DISCUSSION

The *Leucaena leucocephala* (Lam. de Wit) tree mainly used as fodder. The dairy and cattle, poultry and fishery have been supplied with the leaves of this tree. *L. leucocephala* (ipil-ipil) has been found to be a valuable forage and feed for our live stocks. Its leaf and seed meals are in wider use in many tropical countries including Bangladesh. Many reports have discussed its side effects in different domesticated animals and chicken. Rahman and Dewan, (1988) described pathological effects of *Leucaena* seed meal in the duck *(Anas platythyncha domestica)* in Bangladesh.

But from the field experience and some research works (Rahman and Dewan, 1988; Scott, *et. al.* 1969) on this plant indicated that, the leaves and other parts of the tree, which have been used as fodder can do harm to the animals. Although some works have been done on the animals, poultry birds regarding the *L. leucocephala* tree but a little work have so far been done with the fish in this country.

The haematological parameters of the fish reflect the ecological conditions of its habitat, nutritional states, which are found to vary in different fish groups. In the teleosts, these parameters are found to vary from species to species. Any deviation from normal may be a clue to the physiological and pathological state of the animal. During the experiment, the behavioral, physical, haematological and histopathological parameters were changed due to *L. leucocephala* leaf and seed food on *C. punctatus* and *O. niloticus* respectively in different amount for two months.

The test results reviewed that the each and every parts of *Leucaena leucocephala* contains alkaloid, flavonoid, terpinoid , steroid , saponins. Different research study reviewed that the amount of mimosine content among the different species of *Leucaena leucocephala* seed is higher (2.2% to 10%) than the other parts leaf and bark (1.2 to 5.4%), (Ter Meulen U. Struck E, El-Harith EA. 1979).

The effects of *L. leucocephala* leaf on *C. punctatus* and *O. niloticus* was observed in different amount as 25%, 30%, 35% and 40% of total food, which was calculated 2% of the total body weight. The effects of the *L leucocephala* leaf food was considered as behavioral, physical, haematological and histopathological changes observed in

C. punctatus and *O. niloticus* in different amount. Total five study groups were taken. Here one group was taken as control. Ten fishes were kept for each group.

The effects of *L. leucocephala* seed on *C. punctatus* and *O. niloticus* was observed in different amount as 20% 25%, 30%, and 35% of total food, which was calculated 2% of the total body weight. The effects were considered as behavioral, physical haematological and histopathological changes observed in *C. punctatus* and *O. niloticus* in different amount. Total five study groups were taken. Here one group was kept as control. Ten fishes were taken for each group.

Behavioral changes

The effect on behavior of the fish *C. punctatus* due to *L. leucocephala* leaf was loss of appetite and slow in movement. In each group this phenomena was exhibited in several fishes and was compared with the control group. The effect on behavior of the fish *O. niloticus* due to *L. leucocephala* leaf was loss of appetite, loss of normal movement and erratic swimming. In each group this phenomena was exhibited in several fishes and was compared with the control group.

The effects on behavior of the fish *C. punctatus* due to *L. leucocephala* seed was loss of appetite and loss of movement, excessive mucous secretion and accumulation on the skin and gills. The effects on behavior of the fish *O. niloticus* due to *L. leucocephala* seed was erratic swimming, loss of normal movement and loss of appetite. In each group this phenomena was exhibited in several fishes and was compared with the control group.

Different workers have reported their observations about the physical responses of the fishes in their respective media. Latifa *et.al*. (1987, 1988, 1992); Chowdhury (1996) and Begum (1986) have reported increase in the dose level caused a decreased in the reduction time of total loss of equilibrium in the species.

Physical changes

The physical changes, which were observed after feeding different amount of 25%, 30%, 35% and 40% *L. leucocephala* leaf in four groups of *C. punctatus* were lesion on caudal region, erosion of fins, puffed and loose scales, lesions on skin and muscle and scoliosis, exophthalmus. The physical changes, which were observed after feeding different amount of 25%, 30%, 35% and 40% *L. leucocephala* leaf groups of *O. niloticus* were lesion on skin and erosion on caudal fin, exophthalmus, lesions on skin, puffed and loose scales, fading of body color and change in pigment. These changes were exhibited in each group of fishes.

The physical changes, which were observed after feeding different amount of 20%, 25%, 30% and 35% *L. leucocephala* seed groups were lesions on skin, puffed and loose scales, lesions on caudal region, exophthalmus, scoliosis and abnormality in egg structure. The physical changes, which were observed after feeding different amount of 20%, 25%, 30% and 35% *L. leucocephala* seed groups were erosion of caudal fin, lesion on skin, puffed and loose scales, fading of body color and change in pigment, exophthalmus, curvature in preopercle bone. These changes were exhibited in each group of fish. Akhtar (1994) discussed the physical changes of the body, restlessness and slime secretion.

Haematological changes

The blood volume of fishes varies between 2 and 8% of the body volume. One third to one half of the total blood volume in fish consists of blood cells the rest is fluid plasma. There are two types of celluler elements in blood, i.e. red blood corpuscles and white blood corpuscles. The comparative data of haematological analysis of experimental and control fishes are shown in tables.

Erythrocyte

In this investigation total erythrocyte ranged from 3.09 to 3.26 x 10^6/mm^3 for *C. punctatus* and 8.04 to 8.46 x 10^6/mm^3 for *O. niloticus* in case of *L. leucocephala* leaf group. Other investigation of *L. leucocephala* seed food group total erythrocyte ranged from 3.08 to 3.26 x 10^6/mm^3 for *C. punctatus* and 8.28 to 8.56 x 10^6/mm^3 for *O. niloticus.* The highest number of erythrocyte found in the control group of *C.*

punctatus whereas the number was gradually decreasing with the raising percentage of *L. leucocephala* seed group 20%, 25%, 30% and 35% . In present investigation the highest number of erythrocyte of *O. niloticus* found in 20% *L. leucocephala* seed group of whereas the number was gradually decreasing with the raising percentage of *L. leucocephala* seed group 25%, 30% and 35%. Erythrocyte amount was 6.93 $\pm$ 8.28 x 10^6/mm^3 in *O. niloticus* reported by Bittencourt, N. de L. R *et al.* (2003). Difference in count was reported by Shrama & Shandilya, (1982), *Clarias batrachus* with $4.50 \times 10^6 / mm^{-3}$ and with $2.55 \times 10^6 / mm^{-3}$ Geol *et.al.,* (1984) reported higher erythrocyte count in to active fishes among *Mystus tengra*, *Oreochromis niloticus , Labeo bata, Labeo corsa, Notopterus notopterus, Mastacembalus and Channa puctatus* (Joshi, 1989) reported higher erythrocyte count in *China gachua.*

Leucocyte

In case of *L. leucocephala* leaf group total leucocyte ranged from 32.59 to 37.85 x 10^3/mm^3 for *C. punctatus* and 40.01 to 52.28 x 10^3/mm^3 for *O. niloticus.* In other case of *L. leucocephala* seed group total leucocyte ranged from 32.16 to 37.85 x 10^3/mm^3 for *C. punctatus.*and 38.72 to 52.28 x 10^3/mm^3 for *O. niloticus.* In present observation the highest number of leucocyte found in the control group of both cases whereas the number was gradually decreasing with the raising percentage of *L. leucocephala* leaf and seed food group.

Thrombocyte

In case of *L. leucocephala* leaf group total thrombocyte ranged from 43.63% to 45.69% for *C. punctatus* and 54.47% to 57.02% for *O. niloticus.* In other case of *L. leucocephala* seed group total thrombocyte ranged from 43.36% to 45.69% for *C. punctatus* and 54.07% to 57.02% for *O. niloticus.* In present observation the highest number of leucocyte found in the control group of both cases whereas the number was gradually decreasing with the raising percentage of *L. leucocephala* leaf and seed group.

Lymphocyte

In this investigation lymphocyte ranged from 21.19% to 22.51% for *C. punctatus* and 26.83% to 28.08% for *O. niloticus* in case of *L. leucocephala* leaf group. Other investigation of *L. leucocephala* seed group lymphocyte ranged from 21.64% to 22.51% for *Channa punctatus* and 26.63% to 28.08% for *Oreochromis niloticus.* In all the investigation the highest number of lymphocyte found in the control group whereas the number was gradually decreasing with the elevating percentage of *Leucaena leucocephala* leaf and seed group. Vertebrate lymphocytes are highly differentiated cell which responds to immunological stimuli in a variety of ways. White, (1954) called them immune-competent cells.

Monocyte

In this investigation monocyte ranged from 4.30% to 5.60% for *C. punctatus* and 7.20% to 8.30% for *O. niloticus* in case of *L. leucocephala* leaf group. In present observation the lowest percentage of monocyte found in the control group whereas the highest percentage was found in 30% *L. leucocephala* leaf group. The percentage of monocyte was gradually increasing with the raising percentage of *L. leucocephala* leaf group 25%, 30%, whereas the percentage was gradually decreasing in 35% 40% group.Other investigation of *L. leucocephala* seed group monocyte ranged from 4.30% to 5.00% for *C. punctatus* and 6.80% to 8.00% for *O. niloticus* in case of *L. leucocephala* seed group. In present observation the lowest percentage of monocyte found in the control group of *C. punctatus* whereas the percentage was gradually increasing with the raising percentage of *L. leucocephala* seed group 20%, 25%, 30%, and 35%. In present observation the highest percentage of monocyte found in the control group of *O. niloticus* whereas the percentage was gradually decreasing with the raising percentage of *L. leucocephala* seed group 20%, 25%, 30% except 35%.

Neutrophil

In case of *L. leucocephala* leaf group neutrophil ranged from 23.00% to 24.30% for *C. punctatus* and 4.20% to 7.50% for *O. niloticus.* In present observation the highest percentage of neutrophil found in the 25% *L. leucocephala* leaf group of *C. punctatus* whereas the percentage was gradually decreasing with the raising percentage of *L. leucocephala* leaf group 30%, 35% and 40%. In present investigation the lowest percentage of neutrophil found in the control group of *O. niloticus* whereas the percentage was gradually increasing with the raising percentage of *L. leucocephala* leaf group 25%, 30%, 35% except 40%. In other case of *L. leucocephala* seed group neutrophil ranged from 23.50% to 24.60% for *C. punctatus.* and 4.20% to 6.40% for *O. niloticus.* In present observation the lowest percentage of neutrophil found in the control group whereas the percentage was gradually increasing with the raising percentage of *L. leucocephala* seed 20%, 25%, 30%, and 35% . In present investigation the lowest percentage of neutrophil found in the control group of *O. niloticus* whereas the highest percentage found in 20% *L. leucocephala* seed group. The percentage of neutrophil was gradually decreasing with the elevating percentage of *L. leucocephala* seed 25% and 30% except 35%.

Eosinophil

In this investigation eosinophil ranged from 3.60% to 5.70% for *C. punctatus* and 2.30% to 4.20% for *O. niloticus* in case of *L. leucocephala* leaf group. Other investigation of *L. leucocephala* seed group eosinophil ranged from 3.60% to 5.10% for *C. punctatus* and 2.50% to 5.30% for *O. niloticus.* In all the invastigation the lowest number of eosinophil found in the control group whereas the number was gradually increasing with the elevating percentage of *L. leucocephala* leaf and seed group.In case of *C. punctatus* except 40% *L. leucocephala* leaf food group and in *O. niloticus* 35% *L. leucocephala* seed group.

Basophil

In this investigation basophil ranged from 0.40% to 0.60% for *C.punctatus* and 0.40% to 0.20% for *O. niloticus* in case of *L. leucocephala* leaf group. In present observation the lowest percentage of basophil found in the control group of *C. punctatus* whereas the percentage was gradually increasing with the elevating

percentage of *L. leucocephala* leaf group 25%, 30%, 40% except 35% .In present investigation the highest percentage of basophil found in the control group of *O. niloticus* whereas the percentage was gradually decreasing with the raising percentage of *L. leucocephala* leaf group 25%, 30%, 35% and 40%. In other investigation basophil ranged from 0.30% to 0.50% for *C. punctatus* and 0.20% to 0.30% for *O. niloticus.* In present investigation the highest percentage of basophil found in 30% of seed group of *C. punctatus* whereas the percentage was gradually decreasing with the elevating percentage of *L. leucocephala* seed group 20%, 25%, 35% except 30% from control group.In present observation the lowest percentage of basophil found in the control group of *O. niloticus* whereas the percentage was gradually increasing with the raising percentage of *L. leucocephala* seed group 20%, 25%, 30% and 35%.

Effect of *L. leucocephala* leaf and seed on other haematological parameters of *C. punctatus* and *O. niloticus*

Some other haematological parameters i.e. hemoglobin, hematocrit and other calculated haematological parameters MCV, MCH and MCHC were observed after feeding *L. leucocephala* leaf and seed by *C. puctatus* and *O.niloticus*.

Haemoglobin

In this investigation haemoglobin ranged from 11.71 to 12.06 g/dl for *C. punctatus* and 9.84 to 10.22 g/dl for *O. niloticus* in case of *L. leucocephala* leaf group. Other investigation of *L. leucocephala* seed group haemoglobin ranged from 11.82 to 12.06 g/dl for *C. punctatus* and 10.00 to 10.22 g/dl for *O. niloticus.* In all the investigation the highest number of hemoglobin found in the control group whereas the number was gradually decreasing with the elevating percentage of *L. leucocephala* leaf and seed group. Haemoglobin amount was10.52 $\pm$ 3.09 g/dl in *Oreochromis nilotica* reported by Bittencourt *et al.* (2003). According to Ramaswamy and Reddy (1978) it may be reach oxygen stimulating syndrome. Haemoglobin concentration of active *Clarias batrachus* was higher than a sluggish as reported by Chowdhury (1999). A decrease in the number of RBC, WBC and haemoglobin contents was observed in the blood of treated fish *C. punctatus* and *O. niloticus.* These results support the view of Srivastava and Agarawal (1979); Rahman (2001); Chowdhury and Pervin (2002) who have reported similar decreased in the blood parameters.

Hematocrit

In present observation hematocrit ranged from 47.19 to 47.64 for *C. punctatus* and 34.20 to 34.48 for *O. niloticus* in case of *L. leucocephala* leaf group. Another investigation of *L. leucocephala* seed food group hematocrit ranged from 47.10% to 47.64% for *C. punctatus* and 34.26% to 34.48% for *O. niloticus.* In all the investigation the highest number of hematocrit found in the control group whereas the number was gradually decreasing with the raising percentage of *L. leucocephala* leaf and seed group. Hematocrit amount was 31.85 $\pm$ 8.45% in *Oreochromis nilotica* reported by Bittencourt *et al.* (2003).

MCV

In present observation MCV ranged from 147.03 to 153.83fl for *C. punctatus* and 40.81 to 43.51fl for *O. niloticus* in case of *L. leucocephala* leaf group. Another investigation of *L. leucocephala* seed group MCV ranged from 147.03 to 153.16fl for *C. punctatus* and 40.81 to 41.78fl for *O. niloticus.* In all the investigations the lowest number of MCV found in the control group whereas the number was gradually increasing with the raising percentage of *L. leucocephala* leaf and seed group.

MCH

In present observation MCH ranged from 37.13 to 38.11pg for *C. punctatus* and for 12.00 to 12.36pg *O. niloticus* in case of *L. leucocephala* leaf group. Another investigation of *L. leucocephala* seed food group MCH ranged from for 37.19 to 38.44pg *C. punctatus* and 12.00 to 12.05pg for *O. niloticus.* In all the investigations the lowest number of MCH found in the control group whereas the number was gradually increasing with the raising percentage of *L. leucocephala* leaf and seed group.

There was a increasing trend found in the number of MCV and MCH observed in the blood of treated fish *C. punctatus* and *O. niloticus.* These results support the view of Bittencourt *et al.* (2003) who have reported that the erythrocytes count was positively correlated with hemoglobin and negatively correlated with MCV and MCH.

MCHC

In this investigation MCHC ranged from 24.80 to 25.31% for *C. punctatus* and 28.61 to 29.41% for *O. niloticus* in case of *L. leucocephala* leaf group. Other investigation of *L. leucocephala* seed group MCHC ranged from 25.09 to 25.31% for *C. punctatus* and 28.98 to 29.41% for *O. niloticus.* In all the observations the highest number of MCHC found in the control group whereas the number was gradually decreasing with the elevating percentage of *L. leucocephala* leaf and seed group.

Effects of *L. leucocephala* leaf and seed on biochemical parameters of *C. punctatus* and *O. niloticus*

Biochemical parameters *viz.* total protein, albumin, globulin, glucose, urea, creatinine and cholesterol were observed after feeding *Leucaena leucocephala* leaf and seed by *Channa puctatus* and *Oreochromis niloticus*.

Total Protein

In present observations total protein ranged from 2.02 to 2.53 g/dl for *C. punctatus* and 2.48 to 3.18 g/dl for *O. niloticus* in case of *L. leucocephala* leaf group. Another investigation of *L. leucocephala* seed group total protein ranged from 2.02 to 2.38 g/dl for *C. punctatus* and 2.48 to 3.03 g/dl for *O. niloticus.* In all the investigations the lowest amount of total protein found in the control group whereas the number was gradually increasing with the raising percentage of *L. leucocephala* leaf and seed group .The total protein range of 2 to 6g/dl was found in healthy juvenile rainbow trout *Salmo gairdneri.* The variation in the range may be seen possibly as an effect of environmental stretch. Too low level of total protein may be due to infectious disease or kidney damage and too high level may indicate impaired water balance (Wedemeyer and Yasutake, 1977).

Albumin

In present observations albumin ranged from 0.94 to 1.18 g/dl for *C. punctatus* and 1.15 to 1.49 g/dl for *O. niloticus* in case of *L. leucocephala* leaf group. Another investigation of *L. leucocephala* seed group albumin ranged from 0.94 to 1.12 g/dl for *C. punctatus* and 1.15 to 1.41 g/dl for *O. niloticus.* In all the investigations the lowest

amount of albumin found in the control group whereas the number was gradually increasing with the raising percentage of *L. leucocephala* leaf and seed group.

Globulin

In present observation ranged globulin from 1.08 to 1.35 g/dl for *C. punctatus* and 1.33 to 1.69 g/dl for *O. niloticus* in case of *L. leucocephala* leaf group. Another investigation of *L. leucocephala* seed food group globulin ranged from 1.08 to 1.25 g/dl for *C. punctatus* and 1.33 to 1.62 g/dl for *O. niloticus.* In all the investigations the lowest amount of globulin found in the control group whereas the number was gradually increasing with the raising percentage of *L. leucocephala* leaf and seed group.

Glucose

In present observation glucose ranged from 48.78 to 59.80 mg/dl for *C. punctatus* and 64.65 to 80.51 mg/dl for *O. niloticus* in case of *L. leucocephala* leaf food group. Another investigation of *L. leucocephala* seed food group glucose ranged from 48.78 to 60.49 mg/dl for *C. punctatus* and 64.65 to 78.86 mg/dl for *O. niloticus.* In all the investigations the lowest amount of glucose found in the control group whereas the number was gradually increasing with the elevating percentage of *L. leucocephala* leaf and seed group.The estimated rate of glucose in healthy juvenile rainbow trout *Salmo gairdeneri* showed the value 41to 151mg/dl. Too high level of glucose may be resulted from acute or chronic stress (Wedemeyer and Yasutake, 1977).

Urea

In present observation urea ranged from 7.73 to 9.59 mg/dl for *C. punctatus* and 8.48 to 10.20 mg/dl for *O. niloticus* in case of *L. leucocephala* leaf group. Another investigation of *L. leucocephala* seed group urea ranged from 7.73 to 9.43 mg/dl for *C. punctatus* and 8.48 to 9.93 mg/dl for *O. niloticus.* In all the investigations the lowest amount of urea found in the control group whereas the number was gradually increasing with the elevating percentage of *L. leucocephala* leaf and seed group.

Creatinine

In present observation creatinine ranged from 0.17 to 0.21 mg/dl for *C. punctatus* and 0.16 to 0.20 mg/dl for *O. niloticus* in case of *L. leucocephala* leaf group. Another investigation of *L. leucocephala* seed group creatinine ranged from 0.17 to 0.21 mg/dl for *C. punctatus* and 0.16 to 0.20 mg/dl for *O. niloticus.* In all the investigations the lowest amount of creatinine found in the control group whereas the number was gradually increasing with the elevating percentage of *L. leucocephala* leaf and seed group.

Cholesterol

In present observation cholesterol ranged from 64.32 to 73.95 mg/dl for *C. punctatus* and 116.20 to 137.40 mg/dl for *O. niloticus* in case of *L. leucocephala* leaf group. Another investigation of *L. leucocephala* seed group cholesterol ranged from 64.32 to 75.72 mg/dl for *C. punctatus* and 116.20 to 137.48 mg/dl for *O. niloticus.* In all the investigations the lowest amount of cholesterol found in the control group whereas the number was gradually increasing with the elevating percentage of *L. leucocephala* leaf and seed group. Sharma *et.al*. (1982) reported increased serum cholesterol in *Clarias batrachus* and *Heteropneustes fossilis* which were given congo red.

Different haematolocal parameters of *C. punctatus* and *O. niloticus* compared with control group and after feeding *L. leucocephala* leaf and seed

The Correlation coefficient of different haematolocal parameters of *C. punctatus* compared with control group after feeding different amount of 25%, 30%, 35% and 40% *L. leucocephala* leaf. The haematological parameters i.e. length, weight, TEC, TLC, thrombocyte, lymphocyte, monocyte, neutophil, eosinophil and basophil of *C. punctatus* and *O. niloticus* in 25%, 30%, 35% and 40% *L. leucocephala* leaf feeding groups were individually compared with control group. All the haematological parameters of 25% feeding group of *C. punctatus* was positively correlated with control group. Among the groups neutophil of 30%, monocyte and basophil of 35% and thrombocyte, lymphocyte, neutophil and basophil of 40% *L. leucocephala* leaf feeding group showed the negative correlation with the control group. Eosinophil of

all feeding groups, basophil of 25% and 30% and monocyte of 35% and 40% and of *O. niloticus* negatively correlated with the control group.

The haematological parameters i.e. length, weight, TEC, TLC, thrombocyte, lymphocyte, monocyte, neutophil, eosinophil and basophil of *C. punctatus* in 20%, 25%, 30%, and 35% *L. leucocephala* seed feeding groups were individually compared with control group. All the haematological parameters of 20% and 30% feeding groups of *C. punctatus* and 35% of *O. niloticus* was positively correlated with control group. Among the feeding groups monocyte of 25% and basophil of 35% of *C. punctatus* and lymphocyte and eosinophil of 30%, basophil of 20%, 25% and 30% of *O. niloticus* showed the negative correlation with the control group.

Different haematological parameters of *C punctatus* and *O. niloticus* compared with control group and after feeding *L. leucocephala* leaf and seed

The other haematological parameters i.e. TEC, Hb, Hct, MCV, MCH and MCHC of *C. punctatus* and *O. niloticus* compared with control group after feeding different amount of 25%, 30%, 35% and 40% *L. leucocephala* leaf group. Positive correlation was observed among all groups of *C. punctatus* compared with control group. A positive correlation was observed between 35% feeding group of *O. niloticus* and control group. The MCH of 25%, 30% and 40% and MCV of 40% feeding groups of *O. niloticus* negatively correlated with the control group.

The other haematological parameters i.e. TEC, Hb, Hct, MCV, MCH and MCHC of *C. punctatus* compared with control group after feeding different amount of 20%, 25%, 30%, and 35% *L. leucocephala* seed group. A positive correlation was observed among all groups of *C. punctatus* compared with control group except MCHC of 35%. The MCV of 20%, 25% and 30% and MCH of 20% feeding groups of *O. niloticus* were negatively correlated whereas 35% feeding group was positively correlated with the control group.

Different biochemical parameters of *C. punctatus* and *O. niloticus* compared with control group and after feeding *L. leucocephala* leaf and seed

The different biochemical parameters i.e. total protein, albumin, globulin, glucose, urea, creatinine and cholesterol of *C. punctatus* and *O. niloticus* compared with control group after feeding different amount of 25%, 30%, 35% and 40% *L. leucocephala* leaf group. A positive correlation was observed among all groups of *C. punctatus* and *O.niloticus* compared with control group.

The different biochemical parameters i.e. total protein, albumin, globulin, glucose, urea, creatinine and cholesterol of *C. punctatus* and *O. niloticus* compared with control group after feeding different amount of 20%, 25%, 30% and 35% *L. leucocephala* seed group. Positive correlation was observed among all groups of *C. punctatus* and *O. niloticus* compared with control group.

Effects on different haematological and biochemical parameters of *C punctatus* and *O. niloticus* after feeding *L. leucocephala* leaf and seed

The effects of *L. leucocephala* leaf and seed on different haematological, other haematological and biochemical parameters of *C. punctatus* and *O. niloticus* determined by analysis of variance. To determine the levels of significance among different percentage of *L. leucocephala* leaf and seed on *C. punctatus* and *Oreochromis niloticus,* Tukey HSD test was carried out for multiple comparisons. In case of *L. leucocephala* leaf, among the haematological parameters (TEC, TLC, thrombocyte, lymphocyte, monocyte, neutophil, eosinophil and basophil) only lymphocyte and eosinophil were highly significant in *C. punctatus* whereas thrombocyte, lymphocyte, neotrophil and eosinophil were highly significant in *O. niloticus* at the 5% level of significance. In case of *L. leucocephala* seed, weight and eosinophil were highly significant in *O. niloticus* at the 5% level of significance.

The effects of feeding different amount of *L. leucocephala* leaf and seed to the different other haematological parameters i.e. Hb Hct, MCV, MCH and MCHC of *C. punctatus* and *O. niloticus* were not statistically significant except Weight of *O. niloticus* of seed group which was statistically significant.

In case of *L. leucocephala* leaf, among the biochemical parameters (total protein, albumin, globulin, glucose, urea, creatinine and cholesterol) only total protein, albumin, globulin were highly significant in *C. punctatus* whereas total protein, albumin were highly significant in *O. niloticus* at the 5% level of significance but others were not statistically significant. In case of *L. leucocephala* seed, albumin of *C. punctatus* whereas weight of *O. niloticus* were highly significant at the 5% level of significance and total protein of *C. punctatus* and Glucose of *O. niloticus* were significant at the 10 % level of significance.

Effects on number of Eggs in the ovary of *C. punctatus* and *O. niloticus* after feeding *L. leucocephala* leaf and seed

The effects of feeding different amount of *L. leucocephala* leaf and seed on the number of eggs of *C. punctatus* and *O. niloticus* were highly significant . Tukey HSD test was carried out for multiple comparisons of the different percentage of *L. leucocephala leaf* and seed on the number of eggs of *C. punctatus* and *O. niloticus.* In case of *C. punctatus,* it was found that feeding 30%, 35% and 40% leaf whereas 30% and 35% seed were significantly different from the control at the 5% level of significance. In case of *O. niloticus* feeding 25%, 30%, 35% and 40% leaf whereas 25%, 30% and 35% seed were highly significant at the 5% level of significance.

Histopathological changes

The histopathological changes due to the effect of *Leucaena leucocephala* leaf and seed on *Channa punctatus* and *Oreochromis niloticus* was observed. The histopathological changes specially observed on tissues i.e. eye, gill, muscle, stomach, intestine, liver, kidney and gonad of the fishes. The experiment of feeding *Leucaena leucocephala* leaf and seed were conducted during the month of April 2008 to September 2012.

Eye

The eye of fish is composed of three layers; an outer sclera-corneal layer, a medial uveal layer, and an inner retinal layer. Histopathological changes in the eye perforation of sclera and gas bubbles in sclera, gas bubbles and haemorrhages in choroid were the common problems of the *C. punctatus* and *O.niloticus* observed in different groups of *L. leucocephala* leaf and seed groups. The protruding eye is enlarged, but generally no pathological changes in the tissues can be seen, neither can any parasites be found. Prof. Dr. Bruno Hofer states that it may be caused experimentally by blows or pressure on the head, but it can be hardly be likely that such is the natural cause of this disease (Van Duijin 1967). About the year 1930, Schaperclaus investigated several cases of Exophthalmia in fishes living in ponds and found that these were caused by *Aeromonas punctata* and a variety which he distinguished as *forma sacrowiensis.* Millemann and Knapp 1970 described exophthalmos and damage to the retina and cornea due to *Nanophyetus salmincola*, the “salmon poisoning” trematode. Orbital hemorrhages were seen. Kluge 1965, described an exophthalmos produced in mollies by a flavobacterium inducing granulomas in the optic nerve, orbit and retina. About 80% of the fish had unilateral or bilateral exophthalmos.

Gill

The main gill structure included gill bars and gill lamellae. The gill lamellae were attached to the cartilaginous gill bar. In the experimental groups distorted gill cartilages in filaments ovserved in 25% and 30% (fig.7, 8) *L leucocephala* leaf groups of *C. punctatus* and *O. niloticus.* Hypertrophy of epithelium on gill lamellae

observed in 35% and 40% (fig. 9, 10) and edema at bases of lamellae in 40% *L leucocephala* leaf food group (fig. 10) of *C. punctatus* whereas curling of secondary lamellae, of gill lamellae in 35% (fig. 54) and fusions of gill lamellae and swollen epithelium cells observed in 40% (fig. 55) *L. leucocephala* leaf group of *O. niloticus.* Distorted gill cartilages in filaments in 20% and 25% (fig.97, 98), hypertrophy of epithelium on gill lamellae found in 30% and 35% (fig. 99, 100) *L. leucocephala* seed group of *C. punctatus* whereas distorted gill cartilages in filaments in 20% (fig. 142), curling of secondary lamellae in 25% (fig. 143), and swollen epithelium cells in 30% (fig. 144) and fusions of gill lamellae found in 35% (fig. 145) *L. leucocephala* seed group of *O. niloticus*. Halver, Ashley and Smith (1969) studied the ascorbic acid requirements in coho salmon and rainbow trout, found distorted and twisted cartilage of gill filaments in fish fed a diet containing 5mg ascorbic acid per 100 grams of diet for 24 weeks. Wood and Yasutake (1957) described histopathological changes in the gill associated with nutritional gill disease. The authors inferred that shortened, swollen lamellae with very little structural distortion and hyperplasia of basal epithelium, distal fusion of filaments and complete fusion of adjacent filaments are present in fish fed a nutritionally deficient diet. On the other hand, Ghittino (1967) hypotherized that dusty, dry feeds rather than pantothenic acid deficiencies produce gill disease. In this study, fish developed gill disease although they were fed a diet containing adequate B-complex ingredients.

Muscle

The multiple nuclei lay at the periphery of the muscle fibers. Groups of the fibers were surrounded by a large pale area with loose connective tissues, the perimysium. The whole muscle or muscle bundle was surrounded by a denser connective tissue, the epimysium. Histopathological changes in the muscle, fungal hypa, vacuolar degeneration in muscle bundles, splitting of muscle fibers, degeneration in muscle bundles, these problems were common to all groups of *C. punctatus* and *O.niloticus* after feeding feeding *L. leucocephala* leaf and seed.

Stomach

Stomach showed four basic layers from inner to outer mucosa, submucosa, muscularis and serosa. Histopathological changes in the stomach, Granuloma in submucosal or mucosal layer found in all groups of *C. punctatus* and *O. niloticus*

after feeding *L. leucocephala* leaf and seed. Little work had been done on the etiology of nephrocalcinosis, although it has been speculated that it is just a species modification of visceral granuloma (Dunbar and Herman 1971). Water hardness may play a role. Workers in France, Besse *et. al.* 1968 found that after changing food supplies there was a marked reduction in the inadence of the disease, which suggests that handling and storage of food may be a key factor Aktar (1994) noted that the blood capillaries in the submucosal region of *H. fossilis* stomach were distorted and the epithelial cells are slightly degeneration by 50% ethyl alcohol extract (dry root bark) of *B. acutangula*.

Intestine

The intestine exhibits four basic layers from inner to outer: mucosa, submucosa, muscularis and serosa. Histopathological changes in the intestine, irregular shapes in mucosal folds, Less complex array of mucosal folds, complex array of mucosal folds, some degenerative changes in submucosal layer and Granuloma in muscularis layer found in different groups of *C. punctatus* and *O. niloticus* after feeding *L. leucocephala* leaf and seed. Dr. Herman and Mr. Dunbar (1971) followed the course of visceral granuloma rather closely from the very earliest stages of the disease with no evidence of parasites. Dr. Herman and Mr. Dunbar (1971) followed the course of visceral granuloma rather closely from the very earliest stages of the disease with no evidence of parasites. Visceral granuloma has been shown to be a diet-related disease and a strong but not complete correlation has been demonstrated between the incidence of visceral granuloma and the presence of cottonseed meal in the diet (Dunbar and Herman 1971). This disease resembles sarcoidosis, which is considered by Israel and Sons (1961) to be a hyper sensitivity reaction to some unknown widespread excitant. Aktar (1994) reported degeneration of the lining of epithelium, rupture of mucous membrane and also detected few small vacuoles in the tunica muscularis of the intestine caused by the 50% ethyl alcohol extract of dry root bark of *B. acutangula*.

Liver

The liver was composed of numerous polyhedral hepatic cells with prominent and granular cytoplasm and distinct central nucleus. The main hepatic structure included glisson's capsule, hepatic parenchyma cells which were separated by blood sinusoids and blood vessels. Histopathological changes in the liver, vacuolation of parenchyma, hemorrhages, vacuolation and congestion found in different groups of *C. punctatus* and *O. niloticus* after feeding *L. leucocephala* leaf and seed. Lowe (1967) observed clusters of large vacuoles in the deep periportal pancreatic acinar tissue in the liver. The vacuoles appeared to be intracellular, having caused hypertrophy of the acinar cells. He exposed 0.1 ppm sevin for five months (LD50 12days=1.0 ppm) in flowing sea water and have found a possible change in the introheptic pancreatic tissue in the liver. Vacuolation or hepatocytes degeneration in hypothecates in *A. testudineous* was commonly found with Furadan and Padan treatments (Kabir and Ahmed,1979). At 3.36 and 1.68kg/lha dosage Furadan caused degeneration of parenchymal cells when exposed for 7 days. Centrolobular damage was recorded at 15 days exposure to 3.36kg/ha. Focal areas of necrotic tissue were present at 15 days with 1.68kg/ha dosage. At .84 dosage cytoplasmic degeneration was present at 7 days exposure (Kabir and Ahmed, 1979). Aktar (1994) reported slightly scattered distribution of parenchymatous cells and presence of moderately vacuolated hepatic cells caused by the 50% ethyl alcohol extract of dry root bark of *Barringtonia acutangula*.

Kidney

The kidney contained nephrons and interstitial lymphoid tissue. Nephrons consists of two parts, the Bowman's capsule and the renal tubules. Histopathological changes in the kidney pigment deposits occur in the tissues, tubules filled with deposits, Degeneration in the intertubular tissue found in different groups of *C. punctatus* and *O. niloticus* after feeding *L. leucocephala* leaf and seed. Konar (1975) noted degeneration of renal tubules in carp exposed to DDVP. Similar damage of the epithelial cells was observed by the author in Furadan and Padan treated fishes. Renal histopathology of fishes affected by cadmium, copper, zinc, salts of metals and other pollutants were recorded by a number of workers. Eisler *et. al.* (1972), Eisler and Garden, (1973), Akter (1994) reported degeneration of the epithelial cells

of the proximal tubules, slight necrosis of renal tubules and glomerule, focal areas of swollen epithelion cells with granular concentrated clear cytoplasm and the interstitial tissue between the tubules is packed with very few nuclear haematopoietic cells caused by the 50% ethyl alcohol extract of dry root bark of *B. acutangula.*

Testis

In matured fish the testicular lobules were filled up with spermatozoa and spermatids. Histopathological changes in testes, some spermatogonia, some spermocyte, some spermatid and some spermatozoa found in different groups of *C. punctatus* and *O. niloticus* after feeding *L. leucocephala* leaf and seed. Granuloma found in 30% and 35% seed group of *C. punctatus* (fig.129 and 130), (Plate-26) whereas Granuloma found in 35% seed group of *O. niloticus.* There found a decreasing trend of spermatid and spermatozoa with the increasing level of *L. leucocephala* leaf and seed food amount. On the other word there was an inverse relationship between the *L. leucocephala* leaf and seed level and spermaid and spermatozoa.

Ovary

The mature ovary contained a large number of tertiary oocytes. The secondary oocytes were developed into matured tertiary oocytes. Primary and secondary oocytes were present with a few oogonia. Histopathological changes in ovary, tertiary oocyte, a few secondary oocytes and a few primary oocytes and eggs were in absorptive condition were present in all groups of *C. punctatus* and *O. niloticus* after feeding *L. leucocephala* leaf and seed. The histopathological changes in ovaries of *C punctatus* and *O. niloticus* in this experiment at 25%, 30%, 35%, 40% *L. leucocephala* leaf and 20%, 25%, 30% and 35% *L. leucocephala* seed groups showed the decreasing trend of tertiary oocyte with the increasing level of seed. Santiago *et. al.* (1988) determined the effects of dietary *Leucaena* leaf meal on reproductive performance and growth of Nile tilapia. The experiment was conducted with sexually muture Nile tilapia fed with a test diet which had *Leucaena* leaf meal as the protein source for 24 weeks. Fish fed with the *Leucaena* diet lose some weight and had significantly low ($p < 0.05$) gonadosomatic index and fry production compared to those fed with the control diet. *O. niloticus* showed oocyte in yolk vesicle stage (in normal structure) beside many oocytes in ripe stage. also, clear

histological lesions were detected in ovary as coagulation necrosis in yolk granules, separation of follicular layers, degeneration in oocyte (atresia) and focal area of necrosis in ovarian interstitial tissues where the Ovarian interstitial tissues were found to consist of interstitial cells, adipose cells, yolk granules and blood capillaries. Degenerative changes (atretic state) were observed in the ripe oocytes in ovary of *O. niloticus*.

SUMMARY

In Bangladesh *Leucaena leucocephala* (Lam. de Wit) tree mainly used as animal fodder, as are being used in other parts of the world. *Leucaena* has its origin in Central America and the plant has been introduced in Bangladesh in the late 60's. *Leucaena* seed is rich in protein and carbohydrate but low in fat. The dairy, poultry and culture fish have been supplied with the leaves for its high protein contents. The leaves of this tree are used as a component of the meal, or as the source of protein to animals.

L. leucocephala has been found to be a valuable forage and feed for our live stocks (Rahman and Dewan, 1988). Its leaf and seed meals are in wider use in many tropical countries including Bangladesh. Many reports have discussed its side effects in different domesticated animals and chicken. Rahman and Dewan, 1988 described pathological effects of *Leucaena* seed meal in the duck *(Anas platythyncha domestica)* in Bangladesh. Although some works have been done on the land animals including poultry birds and fishes regarding the *L. leucocephala* meal but a little work have so far been done with the fish.

Keeping the prospect of the uses of *L. leucocephala* in the aquaculture industry, the present research is taken to assess the effect of *L.leucocephala* on two species of culture fish, viz. *Channa punctatus* and *Oreochromis niloticus*.

Objectives of the presest study

- Investigating the chemical composition of the leaves and seeds of the *L. leucocephala* (Lam. de Wit).
- Evaluating the usages of leaves and seeds of *L. leucocephala* as fodder of fish.
- To work out the safety dose ranges on the species studied.
- Observing the behavioral and physical changes of *C.punctatus* (Bloch & Schneider) and *O. niloticus* (Linnaeus) fed different amount *o*f *L. leucocephala* leaves and seeds.
- Observing changes in haematological parameters (TEC, TLC and differential count, hemoglobin, hematocrit, MCV, MCH and MCHC) caused due to feeding of *L. Leucephala* leaves and *s*eeds.

- Changing in blood serum parameters i.e. total protein, globulin, albumin, glucose, urea, creatinine and cholesterol after feeding *L. leucocephala* leaves and seeds.
- Histopathological changes of the vital organs, i.e .tissues of eye, gill, muscle, stomach, intestine, liver, kidney and gonad of *C. punctatus* and *O. niloticus* after feeding *L. leucocephala* leaves and seeds.

The experimental fishes were *Channa punctatus* (Bloch &Schneider, 1801), family Channidae and *Oreochromis niloticus* (Linneaeus, 1758), family Cichlidae. Feed was prepared 2% of the total body weight of each group separately. Different amount of *L. leucocephala* leaf (25%, 30%, 35% and 40%) and seed (20%, 25%, 30% and 35%) were mixed with meshed small fish and flour. Hand dough of small balls were prepared to feed the experimental fishes. The experiment was continued for 60 days The experimental trial was replicated thrice for each amount of leaf and seed.

During the present study, blood was drawn from cardiac puncture. Haematological parameters: Erythrocyte (TEC), Leucocyte (TLC), Haemoglobin (Hb), Hematocrit (Hct), MCV, MCH and MCHC were determined by rolling the blood sample in an automatic Cell Counter Mythic (France). For the differential count of the TLC blood smears were made and stained following Klontz (1972), and counting was done with the help of a leucocytometer (Digital 9key. Erma Inc.). Biochemical parameters i.e. total protein, albumin, globulin, glucose, urea, creatinine and cholesterol were determined by analyzing the blood sample in an Automatic Analyzer, Dimension RXL Max DADE Behring Siemen (U.S.A.). Histopathological study: The histopathological slides were prepared at Bangabandhu Sheikh Mujib Madical University Pathology laboratory. Photomicrograph taken by camera Olympus CH30 at 10 X (10-40) and (4-5) X 10 magnification.

All data were presented as mean ± standard deviation. The haemamatological and biochemical parameters of blood were compared between each experimental group and control group by Pearson correlation coefficient and graphs were drawn. The means of haematological and biochemical variables were compared among the experimental food groups were showed by Analysis of variance (ANOVA) followed by Tukey Honest Significant Difference (HSD) Post hoc for multiple comparisons.

Statistical software SPSS version 20 was used to analyze the data. The level of significance was set at 0.05.

For the phyto-chemical investigation of *Leucaena leucocephala* plant parts leaf, seed extracts were chromatographed on silica gel 60G F_{254} as a solid phase and different solvent system as mobile phase representing the active components of the alcoholic extract was determined qualitatively using various test reagents. The test results reviewed that the each and every parts of *Leucaena leucocephala* contains alkaloid, flavonoid, terpinoid , steroid , saponins. The alkaloid which is present in the different species of *Leucaena leucocephala* was characterized as mimosine and dihydroxy pyridine are toxic compounds which are regarded as anti-nutritional factors.

During the experiment, the behavioral, physical, haematological and histopathological changes were due to *L. leucocephala* leaf and seed food on *C. punctatus* and *O. niloticus* respectively in different amount for two months. The temperature of the water was 29°C ± 1.5°C and pH value was found to be 7.25 ± 0.45 during the experimental period.

The effects of *Leucaena leucocephala* leaf on *Channa punctatus* and *Oreochromis niloticus* were observed in different amount i.e. 25%, 30%, 35% and 40% of total food which was calculated 2% of the total body weight. In another case the effects of *L. leucocephala* seed food on *Channa punctatus* and *Oreochromis niloticus* were observed in different amount 20% 25%, 30%, and 35% of total food, which was also calculated 2% of the total body weight. In both cases the *L leucocephala* leaf and seed food was considered as behavioral, physical, haematological and histopathological changes observed in *C. punctatus* and *O. niloticus* in different amount. Total sixteen study groups were taken and four groups were kept as control. Ten fishes were kept for each group.

The effect on behavior of the fish *C. punctatus* due to *L. leucocephala* leaf food was loss of appetite and slow in movement. In each group this phenomena was exhibited in several fishes and was compared with the control group. The effect on behavior of the fish *O. niloticus* due to *L. leucocephala* leaf food was loss of appetite, loss of normal movement and erratic swimming. In each group this phenomena was exhibited in several fishes and was compared with the control group.

The effect on behavior of the fish *C. punctatus* due to *L. leucocephala* seed food was loss of appetite and loss of movement, excessive mucous secretion and accumulation on the skin and gills. The effect on behavior of the fish *O. niloticus* due to *L. leucocephala* seed food was erratic swimming, loss of normal movement and loss of appetite. In each group this phenomena was exhibited in several fishes and was compared with the control group.

The physical changes, which were observed after feeding different amount of 25%, 30%, 35% and 40% *L. leucocephala* leaf in four groups of *C. punctatus* were lesion on caudal region, erosion of fins, puffed and loose scales, lesions on skin and muscle and scoliosis, exophthalmus. The physical changes, which were observed after feeding different amount of 25%, 30%, 35% and 40% *L. leucocephala* leaf groups of *O. niloticus* were lesion on skin and erosion on caudal fin, exophthalmus, lesions on skin, puffed and loose scales, fading of body color and change in pigment. These changes were exhibited in each group of fishes.

The physical changes, which were observed after feeding different amount of 20%, 25%, 30% and 35% *L leucocephala* seed groups were lesions on skin, puffed and loose scales, lesions on caudal region, exophthalmus, scoliosis and abnormality in egg structure. The physical changes, which were observed after feeding different amount of 20%, 25%, 30% and 35% *L. leucocephala* seed groups were erosion of caudal fin, lesion on skin, puffed and loose scales, fading of body color and change in pigment, exophthalmus, curvature in preopercle bone. These changes were exhibited in each group of fish.

The effects of *L. leucocephala* leaf (25%, 30%, 35% and 40%) and *L. lucocephala* seed (20%, 25%, 30% and 35%) on the blood of *C. punctatus* and *O. niloticus* were observed. The morphological elements of the blood of *C. punctatus* and *O. niloticus* which were present in the blood *viz.* erythrocytes, lymphocytes, monocytes, thrombocytes, neutrophils, eosinophils and basophils were observed. There were no detectable changes or abnormalies found in the morphology of blood cells. But there were some remarkable changes found in the number of different cells.

In all the investigation the highest number of erythrocyte found in the control group whereas the number was gradually decreasing with the raising percentage of

Leucaena leucocephala leaf and seed group. In other observation the highest number of leucocyte found in the control group of both cases whereas the number was gradually decreasing with the raising percentage of *L. leucocephala* leaf and seed food group.

In present obsevation the highest number of leucocyte found in the control group of both cases whereas the number was gradually decreasing with the raising percentage of *L. leucocephala* leaf and seed food group.

In all the invastigation the highest number of lymphocyte found in the control group whereas the number was gradually decreasing with the elevating percentage of *L. leucocephala* leaf and seed food group.

In present observation the lowest percentage of monocyte found in the control group whereas the highest percentage was found in 30% *L. leucocephala* leaf group. The percentage of monocyte was gradually increasing with the raising percentage of *L. leucocephala* leaf food group 25%, 30%, whereas the percentage was gradually decreasing in 35% 40% group. The lowest percentage of monocyte found in the control group of *C.punctatus* whereas the percentage was gradually increasing with the raising percentage of *L. leucocephala* seed food. The highest percentage of monocyte found in the control group *O. niloticus* whereas the percentage was gradually decreasing with the raising percentage of *L. leucocephala* seed 20%, 25%, 30% except 35% .

In present observation the highest percentage of neutrophil found in the 25% *L. leucocephala* leaf group of *C. punctatus* whereas the percentage was gradually decreasing with the raising percentage of *L. leucocephala* leaf group. The lowest percentage of neutrophil found in the control group *O. niloticus* whereas the percentage was gradually increasing with the raising percentage of *L. leucocephala* leaf group 25%, 30%, 35% except 40. In present observation the lowest percentage of neutrophil found in the control group whereas the percentage was gradually increasing with the raising percentage of *L. leucocephala* seed. In present investigation the lowest percentage of neutrophil found in the control group of *O. niloticus* whereas the highest percentage found in 20% *L. leucocephala* seed group. The percentage of neutrophil was gradually decreasing with the elevating percentage of *L. leucocephala* seed food 25% and 30% except 35%.

In all the investigation the lowest number of eosinophil found in the control group whereas the number was gradually increasing with the elevating percentage of *L. leucocephala* leaf and seed group except 40% leaf group of *C. punctatus* and 35% seed group of *O. niloticus*.

In present observation the lowest percentage of basophil found in the control group of *C. punctatus* whereas the percentage was gradually increasing with the elevating percentage of *L. leucocephala* leaf group 25%, 30%, 40% except 35%. Tthe highest percentage of basophil found in the control group of *O. niloticus* whereas the percentage was gradually decreasing with the raising percentage of *L. leucocephala* leaf group. In present investigation the highest percentage of basophil found in 30% of seed group of *C. punctatus* whereas the percentage was gradually decreasing with the elevating percentage of *L. leucocephala* seed group 20%, 25%, 35% except 30% . The lowest percentage of basophil found in the control group of *O. niloticus* whereas the percentage was gradually increasing with the raising percentage of *L. leucocephala* seed group.

In all the investigation the highest number of haemoglobin, hematocrit and MCHC found in the control group whereas the number was gradually decreasing with the elevating percentage of *L. leucocephala* leaf and seed group. In other case the lowest number of MCV and MCH found in the control group whereas the number was gradually increasing with the raising percentage of *L. leucocephala* leaf and seed group.

The effects of *L. leucocephala* leaf (25%, 30%, 35% and 40%) and *L. leucocephala* seed (20%, 25%, 30% and 35%) on biochemical parameters *viz.* total protein, albumin, globulin, glucose, urea, creatinine and cholesterol of *C. puctatus* and *O. niloticus* were observed. In all the investigations the lowest amount of total protein, albumin, globulin, glucose, urea, creatinine and cholesterol found in the control group whereas the number was gradually increasing with the raising percentage of *L. leucocephala* leaf and seed group.

The Correlation coefficient of different haematolocal parameters of *C. punctatus* compared with control group after feeding different amount of 25%, 30%, 35% and 40% *L. leucocephala* leaf. The haematological parameters i.e. length, weight, erythrocytes, thrombocytes, lymphocytes, monocytes, neutrophils, eosinophils and

basophils of *C. punctatus* and *O. niloticus* in 25%, 30%, 35% and 40% *L. leucocephala* leaf feeding groups were individually compared with control group. All the haematological parameters of 25% leaf group of *C. punctatus* was positively correlated with control group. Among the groups neutophil of 30%, monocyte and basophil of 35% and thrombocytes, lymphocytes, neutrophils, and basophils of 40% *L. leucocephala* leaf group showed the negative correlation with the control group. Eosinophil of all leaf groups, Basophil of 25% and 30% and Monocyte of 35% and 40% of *O. niloticus* negatively correlated with the control group.

The haematological parameters of *C. punctatus* and *O. niloticus* in 20%, 25%, 30%, and 35% *L. leucocephala* seed groups were individually compared with control group. All the haematological parameters of 20% and 30% seed groups of *C. punctatus* and 35% of *O. niloticus* was positively correlated with control group. Among the feeding groups monocyte of 25% and basophil of 35% of *C. punctatus* and lymphocyte and eosinophil of 30%, basophil of 20%, 25% and 30% of *O. niloticus* showed the negative correlation with the control group.

The other haematological parameters i.e. TEC, Hb, Hct, MCV, MCH and MCHC of *C. punctatus* and *O. niloticus* compared with control group after feeding different amount of 25%, 30%, 35% and 40% *L. leucocephala* leaf. Positive correlation was observed among all groups of *C. punctatus* compared with control group. Positive correlation showed between 35% leaf group of *O. niloticus* and control group whereas negative correlation showed MCH of 25%, 30% and 40% and MCV of 40% leaf groups of *O. niloticus* with the control group.

The other haematological parameters compared with control group after feeding different amount of 20%, 25%, 30%, and 35% *L. leucocephala* seed. A positive correlation was observed among all groups of *C. punctatus* compared with control group except MCHC of 35%. (Table-9) The MCV of 20%, 25% and 30% and MCH of 20% seed groups of *O. niloticus* were negatively correlated whereas 35% seed group was positively correlated with the control group.

The different biochemical parameters i.e. total protein, albumin, globulin, glucose, urea, creatinine and cholesterol of *C. punctatus* and *O.niloticus* compared with control group after feeding different amount of 25%, 30%, 35% and 40% *L.*

leucocephala leaf. A positive correlation was observed among all groups of *C. punctatus* and *O.niloticus* compared with control group.

The different biochemical of *Channa punctatus* and *O.niloticus* compared with control group after feeding different amount of 20%, 25%, 30% and 35% *L. leucocephala* seed group. Positive correlation was observed among all groups of *C. punctatus* and *O. niloticus* compared with control group.

The effects of *L. leucocephala* leaf and seed on different haematological, other haematological and biochemical parameters of *C. punctatus* and *O. niloticus* determined by analysis of variance. To determine the levels of significance among different percentage of *L. leucocephala* leaf and seed on *C. punctatus* and *Oreochromis niloticus,* Tukey HSD test was carried out for multiple comparisons. In case of *L. leucocephala* leaf, among the haematological parameters (length, weight, erythrocytes, thrombocytes, lymphocytes, monocytes, neutrophils, eosinophils and basophils) only lymphocyte and eosinophil were highly significant in *C. punctatus* whereas thrombocyte, lymphocyte, neotrophil and eosinophil were highly significant in *O. niloticus* at the 5% level of significance. In case of *L. leucocephala* seed only thrombocyte was significant in *C. punctatus* at 10% level of significance whereas weight and eosinophil were highly significant in *O. niloticus* at the 5% level of significance.

The effects of feeding different amount of *L. leucocephala* leaf and seed to the different other haematological parameters i.e. Hb Hct, MCV, MCH and MCHC of *C. punctatus* and *O. niloticus* were not statistically significant except weight of *O. niloticus* of seed group which was statistically significant.

In case of *L. leucocephala* leaf, among the biochemical parameters (total protein, albumin, globulin, glucose, urea, creatinine and cholesterol) only total protein, albumin, globulin were highly significant in *C. punctatus* whereas total protein and albumin were highly significant in *O. niloticus* at the 5% level of significance but others were not statistically significant. In case of *L. leucocephala* seed, albumin of *C. punctatus* whereas weight of *O. niloticus* were highly significant at the 5% level of significance and total protein of *C. punctatus* and glucose of *O. niloticus* were significant at the 10 % level of significance.

The effects of feeding different amount of *L.leucocephala* leaf and seed on the number of eggs of *C. punctatus* and *O. niloticus* were highly significant. Tukey HSD test was carried out for multiple comparisons of the different percentages of *L. leucocephala leaf* and seed on the number of *C. punctatus* and *O. niloticus.* In case of *C. punctatus* it was found that feeding 30%, 35% and 40% leaf whereas 35% and 40% seed were significantly different from the control. In case of *O. niloticus* feeding 25%, 30%, 35% and 40% leaf whereas 30%, 35% and 40% seed were significantly different from the control.

The histopathological changes due to the effect of *Leucaena leucocephala* leaf and seed on *Channa punctatus* and *Oreochromis niloticus* was observed. The histopathological changes specially observed on eye, gill, muscle, stomach, intestine, liver, kidney and gonad of the fishes. The experiment of feeding *L. leucocephala* leaf and seed was conducted during April 2008 to September 2012.

Histopathological changes in the eye perforation of sclera and gas bubbles in sclera, gas bubbles and haemorrhages in choroid were the common problems of the *C. punctatus* and *O. niloticus* observed in different groups of *L. leucocephala* leaf and seed groups.

In the experimental groups distorted gill cartilages in filaments ovserved in 25% and 30% *L leucocephala* leaf groups of *C. punctatus* and *O.niloticus.* Hypertrophy of epithelium on gill lamellae ovserved in 35% and 40% and edema at bases of lamellae in 40% *L. leucocephala* leaf food group of C. *punctatus* whereas curling of secondary lamellae, of gill lamellae in 35% and fusions of gill lamellae and swollen epithelium cells ovserved in 40% leaf group of *O. niloticus.* Distorted gill cartilages in filaments in 20% and 25%, hypertrophy of epithelium on gill lamellae found in 30% and 35% seed group of *C. punctatus* whereas distorted gill cartilages in filaments in 20%, curling of secondary lamellae in 25% and swollen epithelium cells in 30% and fusions of gill lamellae found in 35% *L. leucocephala* seed group of *O. niloticus*.

Histopathological changes in the muscle, fungal hypa, vacuolar degeneration in muscle bundles, splitting of muscle fibers, degeneration in muscle bundles, these problems were common to all groups of *C. punctatus* and *O. niloticus* after feeding *L. leucocephala* leaf and seed.

Histopathological changes in the stomach, granuloma in submucosal or mucosal layer found in all groups of *C. punctatus* and *O. niloticus* after feeding *L. leucocephala* leaf and seed.

Histopathological changes in the intestine, irregular shapes in mucosal folds, Less complex array of mucosal folds, complex array of mucosal folds, some degenerative changes in submucosal layer and Granuloma in muscularis layer found in different groups of *C. punctatus* and *O. niloticus* after feeding *L. leucocephala* leaf and seed.

Histopathological changes in the liver, vacuolation of parenchyma, hemorrhages, vacuolation and congestion found in different groups of *C. punctatus* and *O. niloticus* after feeding *L. leucocephala* leaf and seed.

Histopathological changes in the kidney pigment deposits occurred in the tissues, tubules filled with deposits, Degeneration in the intertubular tissue found in different groups of *C. punctatus* and *O. niloticus* after feeding *L. leucocephala* leaf and seed.

Histopathological changes in testis, some spermatogonia, some spermocyte, some spermatid and some spermatozoa found in different groups of *C. punctatus* and *O. niloticus* after feeding *L. leucocephala* leaf and seed. Granuloma found in 30% and 35% seed group of *C. punctatus* whereas Granuloma found in 35% seed group of *O. niloticus.*There found a decreasing trend of spermatid and spermatozoa with the increasing level of *L. leucocephala* leaf and seed amount. On the other word there was an inverse relationship between the *L. leucocephala* leaf and seed level and spermaid and spermatozoa.

Histopathological changes in ovary, tertiary oocyte, a few secondary oocytes and a few primary oocytes and eggs are in absorptive condition were present in all groups of *C. punctatus* and *O. niloticus* after feeding *L. leucocephala* leaf and seed. The histopathological changes in ovaries of *C punctatus* and *O. niloticus* in this experiment at 25%, 30%, 35%, 40% *L.leucocephala* leaf and 20%, 25%, 30% and 35% seed groups showed the decreasing trend of tertiary oocyte with the increasing of percentages of *L. leucocephala* seed level.

CONCLUSION

Fish species in culture system has high dietary protein requirement. Recently, the high cost and short supply of fish meal has made it necessary to substitute with cheaper plant protein sources. Effects of *Leucaena leucocephala* (Lam. de Wit) leaf and seed on *Channa punctatus* (Bloch and Schneider) and *Oreochromis niloticus* (Linnaeus) were observed in respect of behavior, physical, haematological and histopathological changes. In present investigation in behavioral changes, loss of appetite and loss of movement were found in both species. The physical changes that found in *C. punctatus* due to *L. leucocephala* leaf and seed were as same as *O. niloticus,* except scoliosis. In haematological changes, the number of TEC, TLC, haemoglobin, hematocrit and MCHC had the decreasing trend with the increasing amount of *L. leucocephala* leaf and seed whereas the increasing trend found in the case of MCV and MCH observed in both species. The biochemical parameters of all groups of *C. punctatus* and *O. niloticus* showed positive correlation with the control group. The histopathological changes found in tissues i.e. eye, gill, muscle, stomach, intestine, liver, kidney and gonad of the fishes. In the present study the effects were observed specially at higher percentages of the *L. leucocephala* leaf and seed groups. But at the lower dose minimum effects were observed in *C. punctatus* and *O. niloticus* maintaining their normal growth pattern. The effects of feeding different amount of *L. leucocephala* leaf and seed on the number of eggs of *C. punctatus* and *O. niloticus* were highly significant at the 5% level of significance. The present study shows that the low amount of 25% to 30% of *Leucaena Leucocephala* leaf and 20% to 25% of seed can be used as meal in fish feed. It needs to be removed the harmful components from the leaves and seeds of *L. leucocephala,* if higher level of supplementation given in feed. If the harmful components from the leaves and seeds of *L. Leucocephala* can be removed then *L. Leucocephala* will be a very useful source of protein and fish feed in future.

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Output of Analysis of Variance

Appendix-1

Table- *Channa* leaf

ANOVA

L

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	15.817	4	3.954	1.064	.385
Within Groups	167.251	45	3.717		
Total	183.068	49			

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
W	Between Groups	578.680	4	144.670	.587	.674
	Within Groups	11093.900	45	246.531		
	Total	11672.580	49			
TEC	Between Groups	.166	4	.041	.565	.689
	Within Groups	3.298	45	.073		
	Total	3.463	49			

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
TLC	Between Groups	168.168	4	42.042	.734	.573
	Within Groups	2576.544	45	57.257		
	Total	2744.712	49			
THROMBO	Between Groups	28.444	4	7.111	1.743	.157
	Within Groups	183.606	45	4.080		
	Total	212.050	49			
LYMPHO	Between Groups	11.671	4	2.918	2.904	.032
	Within Groups	45.215	45	1.005		
	Total	56.886	49			
MONO	Between Groups	8.920	4	2.230	.872	.488
	Within Groups	115.100	45	2.558		
	Total	124.020	49			
Es	Between Groups	32.520	4	8.130	3.951	.008
	Within Groups	92.600	45	2.058		
	Total	125.120	49			

ANOVA

BASO

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.280	4	.070	.258	.903
Within Groups	12.200	45	.271		
Total	12.480	49			

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) CHANNEL EAF	(J) CHANNEL EAF	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LYMPHO	1	2	.69300	.44828	.539	-.5808	1.9668
		3	1.31300*	.44828	.040	.0392	2.5868
		4	.49400	.44828	.805	-.7798	1.7678
		5	.03200	.44828	1.000	-1.2418	1.3058
	2	1	-.69300	.44828	.539	-1.9668	.5808
		3	.62000	.44828	.641	-.6538	1.8938
		4	-.19900	.44828	.992	-1.4728	1.0748
		5	-.66100	.44828	.584	-1.9348	.6128
	3	1	-1.31300*	.44828	.040	-2.5868	-.0392
		2	-.62000	.44828	.641	-1.8938	.6538
		4	-.81900	.44828	.371	-2.0928	.4548
		5	-1.28100*	.44828	.048	-2.5548	-.0072
	4	1	-.49400	.44828	.805	-1.7678	.7798
		2	.19900	.44828	.992	-1.0748	1.4728
		3	.81900	.44828	.371	-.4548	2.0928
		5	-.46200	.44828	.840	-1.7358	.8118
	5	1	-.03200	.44828	1.000	-1.3058	1.2418
		2	.66100	.44828	.584	-.6128	1.9348
		3	1.28100*	.44828	.048	.0072	2.5548
		4	.46200	.44828	.840	-.8118	1.7358
Es	1	2	-.60000	.64153	.882	-2.4229	1.2229
		3	-1.10000	.64153	.436	-2.9229	.7229
		4	-2.10000*	.64153	.017	-3.9229	-.2771
		5	-2.00000*	.64153	.025	-3.8229	-.1771
	2	1	.60000	.64153	.882	-1.2229	2.4229
		3	-.50000	.64153	.935	-2.3229	1.3229
		4	-1.50000	.64153	.152	-3.3229	.3229
		5	-1.40000	.64153	.205	-3.2229	.4229
	3	1	1.10000	.64153	.436	-.7229	2.9229
		2	.50000	.64153	.935	-1.3229	2.3229
		4	-1.00000	.64153	.531	-2.8229	.8229
		5	-.90000	.64153	.629	-2.7229	.9229
	4	1	2.10000*	.64153	.017	.2771	3.9229
		2	1.50000	.64153	.152	-.3229	3.3229
		3	1.00000	.64153	.531	-.8229	2.8229
		5	.10000	.64153	1.000	-1.7229	1.9229
	5	1	2.00000*	.64153	.025	.1771	3.8229
		2	1.40000	.64153	.205	-.4229	3.2229
		3	.90000	.64153	.629	-.9229	2.7229
		4	-.10000	.64153	1.000	-1.9229	1.7229

*. The mean difference is significant at the 0.05 level.

Output of Analysis of Variance

Appendix-2

Table- *Oreochromis* leaf

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
L	Between Groups	25.020	4	6.255	1.288	.289
	Within Groups	218.500	45	4.856		
	Total	243.520	49			
W	Between Groups	6808.150	4	1702.037	.990	.423
	Within Groups	77351.850	45	1718.930		
	Total	84160.000	49			
TEC	Between Groups	1.277	4	.319	.167	.954
	Within Groups	85.837	45	1.907		
	Total	87.114	49			
TLC	Between Groups	966.663	4	241.666	.594	.668
	Within Groups	18292.638	45	406.503		
	Total	19259.301	49			
THROMBO	Between Groups	81.107	4	20.277	5.058	.002
	Within Groups	180.413	45	4.009		
	Total	261.520	49			

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
LYMPHO	Between Groups	19.676	4	4.919	5.058	.002
	Within Groups	43.767	45	.973		
	Total	63.443	49			
MONO	Between Groups	7.000	4	1.750	.308	.871
	Within Groups	255.500	45	5.678		
	Total	262.500	49			
NEO	Between Groups	85.880	4	21.470	7.353	.000
	Within Groups	131.400	45	2.920		
	Total	217.280	49			
BASO	Between Groups	.280	4	.070	.321	.862
	Within Groups	9.800	45	.218		
	Total	10.080	49			
Es	Between Groups	36.520	4	9.130	4.020	.007
	Within Groups	102.200	45	2.271		
	Total	138.720	49			

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) OREO_ LEAF	(J) OREO_ LEAF	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
THROMBO	1	2	.33500	.89545	.996	-2.2094	2.8794
		3	1.54100	.89545	.432	-1.0034	4.0854
		4	3.35000*	.89545	.004	.8056	5.8944
		5	2.54600*	.89545	.050	.0016	5.0904
	2	1	-.33500	.89545	.996	-2.8794	2.2094
		3	1.20600	.89545	.664	-1.3384	3.7504
		4	3.01500*	.89545	.013	.4706	5.5594
		5	2.21100	.89545	.116	-.3334	4.7554
	3	1	-1.54100	.89545	.432	-4.0854	1.0034
		2	-1.20600	.89545	.664	-3.7504	1.3384
		4	1.80900	.89545	.273	-.7354	4.3534
		5	1.00500	.89545	.794	-1.5394	3.5494
	4	1	-3.35000*	.89545	.004	-5.8944	-.8056
		2	-3.01500*	.89545	.013	-5.5594	-.4706
		3	-1.80900	.89545	.273	-4.3534	.7354
		5	-.80400	.89545	.896	-3.3484	1.7404
	5	1	-2.54600*	.89545	.050	-5.0904	-.0016
		2	-2.21100	.89545	.116	-4.7554	.3334
		3	-1.00500	.89545	.794	-3.5494	1.5394
		4	.80400	.89545	.896	-1.7404	3.3484
LYMPHO	1	2	.16500	.44104	.996	-1.0882	1.4182
		3	.75900	.44104	.432	-.4942	2.0122
		4	1.65000*	.44104	.004	.3968	2.9032
		5	1.25400*	.44104	.050	.0008	2.5072
	2	1	-.16500	.44104	.996	-1.4182	1.0882
		3	.59400	.44104	.664	-.6592	1.8472
		4	1.48500*	.44104	.013	.2318	2.7382
		5	1.08900	.44104	.116	-.1642	2.3422

	3	1	-.75900	.44104	.432	-2.0122	.4942
		2	-.59400	.44104	.664	-1.8472	.6592
		4	.89100	.44104	.273	-.3622	2.1442
		5	.49500	.44104	.794	-.7582	1.7482
	4	1	-1.65000*	.44104	.004	-2.9032	-.3968
		2	-1.48500*	.44104	.013	-2.7382	-.2318
		3	-.89100	.44104	.273	-2.1442	.3622
		5	-.39600	.44104	.896	-1.6492	.8572
	5	1	-1.25400*	.44104	.050	-2.5072	-.0008
		2	-1.08900	.44104	.116	-2.3422	.1642
		3	-.49500	.44104	.794	-1.7482	.7582
		4	.39600	.44104	.896	-.8572	1.6492
NE	1	2	-.40000	.76420	.985	-2.5714	1.7714
		3	-1.80000	.76420	.147	-3.9714	.3714
		4	-3.30000*	.76420	.001	-5.4714	-1.1286
		5	-2.90000*	.76420	.004	-5.0714	-.7286
	2	1	.40000	.76420	.985	-1.7714	2.5714
		3	-1.40000	.76420	.368	-3.5714	.7714
		4	-2.90000*	.76420	.004	-5.0714	-.7286
		5	-2.50000*	.76420	.017	-4.6714	-.3286
	3	1	1.80000	.76420	.147	-.3714	3.9714
		2	1.40000	.76420	.368	-.7714	3.5714
		4	-1.50000	.76420	.300	-3.6714	.6714
		5	-1.10000	.76420	.606	-3.2714	1.0714
	4	1	3.30000*	.76420	.001	1.1286	5.4714
		2	2.90000*	.76420	.004	.7286	5.0714
		3	1.50000	.76420	.300	-.6714	3.6714
		5	.40000	.76420	.985	-1.7714	2.5714
	5	1	2.90000*	.76420	.004	.7286	5.0714
		2	2.50000*	.76420	.017	.3286	4.6714
		3	1.10000	.76420	.606	-1.0714	3.2714
		4	-.40000	.76420	.985	-2.5714	1.7714
ES	1	2	.10000	.67396	1.000	-1.8150	2.0150
		3	-.70000	.67396	.836	-2.6150	1.2150

	4	-1.80000	.67396	.075	-3.7150	.1150
	5	-1.90000	.67396	.053	-3.8150	.0150
2	1	-.10000	.67396	1.000	-2.0150	1.8150
	3	-.80000	.67396	.759	-2.7150	1.1150
	4	-1.90000	.67396	.053	-3.8150	.0150
	5	-2.00000*	.67396	.037	-3.9150	-.0850
3	1	.70000	.67396	.836	-1.2150	2.6150
	2	.80000	.67396	.759	-1.1150	2.7150
	4	-1.10000	.67396	.485	-3.0150	.8150
	5	-1.20000	.67396	.397	-3.1150	.7150
4	1	1.80000	.67396	.075	-.1150	3.7150
	2	1.90000	.67396	.053	-.0150	3.8150
	3	1.10000	.67396	.485	-.8150	3.0150
	5	-.10000	.67396	1.000	-2.0150	1.8150
5	1	1.90000	.67396	.053	-.0150	3.8150
	2	2.00000*	.67396	.037	.0850	3.9150
	3	1.20000	.67396	.397	-.7150	3.1150
	4	.10000	.67396	1.000	-1.8150	2.0150

***. The mean difference is significant at the 0.05 level.**

Output of Analysis of Variance

Appendix-3

Table- *Channa* leaf 2

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
length	Between Groups	15.817	4	3.954	1.064	.385
	Within Groups	167.251	45	3.717		
	Total	183.068	49			
weiht	Between Groups	578.680	4	144.670	.587	.674
	Within Groups	11093.900	45	246.531		
	Total	11672.580	49			
TEC	Between Groups	.179	4	.045	.615	.654
	Within Groups	3.275	45	.073		
	Total	3.454	49			
hb	Between Groups	.911	4	.228	.359	.837
	Within Groups	28.575	45	.635		
	Total	29.486	49			
hct	Between Groups	10.145	4	2.536	.400	.808
	Within Groups	285.312	45	6.340		
	Total	295.457	49			

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
MCV	Between Groups	352.545	4	88.136	2.279	.075
	Within Groups	1740.340	45	38.674		
	Total	2092.885	49			
MCH	Between Groups	4.822	4	1.205	.662	.622
	Within Groups	81.935	45	1.821		
	Total	86.757	49			
MCHC	Between Groups	3.229	4	.807	1.814	.143
	Within Groups	20.029	45	.445		
	Total	23.258	49			

Output of Analysis of Variance

Appendix-4

Table- *Oreochromis* leaf 2

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
length	Between Groups	25.020	4	6.255	1.288	.289
	Within Groups	218.500	45	4.856		
	Total	243.520	49			
weiht	Between Groups	6808.150	4	1702.037	.990	.423
	Within Groups	77350.850	45	1718.908		
	Total	84159.000	49			
TEC	Between Groups	1.277	4	.319	.167	.954
	Within Groups	85.837	45	1.907		
	Total	87.114	49			
hb	Between Groups	.964	4	.241	.076	.989
	Within Groups	142.079	45	3.157		
	Total	143.043	49			
hct	Between Groups	.512	4	.128	.011	1.000
	Within Groups	503.514	45	11.189		
	Total	504.026	49			

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
MCV	Between Groups	46.039	4	11.510	.848	.502
	Within Groups	610.489	45	13.566		
	Total	656.528	49			
MCH	Between Groups	.796	4	.199	.304	.874
	Within Groups	29.451	45	.654		
	Total	30.247	49			
MCHC	Between Groups	4.704	4	1.176	.181	.947
	Within Groups	292.958	45	6.510		
	Total	297.662	49			

Output of Analysis of Variance

Appendix-5

Table- *Channa* leaf 3

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
L	Between Groups	15.817	4	3.954	1.064	.385
	Within Groups	167.251	45	3.717		
	Total	183.068	49			
W	Between Groups	578.680	4	144.670	.587	.674
	Within Groups	11093.900	45	246.531		
	Total	11672.580	49			
TOTAL_PROTIEN	Between Groups	1.533	4	.383	13.508	.000
	Within Groups	1.277	45	.028		
	Total	2.810	49			
ALBUMIN	Between Groups	.332	4	.083	7.733	.000
	Within Groups	.484	45	.011		
	Total	.816	49			
GLOBULIN	Between Groups	.426	4	.106	9.976	.000
	Within Groups	.480	45	.011		
	Total	.906	49			

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
GLUCOSE	Between Groups	727.113	4	181.778	1.304	.283
	Within Groups	6273.614	45	139.414		
	Total	7000.727	49			
UREA	Between Groups	20.354	4	5.089	1.203	.323
	Within Groups	190.333	45	4.230		
	Total	210.687	49			
CREATININE	Between Groups	.009	4	.002	.737	.572
	Within Groups	.139	45	.003		
	Total	.149	49			
CHOLESTEROL	Between Groups	587.576	4	146.894	.526	.717
	Within Groups	12560.290	45	279.118		
	Total	13147.866	49			

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) channa_leaf_2	(J) channa_leaf_2	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
TOTAL_PROTIEN	1	2	-.22200*	.07533	.039	-.4360	-.0080
		3	-.32400*	.07533	.001	-.5380	-.1100
		4	-.41100*	.07533	.000	-.6250	-.1970
		5	-.51100*	.07533	.000	-.7250	-.2970
	2	1	.22200*	.07533	.039	.0080	.4360
		3	-.10200	.07533	.659	-.3160	.1120
		4	-.18900	.07533	.107	-.4030	.0250
		5	-.28900*	.07533	.003	-.5030	-.0750
	3	1	.32400*	.07533	.001	.1100	.5380
		2	.10200	.07533	.659	-.1120	.3160
		4	-.08700	.07533	.776	-.3010	.1270
		5	-.18700	.07533	.113	-.4010	.0270
	4	1	.41100*	.07533	.000	.1970	.6250
		2	.18900	.07533	.107	-.0250	.4030
		3	.08700	.07533	.776	-.1270	.3010
		5	-.10000	.07533	.676	-.3140	.1140
	5	1	.51100*	.07533	.000	.2970	.7250
		2	.28900*	.07533	.003	.0750	.5030
		3	.18700	.07533	.113	-.0270	.4010
		4	.10000	.07533	.676	-.1140	.3140
ALBUMIN	1	2	-.10300	.04636	.190	-.2347	.0287
		3	-.15100*	.04636	.017	-.2827	-.0193
		4	-.19100*	.04636	.001	-.3227	-.0593
		5	-.23800*	.04636	.000	-.3697	-.1063

	2	1	.10300	.04636	.190	-.0287	.2347
		3	-.04800	.04636	.838	-.1797	.0837
		4	-.08800	.04636	.333	-.2197	.0437
		5	-.13500*	.04636	.042	-.2667	-.0033
	3	1	.15100*	.04636	.017	.0193	.2827
		2	.04800	.04636	.838	-.0837	.1797
		4	-.04000	.04636	.909	-.1717	.0917
		5	-.08700	.04636	.344	-.2187	.0447
	4	1	.19100*	.04636	.001	.0593	.3227
		2	.08800	.04636	.333	-.0437	.2197
		3	.04000	.04636	.909	-.0917	.1717
		5	-.04700	.04636	.848	-.1787	.0847
	5	1	.23800*	.04636	.000	.1063	.3697
		2	.13500*	.04636	.042	.0033	.2667
		3	.08700	.04636	.344	-.0447	.2187
		4	.04700	.04636	.848	-.0847	.1787
GLOBULIN	1	2	-.12000	.04619	.088	-.2512	.0112
		3	-.17300*	.04619	.004	-.3042	-.0418
		4	-.22000*	.04619	.000	-.3512	-.0888
		5	-.26800*	.04619	.000	-.3992	-.1368
	2	1	.12000	.04619	.088	-.0112	.2512
		3	-.05300	.04619	.780	-.1842	.0782
		4	-.10000	.04619	.212	-.2312	.0312
		5	-.14800*	.04619	.020	-.2792	-.0168
	3	1	.17300*	.04619	.004	.0418	.3042
		2	.05300	.04619	.780	-.0782	.1842
		4	-.04700	.04619	.846	-.1782	.0842
		5	-.09500	.04619	.257	-.2262	.0362
	4	1	.22000*	.04619	.000	.0888	.3512
		2	.10000	.04619	.212	-.0312	.2312

		3	.04700	.04619	.846	-.0842	.1782
		5	-.04800	.04619	.836	-.1792	.0832
	5	1	.26800*	.04619	.000	.1368	.3992
		2	.14800*	.04619	.020	.0168	.2792
		3	.09500	.04619	.257	-.0362	.2262
		4	.04800	.04619	.836	-.0832	.1792

***. The mean difference is significant at the 0.05 level.**

Output of Analysis of Variance

Appendix-6

Table- *Oreochromis* leaf 3

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
L	Between Groups	25.020	4	6.255	1.288	.289
	Within Groups	218.500	45	4.856		
	Total	243.520	49			
W	Between Groups	6808.150	4	1702.037	.990	.423
	Within Groups	77351.850	45	1718.930		
	Total	84160.000	49			
TOTAL_PROTIEN	Between Groups	2.906	4	.726	3.925	.008
	Within Groups	8.329	45	.185		
	Total	11.235	49			
ALBUMIN	Between Groups	.695	4	.174	3.662	.012
	Within Groups	2.136	45	.047		
	Total	2.832	49			
GLOBULIN	Between Groups	.762	4	.190	2.353	.068
	Within Groups	3.642	45	.081		
	Total	4.404	49			

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
GLUCOSE	Between Groups	1505.532	4	376.383	2.551	.052
	Within Groups	6640.444	45	147.565		
	Total	8145.976	49			
UREA	Between Groups	14.748	4	3.687	1.242	.307
	Within Groups	133.639	45	2.970		
	Total	148.387	49			
CREATININE	Between Groups	.009	4	.002	2.054	.103
	Within Groups	.048	45	.001		
	Total	.057	49			
CHOLESTEROL	Between Groups	2724.422	4	681.105	.527	.716
	Within Groups	58143.029	45	1292.067		
	Total	60867.450	49			

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) oreo_leaf_3	(J) oreo_leaf_3	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
TOTAL_PROTIEN	1	2	-.29900	.19240	.534	-.8457	.2477
		3	-.57100*	.19240	.037	-1.1177	-.0243
		4	-.44200	.19240	.165	-.9887	.1047
		5	-.69900*	.19240	.006	-1.2457	-.1523
	2	1	.29900	.19240	.534	-.2477	.8457
		3	-.27200	.19240	.622	-.8187	.2747
		4	-.14300	.19240	.945	-.6897	.4037
		5	-.40000	.19240	.247	-.9467	.1467
	3	1	.57100*	.19240	.037	.0243	1.1177
		2	.27200	.19240	.622	-.2747	.8187
		4	.12900	.19240	.962	-.4177	.6757
		5	-.12800	.19240	.963	-.6747	.4187
	4	1	.44200	.19240	.165	-.1047	.9887
		2	.14300	.19240	.945	-.4037	.6897
		3	-.12900	.19240	.962	-.6757	.4177
		5	-.25700	.19240	.671	-.8037	.2897
	5	1	.69900*	.19240	.006	.1523	1.2457
		2	.40000	.19240	.247	-.1467	.9467
		3	.12800	.19240	.963	-.4187	.6747
		4	.25700	.19240	.671	-.2897	.8037
ALBUMIN	1	2	-.13800	.09744	.621	-.4149	.1389
		3	-.27800*	.09744	.049	-.5549	-.0011

	4	-.21400	.09744	.200	-.4909	.0629
	5	-.34000*	.09744	.009	-.6169	-.0631
2	1	.13800	.09744	.621	-.1389	.4149
	3	-.14000	.09744	.608	-.4169	.1369
	4	-.07600	.09744	.935	-.3529	.2009
	5	-.20200	.09744	.250	-.4789	.0749
3	1	.27800*	.09744	.049	.0011	.5549
	2	.14000	.09744	.608	-.1369	.4169
	4	.06400	.09744	.964	-.2129	.3409
	5	-.06200	.09744	.968	-.3389	.2149
4	1	.21400	.09744	.200	-.0629	.4909
	2	.07600	.09744	.935	-.2009	.3529
	3	-.06400	.09744	.964	-.3409	.2129
	5	-.12600	.09744	.697	-.4029	.1509
5	1	.34000*	.09744	.009	.0631	.6169
	2	.20200	.09744	.250	-.0749	.4789
	3	.06200	.09744	.968	-.2149	.3389
	4	.12600	.09744	.697	-.1509	.4029

***. The mean difference is significant at the 0.05 level.**

Output of Analysis of Variance

Appendix-7

Table- C*hanna* seed 3

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
L	Between Groups	14.331	4	3.583	.907	.468
	Within Groups	177.678	45	3.948		
	Total	192.009	49			
W	Between Groups	1586.120	4	396.530	1.560	.201
	Within Groups	11441.100	45	254.247		
	Total	13027.220	49			
TEC	Between Groups	.190	4	.047	.598	.666
	Within Groups	3.564	45	.079		
	Total	3.753	49			
TLC	Between Groups	217.109	4	54.277	.813	.524
	Within Groups	3004.618	45	66.769		
	Total	3221.726	49			
THROMBO	Between Groups	35.176	4	8.794	2.368	.067
	Within Groups	167.115	45	3.714		
	Total	202.291	49			

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
LYNPHO	Between Groups	4.996	4	1.249	1.376	.257
	Within Groups	40.842	45	.908		
	Total	45.839	49			
MONO	Between Groups	3.000	4	.750	.273	.894
	Within Groups	123.500	45	2.744		
	Total	126.500	49			
NE	Between Groups	7.720	4	1.930	.439	.780
	Within Groups	197.900	45	4.398		
	Total	205.620	49			
ES	Between Groups	14.680	4	3.670	1.504	.217
	Within Groups	109.800	45	2.440		
	Total	124.480	49			
BASO	Between Groups	.280	4	.070	.274	.893
	Within Groups	11.500	45	.256		
	Total	11.780	49			

Output of Analysis of Variance

Appendix-8

Table- *Oreochromis* seed 3

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
L	Between Groups	7.730	4	1.933	.727	.578
	Within Groups	119.675	45	2.659		
	Total	127.405	49			
W	Between Groups	4806.580	4	1201.645	2.695	.043
	Within Groups	20066.825	45	445.929		
	Total	24873.405	49			
TEC	Between Groups	.461	4	.115	.075	.989
	Within Groups	68.877	45	1.531		
	Total	69.338	49			
TLC	Between Groups	1251.529	4	312.882	2.168	.088
	Within Groups	6493.583	45	144.302		
	Total	7745.113	49			
THROMBO	Between Groups	46.690	4	11.672	2.313	.072
	Within Groups	227.118	45	5.047		
	Total	273.808	49			

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
LYNPHO	Between Groups	11.334	4	2.834	2.314	.072
	Within Groups	55.103	45	1.225		
	Total	66.438	49			
MONO	Between Groups	9.680	4	2.420	.457	.767
	Within Groups	238.100	45	5.291		
	Total	247.780	49			
NE	Between Groups	29.920	4	7.480	1.221	.315
	Within Groups	275.600	45	6.124		
	Total	305.520	49			
ES	Between Groups	64.000	4	16.000	6.000	.001
	Within Groups	120.000	45	2.667		
	Total	184.000	49			
BASO	Between Groups	.120	4	.030	.142	.966
	Within Groups	9.500	45	.211		
	Total	9.620	49			

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) oreo_speed_3	(J) oreo_speed_3	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
W	1	2	23.75000	9.44383	.105	-3.0842	50.5842
		3	26.35000	9.44383	.057	-.4842	53.1842
		4	24.35000	9.44383	.092	-2.4842	51.1842
		5	22.95000	9.44383	.126	-3.8842	49.7842
	2	1	-23.75000	9.44383	.105	-50.5842	3.0842
		3	2.60000	9.44383	.999	-24.2342	29.4342
		4	.60000	9.44383	1.000	-26.2342	27.4342
		5	-.80000	9.44383	1.000	-27.6342	26.0342
	3	1	-26.35000	9.44383	.057	-53.1842	.4842
		2	-2.60000	9.44383	.999	-29.4342	24.2342
		4	-2.00000	9.44383	1.000	-28.8342	24.8342
		5	-3.40000	9.44383	.996	-30.2342	23.4342
	4	1	-24.35000	9.44383	.092	-51.1842	2.4842
		2	-.60000	9.44383	1.000	-27.4342	26.2342
		3	2.00000	9.44383	1.000	-24.8342	28.8342
		5	-1.40000	9.44383	1.000	-28.2342	25.4342
	5	1	-22.95000	9.44383	.126	-49.7842	3.8842
		2	.80000	9.44383	1.000	-26.0342	27.6342
		3	3.40000	9.44383	.996	-23.4342	30.2342
		4	1.40000	9.44383	1.000	-25.4342	28.2342
ES	1	2	-.40000	.73030	.982	-2.4751	1.6751
		3	-1.70000	.73030	.155	-3.7751	.3751
		4	-2.80000*	.73030	.003	-4.8751	-.7249
		5	-2.60000*	.73030	.008	-4.6751	-.5249
	2	1	.40000	.73030	.982	-1.6751	2.4751
		3	-1.30000	.73030	.398	-3.3751	.7751

		4	-2.40000*	.73030	.016	-4.4751	-.3249
		5	-2.20000*	.73030	.033	-4.2751	-.1249
	3	1	1.70000	.73030	.155	-.3751	3.7751
		2	1.30000	.73030	.398	-.7751	3.3751
		4	-1.10000	.73030	.564	-3.1751	.9751
		5	-.90000	.73030	.733	-2.9751	1.1751
	4	1	2.80000*	.73030	.003	.7249	4.8751
		2	2.40000*	.73030	.016	.3249	4.4751
		3	1.10000	.73030	.564	-.9751	3.1751
		5	.20000	.73030	.999	-1.8751	2.2751
	5	1	2.60000*	.73030	.008	.5249	4.6751
		2	2.20000*	.73030	.033	.1249	4.2751
		3	.90000	.73030	.733	-1.1751	2.9751
		4	-.20000	.73030	.999	-2.2751	1.8751

***. The mean difference is significant at the 0.05 level.**

Output of Analysis of Variance

Appendix-9

Table- *Channa* seed 3.2

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
length	Between Groups	14.331	4	3.583	.907	.468
	Within Groups	177.678	45	3.948		
	Total	192.009	49			
weght	Between Groups	1586.120	4	396.530	1.560	.201
	Within Groups	11441.100	45	254.247		
	Total	13027.220	49			
TEC	Between Groups	.190	4	.047	.598	.666
	Within Groups	3.564	45	.079		
	Total	3.753	49			
hb	Between Groups	.372	4	.093	.190	.942
	Within Groups	21.993	45	.489		
	Total	22.365	49			
hct	Between Groups	1.894	4	.474	.084	.987
	Within Groups	254.653	45	5.659		
	Total	256.548	49			

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
MCV	Between Groups	253.573	4	63.393	1.299	.285
	Within Groups	2195.549	45	48.790		
	Total	2449.122	49			
MCH	Between Groups	10.762	4	2.690	.997	.419
	Within Groups	121.380	45	2.697		
	Total	132.142	49			
MCHC	Between Groups	.316	4	.079	.238	.916
	Within Groups	14.948	45	.332		
	Total	15.263	49			

Output of Analysis of Variance

Appendix-10

Table- *Oreochromis* seed 3.2

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
length	Between Groups	7.730	4	1.933	.727	.578
	Within Groups	119.675	45	2.659		
	Total	127.405	49			
weght	Between Groups	4806.580	4	1201.645	2.695	.043
	Within Groups	20066.825	45	445.929		
	Total	24873.405	49			
TEC	Between Groups	.461	4	.115	.075	.989
	Within Groups	68.877	45	1.531		
	Total	69.338	49			
hb	Between Groups	.328	4	.082	.026	.999
	Within Groups	142.847	45	3.174		
	Total	143.175	49			
hct	Between Groups	.318	4	.080	.007	1.000
	Within Groups	504.265	45	11.206		
	Total	504.583	49			

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
MCV	Between Groups	9.424	4	2.356	.357	.838
	Within Groups	296.914	45	6.598		
	Total	306.338	49			
MCH	Between Groups	.141	4	.035	.061	.993
	Within Groups	25.985	45	.577		
	Total	26.126	49			
MCHC	Between Groups	1.398	4	.349	.054	.994
	Within Groups	292.718	45	6.505		
	Total	294.116	49			

Multiple Comparisons

weight

Tukey HSD

(I) oreo_speed_3.2	(J) oreo_speed_3.2	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	23.75000	9.44383	.105	-3.0842	50.5842
	3	26.35000	9.44383	.057	-.4842	53.1842
	4	24.35000	9.44383	.092	-2.4842	51.1842
	5	22.95000	9.44383	.126	-3.8842	49.7842
2	1	-23.75000	9.44383	.105	-50.5842	3.0842
	3	2.60000	9.44383	.999	-24.2342	29.4342
	4	.60000	9.44383	1.000	-26.2342	27.4342
	5	-.80000	9.44383	1.000	-27.6342	26.0342
3	1	-26.35000	9.44383	.057	-53.1842	.4842
	2	-2.60000	9.44383	.999	-29.4342	24.2342
	4	-2.00000	9.44383	1.000	-28.8342	24.8342
	5	-3.40000	9.44383	.996	-30.2342	23.4342
4	1	-24.35000	9.44383	.092	-51.1842	2.4842
	2	-.60000	9.44383	1.000	-27.4342	26.2342
	3	2.00000	9.44383	1.000	-24.8342	28.8342
	5	-1.40000	9.44383	1.000	-28.2342	25.4342
5	1	-22.95000	9.44383	.126	-49.7842	3.8842
	2	.80000	9.44383	1.000	-26.0342	27.6342
	3	3.40000	9.44383	.996	-23.4342	30.2342
	4	1.40000	9.44383	1.000	-25.4342	28.2342

Output of Analysis of Variance

Appendix-11

Table- *Channa* seed 4

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
length	Between Groups	14.331	4	3.583	.907	.468
	Within Groups	177.678	45	3.948		
	Total	192.009	49			
weght	Between Groups	1586.120	4	396.530	1.560	.201
	Within Groups	11441.100	45	254.247		
	Total	13027.220	49			
total_protien	Between Groups	.761	4	.190	2.579	.050
	Within Groups	3.318	45	.074		
	Total	4.079	49			
albumin	Between Groups	.195	4	.049	3.211	.021
	Within Groups	.682	45	.015		
	Total	.877	49			
globulin	Between Groups	.186	4	.046	1.456	.231
	Within Groups	1.434	45	.032		
	Total	1.620	49			

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
glocuse	Between Groups	840.155	4	210.039	1.527	.211
	Within Groups	6190.676	45	137.571		
	Total	7030.831	49			
urea	Between Groups	18.168	4	4.542	1.084	.376
	Within Groups	188.474	45	4.188		
	Total	206.642	49			
creatinine	Between Groups	.011	4	.003	.896	.474
	Within Groups	.136	45	.003		
	Total	.147	49			
cholesterol	Between Groups	839.247	4	209.812	.786	.541
	Within Groups	12018.717	45	267.083		
	Total	12857.965	49			

Multiple Comparisons

albumin

Tukey HSD

(I) channa_seed_4	(J) channa_seed_4	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-.06200	.05506	.792	-.2185	.0945
	3	-.09900	.05506	.387	-.2555	.0575
	4	-.13500	.05506	.120	-.2915	.0215
	5	-.18300*	.05506	.015	-.3395	-.0265
2	1	.06200	.05506	.792	-.0945	.2185
	3	-.03700	.05506	.961	-.1935	.1195
	4	-.07300	.05506	.677	-.2295	.0835
	5	-.12100	.05506	.199	-.2775	.0355
3	1	.09900	.05506	.387	-.0575	.2555
	2	.03700	.05506	.961	-.1195	.1935
	4	-.03600	.05506	.965	-.1925	.1205
	5	-.08400	.05506	.552	-.2405	.0725
4	1	.13500	.05506	.120	-.0215	.2915
	2	.07300	.05506	.677	-.0835	.2295
	3	.03600	.05506	.965	-.1205	.1925
	5	-.04800	.05506	.906	-.2045	.1085
5	1	.18300*	.05506	.015	.0265	.3395
	2	.12100	.05506	.199	-.0355	.2775
	3	.08400	.05506	.552	-.0725	.2405
	4	.04800	.05506	.906	-.1085	.2045

***. The mean difference is significant at the 0.05 level.**

Output of Analysis of Variance

Appendix-12

Table- *Oreochromis* seed 4

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
L	Between Groups	7.730	4	1.933	.727	.578
	Within Groups	119.675	45	2.659		
	Total	127.405	49			
W	Between Groups	4806.580	4	1201.645	2.695	.043
	Within Groups	20066.825	45	445.929		
	Total	24873.405	49			
total_protien	Between Groups	1.814	4	.453	1.818	.142
	Within Groups	11.222	45	.249		
	Total	13.036	49			
albumin	Between Groups	.414	4	.103	1.787	.148
	Within Groups	2.604	45	.058		
	Total	3.018	49			
globulin	Between Groups	.492	4	.123	1.625	.185
	Within Groups	3.405	45	.076		
	Total	3.897	49			

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
glocuse	Between Groups	1212.673	4	303.168	2.105	.096
	Within Groups	6479.882	45	143.997		
	Total	7692.555	49			
urea	Between Groups	10.066	4	2.517	.885	.481
	Within Groups	128.016	45	2.845		
	Total	138.082	49			
creatinine	Between Groups	.004	4	.001	.948	.445
	Within Groups	.052	45	.001		
	Total	.057	49			
cholesterol	Between Groups	2771.733	4	692.933	.518	.723
	Within Groups	60218.575	45	1338.191		
	Total	62990.309	49			

Multiple Comparisons

Weight

Tukey HSD

(I) oreo_speed_4	(J) oreo_speed_4	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	23.75000	9.44383	.105	-3.0842	50.5842
	3	26.35000	9.44383	.057	-.4842	53.1842
	4	24.35000	9.44383	.092	-2.4842	51.1842
	5	22.95000	9.44383	.126	-3.8842	49.7842
2	1	-23.75000	9.44383	.105	-50.5842	3.0842
	3	2.60000	9.44383	.999	-24.2342	29.4342
	4	.60000	9.44383	1.000	-26.2342	27.4342
	5	-.80000	9.44383	1.000	-27.6342	26.0342
3	1	-26.35000	9.44383	.057	-53.1842	.4842
	2	-2.60000	9.44383	.999	-29.4342	24.2342
	4	-2.00000	9.44383	1.000	-28.8342	24.8342
	5	-3.40000	9.44383	.996	-30.2342	23.4342
4	1	-24.35000	9.44383	.092	-51.1842	2.4842
	2	-.60000	9.44383	1.000	-27.4342	26.2342
	3	2.00000	9.44383	1.000	-24.8342	28.8342
	5	-1.40000	9.44383	1.000	-28.2342	25.4342
5	1	-22.95000	9.44383	.126	-49.7842	3.8842
	2	.80000	9.44383	1.000	-26.0342	27.6342
	3	3.40000	9.44383	.996	-23.4342	30.2342
	4	1.40000	9.44383	1.000	-25.4342	28.2342

Output of Analysis of Variance

Appendix-13

Table 1: Number of eggs in the ovary of *C. punctatus* after feeding *L. leucocephala* leaf

ANOVA

negg

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	240.000	4	60.000	15.000	.000
Within Groups	40.000	10	4.000		
Total	280.000	14			

Multiple Comparisons

negg
Tukey HSD

(I) pleaf	(J) pleaf	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	4.000	1.633	.179	-1.37	9.37
	3	6.000*	1.633	.028	.63	11.37
	4	8.000*	1.633	.004	2.63	13.37
	5	12.000*	1.633	.000	6.63	17.37
2	1	-4.000	1.633	.179	-9.37	1.37
	3	2.000	1.633	.738	-3.37	7.37
	4	4.000	1.633	.179	-1.37	9.37
	5	8.000*	1.633	.004	2.63	13.37
3	1	-6.000*	1.633	.028	-11.37	-.63
	2	-2.000	1.633	.738	-7.37	3.37
	4	2.000	1.633	.738	-3.37	7.37
	5	6.000*	1.633	.028	.63	11.37
4	1	-8.000*	1.633	.004	-13.37	-2.63
	2	-4.000	1.633	.179	-9.37	1.37
	3	-2.000	1.633	.738	-7.37	3.37
	5	4.000	1.633	.179	-1.37	9.37
5	1	-12.000*	1.633	.000	-17.37	-6.63
	2	-8.000*	1.633	.004	-13.37	-2.63
	3	-6.000*	1.633	.028	-11.37	-.63
	4	-4.000	1.633	.179	-9.37	1.37

*. The mean difference is significant at the 0.05 level.

Output of Analysis of Variance

Appendix-14

Table 2: Number of eggs in the ovary of *C. punctatus* after feeding *L.leucocephala* seed

ANOVA

neggg

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	177.600	4	44.400	11.100	.001
Within Groups	40.000	10	4.000		
Total	217.600	14			

Multiple Comparisons

neggg
Tukey HSD

(I) seed	(J) seed	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	2.000	1.633	.738	-3.37	7.37
	3	4.000	1.633	.179	-1.37	9.37
	4	6.000*	1.633	.028	.63	11.37
	5	10.000*	1.633	.001	4.63	15.37
2	1	-2.000	1.633	.738	-7.37	3.37
	3	2.000	1.633	.738	-3.37	7.37
	4	4.000	1.633	.179	-1.37	9.37
	5	8.000*	1.633	.004	2.63	13.37
3	1	-4.000	1.633	.179	-9.37	1.37
	2	-2.000	1.633	.738	-7.37	3.37
	4	2.000	1.633	.738	-3.37	7.37
	5	6.000*	1.633	.028	.63	11.37
4	1	-6.000*	1.633	.028	-11.37	-.63
	2	-4.000	1.633	.179	-9.37	1.37
	3	-2.000	1.633	.738	-7.37	3.37
	5	4.000	1.633	.179	-1.37	9.37
5	1	-10.000*	1.633	.001	-15.37	-4.63
	2	-8.000*	1.633	.004	-13.37	-2.63
	3	-6.000*	1.633	.028	-11.37	-.63
	4	-4.000	1.633	.179	-9.37	1.37

*. The mean difference is significant at the 0.05 level.

Output of Analysis of Variance

Appendix-15

Table 3: Number of eggs in the ovary of *O. niloticus* after feeding *L. leucocephala* leaf

ANOVA

oreo_legg

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	248.400	4	62.100	38.812	.000
Within Groups	16.000	10	1.600		
Total	264.400	14			

Multiple Comparisons

oreo_legg
Tukey HSD

(I) oreo_leaf	(J) oreo_leaf	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	5.000*	1.033	.005	1.60	8.40
	3	8.000*	1.033	.000	4.60	11.40
	4	9.000*	1.033	.000	5.60	12.40
	5	12.000*	1.033	.000	8.60	15.40
2	1	-5.000*	1.033	.005	-8.40	-1.60
	3	3.000	1.033	.091	-.40	6.40
	4	4.000*	1.033	.020	.60	7.40
	5	7.000*	1.033	.000	3.60	10.40
3	1	-8.000*	1.033	.000	-11.40	-4.60
	2	-3.000	1.033	.091	-6.40	.40
	4	1.000	1.033	.863	-2.40	4.40
	5	4.000*	1.033	.020	.60	7.40
4	1	-9.000*	1.033	.000	-12.40	-5.60
	2	-4.000*	1.033	.020	-7.40	-.60
	3	-1.000	1.033	.863	-4.40	2.40
	5	3.000	1.033	.091	-.40	6.40
5	1	-12.000*	1.033	.000	-15.40	-8.60
	2	-7.000*	1.033	.000	-10.40	-3.60
	3	-4.000*	1.033	.020	-7.40	-.60
	4	-3.000	1.033	.091	-6.40	.40

*. The mean difference is significant at the 0.05 level.

Output of Analysis of Variance

Appendix-16

Table 4: Number of eggs in the ovary of *O. niloticus* after feeding *L.leucocephala* seed

ANOVA

oreo_segg

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	206.400	4	51.600	12.900	.001
Within Groups	40.000	10	4.000		
Total	246.400	14			

Multiple Comparisons

oreo_segg
Tukey HSD

(I) oreo_seed	(J) oreo_seed	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	2.000	1.633	.738	-3.37	7.37
	3	6.000*	1.633	.028	.63	11.37
	4	8.000*	1.633	.004	2.63	13.37
	5	10.000*	1.633	.001	4.63	15.37
2	1	-2.000	1.633	.738	-7.37	3.37
	3	4.000	1.633	.179	-1.37	9.37
	4	6.000*	1.633	.028	.63	11.37
	5	8.000*	1.633	.004	2.63	13.37
3	1	-6.000*	1.633	.028	-11.37	-.63
	2	-4.000	1.633	.179	-9.37	1.37
	4	2.000	1.633	.738	-3.37	7.37
	5	4.000	1.633	.179	-1.37	9.37
4	1	-8.000*	1.633	.004	-13.37	-2.63
	2	-6.000*	1.633	.028	-11.37	-.63
	3	-2.000	1.633	.738	-7.37	3.37
	5	2.000	1.633	.738	-3.37	7.37
5	1	-10.000*	1.633	.001	-15.37	-4.63
	2	-8.000*	1.633	.004	-13.37	-2.63
	3	-4.000	1.633	.179	-9.37	1.37
	4	-2.000	1.633	.738	-7.37	3.37

*. The mean difference is significant at the 0.05 level.