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THE EFFECT OF WALNUT (*TETRACARPIDIUM CONOPHORUM*) LEAF AND ONION (*ALLIUM CEPA*) BULB RESIDUES ON THE TISSUE BACTERIOLOGICAL CHANGES OF CLARIAS GARIEPINUS JUVENILES

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Running title: Bacteriological changes of Clarias gariepinus

Abstract

In this study, the effect of walnut leaf (WL) and onion bulb (OB) residues on tissue bacteriology of Clarias gariepinus juveniles by dietary intake was investigated. Nine experimental diets: control (0%), OB2 (0.5%), OB3 (1.0%), OB4 (1.5%), OB5 (2.0%), WL6 (0.5%), WL7 (1.0%), WL8 (1.5%) and WL9 (2.0%) were formulated and replicated thrice at 40% crude protein. Fish (mean weight 7.4±0.02g) were fed twice daily at 3% body weight for 12 weeks. Microbiological analyses of water and fish (skin, gill, intestine and liver) and organ index (liver, spleen, kidney and heart) were investigated. Data were analysed using descriptive statistics and ANOVA at p=0.05. Results of enterobacteriacea and total viable count from this study revealed that bacterial loads on the water and fish of the experimental tanks were more affected by *A. cepa* and *T. conophorum* than the control for 4, 8 and 12 weeks and were significantly different (P<0.05) from the control. The values decreased in treated groups as the levels of inclusion (0.5%, 1.0%, 1.5% and 2.0%) increased and as the months increased. Also, organ index showed that the liver, heart, kidney and spleen were not significantly increased in all the treated groups and the control. The results suggest that walnut leaf and onion bulb residues inclusion in the diet of Clarias gariepinus could be a potential, less expensive and promising dietary supplementation that would positively influence growth, reduce and prevent bacterial infections in fish culture.

Keywords: microbial load, walnut leaf, onion bulb, Clarias gariepinus, bacteria

L'EFFET D'UN APPORT ALIMENTAIRE DE RESIDUS DE FEUILLES DE NOYER (TETRACARPIDIUM CONOPHORUM) ET DE BULBES D'ONION (ALLIUM CEPA) SUR LA BACTERIOLOGIE DES TISSUS DE CLARIAS GARIEPINUS JUVENILES

Titre courant : Changements bactériologiques de Clarias gariepinus

Résumé

La présente étude a examiné l'effet d'un apport alimentaire de résidus de feuilles de noyer (WL : walnut leaf) et de bulbes d'oignon (OB : onion Bulb) sur la bactériologie des tissus de Clarias gariepinus juvéniles. Neuf régimes expérimentaux - témoin (0%), OB2 (0,5%), OB3 (1,0%), OB4 (1,5%), OB5 (2,0%), WL6 (0,5%), WL7 (1,0%), WL8 (1,5%) et WL9 (2,0%) - ont été préparés et répétés trois fois avec une teneur en protéines brutes de 40%. Des poissons (poids moyen 7,4 ± 0,02 g) ont été nourris à 3% du poids corporel deux fois par jour pendant 12 semaines. Des analyses microbiologiques de l'eau et des poissons (peau, branchies, intestin et foie) ont été effectuées, et l'indice d'organes (foie, rate, reins et cœur) a été étudié. Les données ont été analysées à l'aide de statistiques descriptives et de l'ANOVA à p = 0,05. Les résultats du dénombrement des enterobactériacées et le total des comptages viables de cette étude ont révélé que les charges bactériennes sur l'eau et les poissons des bassins expérimentaux ont été plus affectées par A. cepa et T. conophorum par rapport au groupe témoin pendant 4, 8 et 12 semaines et étaient significativement différentes (P <0,05) de celles du groupe témoin. Les valeurs ont diminué dans les groupes traités au fur et à mesure de l'augmentation des niveaux d'inclusion (0,5%, 1,0%, 1,5% et 2,0%) et des mois. De plus, l'indice d'organes a montré que le foie, le cœur, les reins et la rate n'avaient pas significativement

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augmenté de volume, que ce soit dans tous les groupes traités ou le groupe témoin. Ces résultats portent à croire que l'inclusion de résidus de feuilles de noyer et de bulbes d'oignons dans le régime alimentaire de Clarias gariepinus pourrait être envisagée comme une supplémentation alimentaire potentielle, moins coûteuse et prometteuse, capable d'avoir une influence positive sur la croissance, de réduire et de prévenir les infections bactériennes dans l'élevage de poissons.

Mots-clés: charge microbienne ; feuille de noyer ; bulbe d'onion ; Clarias gariepinus ; bactéries

Introduction

The main goals of aquaculture industry are to optimize growth and to produce high-quality fish. The outbreak of diseases in fish farming is a major obstacle worldwide and this brought economic loss to the industry. The high susceptibility of fish to stress and the rapid spread of diseases in water have forced fish farmers to concentrate their efforts on maintaining fish against infectious disease in order to achieve sustainable economic performances. The epithelial surfaces of fish, such as those of skin, gills or gastrointestinal tract are the first contact areas for potential pathogens (Iijima *et al.*, 2003, Narvaez *et al.*, 2010). Prophylaxis and treatment using antibiotics in aquaculture have negative impacts, one of which is the emergence of bacterial resistance. Considering the potential threat of diseases to human and animal health, issues associated with the use of antibiotics, disease management should therefore concentrate on environmental-friendly, preventative methods such as the use of immunostimulants.

Using immunostimulants can enhance activities in the non-specific defense mechanism (Anderson, 1992), increase resistance to infectious diseases by enhancing innate humoral and cellular defense mechanisms and indirectly to cause growth improvement in fish (Galindo-Villegas and Hosokawa, 2004). Presently, attention is given to immunostimulants and many different immunostimulants have been found to be effective in various fish species (Gatta *et al.*, 2001, Li *et al.*, 2004, Rairakhwada *et al.*, 2007, Cerezuela *et al.*, 2009). Organic fish culturing has become popular over the last decade and therefore natural immunostimulants have received even more attention. Some researchers observed positive results in the improvement of immune system in fish fed with natural immunostimulants (Dugenci *et al.*, 2003, Divyagnaneswari *et al.*, 2007, Yin *et al.*, 2009)

Walnut leaf and onion bulb as plant immunostimulants could be considered as immunostimulants in cultured fish as they possess high antimicrobial and antibacterial effects (Ajaiyeoba and Fadare 2006, Azu and Onyeagba, 2007). This study was carried out to evaluate the possible effect of walnut leaf and onion bulb residues as potential antimicrobials in the farming of Clarias gariepinus

Materials and Methods

Plant Collection and Identification

Onion bulbs were purchased from Bodija market in Ibadan, Nigeria. Walnut leaf was obtained from a farm at Oka -Akoko, Nigeria. They were authenticated at the herbarium of the Forestry Research Institute of Nigeria (FRIN), Ibadan, where a voucher specimen was deposited under FHI 107515.

Preparation and Extraction of Plant Materials

Onion extraction

The onion bulbs were washed with distilled water and allowed to air dry at ambient temperature (25°C) for one hour. The dry outer coverings of the onions were manually peeled off, washed and extracted as described by Azu and Onyeagba, (2007). 200g of the fresh onion bulbs were blended into fine powder and soaked in 100ml of 95% ethanol for 24hrs. The pulp obtained was left in a clean, sterile glass container, shaken vigorously to allow for proper extraction, filtered using a sterile muslin cloth after which the residue was obtained, air-dried and stored at 4°C until required.

Walnut leaf extraction

The extraction was carried out as described by Ajaiyeoba and Fadare (2006). The air – dried walnut leaves were ground with a hammer mill to fine powder. 200g of the

powder was soaked in 100ml of 80% methanol for 72 hours, properly mixed with methanol, filtered using a sterile muslin cloth after which the extract was obtained. The residue was air – dried and stored at 25°C until required.

Media Preparation

All media used were prepared according to manufacturer's instruction as follows:

- MacConkey agar: This agar was prepared by suspending 52g in 1 litre of distilled water. It was brought to boil to dissolve completely then sterilized by autoclaving at 121°C for 15 minutes.
- Nutrient agar: This agar was prepared by suspending 28g in 1 litre of distilled water and then sterilized by autoclaving at 121°C for 15 minutes.
- Mueller Hinton agar: This agar was prepared by suspending 36g in 1 litre of distilled water and then sterilized by autoclaving at 121°C for 15 minutes.
- Nutrient broth: This broth was prepared by suspending 25g in 1 litre of distilled water and then sterilized by autoclaving at 121°C for 15 minutes.
- Peptone water: This was prepared by suspending 15g in 1 litre of distilled water and then sterilized by autoclaving at 121°C for 15 minutes.

All these media were allowed to cool after sterilization to about 45°C before pouring into Petri dishes.

Preparation of Experimntal Diets

The mean proximate composition of the experimental diet was 40.0%crude protein, 15.9% ether extract, 15.7% ash, 7.4% moisture, and 20.9% NFE. Nine experimental diets were prepared by incorporating walnut leaf and onion bulb residues at the following inclusion levels; 0 (control), 0.5%, 1.0%, 1.5% and 2.0% respectively. Feed ingredients such as fishmeal, soybean, maize, starch, vegetable oil, Di calcium phosphate (DCP), salt and vitamin- mineral premix were added and the dry ingredients mixed thoroughly in a mixer. Water was added and the resulting dough pelletted. The pellets were sun –dried, and stored in airtight containers at room temperature to prevent mould formation until required.

Microbiological analysis

Water samples from the aquaria were collected monthly in sterile glass bottles. Peptone water 0.1% was used for serial dilution. 1ml of water sample was added to 9ml sterile peptone water to 10-1 and then diluted to 10-4. Each diluent (1ml) was poured in two Petri dishes; one received plate count agar for total bacterial count using the pure plate count method according to the standard methods for the examination of water and wastewater (APHA, 1985), the second Petri dish received MacConky agar for total coliforms count according to Hitchins *et al.*, (1995). Petri dishes were gently tapped on the sides for a few times. Petri dish for total coliform count and that of the dishes of total bacterial count were incubated at 37°C for 24h.

Fish samples (skin, liver, gill and intestine) were collected monthly during the experimental period for bacteriological examination with through asepsis. In accordance with the American Public Health Association, 1g of fish sample was grained in 9ml sterile peptone water in the mortar. 1ml of the suspension was diluted by peptone water to 10-4. Each diluent (1ml) was poured in two Petri dishes; one received plate count agar and the other received MacConky agar. The incubation period was 24h at 37°C. After incubation of water and fish sample dishes the colonies were counted using colony counter. Total viable count and enterobacteriacea were determined, the result were expressed in $\log_{10}$CFU/ml and $\log_{10}$ CFU/g for water and fish, respectively.

Organ Index

Three fish from each experimental treatment were killed by rapid cervical chopping and weighed. The liver, kidney, intestine and spleen were removed and weighed and the average was calculated. Moreover, the hepatosomatic and splenosomatic indices were calculated according to Fox *et al.*, (1997)

Organ-somatic index = [organ weight (g)/body weight (g)] ×100.

Table 1: Enterobacteriacea and total viable counts (log 10CFU/ml) of water samples treated with onion bulb and walnut leaf

Treatment	4 Weeks		8 Weeks		12 Weeks	
	Enterobacte-riacea	Total viable counts	Enterobacte-riacea	Total viable counts	Enterobacte-riacea	Total viable counts
Control	5.29±0.01^{f}	5.48±0.01^{c}	5.28±0.05f	5.40± 0.02^{f}	5.28± 0.00^{e}	5.41±0.01^{g}
OB2	5.02±0.02^{e}	5.11±0.03^{a}	4.88±0.00^{d}	5.28± 0.01^{e}	5.00± 0.04^{d}	5.19±0.03f
OB3	4.70±0.05^{c}	5.08±0.00^{a}	4.88±0.07^{d}	5.16± 0.00^{d}	5.00± 0.02^{d}	5.12± 0.02^{e}
OB4	4.60± 0.02^{b}	5.50± 0.00^{c}	4.78± 0.04^{c}	4.93± 0.03^{b}	4.70± 0.00^{c}	4.90± 0.06^{c}
OB5	4.40± 0.03^{a}	5.56± 0.02^{c}	4.18± 0.02^{a}	4.74± 0.02^{a}	4.54±0.04^{b}	4.70± 0.02^{ab}
WL6	5.02± 0.01^{e}	5.22± 0.01^{b}	5.04± 0.01^{e}	5.06± 0.04^{c}	5.00± 0.07^{d}	5.04± 0.01^{d}
WL7	4.78± 0.01^{d}	5.11± 0.03^{a}	5.04± 0.05^{e}	5.04± 0.10^{c}	4.98± .0.08^{d}	5.02± 0.00^{d}
WL8	4.60± 0.02^{b}	5.26± 0.01^{b}	4.65± 0.03^{b}	4.95± 0.03b	4.54± 0.02^{b}	4.78± 0.00^{b}
WL9	4.54± 0.05^{b}	5.56± 0.00^{c}	4.18± 0.02^{a}	4.78± 0.00^{a}	4.40± 0.04^{a}	4.65±0.02^{a}

Key: Mean followed by the same letter is not significantly different ($p > 0.05$)

Table 2a: Enterobacteriacea and total viable counts (log 10CFU/g) of Clarias gariepinus treated with onion bulb

Treatment	Fish sites	4 Weeks		8 Weeks		12 Weeks	
		Enterobac-teriacea	Total viable counts	Enterobacte-riacea	Total viable counts	Enterobacte-riacea	Total viable counts
Control	Skin	4.04± 0.02^{g}	4.11± 0.10^{g}	4.02± 0.00f	4.10± 0.02^{h}	3.71± 0.00^{g}	3.74± 0.02^{g}
	Liver	3.88± 0.04^{g}	3.89±0.00^{g}	3.85± 0.02^{g}	3.87± 0.01^{h}	3.65± 0.02^{g}	3.70± 0.04^{f}
	Gill	3.97± .002^{g}	4.09± 0.04^{g}	3.93± 0.01f	4.06± 0.01^{g}	3.46± 0.01^{h}	3.49± 0.02^{g}
	Intestine	3.85± 0.00h	3.85± 0.05a	3.79± 0.00f	3.82± 0.03^{g}	3.52±0.02^{f}	3.58± 0.06^{f}
OB2							
	Skin	3.76± 0.00f	3.91± 0.07^{f}	3.72± 0.00e	3.89± 0.01^{g}	3.58± 0.09^{f}	3.70± 0.02^{f}
	Liver	3.80±0.02^{f}	3.80± 0.06^{f}	3.74± 0.05^{f}	3.77± 0.00^{g}	3.62±0.10^{e}	3.66± 0.05^{ef}
	Gill	3.72± 0.02^{f}	3.92± 0.02^{f}	3.68± 0.02e	3.91± 0.05^{f}	3.24± 0.01^{h}	3.46± 0.03^{f}
	Intestine	3.81± 0.01^{g}	3.77± 0.00^{a}	3.77± 0.04f	3.74± 0.00^{f}	3.42±0.04^{e}	3.56± 0.01^{ef}
OB3							
	Skin	3.65±0.01^{e}	3.76±0.00^{e}	3.59± 0.01^{d}	3.73± 0.00^{f}	3.49± 0.03^{e}	3.62± 0.00^{e}
	Liver	3.60±0.02^{e}	3.67±0.08^{d}	3.54± 0.01^{e}	3.71± 0.00^{f}	3.43± 0.02^{d}	3.62± 0.01^{e}
	Gill	3.62±0.00^{e}	3.64±0.08^{d}	3.58±0.00^{d}	3.62± 0.01^{d}	3.11± 0.04^{g}	3.44± 0.01^{e}
	Intestine	3.65± 9.09^{f}	3.69±0.01^{a}	3.61± 0.02^{e}	3.68± 0.03^{e}	3.20± 0.09^{d}	3.40± 0.05^{d}
OB4							
	Skin	3.53± 0.00^{d}	3.63±0.01^{d}	3.48± 0.05^{c}	3.59±0.04^{e}	3.41± 0.09^{d}	3.56± 0.04^{d}
	Liver	3.51±0.05^{d}	3.62±0.01^{c}	3.46±0.02^{d}	3.57±0.00^{d}	3.32± 0.07^{d}	3.52± 0.03^{d}
	Gill	3.52±0.06^{d}	3.61±0.02^{d}	3.42±0.02^{c}	3.59± 0.01^{d}	3.09± 0.01^{e}	3.28± 0.00^{c}
	Intestine	3.54±0.01^{e}	3.69±0.01^{a}	3.50±0.01^{d}	3.64± 0.02^{e}	3.13± 0.00^{c}	3.39± 0.05^{d}
OB5							
	Skin	3.48± 0.07^{c}	3.49± 0.02^{b}	3.39±0.04^{b}	3.45± 0.03^{b}	3.18±0.06^{b}	3.46± 0.08^{b}
	Liver	3.46± 0.09^{c}	3.53± 0.02^{b}	3.34±0.03^{c}	3.53± 0.13^{c}	3.28±0.05^{b}	3.41± 0.00^{c}
	Gill	3.40± 0.02^{c}	3.53±0.03^{c}	3.33±0.01^{b}	3.51± 0.02^{c}	2.81± 0.00^{d}	3.24± 0.02^{b}
	Intestine	3.48±0.06^{d}	3.54±0.00^{a}	3.42± 0.05^{c}	3.52± 0.01^{d}	3.06± 0.10^{b}	3.31± 0.01^{c}

Key: Mean followed by the same letter is not significantly different ($p > 0.05$)

Table 2b: Enterobacteriacea and total viable counts (log 10CFU/g) of Clarias gariepinus treated with walnut leaf

Treatment	Fish sites	4 Weeks		8 Weeks		12 Weeks	
		Enterobacte-riacea	Total viable counts	Enterobac-teriacea	Total viable counts	Enterobac-teriacea	Total viable counts
Control	Skin	4.04± 0.02^{g}	4.11± 0.10^{g}	4.02± 0.00f	4.10± 0.02^{h}	3.71± 0.00^{g}	3.74± 0.02^{g}
	Liver	3.88± 0.04^{g}	3.89±0.00^{g}	3.85± 0.02^{g}	3.87± 0.01^{h}	3.65± 0.02^{g}	3.70± 0.04^{f}
	Gill	3.97± .002^{g}	4.09± 0.04^{g}	3.93± 0.01f	4.06± 0.01^{g}	3.46± 0.01^{h}	3.49± 0.02^{g}
	Intestine	3.85± 0.00^{h}	3.85± 0.05a	3.79± 0.00f	3.82± 0.03^{g}	3.52±0.02^{f}	3.58± 0.06^{f}
WL6							
	Skin	3.54±0.01^{d}	3.61± 0.00^{d}	3.48±0.02^{c}	3.59±0.07^{e}	3.51±0.04^{e}	3.63±0.08^{e}
	Liver	3.48±0.00^{cd}	3.70± 0.05^{e}	3.43±0.06^{d}	3.66±0.08^{e}	3.39±0.01f	3.57±0.05^{g}
	Gill	3.60± 0.01^{e}	3.69± 0.07^{e}	3.57±0.04^{d}	3.66±0.00^{e}	3.31±0.03f	3.56±0.03^{d}
	Intestine	3.48± 0.02^{d}	3.53± 0.03a	3.45±0.01^{cd}	3.48±0.02^{c}	3.39±0.09^{e}	3.50±0.01^{e}
WL7							
	Skin	3.46± 0.05^{bc}	3.53± 0.08c	3.41±0.01^{b}	3.50±0.02c	3.32±0.02^{c}	3.51±0.02^{c}
	Liver	3.36± 0.03^{b}	3.53±0.00^{b}	3.29±0.02^{b}	3.48±0.01^{b}	3.11±0.07^{c}	3.38±0.05^{d}
	Gill	3.44± 0.00^{c}	3.57± 0.01^{c}	3.41±0.09^{c}	3.53±0.07^{c}	3.06±0.00^{c}	3.31±0.01^{b}
	Intestine	3.37± 0.04^{c}	3.50± 0.02a	3.31±0.00^{b}	3.46±0.02^{bc}	3.22±0.02^{d}	3.28±0.09^{bc}
WL8							
	Skin	3.43± 0.03^{b}	3.43± 0.01^{a}	3.41±0.03^{b}	3.38±0.06^{a}	3.11±0.01^{b}	3.32±0.02^{a}
	Liver	3.35± 0.00^{b}	3.51± 0.00^{a}	3.36±0.01^{c}	3.46±0.05^{b}	2.93±0.02^{a}	3.31±0.08^{b}
	Gill	3.30± 0.01^{b}	3.46± 0.04^{b}	3.33±0.01^{b}	3.42±0.04^{b}	2.70±0.05^{b}	3.11±0.03^{a}
	Intestine	3.43± 0.01^{b}	3.40± 0.02a	3.24±0.02^{b}	3.37±0.01^{a}	3.11±0.02^{c}	3.26±0.06^{ab}
WL9							
	Skin	3.35± 0.07^{a}	3.42± 0.01^{a}	3.33±0.02^{a}	3.53±0.09^{d}	2.90±0.01^{a}	3.29±0.06^{a}
	Liver	3.20± 0.08^{a}	3.35± 0.09^{a}	3.16±0.05^{a}	3.33±0.04^{a}	2.88±0.01^{a}	3.28±0.04^{a}
	Gill	3.04± 0.01^{a}	3.15± 0.00^{a}	2.93±0.07^{a}	3.06±0.03^{a}	2.70±0.03^{a}	3.06±0.02^{a}
	Intestine	3.18± 0.03^{a}	3.37±0.03^{a}	3.11±0.02^{a}	3.45±0.13^{b}	2.98±0.04^{a}	3.23±0.00^{a}

Key: Mean followed by the same letter is not significantly different ($p > 0.05$)

Table 3: Organ index of Clarias gariepinus treated with onion bulb and walnut leaf residues

Treatments	Liver	Spleen	Kidney	Heart
Control	0.007±0.00^{ab}	0.002±0.01^{a}	0.004±0.00^{ab}	0.002±0.01^{a}
OB2	0.008±0.01^{ab}	0.002±0.01^{a}	0.003±0.02^{a}	0.002±0.00^{a}
OB3	0.011±0.00^{b}	0.002±0.00^{a}	0.002±0.02^{a}	0.002±0.00^{a}
OB4	0.004±0.01^{a}	0.001±0.02^{a}	0.002±0.00^{a}	0.002±0.02^{a}
OB5	0.009±0.02^{b}	0.003±0.00^{a}	0.003±0.01^{a}	0.003±0.00^{a}
WL6	0.009±0.01^{b}	0.002±0.01^{a}	0.007±0.00^{b}	0.002±0.01^{a}
WL7	0.008±0.00^{ab}	0.002±0.00^{a}	0.005±0.00^{ab}	0.002±0.01^{a}
WL8	0.007±0.01^{ab}	0.002±0.01^{a}	0.004±0.01^{ab}	0.002±0.00^{a}
WL9	0.007±0.00^{ab}	0.002±0.01^{a}	0.005±0.01^{ab}	0.002±0.01^{a}

Key: Mean followed by the same letter is not significantly different ($p > 0.05$)

Statistical Analysis

Bacteriological characteristics and organ indices resulting from the experiment were subjected to one-way analysis of variance (ANOVA) using SPSS (Statistical Package for Social Sciences 2006 version 15.0). Duncan's multiple range test was used to compare differences among individual means.

Results

Microbiological Analyses of Water and *Clarias Gariepinus*

The results of Enterobacteriacea and total viable counts of water samples and Clarias gariepinus (skin, liver, intestine and gills) fed diets containing onion bulb and walnut leaf residues are presented on Tables 1 and 2.

Organs Index of *Clarias Gariepinus*

The results of organ index were presented in Table 3.

Discussion

Results of these findings show that enterobacteriacea in water was higher in the control than the treated groups fed diets containing onion bulb and walnut leaf residues. The values decreased in all the treated groups with increasing inclusions of the residues in the diets. The control diet recorded highest enterobacteriacea for 4 weeks, 8 weeks and 12 weeks. The lowest enterobacteriacea was recorded in OB5 in onion bulb residue treatments and WL9 in walnut leaf residue treatments for 4 weeks, 8 weeks and 12 weeks.

Total viable count (TVC) from the water of Clarias gariepinus fed onion bulb and walnut leaves showed that the TVC was higher in the control diets at 4 weeks, 8 weeks and 12 weeks and the lowest TVC was recorded in OB5 in onion bulb residue treatments and WL9 in walnut leaf residue treatments for water at 4 weeks, 8 weeks and 12 weeks. The values of TVC obtained from the present findings decreased in all the treated groups with increasing inclusion levels of the residues.

Results of enterobacteriacea from this study revealed that bacteria load on the water of the experimental tanks was more affected by A. cepa and T. conophorum than the control. Also, the enterobacteriacea and total viable count in water sample for 4, 8 and 12 weeks were significantly different ($P<0.05$) from the control. The findings of this study agree with the work of Shalaby *et al.*, (2006) who obtained decreases in bacterial load of water fed O .niloticus on garlic and chloramphenicol at different graded levels. The report of Sugita *et al.*, (1989) was in agreement with the present work. However, these results contradict those of Al- Harbi and Uddin (2003) who reported that counts of total viable bacteria were in the range of 3.74 – 3.38 $\log_{10}$ CFU/ml in pond water without any treatment; these value were lower than enterobacteriacea in the control of this present work. Also, Nedoluha and Westhoff, (1997) reported 6.80 $\log_{10}$ CFU/ml for fish growing water in tanks with a stocking density of 3g fish/ l; these values were higher than the one obtained in this present study.

However, the results of total viable count (TVC) from water with C. gariepinus fed diets with onion bulb and walnut leaf residues were lower than the water from the control. The TVC in this present study was lower than that reported by McKeon *et al.*, (2001) in pre-filtered water of recirculating systems (106 CFU/100ml), but in filtered water it was 4.20 log10 CFU/100ml which also agreed with result obtained in 8 weeks and 12 weeks of the study.

The antimicrobial effect of walnut leaf and onion bulb residues in diets led to reductions in the microbial load of water of experimental tanks and inhibited the growth of microorganisms or pathogenic bacteria that result in infection of fish. Enterobacteriacea in skin, liver, gills and intestine of C. gariepinus on control diet were higher than the treated groups. The lowest enterobacteriacea was recorded in OB5 in onion bulb residue treatments for skin, gill, liver and intestine at 4 weeks, 8 weeks and 12 weeks and WL9 in walnut leaf residue treatments for skin, gill, liver and intestine at 4 weeks, 8 weeks and 12 weeks. The values decreased in treated groups as the level of inclusion (0.5%, 1.0%, 1.5% and 2.0%) increased and as the months increased.

Moreover, the result of TVC in skin, liver, gills and intestine of C gariepinus of the control was higher than the onion bulb

and walnut leaf residues and TVC in skin, gill, liver and intestine was highest in the control diets at 4 weeks, 8 weeks and 12 weeks. The lowest TVC was recorded in OB5 in onion bulb residue treatments in skin, gill, liver and intestine at 4 weeks, 8 weeks and 12 weeks while the lowest TVC was recorded in WL9 in walnut leaf residue treatments in skin, gill, liver and intestine at 4 weeks, 8 weeks and 12 weeks.

The values decreased in treated groups as the levels of inclusion (0.5%, 1.0%, 1.5% and 2.0%) increased and as the months increased. The findings of enterobacteriacea from this study revealed that bacterial load in skin, liver, gills and intestine of C. gariepinus fed A. cepa and T. conophorum were lower than the control. Enterobacteriacea and total viable count of (skin, liver, gill and intestine) for 4, 8 and 12 weeks were significantly lower ($P<0.05$) than the control. The reason for this might due to the presence of antimicrobial properties present in walnut leaf and onion bulb. Treatment with T. conophorum was more effective in reducing bacteria in skin, liver, gills and intestine. The findings of this study is in agreement with the work of Shalaby et al., (2006) where there were low value in muscles and intestine of O. niloticus fed Allium sativum and chloramphenicol on different graded levels. Also, Shalaby *et al.*, (2006) revealed that coliform count from the intestine of fish fed garlic diet was 4.78 – 5.69 log10 CFU/g and in fish fed on chloramphenicol diet was 3.48 – 5.45 log10 CFU/g this report was in agreement with the present findings.

Organ indices showed that the liver, heart, kidney and spleen i.e. the hepatosomatic and splenosomatic indices were not significantly increased in all the treated groups. This finding agrees with the report of Azza and Abd-El-Rhman, (2009). Fox *et al.*, (1997) reported that the organosomatic indices are indicators of health (hepatosomatic index and splenosomatic index) which could be used to predict the health status of fish. The findings showed no traces of oedema and high variation of the intestinal organs, the inclusion of walnut leaf and onion bulb in the diet of C. gariepinus could therefore, be considered safe and non-toxic for consumption.

In conclusion, since antimicrobial effects of walnut leaf and onion bulb residue resulted in reduction in microbial loads of water and fish the inclusion of these plants as a replacement or additive in fish feed could aid productivity in aquaculture industry. Their use in aquaculture industry is safe since they are highly biodegradable and do not have any side effects (Blumenthal *et al.*, 2000) such as drug resistance that have been generally reported in synthetic antibiotics.

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References

Ajaiyeoba E O and Fadare D A. 2006. Antimicrobial potential of extracts and fractions of the African walnut – Tetracarpidium conophorum African Journal of Biotechnology Vol. 5 (22), pp. 2322-2325

AL-Harbi A H, Uddin N. 2003. Quantitative and qualitative studies on bacterial flora of hybrid tilapia (Oreochromis niloticus X O. aureus) cultured in earthen ponds in Saudi Arabia. Aquaculture Resources, 34, 43-8.

American Public Health Association (APHA) 1985. Standard Methods for the Examination of Water and Wastewater. 16th Edition. Washington: American Public Health Association, American Water Works Association, Water Pollution Control Federation, 1268p.

Anderson D P. 1992. Immunostimulants, adjuvants, and vaccine carriers in fish: *Application to aquaculture. Annu Rev Fish Dis* 2:281-307.

Azu N C and Onyeagba R A. 2007. Antimicrobial properties of extracts of Allium cepa (Onions) and Zingiber officinale (Ginger) on Escherichia coli, Salmonella typhi, and Bacillus subtilis. *The Internet Journal of Tropical Medicine*. ISSN 1540 - 2681 Volume 3 Number 2

Azza M and Abd-El-Rhman M. 2009. Antagonism of Aeromonas hydrophila by propolis and its effect on the performance of Nile tilapia, *Oreochromis niloticus Fish & Shellfish Immunology* Volume 27, Issue 3, Pages 454-459

Blumenthal M, Goldberg A and Brinckmann, J. 2000. Herbal Medicine: Expanded Commission E Monographs. Copyright American Botanical Council. Published by Integrative Medicine Communications, 1029 Chestnut Street, Newton, MA 02464. Pp. 401-403.

Cerezuela R, Cuesta A, Meseguer J and Esteban M A. 2009. Effects of dietary vitamin D3 administration on innate immune parameters of seabream (Sparus aurata L.).

Cyprinus carpio and protection against Aeromonas hydrophila. *Fish & Shellfish Immunology* 26:140-5.

Divyagnaneswari M, Christybapita D, Michael R.D.2007. Enhancement of non-specific Immunity and Disease resistance in Oreochromis mossambicus by Solanum trilobatum leaf fractions. *Fish & Shellfish Immunology* 23:249-59.

Dugenci S K, Arda A and Candan A. 2003. Some medicinal plants as immunostimulants for fish. *Journal of Ethnopharmacology* 88 (1): 99-106.

Fish & Shellfish Immunology 26:243-8

Fox H E, White S. A, Koa, M. F and R.D. Fernald, R.D. 1997. Stress and Dominance in a Social fish, *The Journal of Neuroscience* 16 (17), pp. 6463–6469.

Galindo-Villegas J and Hosokawa H. 2004. Immunostimulants: towards temporary prevention of diseases in marine fish. In: Cruz Suarez LE, Ricque Marie D, Nieto

Gatta PP, Thompson KD, Smullen R, Piva A, Testi S and Adams A. 2001. Dietary Organic chromium supplementation and its effect on the immune response of rainbow trout (Oncorhynchus mykiss), *Fish & Shellfish Immunology* 11:371- 82.

Hitchins A D, Feng P, Watkins W D, Rippey S R and Chandler L A. 1995. Escherichia coli and the coliform bacteria. In: WATKINS WD. Ed. Food and drug administration bacteriological analytical manual. 8. ed. Arlington: *Association of Official Analytical Chemists*, 401-29.

Iijima N, Tanimoto N, Emoto Y, Morita Y, Uematzu K and Murakami T, et al., 2003. Purification and characterization of three isoforms of chrysophsin, a novel antimicrobial peptide in the gills of the red sea bream, Chrysophrys major. European Journal of Biochemistry 270:675-86.

Li P, Lewis DH and Gatlin DM.2004. Dietary oligonucleotides from yeast RNA influence immune responses and resistance of hybrid striped bass (Morone chrysops X Morone saxatilis) to Streptococcus iniae infection. Fish & Shellfish Immunology 16:561-9.

Lopez MG, Villarreal D, Scholz U, Gonzalez M, editors. Memorias del 7 Simposium Internacional de Nutricion Acuicola. 16-19. November, 2004. Hermosillo, Sonora, Mexico. Avances en Nutricion Acuicola, 7: 279-319.

McKeon C, Glenn E, Gerba C P and Fitzsimmons K. 2001. Microbiological hazards of tilapia culture systems. In: *International Scientific Conference*, 2, Mansoura: Mansoura University, p.479-85.

Narvaez E, Berendsen J, Guzmán F, Gallardo JA, Mercado L. 2010. An Immunological method for quantifying antibacterial activity in Salmo salar (Linnaeus, 1758) skin mucus. *Fish & Shellfish Immunology* 28:235-9.

Nedoluha P C and Westhoff D. 1997. Microbiological analysis of striped bass (Morone saxatilis) grown in a recirculating system. *J. Food Prot.*, 60, 948-53.

Rairakhwada D, Pal A K, Bhathena Z P, Sahu N P, Jha, A and Mukherjee S C. 2007. Dietary microbial levan enhances cellular non-specific immunity and survival of Common carp (Cyprinus carpio) juveniles. *Fish & Shellfish Immunology*, 16:1-10.

Shalaby A M, Khattab Y A and 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 of Animal Toxins include Tropical Diseases, volume* 12, 2 Pp 172 – 201.

Sugita H, Iwata J, Miyajima C K T, Noguchi T, Hashimoto K and Deguchi, Y. 1989 Changes in micro flora of a puffer fish, Fugu niphobles, with different water temperatures. *Mar. Biol.*, 101, 299-304.

Yin G, Ardo L, Thompson KD, Adams A, Jeney Z and Jeney G. 2009. Chinese herbs (Astragalus radix and Ganoderma lucidum) enhance immune response of carp,