Effects Of Some Herbal Plants As Natural Feed Additives In Comparison With Antibiotic On Growth Performance And Immune Status Of Nile Tilapia (Oreochromis niloticus)

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Received 16 January 2014; accepted 25 January 2014

Abstract
This study was assigned to evaluate the comparative effects of Echinacea, Ginseng extracts, and antibiotic supplementation on growth performance and immune status in Nile tilapia (Oreochromis niloticus). A total of Four hundred Nile tilapia with an average body weight of 41.57±0.3g were assigned at random to 8 treatments with 2 replicates (25 per replicate) in a randomized complete block design. Dietary treatments consisted of basal diet with no additives (group1 as control) or basal diet supplemented with 1g /kg diet oxytetracycline (group 2), 0.75g /kg diet of Echinacea extract (group 3), 0.75g /kg diet of Echinacea extract plus 1g /kg diet oxytetracycline (group 4), 0.6g /kg diet of Ginseng extract (group5), 0.6 gm/kg diet Ginseng extract plus 1g /kg diet oxytetracycline to (group 6), 0.75g /kg diet of Echinacea extract and 0.6g /kg diet of ginseng extract (group 7) and 0.75g /kg diet of Echinacea extract , 0.6g /kg diet of Ginseng extract and 1g /kg diet of oxytetracycline (group 8).

The results revealed that groups treated with Echinacea or Ginseng extracts, or both alone were significantly higher in weight gain % than groups treated with these extracts with oxytetracycline. the non specific immune parameters revealed that the total leukocytic count of groups treated with Echinacea or Ginseng extracts were significantly higher than groups treated with these extracts with oxytetracycline, and these later groups were also higher than group treated with oxytetracycline alone. In conclusion, this study showed that the addition of Echinacea or Ginseng extracts or both to the Nile tilapia diets could be suggested as effective alternative for oxytetracycline as a growth promoters, also they can be used as immunostimulants for Nile tilapia with respect that Echinacea extract is the most potent one of them.

Keywords: Immunostimulants, Ginseng, Echinacea, Growth Promoters, Oxytetracycline, Oreochromis Niloticus.

Introduction
Antibiotics have been used to prevent diseases and to improve feed efficacy for long time, but there are many problems associated with the use of antibiotics in aquaculture, such as the residues of antibiotics left in the tissue of treated fish, the generation of antibiotic-resistant bacteria and the imbalance of the normal beneficial intestinal flora (1). The restriction on the use of in-feed antibiotics in many countries has fueled the interest in alternative products (2). Common feed additives used in animal diets include immunostimulators, antimicrobials, antioxidants and herbal plants. In recent years, herbal plants serve as a new class of growth promoters that provide an alternative feeding strategy to replace antibiotic growth promoters (3). (4) investigated the effect of feeding with oxytetracycline containing pellets on the immune system of carp and found that the cellular immunity was not affected while the humoral immune response was depressed. (5) reported the immunosuppressive effect of oxytetracycline in carp. It was shown that both cellular and humoral immunity were depressed after feeding or injecting the antibiotic. (6) reported that oxytetracycline and other similar compounds may interfere with normal immunological processes in fish, birds and mammals. (7) concluded that oxytetracycline had a suppressive effect on...
specific and non-specific immune system parameters of rainbow trout (*Oncorhynchus mykiss*), such as leucocyte counts, nitroblue tetrazolium activity, total plasma protein and immunoglobulin levels, and phagocytic activity. There are traditional herbal medicine that used as herbal immune stimulants such as Ginseng. (8) stated that the active ingredients of Ginseng are ginsenosides, mono and polysaccharides.(9) showed that ginsan polysaccharide isolated from the root of panax Ginseng has been shown to be a potent immunomodulator, producing several cytokines (TNF-α, IL-β, IL-2, IL-6, IFNγ, GMCSF) and stimulate lymphoid cells to proliferate. (8) revealed that the steroidal saponins (ginsenosides) enhance both B and T-cell mediated immune responses. (10) examined the immunomodulatory activity of the ethanol-insoluble fraction of an aqueous extract of panax Ginseng and found that it induced proliferation of splenocytes and generated activated killer cells in vitro. (11) investigated the immunomodulatory effect of Ginseng and found that it enhanced lymphocyte proliferation only in the mitogen stimulation assay. (12) investigated the immune response induced by dietary supplementation of 2% panax Ginseng on Nile tilapia for 84 days. These investigation were done at 2, 4, 6, 8, 10 and 12 weeks of supplementation of diet and found that the average body weight, complement activity, bactericidal activity against *Escherichia coli*, lysozyme activity and adherent phagocyte activity were high in fish. (13) stated that Echinacea seems to activate the macrophages and other immunological function in lab animals and humans and there is considerable evidence for the role played by the polysaccharides fraction in the immunostimulating effect of Echinacea preparations, as it is able to activate macrophages and other components of the immune system in mice, rats and humans. (14) investigated the mechanism of action of purple cornflower (Echinacea purpurea (L) Moench) preparation on the immune system by injection of (1 ml/kg) of extracts from over ground parts and roots of purple cornflower into rabbits. They observed that there is significant increase in the number of leucocytes and lymphocytes especially T-lymphocytes with significant increase in phagocytosis. (15) stated that Echinacea preparations are commonly used as non specific immunomodulatory agents and also stated that Echinacea is a wide-spectrum immunomodulator that modulates both innate and adaptive immune responses. (16) studied the effect of Echinacea (Echinacea purpurea) on Nile tilapia and found that fish were fed Echinacea showed an increase in body weight gain, specific growth rate, hematocrite values, lysozyme activities and total leukocytic counts, especially in terms of lymphocytes and eosinophils when compared with control groups. It was found also that the survival rate was significantly increased in Echinacea treated group with and without challenge by I/P inoculation of fish with 0.5 ml suspension culture of the pathogen *Pseudomonas fluorescens* (1X10⁸ bacteria ml⁻¹), while no significant changes in the monocytes numbers and the nitroblue tetrazolium test occurred. This study was aimed to evaluate the effects of Echinacea or Ginseng extracts or both in comparison with oxytetracycline on growth performance and immune parameters of Nile tilapia.

**Material and Methods**

**Experimental fish**

Four hundred Nile tilapia with an average body weight of 41.5±0.3 g, were kept in two concrete pond (3 X 1 X 1 m) for two weeks to be acclimatized before the start of the experiment.

**Supplements:** Muv-Oxytetracycline (muvco), each 100 g of Muv-Oxytetracycline contains Oxytetracycline Hydrochloride 20 g (Equivalent to18.53 Oxytetracycline base). Immunovita capsules (Ema pharm pharmaceuticals), each capsule contains 210 mg Echinacea purpurea root extract that contains polysaccharides, caffic acids (echinacosides), cichoric acid. Ginseng capsules (Pharco pharmaceuticals), each capsule contains 100 mg Ginseng root extract that contains ginsenosides (saponin glucosides).

**Experimental fish design**

As shown in Table 1, These experiment was carried out in Fish Research Center, Fac. of Vet. Med., Zagazig University, Egypt, and extended for 3 months in the summer season (June-September).

<table>
<thead>
<tr>
<th>Measurement of survival and growth performance parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average body weight of fish for each group was weighed after one and two month of the feeding experiment according to (19) and (20) as: Average body weight = the total weight of fish / the number of fish in each group.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 1: Experimental fish design:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group Diet</strong></td>
</tr>
<tr>
<td>Fish number</td>
</tr>
<tr>
<td>Basal diet (32.16%protein)</td>
</tr>
<tr>
<td>Oxytetracycline (1gm/kg diet)</td>
</tr>
<tr>
<td>Echinacea extract (0.75gm/kg diet)</td>
</tr>
<tr>
<td>Ginseng extract (0.6 gm/kg diet)</td>
</tr>
</tbody>
</table>

**Diets and feeding**

A balanced dietary ration formulation was prepared to meet the requirements of Nile tilapia according to (17) (Table 2).The amount of feed (on dry matter basis) delivered per day was adjusted at the beginning and after one month of the experiment as (3% of body wt.) (18).
Weight gain % was calculated according to (21) as: Weight gain % = Final average body weight – Initial average body weight / Initial average body weight x 100.

Specific growth rate (SGR) was determined according to (22) as:
Specific growth rate (SGR) = 100 (ln W2 − ln W1) / T. Where W1 and W2 are the initial and final fish weight, respectively, and T was the number of days in the feeding period.

**Evaluation of feed utilization**
Feed intake was calculated as the total weight diet offered in a given period divided by the number of survival fish. Feed conversion ratio (FCR) was calculated according to (22) as: FCR = (dry feed intake by gm) / (live weight gain by gm).

Condition factor (CF) was calculated according to (23) as:
CF = (body weight by gm) / (total length cm)³ x 100

Survival rate was calculated according to the following formula: Survival % = (No. of fish counted) / (No. of stocked fish) x100.

**Blood and serum samples**
Blood and serum samples were taken after one and two months of the feeding experiment, 6 fish of each replicate were randomly taken and anaesthetized using benzocaine 50 mg/L.

**Evaluation of immunological parameters**

**Non specific immune parameters**
Total leukocytic count was performed using the improved Neubaur chamber, Natt and Herrick's solution as diluting fluid and 1:100 diluted blood according to the method described by (24). Nitroblue tetrazolium activity (NBT) was performed according to (25). Lysozyme activity, true lysozymes have the ability to lyse *Micrococcus lysodeikticus* cells (26). The Lysozyme concentrations in the samples were determined from a plotted standard curve against the corresponding clear zone ring diameter on the linear axis (27).

**Semen bactericidal activity (SBA)**
It examines the ability of the fish's serum that fed some plant extracts and oxytetracycline with its diet to kill the pathogenic organisms. The turbidity of bacterial suspension of *A. hydrophila* was adjusted by comparing with 0.5 McFarland turbidity standards (1.5x10⁻³). The number of viable bacteria was calculated by counting the colonies of resultant incubated mixture (28). The bactericidal activity of the tested serum was expressed as the percentage of colony forming units in test the group to that in the control group (29). Determination of total globulin was estimated by the method of (30).

**Specific immune parameters**
The quantitative determination of IgM was made by using nephelometry technique, according to (31). Lymphocyte transformation test (32) was carried out according to the following steps:

1. **Separation of lymphocytes (33)**
2. **Viability of lymphocytes**: by using 0.4% trypan blue stain according to the technique described by (34) and (35).
3. The number of lymphocytes per ml was calculated according to the following equation, No. of lymphocytes/ml = No. of counted lymphocytes / No. of triple ruled squares x 25 x 10⁻⁷ x dilution factor; where the dilution factor is 2.

2. **Standardization of the lymphocytes concentration**
The required final concentration could be adjusted to reach 2x10⁶ lymphocytic cell/ml by adding RPMI-1640 medium with 10% foetal bovine serum (RPMI-10) (36).

3. **Preparation and standardization of mitogenic solution (non-specific mitogen)**
Non-specific mitogen phytohaemagglutinin (PHA) was prepared by dissolving the content of vial in 5 ml RPMI-0 medium, the required concentration 12 mg/culture.

4. **Glucose consumption assay**

**Evaluation of the lymphocyte transformation test**
The extent of lymphocyte transformation rate was calculated according to (38).

**Challenge test**
The challenge test was done at the end of the feeding experiment where 20 fish from each group(10 fish/replicate) were transferred to glass aquaria, then were
inoculated with 0.5 ml culture suspension (1x10^8 bacteria ml^-1) of pathogenic A. hydrophila via intraperitoneal route. The challenged fish were observed for 7 days in order to record the mortalities (39).

**Statistical analysis**

Data were statistically analyzed using the Analysis Of Variance (ANOVA) and Duncan multiple range test to determine differences between treatments and standard errors of treatment means. Differences were considered significant when (P < 0.05). All statistics were carried out using Statistical Analysis Systems (SAS) program (40).

**Results**

After 4 weeks of the feeding experiment all treated groups showed a significant increase in weight gain % and FCR in comparison with untreated control, while all treated and control untreated groups showed no significant change in SGR (P<0.05). Group treated with oxytetracycline alone showed a significant increase in feed intake in comparison with control untreated group and other groups, while groups treated with Echinacea or Ginseng showed a significant increase in condition factor (CF) as shown in (Table 3). All treated groups showed a significant increase in total leukocytic count except oxytetracycline treated group that showed a significant decrease in comparison with control untreated group (P<0.05). All treated groups showed a significant increase in NBT, lysozyme in comparison with control untreated group (P<0.05). Serum bactericidal activity (SBA) of groups that treated with Echinacea or Ginseng or both extracts against A. hydrophila was significantly higher than that of control untreated group (P<0.05), as shown in (Table 5).

**Table (3): Growth performance parameters of O.niloticus 4 weeks after treatment with Oxytetracycline, Echinacea and Ginseng extracts**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G1</td>
</tr>
<tr>
<td>Initial average b. wt</td>
<td>43.16±0.14</td>
</tr>
<tr>
<td>Final average b. wt</td>
<td>54.5±0.29</td>
</tr>
<tr>
<td>Wt. gain %</td>
<td>13.7±0.05^a</td>
</tr>
<tr>
<td>Specific growth rate</td>
<td>1.32±0.05^a</td>
</tr>
<tr>
<td>Feed intake</td>
<td>21.44±0.28^b</td>
</tr>
<tr>
<td>Feed conversion ratio</td>
<td>3.50±0.00^b</td>
</tr>
<tr>
<td>Condition factor</td>
<td>1.55±0.01^d</td>
</tr>
</tbody>
</table>

*Rows with the same litter are not significant different (P<0.05). G1=control G2= OTC G3= Echinacea G4= Echinacea and OTC G5= Ginseng G6= Ginseng and OTC G7= Echinacea and Ginseng G8= Echinacea, Ginseng and OTC

**Table (4): Growth performance parameter and Survival percentage of O.niloticus 8 weeks after treatment with Oxytetracycline, Echinacea and Ginseng extracts**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatments</th>
</tr>
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<tbody>
<tr>
<td></td>
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</tr>
<tr>
<td>Feed conversion ratio</td>
<td>3.50±0.00^b</td>
</tr>
<tr>
<td>Condition factor</td>
<td>1.55±0.01^d</td>
</tr>
<tr>
<td>Survival %</td>
<td>96 ±2.31^a</td>
</tr>
</tbody>
</table>

*Rows with the same litter are not significant different (P<0.05). G1=control G2= OTC G3= Echinacea G4= Echinacea and OTC G5= Ginseng
Table (5): Non specific immune parameters of *O. niloticus* 4 weeks after treatment with Oxytetracycline, Echinacea and Ginseng extracts

<table>
<thead>
<tr>
<th>Parameter</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
<th>G5</th>
<th>G6</th>
<th>G7</th>
<th>G8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total leukocytic count (10⁶/µL)</td>
<td>67 ± 1.15</td>
<td>61.67 ± 0.88</td>
<td>81.58 ± 0.80</td>
<td>73 ± 0.58</td>
<td>85 ± 0.58</td>
<td>70.08 ± 0.85</td>
<td>70.08 ± 0.85</td>
<td>85.5 ± 1.18</td>
</tr>
<tr>
<td>Nitroblue tetrazolium activity (OD at 620 nm)</td>
<td>0.033 ± 0.002d</td>
<td>0.038 ± 0.002ed</td>
<td>0.073 ± 0.001a</td>
<td>0.046 ± 0.003b</td>
<td>0.045 ± 0.007c</td>
<td>0.047 ± 0.002c</td>
<td>0.042 ± 0.002cd</td>
<td>0.045 ± 0.0023</td>
</tr>
<tr>
<td>Lysozyme activity (µg/ml)</td>
<td>110.13 ± 1.72d</td>
<td>129.31 ± 0.42a</td>
<td>129.19 ± 1.2a</td>
<td>122.36 ± 2b</td>
<td>120.27 ± 2.3c</td>
<td>117.23 ± 1.11cd</td>
<td>131.31 ± 0.82a</td>
<td>115.36 ± 1.09d</td>
</tr>
<tr>
<td>Serum bactericidal activity (% of CFU/control)</td>
<td>40 ± 0.23b</td>
<td>45 ± 0.17a</td>
<td>21 ± 0.17d</td>
<td>37 ± 0.23b</td>
<td>27 ± 0.15c</td>
<td>24 ± 0.15c</td>
<td>45 ± 0.08a</td>
<td></td>
</tr>
<tr>
<td>Total globulin (gm/dl)</td>
<td>2.18 ± 0.02d</td>
<td>2.55 ± 0.04d</td>
<td>4.52 ± 0.34a</td>
<td>2.43 ± 0.06d</td>
<td>2.77 ± 0.05c</td>
<td>2.75 ± 0.06c</td>
<td>3.58 ± 0.08b</td>
<td>2.57 ± 0.16ad</td>
</tr>
</tbody>
</table>

*Rows with the same litter are not significant different (P<0.05).
G1=control G2= OTC  G3= Echinacea  G4= Echinacea and OTC  G5= Ginseng G6= Ginseng and OTC  G7= Echinacea and Ginseng  G8= Echinacea, Ginseng and OTC

Groups treated with Echinacea or Ginseng or both extracts showed a significant increase in total globulin and lymphocyte stimulation index compared to control untreated group and groups treated with oxytetracycline alone or with these extracts (P<0.05) as shown in (Table 4). Groups treated with Echinacea or Ginseng extracts showed a significant increase in total leukocytic count, lysozyme activity, SBA, total globulin, lymphocyte stimulation index and NBT in comparison with control untreated group (P<0.05) as shown in (Tables 5 and 7).

Table (6): Non specific immune parameters of *O. niloticus* 8 weeks after treatment with Oxytetracycline, Echinacea and Ginseng extracts

<table>
<thead>
<tr>
<th>Parameter</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
<th>G5</th>
<th>G6</th>
<th>G7</th>
<th>G8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total leukocytic count (10⁶/µL)</td>
<td>67 ± 1.15</td>
<td>51.5 ± 1.76c</td>
<td>87.5 ± 1.44a</td>
<td>63.17 ± 0.72d</td>
<td>80 ± 1.14b</td>
<td>71.33 ± 1.59c</td>
<td>73.76 ± 0.88a</td>
<td>70 ± 1.15c</td>
</tr>
<tr>
<td>Nitroblue tetrazolium activity (OD at 620 nm)</td>
<td>0.033 ± 0.002d</td>
<td>0.034 ± 0.003d</td>
<td>0.086 ± 0.0007a</td>
<td>0.039 ± 0.001d</td>
<td>0.063 ± 0.008b</td>
<td>0.042 ± 0.005b</td>
<td>0.053 ± 0.004b</td>
<td>0.052 ± 0.003b</td>
</tr>
<tr>
<td>Lysozyme activity (µg/ml)</td>
<td>110.13 ± 1.72d</td>
<td>113.38 ± 1.24c</td>
<td>130.69 ± 1.23s</td>
<td>120.81 ± 1.82s</td>
<td>135.28 ± 1.34c</td>
<td>113.49 ± 1.21c</td>
<td>132.65 ± 1.87c</td>
<td>111.68 ± 1.6c</td>
</tr>
<tr>
<td>Serum bactericidal activity (% of CFU/control)</td>
<td>40 ± 0.23d</td>
<td>63 ± 0.18a</td>
<td>23 ± 0.08f</td>
<td>47 ± 0.25b</td>
<td>34 ± 0.15c</td>
<td>42 ± 0.21bc</td>
<td>33 ± 0.12c</td>
<td>39 ± 0.20cd</td>
</tr>
<tr>
<td>Total globulin (gm/dl)</td>
<td>2.18 ± 0.02d</td>
<td>2.69 ± 0.10c</td>
<td>4.39 ± 0.05d</td>
<td>2.29 ± 0.04b</td>
<td>2.88 ± 0.18b</td>
<td>2.76 ± 0.04b</td>
<td>3.41 ± 0.08b</td>
<td>2.30 ± 0.08d</td>
</tr>
</tbody>
</table>

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Table (7): Specific immune parameters of *O. niloticus* 4 and 8 weeks after treatment with Oxytetracycline, Echinacea and Ginseng extracts

<table>
<thead>
<tr>
<th>Parameter</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
<th>G5</th>
<th>G6</th>
<th>G7</th>
<th>G8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocyte stimulation index (4 week)</td>
<td>1.15 ± 0.02d</td>
<td>1.12 ± 0.01d</td>
<td>2.88 ± 0.06b</td>
<td>1.96 ± 0.02b</td>
<td>2.27 ± 0.12b</td>
<td>1.54 ± 0.02ed</td>
<td>2.67 ± 0.06ab</td>
<td>2.68 ± 0.06ab</td>
</tr>
<tr>
<td>Lymphocyte stimulation index (8 week)</td>
<td>1.15 ± 0.01c</td>
<td>1.32 ± 0.03c</td>
<td>3.28 ± 0.36a</td>
<td>2.12 ± 0.03d</td>
<td>3.26 ± 1.08s</td>
<td>2.48 ± 0.06c</td>
<td>2.99 ± 0.04b</td>
<td>2.64 ± 0.03ab</td>
</tr>
<tr>
<td>Ig M (gm/L)</td>
<td>0.153 ± 0.003d</td>
<td>0.165 ± 0.008b</td>
<td>0.238 ± 0.005b</td>
<td>0.219 ± 0.005a</td>
<td>0.249 ± 0.001a</td>
<td>0.233 ± 0.002b</td>
<td>0.227 ± 0.001b</td>
<td>0.164 ± 0.002b</td>
</tr>
</tbody>
</table>

*Rows with the same litter are not significant different (P<0.05).
G1=control G2= OTC  G3= Echinacea  G4= Echinacea and OTC  G5= Ginseng
After challenge with pathogenic *A. hydrophila* all treated groups showed a significant increase in the amount of IgM in comparison with control untreated group (P<0.05) while groups treated with Echinacea or Ginseng extract with oxytetracycline showed a significant increase in IgM than that treated with oxytetracycline alone (P<0.05) (Table 7).

After challenge with pathogenic *A. hydrophila* the mortalities were recorded for 7 days, groups that received oxytetracycline alone showed no significant change in comparison with control untreated group, while groups received Echinacea or Ginseng or both extracts with or without oxytetracycline showed a significant decrease in mortalities than that treated with oxytetracycline alone or control untreated group (P<0.05) (Table 8).

**Discussion**

In this study, Echinacea or Ginseng or both extracts were used as feed additives to evaluate its role as immunomodulators and alternatives to the oxytetracycline as growth promoters. The weight gain % of group that received Echinacea extract was significantly higher than other groups, these findings was consistent with (16) (41) and (42). All groups that received Echinacea or Ginseng extract or both alone or combined with oxytetracycline were significantly higher in weight gain % than group that received oxytetracycline alone, these agree with (43) and (12), while disagree with (44). The specific growth rate of all treated groups in our work was significantly higher than control untreated group, these results correspond with (43) while disagree with (45) who examined the effect of feeding oxytetracycline on the growth of catfish for 11 weeks and found that the specific growth rate showed no significant change in the oxytetracycline treated fish. Group received Echinacea extract showed a significantly higher value in comparison with other groups, these work in with (16) and (41) and (42). The best feed utilization was for groups received Echinacea or Ginseng extracts or both with oxytetracycline followed by groups received Echinacea or Ginseng extracts then group received oxytetracycline alone and finally the control group. These findings consistent with (46) and (43). On the other hand conflict with (44) and (45). As regard the condition factor of all groups was significantly higher than control group. These data work in with (45) and contradict with (16) who recorded no significant change in the condition factor of Nile tilapia fed Echinacea at a rate of 0.25 ppt on a dry weight basis for 6 months. The survivability of all groups showed no significant change in comparison with control group. These results agree with (16). The ability of plant extracts and oxytetracycline to improve the growth performance of Nile tilapia was varied and may be attributed to the possible mode of action of them as growth promoters as mentioned by (47).

The total leukocytic count of all treated groups were significantly increased in comparison with control untreated group except group received oxytetracycline alone that showed a significant decrease, these findings correspond with (6) and (7). However, it conflict with (48). Groups received Echinacea or Ginseng extracts showed a significant increase in NBT than those with oxytetracycline, these findings agree with (15) and disagree with (16). Groups received Echinacea or Ginseng extracts or both showed a significant increase in lysozyme activity in comparison with groups received these extracts with oxytetracycline or group received oxytetracycline alone. These findings agree with (51) and (15). Groups received Echinacea or Ginseng extracts showed a significant increase in SBA in comparison with groups received these extracts with oxytetracycline, and these later groups showed a significant increase in SBA in comparison with groups fed oxytetracycline alone. These findings agree with (12) and (52). The total globulin of all groups showed a significant increase in comparison with control untreated group, these findings interfere with (7) and (53). The results revealed a potential enhancement of humoral immune response, these agree with (8). Groups that were fed Echinacea or Ginseng or both extracts showed a significant increase in total globulin than that were fed oxytetracycline alone or with these extracts. The level of IgM after the challenge with pathogenic *A. hydrophila* was significantly increased in all treated groups in comparison with control untreated group, these findings disagree with (7). The lymphocyte stimulation index of all treated groups showed a significant increase except group received oxytetracycline alone that showed no significant change in comparison with control untreated group, these findings agree with (11).

After challenge with pathogenic *A. hydrophila* groups that received Echinacea or Ginseng or both extracts with or without oxytetracycline showed a significant decrease in mortalities than that treated with oxytetracycline alone or control untreated group that indicates a good disease resistance, these findings was explained by (15). Group that received oxytetracycline alone showed a non significant

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**Table 8:** Mortality percentage of *O.niloticus* treated with Oxytetracycline, Echinacea and Ginseng extracts for 8 weeks then challenged with pathogenic *A. hydrophila*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
<th>G5</th>
<th>G6</th>
<th>G7</th>
<th>G8</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of fish</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>No. of mortality</td>
<td>10</td>
<td>12</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Mortality %</td>
<td>50%</td>
<td>60%</td>
<td>10%</td>
<td>10%</td>
<td>10%</td>
<td>10%</td>
<td>10%</td>
<td>15%</td>
</tr>
</tbody>
</table>

*Rows with the same litter are not significant different (P<0.05).*

G1= control G2= OTC G3= Echinacea G4= Echinacea and OTC G5= Ginseng
G6= Ginseng and OTC G7= Echinacea and Ginseng G8= Echinacea, Ginseng and OTC
change in mortalities in comparison with control untreated group, this may be due to the effect of oxytetracycline and other similar compounds that may interfere with normal immunological processes in fish, as mentioned by (6).

Conclusion

From obtained results we concluded that, using of Echinacea or Ginseng extracts or both as feed additives for Nile tilapia diets for 8 weeks can improve growth performance parameters and stimulate its specific and non-specific immune parameters. So addition of Echinacea or Ginseng extracts or both to the Nile tilapia diets can be served as a natural alternatives for oxytetracycline as a growth promoters, also they can be used as immunostimulants for Nile tilapia with respect that Echinacea extract is the most potent one of them.

Acknowledgment

The authors thank Prof. Dr. Yousef Abd El Galil Ahmed, Prof. Dr. Ahmed Ammar and Prof. Dr. Gamal El Noby, for their generous support without which this work would not have been possible.

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purpurea stimulates cellular immunity and antibacterial defense independently of the strain of mice.


Source of support: Nil; Conflict of interest: None declared