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Research Article

Effect of plant-based feed on the antioxidant enzymes, biochemical and hematological parameters of *Oreochromis niloticus*

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ABSTRACT

Due to the amino acid profile, high digestibility and palatability, fish meal plays a vital role in the aqua feed. However, stress on aquaculture production has intensified due to rising demand and uncertain fish meal supplies. As a result establishing a sustainable protein source is required to replace fish meal in the aquafeed. *Chenopodium quinoa* seeds were utilized to replace fish meal in aqua feed. This experiment revealed the effect of varying levels (0% (control), 15%, 30%, 45% and 60%) of quinoa seed meal diet on antioxidant enzymes, biochemical and haematological parameters of *Oreochromis niloticus*. The experiment was carried out with constant water pH (7.5), temperature (30°C) and hardness (300 mg/L) for 8 weeks. Results showed a significantly ($p<0.05$) higher value of superoxide dismutase (SOD) and glutathione S transferase (GST) in the liver than the kidney of fish. The activities of SOD and GST in the selected organs of *O. niloticus* were significantly ($p<0.05$) increased with the increased inclusion of quinoa seed in the diet of fish. Mean ALT, AST and ALP values were 78.92±35.18 99.33±20.57 and 70.70±6.76 IU/L, respectively. A significant ($p<0.05$) increase in the concentration of haemoglobin content was observed in the fish, *O. niloticus* fed on a T3 diet. An increase in the values of the parameters was observed in the normal range in *O. niloticus* fed with *C. quinoa*-based diet at different treatment levels. Hence, *C. quinoa* seed meal appeared an excellent replacement for fish meal due to its high-quality protein and complete nutritional characteristics.

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Introduction

Recently world's population is 7.3 billion that is continuously increasing and expected to touch a figure of 9.7 billion by the end of 2050 (Mirghaed et al. 2017). Requirement for feed ingredients rich in protein is expected to enhance accordingly. Production of aquaculture is growing fastly in contrast to production of other animal sectors. Innovative approaches are essential for sustainable aquafeed production to improve the nutritional quality of plant protein ingredients for effective use. Protein separation isolates the pure protein fractions from other non-proteinaceous components in the ingredients involving anti-nutritional factors to improve the utilization of nutrients and digestibility (Fawole et al. 2016).

Green plants have been proved as the cheapest and most abundant protein source because of their ability to synthesize amino acids from the plant-derived ingredients. Any reduction in the feed cost directly affects profitability (Aguado-Gimenez 2020). Several studies have shown that plant protein sources can provide a high quantity of proteins for fish growth. Legumes and seeds have immense potential to be used because they contain a considerable amount of vitamins, carbohydrates, protein, lipids, minerals and certain fibers. But plant's products with high contents of proteins also have some anti-nutrients; imbalance of amino acid and their poor digestibility relative to fish meal are some of the main concerns. Therefore, plant products need some pre-treatments before using aquafeed ingredients (Drew et al. 2007).

Quinoa is known as pseudo-cereal, which has been gaining popularity continuously. It has also been described as grains of 21st century (Schmidt 2021). Protein found in quinoa has high nutritional value due to higher lysine levels than cereals like wheat and rice. It contains variable protein contents between 13.8 to 16.5% in the dry matter of quinoa, while on the average, it is reported as 15% (Navruz-Varli and Sanlier 2016).

Biochemical parameters such as alkaline phosphatase (ALP), alanine transaminase (ALT) and aspartate transaminase (AST) are important serum markers for investigating the health of animal species. In toxicological studies, changes in concentration and enzyme activities often directly indicate cell or organ damage in specific organs (Mirghaed et al. 2017). AST and ALT are released into plasma when cells are destroyed due to some diseases and injuries, and their high concentrations are found as indicator of abnormal physiology (Qadir et al. 2014). ALP is a poly functional enzyme that acts as transphosphorylase at basic pH and plays a central role in the mineralization of fish skeletons (Rahimikia 2017). Serum biochemical parameters, ALT, AST and ALP represent the correlation between altered physiological fitness and environmental stress. The activity of these enzymes, AST and ALT in the *O. niloticus* was lowered with the utilization of dietary protein with the increase in dietary soya bean level and observed liver damage to a certain extent (Lin et al. 2007). So, biochemical parameters provide a clear indication of any change in body physiology.

Fish haematology is receiving increasing importance in fish culture because of its significance in monitoring the health status of fish. Any change in the constituent component of the blood sample could clarify the metabolic state of animals and the state of health when compared to normal values (Ahmed et al. 2020). Stress is an important factor that leads to changes in the homeostasis of fish like other higher animals (Chowdhury and Saikia 2020), which is caused by certain factors, including contaminants, environmental conditions (Temperature and (DO) dissolved oxygen), diseases and parasites, (Movahed et al. 2012). Variations in haematological parameters explain these changes include pack cell volume (PVC), haemoglobin content, white blood cell (WBC) count, red blood cell (RBC) count, mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) (Jimoh et al. 2015). In healthy adults, red blood cells constitute approximately 40 to 48%, whereas newborns may have haematocrits of up to 60% (Crawford et al. 2019). The distinction in the haematological parameters is directly related to the changes in feed constituents that may be due to the presence of a wide variety of nutritional and anti-nutritional factors (Rahate et al. 2020). The haemoglobin, packed cell volume, and RBC concentration values vary with the diet and temperature, season, and nutritional status of the fish (Sharma and Shukla 2021). The present study aims to investigate the beneficial effects of quinoa seeds on fish health and nutrition when used in fish feed.

Materials and Methods

Experimental site

The experiment was conducted in the research laboratory of the Department of Zoology Government College Women University, Faisalabad Pakistan, in the glass aquaria for 60 days. *C. quinoa* seeds were used to replace fish meal in fish feed.

Experimental design and feeding trial

The fish specimens for experiments were collected from a local fish seed hatchery. Healthy fish specimens of *O. niloticus* with equal size and weight were stocked in glass aquaria filled with 70-litre water. The fish was acclimatized for 15 days to laboratory conditions before the experiment. Before the experiment, fish was starved for 24 h, so weight was maintained. The fish were being fed at a rate of 3% of body weight twice daily. During the present experiment, fish meal, used in fish feed, was replaced by quinoa seed meal and its effects were determined on antioxidant enzymes, biochemical and haematological parameters of *O. niloticus* at different replacement levels, viz. control (0%), T1(15%), T2(30%), T3(45%) and T4(60%). Water quality parameters (pH, hardness, temperature, and dissolved oxygen) were maintained according to standard requirements for wellbeing of experimental animals. The mean standard weight and length of fish in each tank were measured at start of experiment.

Antioxidant enzyme parameters

Superoxide dismutase (SOD)

Liver and kidney samples from fish were processed to determine SOD activity (McCord and Fridovich 1969).

After dissection of fish, liver and kidney were separated. Organs were washed thoroughly with phosphate buffer saline (PBS) having pH 6.5 to remove RBCs. Following washing, organs were weighed and homogenized in PBS. The organs homogenates were centrifuged (@10000 rpm for 15 min at 4C°) and clear supernatant was obtained to measure the activity of SOD. The SOD activity was measured by following Giannopolitis and Ries (1977) with minor modifications. The SOD can inhibit photo-reduction of nitro-blue tetrazolium (NBT). Unit of superoxide dismutase can defined as the amount of enzyme that causes 50% of the maximum inhibition of NBT reduction.

The calculation was made by the following formula:

$$\%\text{age inhibition} = \frac{\text{Blank (Abs)} - \text{sample (Abs)}}{\text{Blank (Abs)}} \times 100$$

Glutathione S-transferase (GST)

After extract preparation, the final reaction mixture (1mM CDNB, 1mM glutathione in 50mM PBS (pH 7.4)) was formulated. Reaction was started by adding 50 µl sample (organ extract). Activity of GST was observed following the change in absorbance of CDNB/min at 340 nm.

Biochemical parameters

The activities of AST and ALT were executed using the method described by Reitman and Frankel (1957), while the activity of ALP was determined following the colorimetric method of Rosalki et al. (1993).

To determine biochemical parameters, blood was taken from caudal vein of fish. For sample collection, disposable syringe was used without anticoagulant. Blood sample was put into a gel containing yellow top vacutainer, centrifuged at 3000 rpm for 10

minutes. The separated serum was used for determination of ALT, AST and ALP.

Haematological parameters

Blood samples were taken fortnightly from each fish species for haematological studies. Fish was anaesthetized with buffered MS222 (Tricaine methanesulphonate) 50 mg/l. Then blood sample was taken from the caudal vein of an anaesthetized fish with a sterilized syringe. The blood sample was collected in vacutainer tubes containing ethylene diamine tetraacetic acid (EDTA) as anticoagulant. This anti-coagulant was chosen as being currently used in routine haematological investigations. This anti-coagulant is preferably used because of its ability to preserve the blood components over a long time and its effective anti-coagulant action (Blaxhall 1972). The blood sample was mixed gently to allow thorough mixing of its contents and then stored at -4°C in the refrigerator before analyses. The haematological parameters studied include haemoglobin, RBC, WBC, PCV, MCV, MCH and MCHC. A haemocytometer was used to count the red blood cells and white blood cells, which consisted of blood cells counting chamber, a cover slit, and a plastic mouthpiece. The numbers of WBCs were investigated by the process explained by Dacie and Lewis (2001). The unified method of Svobodova et al. (1991) was applied to prepare the diluting fluid of blood. Blood sample was diluted and number of cells was counted using microscope and reported as number of cells/mm^3.

Statistical analysis

Obtained data were subjected to one-way analysis of variance (ANOVA) using MSTAT C computer software. Duncan's Multiple Range Test was used to compare the means of treatments by the significance level.

Results

During the present experiment, fish meal, used in fish feed, was replaced by quinoa seed meal and its effects were determined on antioxidant enzymes, biochemical and haematological parameters of *O. niloticus* at different replacement levels, viz control (0%), T1(15%), T2(30%), T3(45%) and T4(60%).

Effect of *C. quinoa* seed meal on the SOD activity in the organs of *O. niloticus*

Liver

The SOD activity (Umg^{-1} protein) at 60% inclusion of quinoa-based diet was observed significantly highest with the mean value of 42.29±6.87 Umg^{-1} protein. On the other hand, the mean value of SOD activity at 15% inclusion of quinoa-based diet was observed lowest with the mean value of 23.91±5.87 Umg^{-1} protein, following 24.57±5.17 and 39.73±7.86 Umg^{-1} protein at 30 and 45% inclusion of quinoa-based diet, respectively. These results showed that the mean value of SOD increased statistically with the increased inclusion percentage of quinoa seed meal compared to the control group with the value of 14.62±0.17 Umg^{-1} protein in the liver of *O. niloticus*. Among fortnights, statistically highest and lowest values of SOD were observed at the 4th and 1st fortnight with the mean value of 35.14±14.75 and 23.38±9.11 Umg^{-1} protein, respectively (Table 1).

Kidneys

On the other hand, in the kidney similar trend was observed. The highest (41.35±4.77 Umg^{-1} protein) SOD activity was observed at 60% of the quinoa-based diet while the lowest (12.74±4.31 Umg^{-1} protein) SOD activity was observed at 15% inclusion of quinoa-based diet. At 30 and 45%, the SOD activity is shown with the value of 16.25±4.28 Umg^{-1} protein and 23.31±4.33 Umg^{-1} protein, respectively. Among fortnights, significant variation was observed in SOD activity in kidney of fish. The SOD activity in kidney followed the order: 4th fortnight (24.12±14.95 Umg^{-1} protein) > 3rd fortnight (21.21±13.60 Umg^{-1} protein) > 2nd fortnight (18.77±13.41) > 1st fortnight (15.45±11.77 Umg^{-1} protein) (Table 1).

Comparison of organs represents higher activity of SOD in liver than kidney for all the inclusion levels of quinoa seed meal in fish feed except T4 (Fig. 1).

Effect of *C. quinoa* seed meal on the GST activity in the liver and kidney of *O. niloticus*

Liver

Results showed that the GST activity (nmol mg^{-1} protein) under 60% of quinoa-based diet was statistically highest with the mean value of 63.67±6.93 nmol mg^{-1} protein. On the other hand, the value of GST activity at 15% inclusion of quinoa-based diet was observed to be statistically lowest with the mean value of 44.21±5.21 nmol mg^{-1} protein, followed by that of 49.70±6.61 and 56.26±6.40 nmol mg^{-1} protein at 30 and 45% inclusion of quinoa-based diet, respectively. These results showed that the mean value of GST increased statistically with the increased inclusion percentage of quinoa seed meal compared to the control group with the mean value of 39.48±0.01 nmol mg^{-1} protein in the liver of *O. niloticus*. In accordance with the fortnight, the statistically highest GST value was observed at the 4th fortnight with the mean value of 56.35±12.44 nmol mg^{-1} protein. While statistically lowest value of GST was observed with the mean value of 44.74±7.59 nmol mg^{-1} protein in 1st fortnight. In the 2nd and 3rd fortnight, the value of GST was observed 48.77±8.49 and 52.77±10.47 nmol mg^{-1} protein, respectively (Table 2).

Kidneys

Highest activity (51.48±7.15 nmol mg^{-1} protein) of GST was observed at 60% inclusion level of a quinoa seed meal in fish feed. On the other hand, a statistically lowest value of GST activity (33.99±5.44 nmol mg^{-1} protein) was observed at 15% inclusion of a quinoa seed meal in fish feed. At 30 and 45% inclusion level, the activity of GST was observed as 40.75±5.93 and 46.20±5.44 nmol mg^{-1} protein. In case of fortnights, the statistically highest activity of GST was observed at the 4th fortnight with the mean value of 45.43±9.75 nmol mg^{-1} protein. While statistically lowest value of GST was observed with the mean value of 34.68±6.73 nmol mg^{-1} protein in 1st fortnight (Table 2).

Comparison of fish organs showed highest activity of GST in liver while kidney showed the least activity

with the mean value of 63.67±6.93 and 51.48±7.15 nmol mg^{-1} protein, respectively (Fig. 2).

Biochemical Parameters

The results of the blood serum enzymes of *Oreochromis niloticus* showed a significant ($p<0.05$) difference when fed on different replacement levels of *C. quinoa*.

Alanine transaminase

The ALT level was significantly higher ($p<0.05$) in the fish fed on T3 and T4 diet compared to the control group. Fish fed on T1 and T2 diets showed ALT values less than the control (Table 3).

Aspartate transaminase

Fish showed a significantly different response to different levels (T0, T1, T2, T3 and T4) of quinoa seed meal-based diet in terms of AST. AST values observed at the end of exposure period followed the order: T4 (126.50±54.39 IU/L) > T3 (112.25±14.36 IU/L) > T2 (94.50±11.45 IU/L) > T0 (90.00±0.54 IU/L) > T1 (73.25±4.79 IU/L) (Table 3).

Alkaline phosphatase

Values of ALP in fish fed on a quinoa-based diet were significantly higher in all treatments except T3 as compared to control. Overall means showed that the value of ALP was significantly higher for fish fed on the T2 diet, whereas a lower value was observed for the fish fed on the T3 diet with the mean values of 77.50±6.45 and 62.25±26.55 IU/L, respectively. However, the mean value of ALP in fish fed on T2 was observed statistically similar to the mean value obtained at the T4 dietary level (Table 3).

Haematological Parameters

The results obtained for the haematological parameters of *Oreochromis niloticus* showed significant difference for the fish fed on different replacement levels of *Chenopodium quiona* for four fortnights.

Results for haematological parameters for the fish, *O. niloticus* fed with *C. quinoa*-based diet at different treatment levels are shown in (Table 4). A significantly ($p<0.05$) higher mean value ($153.70±16.46×10^3/μl$) of WBC was observed for the fish feeding on the T4 diet, while a substantially lower mean value ($120.52±0.10×10^3/μl$) was observed for the control group. The mean value of RBC ($2.05±0.25×10^6/μl$) showed that the fish fed on the T4 diet recorded significant increases in the number of RBC, followed by that of T3, T2, T1 and control with mean values of 1.98±0.27, 1.78±0.12, 1.34±0.18 and 1.13±0.06 $×10^6/μl$, respectively. The mean value of haemoglobin (8.05±0.90g/dl) for the fish fed on the T4 diet appeared significantly higher as compared to control fish (7.50±0.07g/dl). The mean values obtained for PCV appeared slightly higher (29.75±2.80%) for the fish fed on T4 diet, while; a minimum value (15.50±0.08%) was observed in control fish. The value of MCV was slightly higher for fish fed on T2 dietary level, followed by that of T4, T3, T1 and control with the mean values of 145.92±7.36, 143.80±7.42, 140.86±17.85 and 137.20±0.25 pg, respectively. The value of MCH and MCHC significantly decreased as the inclusion level increased compared to the control. The least mean values for MCH and MCHC were observed in the fish fed with a T4 diet, while the higher values were obtained in the control group (Table 4).

Discussion

Fish is considered a vital source of animal protein. Almost 16% of animal protein consumed the world over is obtained from fish. Nearly one billion people in the world depend on fish as their primary source of animal protein. Due to high nutrient contents, balanced amino acid profile and good quality protein, fish meal is considered a significant source of animal protein generally used for all types of fish feed. Fish meal is also considered the most commonly used major source of protein, for livestock, poultry and commercial aquafeeds. So, there is a need to find more protein sources for animal feed. During the present investigation, fish meal was replaced with quinoa seed meal in fish feed to determine the effects of quinoa-based diet on antioxidant enzymes, biochemical and haematological parameters of *Oreochromis niloticus*. The antioxidant enzyme activities increased with the increased inclusion level of quinoa seed meal in fish feed. When fish was fed with the diet T1 containing 15% of quinoa seed meal it significantly lowered the activity of SOD and glutathione s-transferase (GST) compared to the fish fed with the other dietary inclusion level of quinoa seed meal. It was observed that decreased SOD activity in the liver of control fish and at the lower dietary level might be due to oxidative stress. The decreased SOD activity pointed out the accumulation of H_2O_2, a toxic product that may be formed as a result of low CAT level. Accumulation of superoxide anions may also lead to low SOD activity (Bagnyukova et al. 2006). Increased level of GST at a higher level of quinoa seed meal implies that this enzyme stimulates the biotransformation of xenobiotics; and thus reduces the production of reactive oxygen species. At a lower level of quinoa seed-based diet, the activity of GST may be decreased due to the reduced detoxification of xenobiotics, which is due to the increased status of oxidative stress at a low dietary level of quinoa seed meal.

Serum biochemical parameters, ALT, AST and ALP were measured to see the correlation between altered physiological fitness and environmental stress. The activity of these enzymes, AST and ALT in the *O. niloticus* was reported (Lin et al. 2007), which found a reduction in the activity of enzymes with the utilization of dietary protein with the increase in dietary soya bean level and observed liver damage to a certain extent. Kim et al. (2008) described that increase in the activity of serum ALT might suggest a defect in liver function. The present experiment showed a higher value of ALP for fish (*O. niloticus*) fed on the T2 diet, whereas a lower value was observed for the fish fed on the T3 diet with the mean values of 77.50±6.45 and 62.25±26.55 IU/L, respectively. According to which increased phosphatase activity indicates a higher breakdown of reserved energy utilized for the growth and survival of fish.

Blood parameters have been proved to be good physiological indicator and important tool to

diagnose the health status of fish (Seriani et al. 2012). The effect of *C. quinoa*-based diet on the fish *O. niloticus* was checked. In general, results showed significant RBC, WBC, and Hb values at all treatment levels of *C. quinoa* for fish species, *O. niloticus*, compared to the control fish group. A higher mean value (153.70±16.46×10^3/µl) of WBC was observed for *O. niloticus* fed on T4 diet as compared to the control fish with a mean value (120.52±0.10×10^3/µl). The numbers of WBCs are highly inconsistent in fish blood, even among members of the same species in similar conditions, just because of dependence on many factors (Ighwela et al. 2012). An increase in immunity due to the rise in the WBCs was observed by Bbole et al. (2016) in the fish species of *O. niloticus* fed on *Moringa oleifera*. This increase eventually improves the capacity of the fish to fight against diseases and infections (Hrubec et al. 2000). The rise in WBCs might be linked with enhancing non-specific responses (Soltanian and Fereidouni 2016).

The mean value of RBCs (2.05±0.25×10^6/µl) for *O. niloticus* fed on the T4 diet recorded significant increases in the number of RBC followed by that of T3, T2, T1 and control with mean values of 1.98±0.27, 1.78±0.12, 1.34±0.18 and 1.13±0.06 (×10^6/µl), respectively. Red blood cell counts greater than 1.0×$10^6$$mm^{-3}$ is measured high and indicate the high oxygen-carrying capacity of the blood which is the characteristic of fishes capable of aerial respiration and increased metabolic activity (Jimoh et al. 2015). The highest value of RBC was also observed in fish *O. niloticus* fed with 0.5% sage oil, and the lowest was observed in fish fed with 0% without sage oil reported by Aydin and Harmantepe (2018).

The present results showed that the mean value of haemoglobin (8.05±0.90g/dl) for *O. niloticus* fed on a T2 diet appeared significantly higher as compared to control fish (7.50±0.07g/dl). Similarly, a higher haemoglobin value was observed in the fish *O. niloticus* fed on a 35% maltose diet (Ighwela et al. 2012). Jimho et al. (2015) explained a higher value of Hb for *O. niloticus* fed on 30% watermelon seed meal. According to Naeem et al. (2020), a reduction in the haemoglobin, red blood cells, and packed cell volume causes anaemia. While Zafar and Khan (2018) associated the high haemoglobin content with the high feed conversion efficiency. Haematocrite (PCV) value was considered an important tool to determine fish's health status (Baba et al. 2016). In the present study, a significant increase in the value of packed cell volume (PCV) was recorded with increasing dietary inclusion levels of *C. quinoa* in the fish species *O. niloticus* compared to the control group.

Table 1: Effect of quinoa seed meal on the SOD (Umg^{-1} protein) activity in the liver and kidney of *Oreochromis niloticus*

Organs	Fortnight	Treatment					
		T1 (15%)	**T2 (30%)**	**T3 (45%)**	**T4 (60%)**	**T0 (control)**	***Overall Mean±SD**
Liver	1st	17.59±0.30**d**	18.58±0.37**c**	31.68±0.31**b**	34.64±0.45**a**	14.44±0.15**e**	23.38±9.11**d**
	2nd	21.35±0.45**c**	22.31±0.32**c**	35.09±0.23**b**	38.90±0.37**a**	14.58±0.10**e**	26.45±10.17**c**
	3rd	25.31±0.31**d**	27.12±0.31**c**	43.01±0.45**b**	45.51±0.53**a**	14.62±0.14**e**	31.11±12.95**b**
	4th	31.33±0.26**c**	30.28±0.48**c**	49.12±0.53**b**	50.12±0.27**a**	14.85±0.22**e**	35.14±14.75**a**
Overall Mean±SD		**23.91±5.87c**	**24.57±5.17c**	**39.73±7.86b**	**42.29±6.87a**	**14.62±0.17d**	
Kidney	1st	7.77±0.23**d**	12.02±0.61**c**	18.20±0.31**b**	35.34±0.41**a**	6.42±0.31**e**	15.45±11.17**d**
	2nd	10.73±0.23**d**	14.19±1.03**c**	21.70±0.23 **b**	40.62±0.68**a**	6.59±0.30**e**	18.77±13.41**c**
	3rd	15.14±0.23**d**	17.10±0.65**c**	25.05±0.32 **b**	42.61±0.23**a**	6.60±0.23**e**	21.21±13.60**b**
	4th	17.31±0.32**d**	21.68±0.62**c**	28.27±0.65 **b**	46.82±0.62**a**	6.53±0.33**e**	24.12±14.95**a**
Overall Mean±SD		**12.74±4.31e**	**16.25±4.28d**	**23.31±4.33 b**	**41.35±4.77a**	**6.54±0.08e**	

Means with similar letters in each row and *overall column means are statistically similar at $p < 0.05$.

Table 2: Effect of quinoa seed meal on the GST (nmol mg-1 protein) activity in the liver and kidney of *Oreochromis niloticus*

Organs	Fortnight	Treatment					
		T1 (15%)	**T2 (30%)**	**T3 (45%)**	**T4 (60%)**	**T0 (control)**	***Overall Mean±SD**
Liver	1st	38.03±0.01**d**	41.58±0.02**c**	48.33±0.02**b**	56.33±0.01**a**	39.44±0.01**e**	44.74±7.59**d**
	2nd	42.22±0.01**d**	47.74±0.02**c**	54.43±0.02**b**	60.00±0.02**a**	39.47±0.02**e**	48.77±8.49**c**
	3rd	46.58±0.01**d**	52.43±0.01**c**	59.04±0.02**b**	66.32±0.01**a**	39.46±0.02**e**	52.77±10.47**b**
	4th	50.02±0.02**d**	57.03±0.02**c**	63.25±0.03**b**	72.01±0.02**a**	39.46±0.03**e**	56.35±12.44**a**
Overall Mean±SD		**44.21±5.21d**	**49.70±6.61c**	**56.26±6.40b**	**63.67±6.93a**	**39.48±0.01e**	
Kidney	1st	27.47±0.01**d**	33.64±0.02**c**	39.64±0.01**b**	43.34±0.01**a**	29.32±0.02**e**	34.68±6.73**d**
	2nd	32.02±0.01**d**	38.44±0.01**c**	44.32±0.02**b**	48.63±0.01**a**	29.33±0.01**e**	38.55±8.10**c**
	3rd	36.43±0.01**d**	43.86±0.03**c**	48.61±0.02**b**	53.93±0.01**a**	29.38±0.02**e**	42.43±9.75**b**
	4th	40.02±0.02**d**	47.06±0.02**c**	53.23±0.02**b**	60.02±0.02**a**	29.35±0.01**e**	45.94±11.85**a**
Overall Mean±SD		**33.99±5.44d**	**40.75±5.93c**	**46.20±5.44b**	**51.48±7.15a**	**29.35±0.03e**	

Means with similar letters in each row and *overall column means are statistically similar at $p < 0.05$.

Table 3: Comparison of serum biochemical enzymes of *Oreochromis niloticus* fed on different levels of *C. quinoa*

Parameters	Treatments				
	T0 (Control)	**T1 (15%)**	**T2 (30%)**	**T3 (45%)**	**T4 (60%)**
ALT (IU/L)	55.16±0.49**c**	51.25±2.99**d**	54.63±1.49**c**	108.5±15.67**b**	125.25±32.10**a**
AST (IU/L)	90.16±0.35**d**	73.25±4.79**e**	94.50±11.45**c**	112.25±14.36**b**	126.50±54.39**a**
ALP (IU/L)	66.16±0.41**c**	70.50±3.32**b**	77.50±6.45**a**	62.25±26.55**d**	77.25± 5.74**a**

Means with similar letters in each row are statistically similar at $p < 0.05$.

Table 4: Means of haematological parameters of *O. niloticus* fed with different C. quinoa-based diet levels.

Parameters	Treatments				
	T0 (Control)	**T1 (15%)**	**T2 (30%)**	**T3 (45%)**	**T4 (60%)**
WBC (10^3/µl)	120.20±0.32 **e**	135.20±27.34**d**	147.78±17.90**c**	151.38±17.19**b**	153.70±16.46**a**
RBC (10^6/µl)	1.15±0.01**e**	1.34±0.18**d**	1.78±0.12**c**	1.98±0.27**b**	2.05±0.25**a**
Hb (g/dL)	7.58±0.08**b**	6.03±1.24**c**	7.35±0.90**b**	8.00±0.77**a**	8.05±0.13**a**
PCV (%)	15.65±0.16**d**	19.75±3.65**c**	27.60±2.32**b**	28.25±2.45**b**	29.75±2.80**a**
MCV (Fl)	136.69±0.60**e**	140.86±17.85**d**	155.33±2.93**a**	143.80±7.42**c**	145.92±7.36**b**
MCH (pg)	66.16±0.18**a**	44.59±3.22**b**	45.23±2.11**b**	41.12±7.61**c**	36.38±5.04**d**
MCHC (g/dl)	48.40±0.23**a**	30.45±0.93**b**	29.10±0.83**c**	28.51±3.99**d**	24.92±2.93**e**

Means with similar letters in each row are statistically similar at $p < 0.05$.

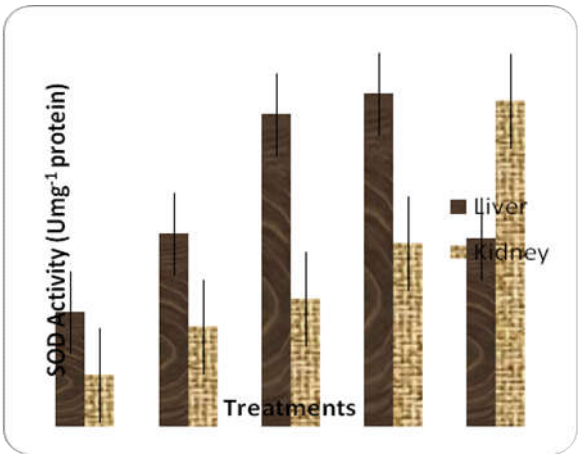

Fig. 1: Comparison of fish organs for SOD activity **(Umg^{-1} protein)**

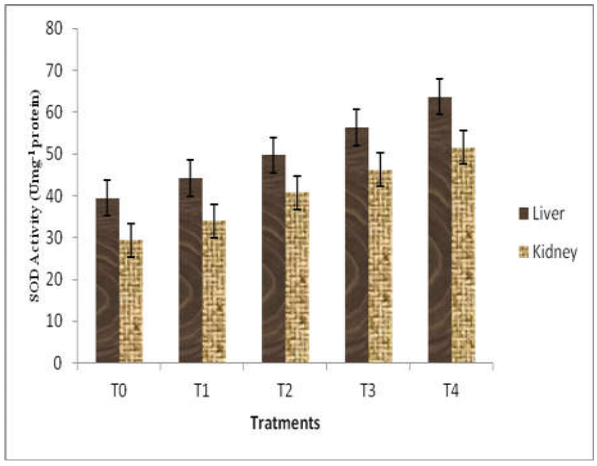

Fig. 2: Comparison of fish organs for GST activity **(Umg^{-1} protein)**

Conclusions

Results obtained from the present investigation showed that *O. niloticus* fed on different inclusion levels of *C. quinoa* in feed exhibited effective antioxidant activity and immune response. Among four studied inclusion levels, T_2 and T_3 give better results showing that 30 and 45 % replacement of fish meal with quinoa seed meal serves as a better alternative to fish meal in fish feed. It is concluded from this study that the use of this super plant as an immune stimulant represents a cost-effective alternative to fish meal in *O. niloticus* culture system.

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