Nig. J. Anim. Prod. 2023, 50(1):1 - 12 https://doi.org/10.51791/njap.v50i1.3899. Nigerian Journal of Animal Production

Survival, Morphometric Indices and Intestinal Histomorphology of *Clarias gariepinus* Fingerlings Vaccinated, fed Turmeric (*Curcuma Longa*) Rhizome and Soursop (*Annona Muricata*) Leaves Supplemented Diets

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Abstract

This study was conducted to assess the effectiveness of the vaccine (monovalent aqueous, AqMV and adjuvant, AdMV), turmeric (Curcuma longa) rhizome (TR) and soursop (Annona muricata) leaves (SL) on survival, morphometric indices and intestinal histomorphology of Clarias gariepinus fingerlings. Experimental diets were composed of Control (0%), AqMV2, AdMV3, TR4(0.5), TR5(1.0), TR6(2.0), SL7(0.5), SL8(1.0), and SL9(2.0%). A total of 360 Clarias gariepinus with an average mean weight of 4.91± 0.01g were replicated twice in a completely randomized design with 20 fish per replicate and were fed twice daily at 40% crude protein for 8 weeks. The survival, condition factor, morphometric indices and intestinal histomorphology were measured using standard methods. Data were analyzed using descriptive statistics and ANOVA at P= 0.05. The result of the study revealed that the survival rate of the fish was better in the treated groups compared to the control and there was a significant difference (P<0.05) among the dietary groups. The result of the morphometric indices shows that there was a general increase in the values of total length, standard length, dorsal width, height and a head length of C. gariepinus fingerlings. Also, it was observed that the weight increased as the week of the feeding trial increased and the treated groups perform better than the control group. The result of intestinal histomorphology obtained at the end of the experiment showed that the treated groups perform better in the area of absorption, goblet cells, villi width, and cryptal depth when compared to the control and there were significantly differences (P<0.05) among the dietary groups. However, the treatment with turmeric rhizome perform better than the soursop groups when compared to the control. Conclusively, the incorporation of vaccine, turmeric rhizome and soursop leaves-based diet positively impacted on survival, morphometric indices and intestinal histomorphology of C. gariepinus fingerlings.

Keywords: *Clarias gariepinus*; Morphometric indices; Intestinal histomorphology; Turmeric rhizome; Soursop leaves

Survie, Indices Morphometriques et Histomorphologie Intestinale des Alevins de Clarias Gariepinus Vaccines, Nourris avec des Feuilles de Curcuma (*Curcuma Longa*), de Rhizome et de Corossol (*Annona Muricata*) Regimes Supplementes

Résumé

Cette étude a été menée pour évaluer l'efficacité du vaccin (aqueux monovalent, AqMV et adjuvant, AdMV), du rhizome de curcuma (Curcuma longa) (TR) et des feuilles de corossol (Annona muricata) (SL) sur la survie, les indices morphométriques et l'histomorphologie intestinale des Alevins de Clarias gariepinus. Les régimes expérimentaux étaient composés de contrôle (0 %), AqMV2, AdMV3, TR4 (0,5), TR5 (1,0), TR6 (2,0), SL7 (0,5), SL8 (1,0) et SL9 (2,0%). Un total de 360 Clarias gariepinus avec un poids moyen de 4,91 ± 0,01g ont été répétés

deux fois dans un plan complètement randomisé avec 20 poissons par répétition et ont été nourris deux fois par jour avec 40 % de protéines brutes pendant 8 semaines. La survie, le facteur de condition, les indices morphométriques et l'histomorphologie intestinale ont été mesurés à l'aide de méthodes standards. Les données ont été analysées à l'aide de statistiques descriptives et d'ANOVA à P = 0,05. Le résultat de l'étude a révélé que le taux de survie des poissons était meilleur dans les groupes traités que dans les groupes témoins et qu'il y avait une différence significative (P < 0,05) entre les groupes alimentaires. Le résultat des indices morphométriques montre qu'il y a eu une augmentation générale des valeurs de longueur totale, de longueur standard, de largeur dorsale, de hauteur et de longueur de la tête des alevins de C. gariepinus. En outre, il a été observé que le poids augmentait à mesure que la semaine de l'essai d'alimentation augmentait et que les groupes traités obtenaient de meilleurs résultats que le groupe témoin. Le résultat de l'histomorphologie intestinale obtenu à la fin de l'expérience a montré que les groupes traités ont de meilleurs résultats dans le domaine de l'absorption, des cellules caliciformes, de la largeur des villosités et de la profondeur cryptale par rapport au groupe témoin et qu'il y avait des différences significatives (P <0,05) entre les groupes diététiques. Cependant, le traitement avec le rhizome de curcuma donne de meilleurs résultats que les groupes de corossol par rapport au groupe témoin. En conclusion, l'incorporation d'un vaccin, d'un régime à base de rhizome de curcuma et de feuilles de corossol a eu un impact positif sur la survie, les indices morphométriques et l'histomorphologie intestinale des alevins de C. gariepinus.

Mots-clés : Clarias gariepinus; Indices morphométriques ; Histomorphologie intestinale ; Rhizome de curcuma ; Feuilles de corossol

Introduction

Fish is one of the cheapest sources of animal protein in Nigeria and constitutes about 40% of animal protein intake by the average Nigerian. The consumption of fish provides an important nutrient to a large number of people worldwide and thus makes a very significant contribution to nutrition (Azam *et al*., 2004). Unlike protein supplies from terrestrial sources which are derived mainly from livestock farming, fish supplies are heavily reliant on natural sources. For many, the most reliable source of protein compared with beef is fish, yet millions of people who depend on fish are faced daily with the fear of food shortage (World Fish Centre, 2003). With the population of Nigeria on the rise, there is a corresponding demand for fish consumption. Thus, there is a need for a suitable method of fish seed production to meet the increasing demand for food. Nigeria, like most third-world countries, is not able to meet its animal protein requirement of meat, fish and their respective products. This is traceable to our fish production which has fallen below expectations and yet fish plays an important role in world protein supplies, especially in developing countries (Dada and Fagbenro, 2008). With the global population explosion, the demand for high-quality animal protein especially from aquatic sources is rising dramatically. Increased aquaculture production is clearly to meet this demand in the third millennium because capture fishery is showing a precipitous decline due to overfishing, habitat destruction and pollution. Further increase in capture fisheries is not anticipated under the secure global conditions (Dunham *et al.,* 2001).

However, the soaring cost of feed is observed as one of the problems hampering the

development of aquaculture in Nigeria (Gabriel *et al.,* 2007). This is a result of high crude protein content (35-50%) which is the most critical and/or costly ingredient in catfish feeds where protein sources represent about 60 per cent or most of the cost of fish feeds. The primary source of protein in fish feed is fishmeal which is facing much pressure from both the livestock and aquaculture industry. Natural alternatives such as the inclusion of plant materials in fish feed formulation do not only have antimicrobial potential but also have been found to have other properties such as digestive stimulant, anti-inflammatory, antioxidant and anti-carcinogenic (Olusola *et al*., 2013), these potential are attributed to the predominant polyphenol compounds in these plant materials.

Soursop belongs to the family Annonaceae and is found to be the most important tropical fruit that contributes much to the wide economic growth of some of the tropical countries such as tropical America, Australia, Africa and Malaysia. The soursop fruits possesses a maximum of 114 volatile compounds that are found to be responsible for the whole aroma profile, 44 esters, 25 terpenes, 10 alcohols, 9 aldehydes and ketones, 7 aromatic compounds, 5 hydrocarbons, 3 acids, 3 lactones and 8 other miscellaneous compounds. Soursop is high in vitamins B1, B2 and C, antibacterial, anticancer, antioxidant, antiparasitic, antitumor, antispasmodic, stomachic, astringent, cytotoxic, febrifuge, hypotensive, insecticide, pesticide, sedative, vasodilator and vermifuge (Omage, 2015; Gajalakshmi *et al*., 2012). Turmeric is a rhizomatous herbaceous perennial plant of the ginger family, Zingiberaceae. Highly branched, yellow to orange, cylindrical, aromatic rhizomes are found. The leaves are alternate and arranged in two rows. They are divided into leaf sheath, petiole, and leaf blade. It enriches many therapeutic compounds used for the treatment of several diseases such as cancer, haemorrhoids, asthma, inflammation and leprosy (Omage, 2015). But there is little or no record of the utilization of turmeric rhizome and soursop leaves on the physiology of *C. gariepinus*. This study was evaluated to assess the efficacy of the turmeric (*Curcuma longa)* rhizome and soursop leaves (*Annona muricata)* leaves on the survival, morphometric index and gut morphology of *Clarias gariepinus* fingerlings.

Materials and Methods

Plant Collection, Identification and Preparation

Turmeric rhizome was purchased from the Okitipupa market in Ondo State, Nigeria. Soursop leaves were obtained from a farm at Igodan-Lisa, Okitipupa, Ondo State, Nigeria. They were identified at the Department of Biological Sciences (Botany Programme), Olusegun Agagu University of Science and Technology, Okitipupa. The dry outer coverings of the turmeric rhizome were manually peeled off, washed with distilled water, drained and sun dried for a week. 200 g of the dried turmeric rhizome were taken to a feed mill and blended into a fine powder while the soursop leaves obtained was air–dried for 4 weeks and 200g of the air–dried soursop leaves were ground with a hammer mill to a fine powder and kept until required.

Experimental System

The experiment was carried out in eighteen (18) experimental tanks for 8 weeks in the research laboratory of the Department of Fisheries and Aquaculture Technology. The water level in each tank was maintained at a volume of 40 litres throughout the experimental period. Water in each tank was replaced every three (3) days throughout the experiment to maintain relatively uniform physiochemical parameters and also to prevent fouling that may result from feed residues. The source of water was the Institution's water station and each experimental tank was well aerated using air stone and aerator pumps (Lawson, 1995).

Experimental Procedure, Design and Feeding Trials

The experimental design was completely randomized design with 20 fish per replicate and replicated twice with a mean initial body weight of 4.91± 0.01g. The fish was acclimated for fourteen days in the tank before the experiment and during the acclimatization period the fish were fed with commercial diets (Coppens, 2 mm) of 40% crude protein twice daily at 3% body weight. Uniform-sized fish was selected from 350 fingerlings, weighed and distributed into the experimental tanks. The experiment lasted for 8 weeks during which the fish were fed at 3% body weight daily. The diet per day was divided into two; 1.5% was given in the morning by 8.00 – 9.00 am and 1.5% in the evening by 5.00 pm. Measurement of the weight changes was performed fortnightly and the feeding rate was adjusted fortnightly according to the new body weight.

Diet Formulation

Experimental diets were composed of Control (0%), AqMV2, AdMV3, TR4 (0.5), TR5 (1.0), TR6 (2.0), SL7 (0.5), SL8 (1.0) and SL9 (2.0%) were mixed with feed ingredients such as fish meal, soybean, yellow maize, vitamin premix, dicalcium phosphate, vegetable oil and starch that was obtained from feed mill at Odogbolu, Ogun State to formulate 40% crude protein diet, 19.88% ash, 7.94% crude fibre, 6.98% ether extract, 5% moisture, and 20.2% nitrogen-free extract. Each diet mixture treated separately was extruded through a 2mm mincer of Hobart A-200T pelleting machine to form a noodle-like strand which was mechanically broken into suitable sizes for the *C. gariepinus* fingerlings. The pelleted diets were sun-dried, packed in a labelled polythene bag and stored until required.

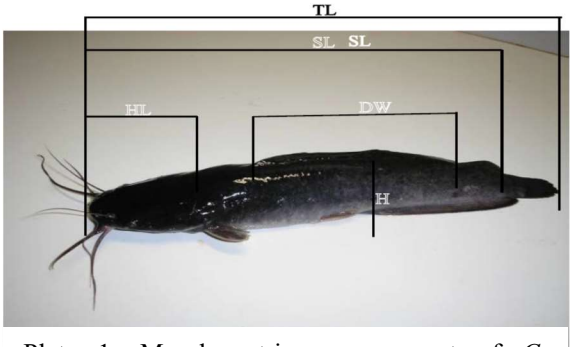

Plate 1: Morphometric measurement of *C. gariepinus*

Performance Evaluation

Fish were evaluated as described by Olusola and Olorunfemi, (2017) as follows: survival rate (%) = initial number of fish stocked – mortality/ initial number of fish stocked x 100; Condition factor (K) = $100W/L^3$ Where: W =Weight of fish (g), L = Standard length (cm)

Histomorphology Evaluation

The organ (intestine) of *C. gariepinus* was taken for histology and this was carried out in the histopathology Laboratory of the Department of Veterinary Medicine, University of Ibadan, Ibadan. The organs and tissues were prepared for histology based on the Culling (1974) and Drury *et al*., (1967). Measurements of cryptal depth, area of absorption, muscle thickness, goblet cell, villi height, villi length and width were taken using a microscope with a micrometre rule as described by Bello *et al*., (2012). Four different samples were measured in each slide per parameter, recorded and an average value was calculated.

Morphometric Analysis

Morphometric characteristics were carried out by using three fish samples from each treatment at the end of the experiment to measure the Total length (TL), Standard length (SL), Head length (HL), Dorsal Width (DW), and Height (H) as described below in plate 1

Bacterial Isolation and Vaccine Preparation

The bacterial strain (*Pseudomonas aeruginosa)* was isolated from diseased *Clarias gariepinus.* The bacterial stock was streaked onto a tryptic soy broth (TSB) agar plate supplemented with 2.5% NaCl. After incubation at 28^0C for 24 h, single colonies were picked up into liquid TSB supplemented with 2.5% NaCl, and incubation continued under rotation at 110-120 r/min for another 28 h. The aqueous monovalent vaccine

and adjuvant vaccine were prepared as described by Zheng *et al*., (2012).

Procedures of Vaccination

All fish were starved for 24 h before vaccination and were anaesthetized using 2- phenoxy-ethanol before vaccination. For monovalent aqueous vaccine (AqMV) and adjuvant vaccine (AdMV), 20 *Clarias gariepinus* fingerlings were vaccinated by water bath using 0.1 ml (1x 10^8 cells per fish) for two (2) hours. In addition, the control group represented by 20 *Clarias gariepinus* were water bath with 0.1 ml per fish of PBS (pH 7.2) for 2 hours. After an incubation period of 2 hours, the water containing the bacterial suspension and PBS (pH 7.2) was removed and 40 litres of fresh water was added to the experimental tank of 45 litres capacity.

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 Test was used to compare differences among individual means.

Results

Performance Evaluation of C. gariepinus Fingerlings fed Experimental Diets for 8 Weeks

The result of the study shows that the value of survival rate was highest in AqMV 2 and SL 8 (80%) and lowest was recorded in the control (70%) and there was a significant difference ($P<0.05$) among the dietary groups. The value of final condition factor in the study was highest in TR 6 (1.93) and the lowest in TR 5 (1.44), there was a significant difference ($P<0.05$) among the dietary groups (see table 1).

Table 1: Survival Rate and Condition Factor of *C. gariepinus* Fingerlings fed Experimental Diets for 8 Weeks

	Survival rate	ICF	FCF	DCF
Control	70.00 ± 0.01^{a}	1.37 ± 0.01^{a}	1.55 ± 0.02^{b}	0.18 ± 0.01^{b}
$AqMV_2$	80.00 ± 0.03^{b}	1.36 ± 0.00^{a}	1.79 ± 0.03^{e}	0.43 ± 0.02^{e}
$AdMV_3$	75.00 ± 0.00^{ab}	1.36 ± 0.01^{a}	1.63 ± 0.02^{d}	0.27 ± 0.01^{d}
TR 4 (0.5)	75.00 ± 0.05^{ab}	1.37 ± 0.02^{a}	1.62 ± 0.01^{cd}	0.25 ± 0.01^{cd}
TR 5 (1.0)	75.00 ± 0.03^{ab}	1.36 ± 0.01^{a}	1.44 ± 0.00^{a}	0.08 ± 0.00^{a}
TR 6 (2.0)	77.50 ± 0.01^{b}	1.37 ± 0.02^{a}	1.93 ± 0.05^{g}	0.56 ± 0.04^{f}
SL 7 (0.5)	77.50 ± 0.01^{b}	1.37 ± 0.01^{a}	1.47 ± 0.01^{a}	0.10 ± 0.01^{a}
SL 8 (1.0)	80.00 ± 0.02^{b}	1.37 ± 0.02^{a}	1.56 ± 0.02^{bc}	0.19 ± 0.02^{bc}
SL 9 (2.0)	75.00 ± 0.02^{ab}	1.37 ± 0.02^{a}	1.86 ± 0.05^{f}	0.49 ± 0.03^{e}

Means (n= 2) in the same row with similar superscripts are not significantly different ($p>0.05$), SL= Soursop leaves, TR = Turmeric rhizome, AqMV = Aqueous Monovalent Vaccine, AdMV = Adjuvant Monovalent Vaccine, ICF= initial condition factor, FCF= final condition factor, DCF= difference between final condition factor and initial condition factor.

Morphometric Characteristics of C. gariepinus Fingerlings fed with Experimental Diets for 8 Weeks

The result of the present study shows that all the dietary groups had better values of standard length, dorsal width, total length, head length, and height at the end of 8 week feeding trial when compared to the value obtained before the experiment. These values increased as week of feeding trials increased, there was a significant difference ($P<0.05$) among dietary groups (Table 2A –E)

Table 2A: Mean Standard Length (cm) of *C. gariepinus* Fingerlings fed with Experimental Diets for 8 Weeks

	2 weeks	4 weeks	6 weeks	8 weeks
Control	8.13±0.30^{a}	9.08±0.25^{ab}	9.34±0.34^{a}	11.25±0.08^{a}
$AqMV_2$	8.92±0.42^{a}	11.34±0.34^{c}	12.84±0.17^{b}	11.17±0.05^{a}
$AdMV_3$	9.17±0.17^{a}	7.92±0.09^{a}	10.34±0.34^{a}	10.58±0.09^{a}
TR 4 (0.5)	8.42±0.42^{a}	9.33±0.00^{b}	11.09±0.59^{ab}	11.99±0.59^{a}
TR 5 (1.0)	8.33±0.00^{a}	9.67±0.00^{b}	10.63±0.20^{a}	11.34±1.67^{a}
TR 6 (2.0)	9.05±0.05^{a}	9.33±1.00^{b}	10.25±0.25^{a}	11.17±0.50^{a}
SL 7 (0.5)	8.84±0.17^{a}	9.67±0.34^{b}	10.00±0.00^{a}	11.30±1.30^{a}
SL 8 (1.0)	9.05±0.05^{a}	10.00±0.33^{b}	10.00±0.00^{a}	11.17±0.05^{a}
SL 9 (2.0)	8.55±0.55^{a}	9.25±0.25^{ab}	11.34±1.67^{ab}	10.47±0.35^{a}

Means (n= 2) in the same column with similar superscripts are not significantly different (p>0.05)**,** SL= Soursop leaves, TR = Turmeric rhizome, AqMV = Aqueous Monovalent Vaccine, AdMV = Adjuvant Monovalent Vaccine,

Table 2B: Mean Dorsal Width (cm) of *C. gariepinus* Fingerlings fed with Turmeric rhizome and Soursop Leaves for 8 weeks

	2 weeks	4 weeks	6 weeks	8 weeks
Control	5.42±0.59^{a}	7.50±0.17^{a}	8.25±0.42^{a}	8.67±0.00^{ab}
$AqMV_2$	6.59±0.09^{b}	7.50±0.33^{a}	8.84±0.84^{abc}	8.83±0.50^{ab}
$AdMV_3$	6.25±0.42^{ab}	7.17±0.17^{a}	8.33±1.00^{a}	9.17±0.17^{b}
TR 4 (0.5)	5.84±0.17^{ab}	6.82±0.49^{a}	8.67±0.34^{ab}	8.67±0.34^{ab}
TR 5 (1.0)	6.17±0.17^{ab}	8.00±0.33^{a}	8.42±0.90^{a}	9.17±0.50^{b}
TR 6 (2.0)	6.25±0.42^{ab}	6.74±0.57^{a}	10.50±0.00^{bc}	10.50±0.50^{c}
SL 7 (0.5)	6.25±0.42^{ab}	7.00±0.67^{a}	10.08±0.75^{abc}	7.66±0.34^{a}
SL 8 (1.0)	5.92±0.09^{ab}	6.92±0.42^{a}	10.67±0.00^{c}	8.83±0.50^{ab}
SL 9 (2.0)	5.67±0.00^{ab}	6.83±0.50^{a}	9.17±0.50^{abc}	8.67±0.34^{ab}

Means (n= 2) in the same column with similar superscripts are not significantly different (p>0.05)**,** SL= Soursop leaves, TR = Turmeric rhizome, AqMV = Aqueous Monovalent Vaccine, AdMV = Adjuvant Monovalent Vaccine,

Table 2C: Head Length (cm) of *C. gariepinus* Fingerlings fed with Turmeric rhizome and Soursop Leaves

	2 weeks	4 weeks	6 weeks	8 weeks
Control	1.27±0.10^{a}	1.92±0.09^{a}	2.25±0.25^{a}	3.67±0.00^{a}
$AqMV_2$	1.58±0.09^{a}	3.59±0.09^{b}	4.75±0.92^{b}	2.15±0.02^{a}
$AdMV_3$	22.25±0.58^{b}	2.84±0. 17^{b}	2.97±0.30^{a}	2.50±0.33^{a}
TR 4 (0.5)	3.07±0.00^{c}	3.09±0.02^{b}	3.00±0.17^{a}	2.50±0.33^{a}
TR 5 (1.0)	3.10±0.07^{c}	3.17±0.00^{b}	3.50±0.33^{ab}	3.00±1.00^{a}
TR 6 (2.0)	3.04±0.04^{c}	3.59±0.59^{b}	3.75±0.25^{ab}	3.27±0.27^{a}
SL 7 (0.5)	3.00±0.00^{c}	3.20±0.13^{b}	3.20±0.20^{ab}	2.50±0.67^{a}
SL 8 (1.0)	2.89±0.12^{bc}	3.12±0.12^{b}	2.87±0.14^{a}	2.17±0.00^{a}
SL 9 (2.0)	3.00±0.00^{c}	3.39±0.12^{b}	2.92±0.92^{a}	2.67±0.17^{a}

Means (n= 2) in the same column with similar superscripts are not significantly different (p>0.05)**,** SL= Soursop leaves, TR = Turmeric rhizome, AqMV = Aqueous Monovalent Vaccine, AdMV = Adjuvant Monovalent Vaccine,

Table 2D: Mean Height (cm) of *C. gariepinus* fingerlings fed with Turmeric rhizome and Soursop leaves for 8 weeks

	2 weeks	4 weeks	6 weeks	8 weeks
Control	1.20±0.13^{a}	1.34±0.17^{ab}	2.00±0.00^{a}	3.67±0.00^{a}
$AqMV_2$	1.50±0.17^{a}	1.50±0.33^{ab}	3.59±0.42^{b}	2.17±0.00^{a}
$AdMV_3$	1.45±0.22^{a}	1.09±0.09^{a}	2.00±0.17^{a}	2.50±0.33^{a}
TR 4 (0.5)	1.43±0.00^{a}	1.35±0.20^{ab}	2.50±0.33^{ab}	2.50±0.33^{a}
TR 5 (1.0)	1.50±0.33^{a}	2.00±0.17^{b}	2.84±0.34^{ab}	3.00±1.00^{a}
TR 6 (2.0)	1.45±0.22^{a}	1.92±0.09^{b}	2.25±0.25^{ab}	3.26±0.27^{a}
SL 7 (0.5)	1.22±0.02^{a}	1.42±0.09^{ab}	3.50±0.50^{ab}	2.50±0.67^{a}
SL 8 (1.0)	1.22±0.02^{a}	1.45±0.15^{ab}	2.17±0.17^{ab}	2.17±0.00^{a}
SL 9 (2.0)	1.15±0.05^{a}	1.58±0.35^{ab}	3.00±1.00^{ab}	2.67±0.17^{a}

Means (n= 2) in the same column with similar superscripts are not significantly different (p>0.05)**,** SL= Soursop leaves, TR = Turmeric rhizome, AqMV = Aqueous Monovalent Vaccine, AdMV = Adjuvant Monovalent Vaccine

Table 2E: Mean Total Length (cm) of *C. gariepinus* Fingerlings fed with Turmeric and Soursop Leaves for 8 weeks

	2 weeks	4 weeks	6 weeks	8 weeks
Control	9.34±0.17^{a}	10.08±0.25^{ab}	11.50±0.50^{a}	13.58±0.25^{a}
$AqMV_2$	10.33±0.50^{ab}	12.39±0.29^{c}	14.50±0.83^{b}	12.67±0.00^{a}
$AdMV_3$	10.09±0.19^{ab}	9.80±0.20^{a}	11.67±1.00^{ab}	12.09±0.59^{a}
TR 4 (0.5)	9.92±0.42^{ab}	11.15±0.15^{abc}	12.09±0.59^{ab}	12.09±0.59^{a}
TR 5 (1.0)	10.50±0.50^{b}	12.17±0.17^{c}	12.09±0.65^{ab}	13.67±1.67^{a}
TR 6 (2.0)	10.09±0.19^{ab}	12.67±1.00^{c}	12.59±0.92^{ab}	14.17±0.84^{a}
SL 7 (0.5)	10.10±0.20^{ab}	11.00±0.33^{abc}	12.00±0.00^{ab}	12.17±1.50^{a}
SL 8 (1.0)	10.09±0.19^{ab}	11.50±0.17^{abc}	12.17±0.17^{ab}	12.67±0.00^{a}
SL 9 (2.0)	9.50±0.00^{ab}	11.67±1.00^{bc}	13.67±1.67^{ab}	11.75±0.25^{a}

Means (n= 2) in the same column with similar superscripts are not significantly different (p>0.05)**,** SL= Soursop leaves, TR = Turmeric rhizome, AqMV = Aqueous Monovalent Vaccine, AdMV = Adjuvant Monovalent Vaccine

Intestinal Histomorphology of C. gariepinus Fingerlings fed Experimental Diets for 8 Weeks

The intestinal histomorphology measurements of the villi length, villi width, cryptal depth, area of absorption, goblets cell and muscular thickness of *C. gariepinus* fingerlings vaccinated, fed graded levels of turmeric rhizome and soursop leaves were presented in Table 3

Discussion

The result of this study shows that all the treated groups had better survival rates when compared to the control. The highest survival rate was recorded in AqMV2 and SL8 (1.0% inclusion) (80%) respectively and the least in the control (70%), there was a significant difference (P<0.05) among the dietary groups. Ayebidun *et al*., (2022) reported a better survival rate in *C. gariepinus* fed different graded levels of giant milkweed when compared to the control which was in accord with this present study. The general well–being of the fish vaccinated, fed different graded levels of turmeric rhizome and soursop leaves – based diets as expressed by the condition factor (k) was best in the treated groups when compared to the control except for TR 5 (1.0 inclusion) and SL 7 (0.5% inclusion). There were significant differences (P< 0.05) among the dietary groups. All the fish were in better state of well- being at the end of the experiment than they were at the beginning (see Table 1). The presence of essential nutrients such as vitamin C as well as having antioxidant properties in the plants of the treated groups compared to the control could have resulted in better survival and well-being of the fish.

The result obtained in this study showed that there was an increase in head length, standard length, total length, dorsal width, and height in the treated groups when compared to the value obtained before the experiment. The result indicates that the diet supported the growth of fish as the increase in head length, standard length, total length, dorsal width, and height were observed as the period of exposure increased. However, treated groups with aqueous monovalent vaccine, turmeric rhizome, and soursop leaves inclusion

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Table 3: Mean Intestinal Histomorphology *C. gariepinus* Fingerlings fed with Turmeric Rhizome and Soursop Leaves for 8 weeks

	Villi height (µm)	Villi width (µm)	Area of absorption (mm^2)	Cryptal depth (µm)	Cryptal width (µm)	Muscular thickness (µm)	Goblet cells (mm^2)	Villi height: cryptal depth ratio
Control	2263.84±1.78^{b}	227.46±1.96^{ab}	514929.38±47.69^{bc}	1077.73±41.71^{b}	222.81±1.10^{bc}	858.03±71.16^{d}	4.33±2.33^{ab}	2.11±0.09^{a}
$AqMV_2$	1882.15±346.31^{a} b	222.85±1.50^{ab}	419408.29±77.10^{ab} c	773..25±166.63^{a} b	203.42±18.42^{ab} c	297.00±34.00^{a}	8.00±1.15^{ab}	2.82±0.89^{a}
$AdMV_3$	1546.97±355.24^{a} b	222.07±0.02^{ab}	343513.87±78.93^{ab}	1078.48±41.57^{b}	116.74±0.31^{a}	333.61±62.92^{a} b	11.33±2.40^{ab}	1.47±0.40^{a}
TR_4 (0.5)	1903.86±3.55.18 ab	153.46±36.59^{a}	266171.66±22.35^{a}	960.41±73.64^{b}	152.90±34.95^{ab}	811.38±35.92^{d}	14.33±2.03^{bc}	1.98±0.35^{a}
TR_5 (1.0)	1899.99±358.77^{a} b	334.14±2.24^{c}	634698.85±11.80^{c}	883.83±64.69^{ab}	261.02±34.80^{c}	812.89±132.04^{d}	7.33±3.71^{ab}	2.12±0.31^{a}
TR_6 (2.0)	2261.87±18.20^{b}	190.03±36.63^{a} b	429752.40±82.12^{ab} c	920.43±130.91^{b}	188.09±35.50^{ab} c	578.51±74.27^{b} c	23.33±6.51^{c}	2.58±0.42^{a}
SL_7 (0.5)	1521.37±344.45^{a} b	224.71±1.78^{ab}	343691.92±81.60^{ab}	889.83±168.68^{ab}	223.13±1.64^{bc}	662.00±62.30^{c} d	3.00±1.53^{a}	1.79±0.37^{a}
SL_8 (1.0)	1164.30±15.00^{a}	189.37±35.20^{a} b	221334.18±42.38^{a}	556.01±108.41^{a}	153.74±36.41^{ab}	296.04±34.61^{a}	4.33±1.20^{ab}	2.31±0.55^{a}
SL_9 (2.0)	2218.52±3.77^{b}	261.03±38.50^{b}	579265.75±85.89^{bc}	919.08±74.53^{b}	155.16±34.98^{ab}	408.15±38.44^{a} b	7.00±3.51^{ab}	2.45±0.22^{a}

Means (n= 2) in the same column with similar superscripts are not significantly different (p>0.05)**,** SL= Soursop leaves, TR = Turmeric rhizome, AqMV = Aqueous Monovalent Vaccine, AdMV = Adjuvant Monovalent Vaccine,

difference (P <0.05) among the dietary groups. This result was in agreement with Nugroho *et al.,* (2019) who reported an increase in the values of head length, standard length, total length, dorsal width, and height in the treated groups when compared to the control of the fish fed with 1% *Myrmeco diatuberosa.* Also, Olusola *et al.*, (2022) reported better standard length, dorsal width, height, total length and head length in *C. gariepinus* juveniles fed different graded levels of Cinnamon bark which was in agreement with this showed higher performance compared to the control and before the experiment and there was a significant present study

Intestinal histomorphometry is used to evaluate the absorption of nutrients in an organism. The result indicated that an increase in the villi height of the diet supplemented with turmeric rhizome and soursop leaves compared to the control may indicate an increase in the absorption surface area of the intestine and thus improved absorptive capacity, which may enhance the growth rate in the treated groups compared to the control. There was a significant difference (P<0.05) in cryptal depth and villi height ratio in the treated groups when compared to the control may signify enhanced digestion as cryptal depth is responsible for the regeneration of the villi and secretion of electrolytes which enhances water secretion into the intestinal lumen for digestion and absorption in the fish. This study aligned with the work of Zhou *et al.*, (2010) who reported that a higher cryptal depth indicates efficient digestibility and absorption in the ingested feed. However, the result revealed that a higher area of absorption recorded in the treated groups compared to the control may be due to the higher proliferation of intestinal mucosal activity in the fish which contributes to increased nutrient absorption and may enhance the metabolism of the fish. This report agrees with the work of Bello *et al*. (2012) who reported higher values in cryptal depth, villi length and villi width of the treated groups than the control of *C. gariepinus* fed onion bulb and walnut leaves-based diets. Also, this report is in accord with the report of Selim and Reda (2015) who observed a higher goblet cell number in Nile tilapia-fed diets containing probiotics when compared to the control.

The goblet cells protect the mucosal layer of the intestine by the secretion of mucus and antibacterial substances which improve digestion and decrease the number of pathogenic bacteria (Pirarat *et al*., 2015). The results of this study indicated that fish vaccinated, and fed different graded levels of turmeric rhizome and soursop leaves had a higher number of goblet cells when compared to the control and there was a significant difference (P<0.05) among the dietary groups which confirm the beneficial role of turmeric rhizome and soursop leaves supplemented diet in the *C. gariepinus* diet. This study supports the report of Magouz, *et al.*, (2020) who reported higher goblets cells in common carp, *Cyprinus carpio* fed supplementation of Aquagest® at different graded levels when compared to the control.

Conclusion

This result indicated that dietary inclusion of turmeric rhizome and soursop leaves positively affect survival rate, Morphometric indices and enhanced the intestinal histomorphology of *C. gariepinus* fingerlings.

ACKNOWLEDGEMENTS

We wish to appreciate the efforts of Mr T. A. Fatokun for his technical assistance during the study. Also, our appreciation goes to Tertiary Education Trust Fund (TETFUND) for providing fund for this study.

CONFLICT OF INTEREST

The authors declare that no conflict of interest was observed during the study.

FUNDING

This work was supported by a grant from the Tetfund 2020 merged. The funding body had no role in the design of the experiment or the interpretation of the data.

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