the industry. Hence, the need for underutilized feed ingredients that promote growth and health such as onion bulbs and walnut leaves. The plant *T. conophorum* belongs to the family Euphorbiaceae. It is a climber found in Southern Nigeria and West Africa in general. In Nigeria, the plant is known as "Ukpa" in Ibo, "Asala" in Yoruba, Okhue or Okwe in Edo and "Kaso" in Cameroon. The fruits are edible with four round seeds in each fruit and the plant is medicinal and used for various purposes such as antifungal, antibacterial, anti-inflammatory, antiviral, and immune response in animals (Blumenthal *et al.*, 2000).
Onion belongs to the family Amaryllidaceae and forms a large genus of about 700 species of strong–smelling, bulbous or rhizomatous biennials and perennials (Deni, 1996). The bulb is composed of shortened, compressed, underground stems surrounded by fleshy modified scale (leaves) that envelop a central bud at the tip of the stem. In traditional medicine, onion is used to treat anti-inflammatory, anti-cholesterol, antibacterial, and anti-fungal and stimulate the immune system (Bello, 2014). Several studies have been conducted on onion bulbs and walnut leaves but their toxicity effects on the organ of *C. gariepinus* have not been well documented. Hence, the present study investigated the health status, production performance index and histopathological changes in *Clarias gariepinus* juveniles fed onion bulb and walnut leaves supplemented diets

MATERIALS AND METHODS
Plant Collection, Identification and Preparation
Onion bulbs were purchased from the Oja Oba market in Ondo, Ondo State. Walnut leaves were obtained from a farm at Ikire, Osun State. They were identified at the Department of Biological Sciences (Botany unit), Olusegun Agagu University of Science and Technology, Okitipupa. The dry outer coverings of the onions were manually peeled off, and washed and 200g of the fresh onion bulbs were macerated and soaked in 100 ml of 95% ethanol for 48hrs (Bello, 2014). The pulp obtained was

left in a clean, sterile glass container, shaken vigorously to allow for proper extraction, and filtered using a sterile muslin cloth after which the extract was obtained, air-dried and stored (4^{o}C) until required. Walnut leaves obtained were air–dried in a room for 4 weeks and 200g were extracted by maceration at room temperature (25^{0}C) in 100ml aqueous methanol (20:80) for 72 hours as described by Bello, (2014). After the removal of solvents, yields of extracts were obtained and the extracts were stored in the refrigerator until required.

Source of Experimental Fish and Design

Three hundred and sixty (360) healthy and active juveniles of *C. gariepinus* (6.41±0.01g) were obtained from Lokoabata Farm, Okitipupa. The fish were acclimatized for one week in outdoor holding tanks at the Fisheries and Aquaculture Technology Laboratory, Olusegun Agagu University of Science and Technology, Okitipupa. During the period the fish were fed with commercial diets (blue crown, 2 mm) of 40% crude protein twice daily at 3% body weight. Water was sourced from the University borehole and the experimental tanks were filled with 40 litres of water *C. gariepinus* juveniles (20 fish per bowl) were stocked into 9 experimental tanks and replicated twice. The fish were allocated randomly to the tank and the dietary groups of fish were fed at 8.00 am and 5:00 pm for 12 weeks.

Preparation of Experimental Diet

The ground leaves extract of walnut and onion bulb at different inclusion levels 0%, 0.5%, 1.0%, 1.5% and 2.0% were mixed separately with feed ingredients such as fish meal, soybean, groundnut cake, yellow maize, millets, vitamin premix, vegetable oil and starch that was obtained from feed mill at Odogbolu, Ogun State to formulate 40% crude protein diet, 15% ash, 7% crude fibre, 10% ether extract, 6% moisture, and 22% nitrogen-free extract. Each diet mixture treated separately was extruded through a 2mm mincer pelleting machine to form a noodle-like strand which was mechanically broken into suitable sizes for the *C. gariepinus* juveniles. The pelleted diets were sun-dried, packed in a labelled polythene bag and stored until required.

Biological Evaluation

Fish were evaluated as follows: weight gain = final body weight - initial body weight; survival rate (%) = initial number of fish stocked – mortality/ initial number of fish stocked x 100; production performance index = survival rate x weight gain / experimental days; Condition factor (K) = $100W/L^3$
Where: W =Weight of fish (g), L = Standard length (cm)

Histopathological Analysis

The organs (muscle, liver, intestine, kidney, gill and testis) of the fish were taken for histological examination and this was carried out in the Histopathology Laboratory of the Department of Veterinary Pathology, University of Ibadan, Ibadan. The slides were prepared for histopathology according to Olusola *et al.*, (2021) method for the organs or tissues after the experiment. Each slide was observed under a microscope (Olympus CX21, Japan).

Statistical Analysis

Data were statistically analyzed using one–way analysis of variance (ANOVA) using SPSS (Statistical Package for Social Sciences 2006 version 20.0). Duncan multiple range tests were used to compare differences among individual means.

RESULTS

Performance Evaluation of* C. gariepinus *fed Onion Bulb and Walnut Leaves Based Diets

The results of the experiment revealed that better weight gain, survival rate, production performance index and condition factor were recorded in the treated groups when compared to the control. There were significant differences ($p>0.05$) among the parameters except for initial body weight and initial condition factor.

Table 1: Performance Evaluation of *C. gariepinus* fed Onion Bulb and Walnut Leaves Based Diets

	IBW	**FBW**	**WG**	**SR**	**PPI**	**ICF**	**FCF**	**DCF**
Control	6.41±0.01^{a}	52.85±0.02^{a}	46.44±0.01^{a}	80±0.01^{a}	44.23±0.02^{a}	0.59±0.00^{a}	2.10±0.03^{f}	1.51±0.01^{f}
OB 2	6.41±0.00^{a}	54.17±0.05^{e}	47.76±0.01^{e}	97±0.00^{c}	55.15±0.04^{f}	0.60±0.01^{a}	1.71±0.01^{c}	1.11±0.02^{bc}
OB 3	6.41±0.00^{a}	55.49±0.07^{f}	49.08±0.04^{f}	87±0.01^{b}	50.83±0.01^{e}	0.59±0.01^{a}	1.85±0.02^{d}	1.26±0.03^{d}
OB 4	6.41±0.01^{a}	56.72±0.08^{g}	50.31±0.06^{g}	93±0.01^{bc}	55.70±0.06^{g}	0.59±0.00^{a}	1.74±0.01^{c}	1.15±0.02^{c}
OB 5	6.41±0.00^{a}	53.24±0.03^{c}	46.83±0.03^{c}	90±0.00^{bc}	50.18±0.01^{c}	0.59±0.01^{a}	1.94±0.01^{e}	1.35±0.04^{e}
WL 6	6.41±0.01^{a}	53.70±0.01^{d}	47.29±0.02^{d}	90±0.00^{bc}	50.67±0.04^{d}	0.60±0.01^{a}	1.39±0.00^{a}	0.79±0.00^{a}
WL 7	6.41±0.00^{a}	56.82±0.04^{h}	50.41±0.05^{h}	93±0.01^{bc}	55.81±0.06^{h}	0.59±0.00^{a}	1.64±0.01^{b}	1.05±0.01^{b}
WL 8	6.41±0.01^{a}	61.22±0.09^{i}	54.81±0.08^{i}	97±0.01^{c}	63.29±0.09^{i}	0.60±0.01^{a}	2.10±0.05^{f}	1.50±0.04^{f}
WL 9	6.41±0.01^{a}	53.10±0.03^{b}	46.69±0.01^{b}	90±0.00^{bc}	50.03±0.02^{b}	0.59±0.01^{a}	1.98±0.02^{e}	1.39±0.03^{e}

IBW= Initial body weight, FBW= final body weight, WG= weight gain, SR= survival rate, PPI= production performance index, ICF= initial condition factor, FCF= final condition factor, DCF= difference between final condition factor and initial condition factor. The above values are means of duplicate data, mean values in each column with similar superscripts are not significantly different (p > 0.05)

Histopathological Changes in *C. gariepinus* Juveniles Fed Onion Bulb and Walnut Leaves Diets

The result of the experiment shows no visible lesion in the intestine and testis of *C. gariepinus* in all the treatments, generalized fatty congestion and multifocal mild necrosis with mononuclear infiltration were observed in all the treatments while focal necrosis on the skin was observed in control, OB_2 and WL_6 and degeneration with vacuolation were observed in the gill of control diet (Table 2).

DISCUSSION

Table 2: Histopathological changes in *C. gariepinus* juveniles fed onion bulb and walnut leaves diets

Organs and tissues	Histological changes	Control	OB2	OB3	OB4	OB5	WL6	WL7	WL8	WL9
Skin + muscle	Focal necrosis on the skin, more aggregate and focal SALT	½	½	-	-	-	½	-	-	-
Intestine	No visible lesion	-	-	-	-	-	-	-	-	-
Gill	Degeneration with vacuolation	½	-	-	-	-	-	-	-	-
Liver	Generalized fatty degeneration, multifocal mild necrosis with mononuclear infiltration.	½	½	½	½	½	½	½	½	½
Kidney	Highly congested	½	-	-	-	-	-	-	-	-
Testis	No visible lesion	-	-	-	-	-	-	-	-	-

Legend: ½ = present but less marked (mild) than usual, - = no lesion and morphological changes in organ and tissue

The results of this study show that weight gain, survival rate (SR), production performance index (PPI) and condition factor (CF) was better in the treated groups when compared to the control. However, walnut leaves at a 1.5% inclusion level had the best performance in terms of SR, PPI and CF when compared to other treated dietary groups. The reason for better performance in the treated groups might be a result of phytoconstituents present in these plants such as tannins, saponins and flavonoids. This study was aligned with the report of Ayebidun *et al*., (2022) who reported better condition factors and survival rates in the *C. gariepinus* fingerlings fed graded levels of giant milkweed leaves. The liver of *C. gariepinus* in this study had brown colouration which revealed marked diffuse (fatty change) vacuolation of hepatocytes among the treatments (control – WL 9) after the experiment. This observation may be due to environmental stress and the age of fish may cause changes in cellular function that alter the physiology of organ systems in the fish as reported by Vanvuren *et al.,* (1994). There was no visible lesion in the testis and intestine in all the treatments. The result of the study revealed that onion bulb and walnut leaves extract–based diets could not alter the gastrointestinal tract and testis of *C. gariepinus* which means that the inclusion of onion bulb and walnut leaves in the diet of *C. gariepinus* may not have a residual effect on the fish. This was in accord with Pyle *et al.*, (2002) that homeostatic regulation of intestinal diets by fish may not be toxic to fish.

The observed focal and more aggregate of skin-associated lymphoid tissue (SALTS), in the treatments except for OB_2 and WL_6 is suggestive of an enhanced mucosal immune response which can be explored in enhancing vaccination. The result of the experiment shows that there is no visible lesion in the kidney of the fish among the treatments which further strengthens the safety of the plants being used as feed additives. Histopathological examination of the gills fish, *C. gariepinus* from the treatment groups showed no visible lesion except OB_2 which showed slight degeneration with vacuolation. The observed changes were similar to Shaw and Handy, (2006) who reported no visible lesion in tilapia fed 2000Cu/kg diet for 42 days.

CONCLUSION

The result of this study shows positive growth, better health conditions and no alteration in the organs and tissues of the fish. This means that walnut leaves and onion bulb extracts are safe to be used as feed additives in the production of *C. gariepinus*

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