

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

Bello^{1**} O. S, Olaifa¹, F. E and Emikpe² B. O

1. Department of Aquaculture and Fisheries Management, University of Ibadan, Nigeria

2. Department of Veterinary Pathology, University of Ibadan, Nigeria

ABSTRACT

Juvenile *Clarias gariepinus* were fed diets containing Onion Bulb (OB) and Walnut Leaf (WL) residues at different graded levels: 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%). Packed Cell Volume (PCV), Haemoglobin (Hb) content, Red Blood Cells (RBC) and White Blood Cells (WBC) counts were measured in test fish after 84 days of feeding. Biochemical indices such as total protein, Albumin as well as blood serum, aspartate amino-transferase and alanine amino-transferase were investigated before and after the experiment. Data were analyzed using descriptive statistics and ANOVA at $P = 0.05$. The results obtained showed that packed cell volume and haemoglobin content were significantly different ($p < 0.05$) among the treatments while red blood cell, white blood cell and mean cell volume and mean cell haemoglobin were not significantly different ($p > 0.05$) among the dietary groups. There were increases in total protein and albumin but values of aspartate amino-transferase and alanine amino-transferase decreased though not significantly ($p > 0.05$) among the treatments. However, fish fed the walnut leaves and onion bulbs residue- based diets recorded higher values in PCV, Hb, WBC and total protein compared to the values obtained before experiment and the control. The results of this study suggested that the dietary supplementation of walnut leaves and onion bulbs residues could be a potential, less expensive and positively affected haematological factor and boost immune response of cultured *Clarias gariepinus* juveniles.

Keywords: Walnut leaf, Onion bulb, Haematology, *Clarias gariepinus*

* Tel.: +xx xx 265xxxxx; fax: +xx aa 462xxxxx.

E-mail address: xyz@abc.com.

19 ** Corresponding author: +2348034110139 E-mail: belloolus@yahoo.com
20

21
22

23 1. INTRODUCTION

24 Aquaculture production has increased tremendously in Nigeria and *Clarias*
25 *gariepinus* is an economically important cultured fish species. Nutrition is a major factor that
26 determines the physiological well being of an animal. Use of natural feed additives is
27 becoming more important for fish feeding rather than chemical feed additives due to the
28 cumulative effects of the chemical components on animal and human health. Therefore,
29 using immunostimulants seems to be an attractive alternative to control fish diseases and
30 enhance growth (Raa, 1996; Secombes, 1994). These substances play a promising role in
31 aquaculture by enhancing the resistance of cultured fish against diseases (Raa, 1996).

32 Recent studies have shown successful use of medicinal plants and natural herbs in
33 fish nutrition including marjoram, licorice roots, black seeds, peppermint, caraway seed,
34 fennel seed and fenugreek seeds (Abdel-Monem *et al.*, 2002; Sakr, 2003; Shalaby *et al.*,
35 2003; El-Dakar *et al.*, 2004a&b; El-Dakar, 2004; Shalaby, 2004; El-Dakar *et al.*, 2007 and El-
36 Dakar *et al.*, 2008). Also, a number of studies have shown that differences in blood cell
37 formation and function can also be indicative of dietary manipulations.

38 Use of natural herbs, medicinal and aromatic plants in fish feeding is still limited both
39 on the experimental and commercial scales and there is scarce information on walnut leaf
40 and onion bulb as plants immunostimulants for treating fish diseases or boost immune
41 response in fish. This study is aimed at investigating the effects of different levels of
42 inclusion of walnut leaf and onion bulb residues in the diets and haematology of *Clarias*
43 *gariepinus* juveniles.

44

45

46 2. MATERIALS AND METHODS

47 2.1 Plant Collection, Identification, Preparation and Extraction:

48 Onion (*A. cepa*) bulbs were purchased from a market in Ibadan while walnut (*T.*
49 *conophorum*) leaves were harvested from a farm at Oka -Akoko, Ondo State in Nigeria.
50 They were authenticated at the herbarium of the Forestry Research Institute of Nigeria
51 (FRIN), Ibadan, where a voucher specimen was deposited under FHI 107515.

52 **Onion extraction:**

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

60 **Walnut leaf extraction:**

61 The extraction of walnut leaves was as described by Ajaiyeoba and Fadare (2006).
62 The air – dried walnut leaves were ground with a hammer mill to fine powder and 200 g of
63 the powder mixed thoroughly with methanol and filtered using a sterile muslin cloth. The
64 residue obtained after extraction was air – dried and stored in a refrigerator at (25°C) until
65 required.

66 **2.2 EXPERIMENTAL SYSTEM**

67 The experiment was carried out in twenty seven plastic experimental tanks
68 (50x34x27cm) for 12 weeks in the Fisheries Laboratory, University of Ibadan, Nigeria. The
69 water level in each tank was maintained at volume of 35 litres throughout the study period.
70 Water in each tank was replaced every three (3) days throughout the period to maintain
71 relatively uniform physiochemical parameters and prevent fouling resulting from feed
72 remnants. The source of water was from University of Ibadan water station and each tank
73 was well aerated using aerators (Lawson, 1995). The mean water temperature, pH and

74 dissolved oxygen were recorded as $26.79 \pm 1.25^{\circ}\text{C}$, 7.83 ± 0.26 and 6.97 ± 0.09 mg/l
75 respectively

76 **2.3 EXPERIMENTAL PROCEDURE & FEEDING TRIALS**

77 There were nine experimental diets during this study. Each treatment had three
78 replicates with 20 fish per replicate and mean initial body weight of 7.39 ± 0.02 g. The fish
79 were acclimatized for fourteen days before the experiment and fed at 3% body weight daily.
80 Daily feed was divided into two rations of 50% of total feed weight and was given in the
81 morning (between 8.00am and 9.00am) and in the evening (at 5.00p.m.)

82 **2.4 PREPARATION OF EXPERIMENTAL DIETS**

83 The mean proximate composition of the experimental diet was $40.00 \pm 0.01\%$ crude
84 protein, $15.90 \pm 0.04\%$ ether extract, $15.70 \pm 0.02\%$ ash, $7.40 \pm 0.08\%$ moisture, and
85 $20.90 \pm 0.01\%$ Nitrogen Free Extract. Nine experimental diets were prepared by incorporating
86 walnut leaf and onion bulb residues at the following inclusion levels; 0 (control), 0.5%, 1.0%,
87 1.5% and 2.0% respectively. Feed ingredients such as fishmeal, soybean, maize, starch,
88 vegetable oil, Di-calcium phosphate (DCP), salt and vitamin- mineral premix were added.
89 The dry ingredients were mixed thoroughly in a mixer, water was added and the resulting
90 dough pelletized. The pellets were sun -dried, and stored in airtight containers at room
91 temperature until required.

92 **2.5 HAEMATOLOGICAL AND BIOCHEMICAL ANALYSES**

93 Haematological analysis of the fish was carried out before and after the experiment
94 at the Haematological Laboratory of Veterinary Pathology Department, University of Ibadan
95 within 30 minutes of sampling. 3 – 4cm from the genital opening of each fish was punctured
96 and wiped with dry tissue paper to avoid contamination with mucus. The needle was inserted
97 at right angle to the vertebral column of the fish. The blood was taken under gentle
98 aspiration until about 3ml had been obtained. The needle was gently withdrawn and the
99 blood transferred into heparinized plastic containers and complete haematology was done
100 as described by the methods of Blaxhall and Daisley (1973) using modified hyme's dilution

101 fluid. The collected blood was introduced into Neubauer Counting Chamber (Neubauer
102 improved bright line Marienfield, Germany 0.100mm, 0.0025mm²) and the cells were
103 counted under the microscope at 100 x objective.

104 .Blood samples for biochemical analysis were centrifuged for 5 minutes at 3000rpm
105 with Hawsley minor bench centrifuge (P spectra, Centromix no 231254 CD7000549, Spain).

106 The derived was stored at – 20 °C and analyzed at the Haematology Laboratory of Veterinary
107 Pathology Department, University of Ibadan for total protein, albumin, globulin, albumin –
108 globulin ratio and blood serum enzymes such as Aspartate amino transferase (AST) and
109 Alanine amino transferase (ALT) as described by the methods of Blaxhall and Daisley
110 (1973).

111

112 **2.6 STATISTICAL ANALYSIS**

113 Haematology, biochemical indices and blood serum resulting from the experiment
114 were subjected to one-way analysis of variance (ANOVA) using SPSS (Statistical Package
115 for Social Sciences 2006 version 15.0). Duncan multiple range test was used to compare
116 differences among individual means.

117
118
119
120
121
122
123
124
125
126
127
128
129
130
131
132
133
134
135
136
137

138 3. RESULTS

139 3.1 HAEMATOLOGY OF *Clarias gariepinus* BEFORE AND AFTER FEEDING TRIALS

140 There was increased in values of some haematological parameters after the feeding experiment when compared with the values
 141 obtained before the experiments. Packed cell volume and haemoglobin content were significantly different ($p<0.05$) among the treatments
 142 while red blood cell, white blood cell and mean cell volume and mean cell haemoglobin were not significantly different ($p>0.05$) among the
 143 dietary groups (Table 1)

144 **Table 1: Mean Values of Some Haematological Parameters of African Catfish *Clarias gariepinus* Juveniles before and after the**
 145 **feeding experiment**

Parameters	Before experiment	After feeding experiment								
		CONTROL	OB2	OB3	OB4	OB5	WL6	WL7	WL8	WL9
PCV (%)	12.50±2.50 ^a	26.00±4.00 ^{bc}	24.00±1.41 ^{bc}	28.00±2.83 ^{bc}	22.00±0.89 ^{bc}	20.00±1.70 ^b	29.50±2.12 ^{bc}	25.00±4.24 ^{bc}	28.00±1.10 ^{bc}	34.50±0.71 ^c
Hb (gm %)	4.10±0.45 ^a	8.30±1.98 ^{bc}	7.25±0.64 ^{bc}	8.80±1.41 ^{bc}	6.70±2.97 ^{bc}	5.65±0.07 ^b	9.35±0.92 ^{bc}	7.70±1.41 ^{bc}	9.10±3.25 ^{bc}	10.65±0.07 ^c
RBC x10 ^{12/L}	1.07±0.05 ^a	2.47±1.12 ^a	2.02±0.50 ^a	2.90±0.59 ^a	2.38±1.75 ^a	1.75±0.05 ^a	2.90±0.52 ^a	2.20±0.53 ^a	2.55±1.26 ^a	3.39±0.03 ^a
WBC x10 ^{9/L}	15,250±5.50 ^a	11,650±2.10 ^a	16,025±3.64 ^a	17,325±8.24 ^a	14,875±3.14 ^a	13,400±4.10 ^a	17,050±2.68 ^a	16,050±9.68 ^a	13,625±1.87 ^a	13,500±3.82 ^a
Platelet	133,000±1.10 ^{ab}	99,500±4.94 ^a	129,000±3.50 ^{ab}	203,000±4.10 ^b	135,500±1.06 ^{ab}	112,000±4.24 ^a	123,000±1.55 ^a	128,500±2.89 ^{ab}	160,000±4.81 ^{ab}	107,000±9.89 ^a
ESR (mm/hr)	0.20±0.14 ^{ab}	0.3±0.14 ^b	0.15±0.07 ^{ab}	0.10±0.00 ^a	0.25±0.07 ^{ab}	0.15±0.07 ^{ab}	0.20±0.00 ^{ab}	0.20±0.14 ^{ab}	0.10±0.00 ^a	0.10±0.00 ^a
MCV (fL)	118.17±0.38 ^a	111.49±0.62 ^a	122.02±0.33 ^a	97.60±0.70 ^a	95.24±0.75 ^a	114.66±0.23 ^a	102.88±0.60 ^a	113.84±0.13 ^a	112.57±0.40 ^a	101.76±0.68 ^a
MCH (Pg)	3.91±0.86 ^a	3.55±0.80 ^a	3.68±0.60 ^a	3.05±0.14 ^a	2.76±0.050 ^a	3.24±0.05 ^a	3.26±0.26 ^a	3.50±0.20 ^a	3.71±0.56 ^a	3.15±0.07 ^a
MCHC (%)	34.00±0.04 ^b	32.00±0.00 ^b	31.00±0.01 ^{ab}	32.00±0.02 ^b	31.00±0.01 ^{ab}	29.00±0.01 ^a	32.00±0.04 ^b	31.00±0.01 ^{ab}	33.00±0.01 ^b	31.00±0.00 ^{ab}
Lym (%)	69.00±1.00 ^a	66.00±0.07 ^a	68.00±0.00 ^a	66.00±0.07 ^a	67.50±0.71 ^a	67.00±0.41 ^a	67.00±0.65 ^a	68.00±5.66 ^a	70.00±0.07 ^a	68.50±0.49 ^a
Hetero (%)	25.00±2.00 ^a	29.50±2.12 ^a	25.00±1.40 ^a	30.00±8.49 ^a	30.00±1.41 ^a	32.00±2.83 ^a	31.00±2.83 ^a	36.00±7.07 ^a	33.50±7.78 ^a	31.00±8.49 ^a
Mono	3.00±0.00 ^a	1.50±0.71 ^a	2.50±0.07 ^a	2.00±1.41 ^a	1.50±0.71 ^a	1.50±0.71 ^a	2.50±0.07 ^a	1.50±0.11 ^a	2.00±1.41 ^a	1.50±0.71 ^a
Eos	3.00±1.00 ^a	2.50±2.12 ^a	4.50±0.71 ^a	2.00±0.00 ^a	1.00±0.00 ^a	1.50±2.12 ^a	1.50±2.12 ^a	1.50±0.71 ^a	2.50±0.71 ^a	2.50±2.12 ^a

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

147 **NOTE:** PCV = packed cell volume, Hb =Haemoglobin, RBC = Red Blood Cell, WBC = White Blood Cell, ESR = Erythrocytes sedimentation
148 rate, MCV =Mean Cell Volume, MCH = Mean Cell Haemoglobin, MCHC = Mean Cell Haemoglobin Concentration, Lym =Lymphocytes,
149 Hetero =Heterophil, Eos = Eosunophil, Mono = Monocytes

151 3.2 PLASMA BIOCHEMISTRY OF *Clarias gariepinus* BEFORE AND AFTER FEEDING TRIALS

152 The result of plasma biochemistry (total protein, albumin, globulin and albumin and globulin ratio) shows variation in the values.
153 Albumin values was not significant (p>0.05) difference among the treatments but, total protein, globulin and albumin and globulin ratio were
154 significantly different (p<0.05) among the treatments (Table 2)

155 **Table 2: Mean Plasma Biochemistry Parameters of African Catfish *Clarias gariepinus* Juveniles before and after the feeding**
156 **experiment**

157

158

Parameters	Before experiment	After feeding experiment								
		CONTROL	OB2	OB3	OB4	OB5	WL6	WL7	WL8	WL9
Total protein	3.10±0.14 ^a	2.85±0.49 ^a	4.60±0.05 ^{ab}	3.95±1.06 ^{ab}	2.60±0.85 ^a	4.00±0.57 ^{ab}	2.90±0.14 ^a	5.70±0.99 ^b	4.15±0.92 ^{ab}	3.60±1.99 ^{ab}
Albumin	0.95±0.35 ^a	2.35±0.21 ^a	2.25±0.07 ^a	2.30±0.00 ^a	1.90± 1.27 ^a	2.60±0.57 ^a	2.50±0.28 ^a	2.75±0.07 ^a	2.45±0.64 ^a	1.95±1.06 ^a
Globulin	2.15±0.49 ^{bc}	0.50±0.28 ^a	2.35±0.07 ^{bc}	1.65±1.06 ^{abc}	0.70±0.42 ^a	1.40±0.00 ^{ab}	0.40±0.14 ^a	2.95±1.06 ^c	1.50±0.00 ^{abc}	1.65±0.92 ^{abc}
A.G Ratio	0.50±0.28 ^{ab}	0.20±0.14 ^a	1.00±0.00 ^b	0.65±0.49 ^{ab}	0.55±0.64 ^{ab}	0.55±0.07 ^{ab}	0.15±0.07 ^a	1.00±0.42 ^b	0.65±0.21 ^{ab}	0.80±0.00 ^{ab}

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

160

161

162

163

164 **3.3 BLOOD SERUM ENZYMES OF *Clarias gariepinus* BEFORE AND AFTER FEEDING TRIALS**

165 The result of the experiment shows that highest value of AST was recorded in OB2 and lowest in WL 8 while highest ALT was
 166 recorded in OB3 and lowest in WL 8, these values were lower than the one obtained before the experiment.

167 **Table 3: Blood Serum of African Catfish *Clarias gariepinus* Juveniles before and after the feeding experiment**

168

Parameters	Before experiment	After feeding experiment								
		CONTROL	OB2	OB3	OB4	OB5	WL6	WL7	WL8	WL9
AST(IU/l)	151.00±1.31 ^a	132.00±4.24 ^a	136.00±2.83 ^a	141.50±9.19 ^a	135.00±9.90 ^a	131.00±9.89 ^a	131.00±4.14 ^a	139.00±9.90 ^a	129.50±6.26 ^a	137.50±10.61 ^a
ALT(IU/l)	68.00±8.49 ^b	24.00±4.14 ^a	22.00±7.07 ^a	26.00±5.66 ^a	19.00±2.83 ^a	20.00±2.83 ^a	20.00±7.07 ^a	21.50±2.12 ^a	18.00±2.83 ^a	22.50±3.84 ^a

169

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

171 Note: AST =Aspertate amino transferase ALT =Alanine amino transferase

172

173

174

175

176

177

178

179

180

181

182

183

184 4. DISCUSSION

185 As immunological studies continue to include dietary manipulation, haematology
186 becomes a necessary research tool for further interpretation of dietary effects. Examination
187 of the fish before and after the experiment revealed that the fish were in good condition free
188 from disease and infections. However, there was decrease in some haematological and
189 biochemical parameters after the experiment when compared with the values recorded
190 before the experiment. This could be attributed to stress encountered during sampling,
191 capture and handling procedure, which must have increased the catecholamine secretion.
192 The mean PCV(%) values of *Clarias gariepinus* juveniles fed different graded level of onion
193 bulb (OB) and walnut leaf (WL) of 0.5%, 1.0%, 1.5% and 2.0% respectively showed general
194 increase in values when compared with the values obtained before the experiment

195 This study revealed that use of onion bulb and walnut leaf extract residues in fish
196 feed induced increases in the blood parameters like (erythrocyte count, haemoglobin content
197 and hematocrit values. From the result obtained in PCV, Hb and RBC during the study
198 showed that the onion bulb and walnut leaf residues inclusion does not result into anaemia
199 and this could support its use in aquaculture as safe plant immunostimulants. Reduction in
200 packed cell volume and red blood cell values are indicative of low protein intake or mild
201 anaemia (Nuhu, 2010). Reduction in the concentration of PCV in the blood usually suggests
202 the presence of a toxic factor (e.g. haemagglutinin) which has adverse effect on blood
203 formation (Oyawoye and Ogunkunle, 1998)). Also, Akinmutimi, 2004 reported that both the
204 biochemical and haematological blood components are influenced by the quantity and
205 quality of feed and also the level of anti-nutritional elements or factors present in the feed).

206 This suggests that walnut leaf and onion bulb residues could enhance non- specific
207 immune responses. The result also, shows that white blood cell recorded in the treated
208 groups were higher in values compared to the control which reflected that fish fed walnut
209 leaf and onion bulb diet were able to build immunity against pathogens. The findings of this
210 study were similar to the results of Shalaby *et al.*, (2006) who reported a significant ($p<0.05$)

211 increase in erythrocyte count, heamoglobin content and hamatocrit value in *O. niloticus* fed
212 different graded levels of garlic (*Allium sativum*) and chloramphenicol. Also, the results of
213 Martins *et al.*, (2002) showed that addition of *Allium sativum* to fish diets increased
214 erythrocyte number, heamoglobin content, hematocrit value, leucocytes and thrombocytes.
215 Also, Azza *et al.*, (2009) reported that the erythrocyte-count was significantly higher in
216 *Oreochromis niloticus* fed with probiotics (*Micrococcus luteus* and *Pseudomonas species*)
217 than the control. Aly *et al.*, (2008) evaluated the uses of probiotics (*Bacillus subtilis* and
218 *Lactobacillus acidophilus*) in the diets of *Oreochromis niloticus* and reported that the
219 hamatocrit values were significantly higher in the group fed on diet supplemented with
220 probiotic, when compared with the control group. Das *et al.*, (2009) evaluated the uses of
221 *Euglena viridis* in the diets of *Labeo rohita* and reported that a significant ($p<0.05$) increase
222 in WBC and RBC was observed in group fed treated diet of different graded level of *Euglena*
223 *viridis* compared to the control. Onion bulb (*Allium cepa*) and walnut leaves (*Tetracarpidium*
224 *conophorum*) have some constituents (alkaloids, flavonoids, tannin and thiosulfinates) that
225 may play a role in the immune system stimulation and in the function of organs related to
226 blood cell formation such as thymus, spleen and bone marrow.

227 Blood indices (MCV, MCH and MCHC) are particularly important for the diagnosis of
228 anaemia in most animals (Coles, 1986). There were decreases of MCV, MCH and MCHC in
229 fish fed onion bulb and walnut leaves residue –based diets which is in agreement with the
230 work of Shalaby *et al.*, (2006) who reported decrease of MCV, MCH and MCHC in
231 *Oreochromis niloticus* fed graded levels of *Allium sativum*. The differential leucocytic-count is
232 an indicator of health in fish (Fox *et al.*, 1997). The current study showed insignificant
233 changes in the counts of the large lymphocytes: heterophils and monocytes among the
234 experimental groups. This is in agreement with the results of Talpur and Ikhwanuddin
235 (2013) who reported insignificant change in the count of the large lymphocytes, heterophils
236 and monocytes among the experimental groups. Cuesta *et al.*, (2005) reported that water

237 and ethanolic-extracts of propolis increased the percentage of phagocytes (monocyte-
238 macrophages and acidophilic granulocytes) of gilthead seabream.

239 Result obtained from this study shows an increase in total protein, albumin, globulin
240 and albumin – globulin ratio. These findings were similar to results obtained by Sahoo and
241 Mukherjee, (2001) in which total protein levels remained unchanged irrespective of aflatoxin
242 / levamisole exposure, although the increased albumin / globulin ratio noted due to aflatoxin
243 treatment was restored by levamisole feeding. Another study by EL-Boushy and EL-Ashram,
244 (2002) in African catfish reported that levamisole was found to restore the total protein level
245 in immune-compromised fish and increasing γ -globulin levels in healthy fish.

246 However, Das *et al.*, (2009) reported that the serum total protein after long term
247 feeding with *E. viridis* increased in comparison to the control diet which supported the
248 present findings. Also, Siwicki (1989) observed an increase in total protein content after
249 feeding of β -glucan (0.2%) and chitosan (0.5%) in the diet. Serum albumin and globulin
250 values in fish fed with *E. viridis* were higher than the control. Wiegertjes *et al.*, (1996)
251 reported that increases in serum protein, albumin and globulin levels may be a result of
252 immune response to certain constituents of the extracts. Hussein *et al.*, (2001) also showed
253 that serum total protein content was elevated in male Albino rats after administration of garlic
254 oil. Blood serum protein is a fairly labile biochemical system, precisely reflecting the
255 condition of the organism and the changes happening to it under influence of internal and
256 external factors (Shalaby *et al.*, 2006).

257 Results of this study showed that serum enzymes Aspartate Amino Transferase
258 (AST) and Alanine Amino Transferase (ALT) activities decreased in the fish group fed on all
259 levels of inclusion of *A. cepa* and *T. conophorum* compared to control. There were no
260 significant differences ($P < 0.05$) among the treatments in terms of AST and ALT. These
261 findings were similar to Shalaby *et al.*, (2006) who reported that serum AST and ALT
262 activities decreased significantly in the fish group fed on all levels of *Allium sativum* and
263 chloramphenicol. This work also agreed with those reported by El-Shater *et al.*, (1997) and

264 Augusti *et al.*, (2001), who found that the lipid parameters and enzyme activities (AST, ALT,
265 and ALP) in serum of rats decreased significantly when they were fed on a diet containing
266 5% *Allium sativum*. The results of this study revealed *Allium cepa* and *T. conophorum*, which
267 may cause stabilized cell membrane and protect the liver against deleterious agents and
268 free radical-mediated toxic damage to the liver cells. This is reflected in the reduction of liver
269 enzymes. *Allium cepa* and *T. conophorum* help the liver to maintain its normal function by
270 accelerating the regenerative capacity of its cells.

271 In conclusion, this work revealed that the immune response studied in fish can be
272 affected by dietary feed intake. Therefore, dietary supplementation of walnut leaf and onion
273 bulb residues in African catfish, *Clarias gariepinus* feeds should be encouraged since the
274 values obtained were within acceptable limits for cultured fish and no traces of infection such
275 as anaemia were observed in the cultured fish, *Clarias gariepinus*. The extracts of walnut
276 leaf and onion bulb will be useful in fish diseases to stimulate immune response.

277

278 REFERENCES

- 279 Abdel-Monem A, Shalaby SM, El-Dakar AY. Response of red tilapia to different levels of
280 some medicinal plants by-products black seed and roquette seed meals. 1st Conference in
281 Aquaculture. *Egyptian Aquaculture Society, El-Arish, Egypt*, 2002, pp. 247-280.
- 282 Ajaiyeoba EO, Fadare DA. Antimicrobial potential of extracts and fractions of the African
283 walnut – *Tetracarpidium conophorum* *African Journal of Biotechnology* 2006, Vol. 5 (22):
284 2322-2325
- 285 Akinmutimi AH. Evaluation of sword bean (*Canavalia gladiata*) as an alternative feed
286 resource for broiler chickens. Ph.D. Thesis, Department of Non-ruminant Animal Production,
287 Michael Okpara University of Agriculture, Umudike, Nigeria., 2004.
- 288 Aly SM, Ahmed Y, Ghareeb AA, Mohammed MF. Studies on *Bacillus subtilis* and
289 *Lactobacillus acidophilus*, as potential probiotics, on the immune response and resistance of

290 Tilapia nilotica (*Oreochromis niloticus*) to challenge infections, *Fish and Shellfish*
 291 *Immunology* 2008, **25**: 128–136.

292 Augusti KT, Narayanan A, Pillai LS, Ebrahim RS, Sivadasan R, Sindhu KR, Subha I, Abdeen
 293 S, Nair SS. Beneficial effects of garlic (*Allium sativum* Linn) on rats fed with diets containing
 294 cholesterol and either of the oil seeds, coconuts or groundnuts. *Indian J. Exp. Biol.*, 2001,
 295 39: 660-667.

296 Azu NC, Onyeagba RA. Antimicrobial properties of extracts of *Allium cepa* (Onions) and
 297 *Zingiber officinale* (Ginger) on *Escherichia coli*, *Salmonella typhi*, and *Bacillus subtilis*. *The*
 298 *Internet Journal of Tropical Medicine*. 2007, Volume 3 (2), 8pp ISSN: 1540 - 2681

299 Azza M, Abd El-Rhman AM, Yassir AE. Khattab YAE, Adel ME, Shalaby AME. *Micrococcus*
 300 *luteus* and *Pseudomonas* species as probiotics for promoting the growth performance and
 301 health of Nile tilapia, *Oreochromis niloticus*, *Fish & Shellfish Immunology*, 2009, Volume
 302 27(2):175-180

303 Blaxhall PC, Daisley KW. Routine haematological methods for use with fish blood. *Journal of*
 304 *Fish Biology* 1973, 5, 771pp

305 Coles EH. Veterinary Clinical Pathology. Philadelphia: Saunders, 1986, 615p.

306 Cuesta A, Rodri A, Esteban MA and Meseguer J. *In vivo* effects of propolis, a honeybee
 307 product, on gilthead sea bream innate immune responses, *Fish and Shellfish Immunology*,
 308 2005, **18**: 71–80

309 Das BK, Pradhan J, Sahu S. The effect of *Euglena viridis* on immune response of rohu,
 310 *Labeo rohita* (Ham.) *Fish and Shellfish Immunology*, 2009, 26: 871–876.

311 El-Boushy ME, EL-Ashram AMM. Immunostimulating effect of levamisole on non-specific
 312 immunity of African cat fish (*Clarias gariepinus*) after immunosuppression induced by
 313 Malathion. *Mansoura Veterinary Medicine Journal* 2002, Vol.4 (1): 51-64.

314 El-Dakar AY Growth response of hybrid tilapia, *Oreochromis niloticus* x *Oreochromis aureus*,
 315 fingerlings to diets supplemented with different levels of caraway seeds. *Mansoura*
 316 *University Journal of Agricultural Sciences*. 2004, 29: 6083-6094.

317 El-Dakar AY, Hassanien GD, Gad SS, Sakr SE. Use of dried basil leaves as a feeding
 318 attractant for hybrid Tilapia, *Oreochromis niloticus* X *Oreochromis aureus*, fingerlings.
 319 *Mediterranean Aquaculture Journal*. 2008, 1 (1): 35-44.

320 El-Dakar AY, Shalaby SM, Saoud IP. 2007. Assessing the use of a dietary probiotic/prebiotic
 321 as an enhancer of spinefoot rabbitfish *Siganus rivulatus* survival and growth. *Aquaculture*
 322 *Nutrition*. 2007, **13** (6): 407-412.

323 El-Dakar AY, Hassanein GDI, Gad SS, Sakr SE. Use of medical and aromatic plants in fish
 324 diets: 1- Effect of dried marjoram leaves on performance of hybrid tilapia, *Oreochromis*
 325 *niloticus* x *Oreochromis aureus*, fingerlings. *J.Egypt.Acad.Soc.Environ.Dev.B (Aquaculture)*.
 326 2004a, 5 (1): 67-83.

327 El-Dakar AY, Shalaby SM., Abdel-Monem AI, Wahby OM. Enhancement of performance
 328 using fennel seeds meal as feed additive for Nile tilapia, *Oreochromis niloticus*.
 329 *J.Egypt.Acad.Soc.Environ.Dev.B (Aquaculture)*. 2004b, 5: 43-67.

330 EL-Shater MA, Sobhhy MH, Shehab JG. Some pharmacological and pathological studies on
 331 *Allium sativum* (garlic). *Applied Veterinary Medicine Journal*, 1997, 36: 264 - 83.

332 Fox HE, White SA, Koa MF, Fernald RD. Stress and dominance in a social fish, *The Journal*
 333 *of Neuroscience* 1997, **16** (17): 6463–6469.

334 Hussein SA, ABD-EL-Maksoud H, Azab ME. Certain biochemical effect of garlic oil on
 335 normal and experimentally induced hyperlipidemia in male albino rats. In: *International*
 336 *Scientific conference*, Mansoura: Mansoura University, 2001, 2:103-29.

337 Lawson TB. Fundamental of Aquaculture Engineering (Chapman and Hall 1995)
 338 International Thamson publishing Inc., 1995, Pp 28-39.

339 Martins ML, Moraes FR, Miyazaki DM., Brum CD, Onaka EM., Fenerick Jr. J, Bozzo FR.
 340 Alternative treatment for *Anacanthorus penilabiatus* (Monogenea: Dactylogyridae) infection
 341 in cultivated pacu, *Piaractus mesopotamicus* (Osteichthyes: Characidae) in Brazil and its
 342 haematological effects. *Parasite*, 2002, 9, 17180

343 Nuhu, F. Effect of Moringa leaf meal (MOLM) on nutrient digestibility, growth, carcass and
 344 blood indices of weaner rabbits. M. Sc project, Kwame Nkrumah University of Science and
 345 Technology, Kumasi, 2010.

346 Oyawoye EO, Ogunkunle M. Physiological and biochemical effects of raw jack beans on
 347 broilers. Proceedings of annual Conference of Nigerian Society of Animal Production, 1998,
 348 23: 141-142.

349 Raa J. The use of immunostimulatory substances in fish and shellfish farming. *Revision and*
 350 *Fisheries Science*. 1996, 4:229-88.

351
 352 Sahoo PK, Mukherjee SC. Effect of dietary β -1–3-glucan on immune responses and disease
 353 resistance of healthy and aflatoxin B1 induced immunocompromised rohu (*L. rohita*). *Fish*
 354 *and Shellfish Immunology*, 2001, 11 (8): 683–695

355 Sakr SS. Studies on the feeding attractants for fish. M.Sc., Faculty of Environmental
 356 Sciences, Suez Canal University, El-Arish, Egypt, 2003.

357 Shalaby AM, Khattab YA, Abdel -Rahman AM. Effects of garlic (*Allium sativum*) and
 358 chloramphenicol on growth performance, physiological parameters and survival of Nile
 359 tilapia. *Journal of Venomous of Animal Toxins include Tropical Diseases*, volume, 2006,
 360 12(2): 172 – 201

361 Shalaby SM. Response of Nile tilapia, *Oreochromis niloticus*, fingerlings to diets
 362 supplemented with different levels of fenugreek seeds (Hulba). Mansoura University *Journal*
 363 *of Agricultural Sciences*, 2004, 29 (5): 2231-2242.

364 Shalaby SM, Abdel-Monem AI, El-Dakar AY. Enhancement of growth performance, feed and
 365 nutrient utilization of Nile tilapia (*Oreochromis niloticus*), using of licorice roots (Erksous) as
 366 a feed attractant. *J.Egypt.Acad.Soc.Environ.Dev.B (Aquaculture)*. 2003, 4: 119-142.

367 Secombes CJ. Enhancement of fish phagocyte activity. *Fish and Shellfish Immunology*,
 368 1994, 4: 421-36.

369 Siwicki AK. Immunostimulating influence of levamisole on non-specific immunity in carp
 370 (*Cyprinus carpio*). *Development and Comparative Immunology*, 1989, 13:87–91.

371 Talpur AD, Ikhwanuddin M. *Azadirachta indica* (Neem) leaf dietary effects on the immunity
372 response and disease resistance of Asian seabass, *Lates calcarifer* challenged with *Vibrio*
373 *harveyi*. *Fish and Shellfish Immunology*, 2013, 34: 254-264

374 Wiegertjes GF, Stet RJM, Parmentier HK, Vas Muiswinkel WB. Immunogenetics of disease
375 resistance in fish: a comparable approach. *Development and Comparative Immunology*,
376 1996, 20:365–81

377 Witeska M. Stress in fish-haematological and immunological effects of heavy metals,
378 *Electronic Journal of Ichthyology*, 2005, 1: 35–41.

379

380

381

382 **COMPETING INTERESTS**

383

384 Authors have declared that no competing interests exist.

385

386 **AUTHORS' CONTRIBUTIONS**

387

388 Author A, B and C designed the study and wrote the protocol, author B and C supervised the
389 study. Author A performed the experiment and the statistical analysis as well as managed
390 the literature searches. Also, Author A wrote the first draft of the manuscript. All authors read
391 and approved the final manuscript.

392

393