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STUDY ON EFFECT OF *CHENOPODIUM QUINOA* ON GROWTH PERFORMANCE, CARCASS COMPOSITION AND ANTIOXIDANT ACTIVITY OF *OREOCHROMIS NILOTICUS* AND *CYPRINUS CARPIO*

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ABSTRACT

Plant-based protein sources are suitable and profitable for aquaculture. *Chenopodium quinoa* is one of the pseudo-cereal plants and promising source of protein. A 60-day feeding experiment was designed to check the impacts of *C. quinoa* seed meal (CQSM) on growth indices, carcass composition and antioxidant activity of *Oreochromis niloticus* and *Cyprinus carpio*. Six test diets as I, II, III, IV, V and VI were formed by using CQSM as replacement of fishmeal (FM) at 0%, 10%, 20%, 30%, 40% and 50%, respectively. Triplicate tanks having 15 juveniles in each were used and juveniles were fed at a diet equivalent to 5% of their live wet weight. Digestibility was measured with the help of feces. In *O. niloticus* and *C. carpio*, maximum results for weight gain (13.65g), (14.12g), feed conversion ratio (FCR) (0.98), (0.92), weight gain% (168.80), (199.15), and specific growth rate (SGR) (1.63), (1.21), respectively, were seen at level-III (20%) replacement of FM with *C. quinoa* seed meal based diet. For nutrient digestibility, best results in *O. niloticus* and *C. carpio* were also noticed at 20% replacement of FM with CQSM as crude protein (CP) (68.59), (66.30), crude fat (CF) (65.82) (65.65) and gross energy (GE) (72.36), (65.60), respectively. The antioxidant activity was maximum: 6.56 for *O. niloticus* and 7.13 for *C. carpio* at 20% replacement of FM. The findings indicated that the substitution of FM with CQSM up to 20% has a positive effect on fish growth, carcass composition, antioxidant activity and nutrient digestibility.

Keywords: Quinoa seed meal, Pseudocereal plants, Fishmeal, Growth performance, Antioxidant activity.

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INTRODUCTION

Unlike the beef, pork, and poultry sectors, aquaculture has been growing at an exponential rate and currently provides over half of global animal protein consumption (Galkanda-Arachchige *et al*., 2020). Global aquaculture production increased by 5.3% annually on average while fishmeal (FM) production decreased from 2000 to 2020 (Bianchi *et al*., 2014). There is approximately 7% rise in the aquaculture industry each year (Chen *et al*., 2019). The growth of aquaculture farming has increased the demand for fish feed, making it essential for fish farmers to have access to sustainable and affordable feed options. Therefore, there is a pressing need to identify and develop environmentally friendly and cost-effective feed sources (Mensah *et al*., 2018; Selamoglu, 2021a). The dependence of the worldwide FM supply on overfished pelagic marine fish stocks makes it unsustainable (FAO, 2018). The historic collapse of Peruvian anchovy populations and ongoing declines in wild fish stocks have led to an artificial shortage of FM, driving up prices and underscoring the urgent need for sustainable alternatives (Ezewudo *et al*., 2015; Ferguson-Cradler, 2018; Selamoglu, 2021b, Mesut,S. 2021). Despite the vast global fish supply, only a small fraction (10%) is utilized as FM in aquaculture, failing to meet the industry's soaring demand for fish feed production (FAO, 2012). Scientists emphasize the necessity of using plant proteins as an alternate protein source to FM to secure the sustainability of the aquaculture sector due to rising demand, high costs, and unpredictable supply (Agupugo *et al*., 2022). Because of their great availability, favorable nutritional profile, and competitive pricing, plant-based proteins emerged as a promising alternative (Hardy, 2010).

Chenopodium quinoa is a member of the group of pseudo-cereals. Quinoa has exceptional nutritional qualities because of its good amino acid profile, unsaturated fatty acids, vitamins, dietary fibre, and minerals (D'Amico *et al*., 2017). This crop is resilient and can thrive in saline soil conditions, making it an ideal choice for Pakistan, where approximately one-third of the cultivated land is salinized. Quinoa can be cultivated in hilly areas like Hunza valley and in lower plains like Bahawalpur. The growing season of quinoa in Pakistan is October-March. Quinoa belongs to the family Chenopodiaceae of group Dicotyledoneae, genus Chenopodium and species quinoa. There are over 250 species of chenopodium and either the entire plant or specific plant components are employed. The cultivation of *C. quinoa* has expanded rapidly, from just eight countries in 1980 to forty in 2010, and is projected to reach over a hundred countries by 2021, marking a significant increase in its global adoption (Ruiz *et al*., 2014). More than 80% of the world's quinoa is produced by three countries in South America - Bolivia, Ecuador, and Peru (Bazile *et al*., 2016). The crop is highly adaptable to agro-ecological extremes (soils, rainfall, temperature, and altitude) due to its high levels of genetic diversity (Ruiz *et al*., 2016). Quinoa seeds, irrespective of their source or variety, have a high content of protein (14% on average) and a rich profile of amino acids (especially lysine and histidine). Due to their well-balanced composition of essential amino acids, quinoa proteins appear to be a feasible option (Pereira *et al*., 2019, Wang *et al*., 2020). Additionally, it contains dietary fibers, powerful antioxidants including polyphenols and unsaturated fatty acids like linolenic acid that have been demonstrated to be crucial for disease prevention and has strong antioxidant activity (Pellegrini *et al*., 2018).

The main edible component of *C. quinoa* is its grains, which are gluten-free and has high protein content, valuable amino acids, vital minerals, and vitamins. Often termed as a "superfood," it has unique nutritional properties and numerous health benefits. In recognition of quinoa's importance, the United Nations General Assembly designated 2013 as the "International Year of Quinoa" (Pathan and Siddiqui, 2022). Additionally, quinoa leaves have high concentrations of potassium, manganese, and copper, as well as average concentrations of calcium, phosphorus, salt, and zinc (Adamczewska-Sowińska *et al*., 2021). Young (30-45 days old) dried quinoa leaves had a protein value of 28.2-37.0 g 100 g^{-1}, whereas quinoa grains had a protein level of 9.1-15.7 g 100 g^{-1}, both on a dry weight (DW) basis. Green quinoa leaves had a lower fat content than quinoa grains (4.0 to 7.6%), ranging from 2.4 to 4.5%. Fiber content varies from 6.9% in quinoa leaves and 7.8% in quinoa sprouts to 7.1% in quinoa grains. Quinoa leaves' total carbohydrate content (34.0%) was considerably lower than that of the grain (48.5-69.8%) (Pathan and Siddiqui, 2022). The methionine and lysine content in quinoa is 40–100 and 510–640 mg 100 g^{-1}, respectively, due to which it is considered as a "complete protein". According to reports, it has a lysine level that is 25% greater than rice and nearly twice as high as that of wheat and corn. It could potentially be researched as a FM replacement due to its high nutritional content.

One of the most significant warm-water fishes raised in aquaculture is tilapia (Kumar and Engle, 2016). With a global production of 4 tonnes in 2018, Nile tilapia (*Oreochromis niloticus*) is one of the most widely farmed fish, ranking third in global aquaculture production, accounting for 8.3% of the most significant aquaculture species (FAO, 2018). Nile tilapia has an overall brownish or greyish color, frequently have hazy body banding, and have vertical tail stripes. Furthermore, this species is also known by various commercial names, including Mango Fish, Nilotica, and Boulti. Nile tilapia often feeds throughout the day, therefore it resembles trout and salmon in this aspect (Munguti *et al*., 2022). *O. niloticus* contributes 62% (about 15,000 tonnes) of the overall aquaculture production each year. Tilapia was regarded as a culinary fish in ancient Egypt (Soliman and Yacout, 2016) and it is a well-known supermarket fish in the United States. Nile tilapia, which is available all year round, is the most prevalent fish in several South Indian lakes. In a given time, *O. niloticus* grows more rapidly and attains larger size. Tilapia mainly feeds on debris. Its widespread availability and affordability make it a highly sought-after food source, particularly among low-income communities that can easily afford it (Khan and Panikkar, 2009).

The common carp (*Cyprinus carpio*) belongs to the family Cyprinidae. Carp are native to slow-moving rivers and marshes, typically inhabiting weedy and muddy areas. During their early life stages, they feed on zooplankton, such as copepods and rotifers, but as they mature, they transition to a benthic diet, consuming organisms and organic matter. Since the beginning of time, people have farmed cyprinids, and today they are without a doubt the most significant teleost family raised on a global scale, with a current production rate of over 13 million tonnes annually (Takeuchi *et al*., 2002). It is widely available in almost every country, but is particularly popular in Asia and several European countries (Parkos, 2003). The third most often cultured species worldwide is the common carp (Rahman, 2015). The main focus of this study was the replacement of FM with CQSM in *O. niloticus* and *C. carpio* and to assess fish growth, carcass composition, antioxidant activity and nutrient digestibility.

MATERIALS AND METHODS

Fish and experimental condition: Ninety juveniles of *O. niloticus* and *C. carpio* were brought from the Government Fish Seed Hatchery. To prevent fungal infections and remove external parasites, the juveniles were immersed in a 5 g/L sodium chloride (NaCl) solution for 2 minutes, prior to the experiment (Rowland and Ingram, 1991). V-shaped tanks were used to adapt the fish juveniles to laboratory conditions over the time course of two weeks and these tanks are specifically created for collecting feces from water. The juveniles were fed on the basal diet two times a day for this duration. In order to maintain oxygen level, aeration throughout the day was provided by pumping via the capillary system to all experimental tanks. Daily monitoring was done for physical factors including temperature, pH, and dissolved oxygen.

Ingredients of feed and experimental diet: Six experimental diets including one control and five CQSM based diets were prepared. As inert marker, chromic oxide was added in the recommended diet at 1% inclusion rate. All the diet ingredients and nutrient composition are shown in Table 1. The 0.5 mm sieve size was used after the feed ingredients were ground. After 5 minutes of blending of all the ingredients, fish oil was gradually added. 10-15% distilled water was used to make a proper dough. Finally, a pelleting machine was used to create feed pellets measuring 3 mm in size. The pellets were then dried for 12 hours at 105 °C in the oven.

Feeding method and collection of samples: The recommended diets were fed at a rate of 5% to *O. niloticus* and *C. carpio* twice daily. Three replicates were assigned for each experimental diet, and each tank was stocked with 15 fish. After 2 hours of feeding session, the valves of each tank were opened to drain the uneaten feed. The feces were subsequently removed from the tank's fecal collection tubes by opening valves I and II, twice daily. Feces were dried and preserved for chemical analysis for each treatment. The duration of this feeding trial was eight weeks. The oven was used to dry feces at 60 °C, ground and then preserved for the digestibility estimation.

Carcass analysis: At the completion of the experiment, three fish were randomly selected from each tank, and their complete bodies were chemically analyzed. The fish samples were homogenized with a mortar and pestle. These samples were analyzed by the established standard methods (AOAC, 2005). The crude protein (CP) (N×6.25) was measured using a micro Kjeldahl device, while the moisture content was evaluated by oven drying for 12 hours at 105°C. Petroleum ether extraction was used to get the ether extract (EE) using the Soxtec HT2 1045 equipment and the gross energy (GE) was calculated through oxygen bomb calorimeter. Chromic oxide was added to test diets to assess the nutritional digestibility.

Calculation of digestibility: The apparent digestibility of GE, crude fat (CF) and CP of test diets was determined indirectly by using an inert marker i.e. chromic oxide (NRC, 1993).

Study of Growth performance: To measure growth, fish were taken from each tank at the completion of each experiment. To calculate the growth performance of these fingerlings, standard formulae were utilized.

$$\text{Weight Gain \%} = \frac{\text{Final Weight} - \text{Initial Weight}}{\text{Initial Weight}} \times 100$$

$$\text{FCR} = \frac{\text{Total dry feed intake (g)}}{\text{Wet weight gain}}$$

Evaluation of Antioxidant Activity: DPPH method was used to measure antioxidant activity of feed and fish using 1,1-diphenyl-2-picrylhydrazylradical scavenging assay as with some alterations used by Brand-Williams (1995). 2 ml of liquid methanol was used to dissolve 2 g of the ingredients, which were then kept at standard temperature for 10 mins. The homogenized material was centrifuged (5000 rpm, 4^0C, 10 mins) and allowed to shift it through a 0.45 nm filter having syringe filter (Whatman) before analysis. After that, 100 µl of shifted material was kept in 1.5 ml cuvette and 900 µl DPPH methanol solution (100 µM) was added to get the final volume of 1 ml by the use of spectrophotometer. For ten minutes, the blended material's absorbance at 517 nm was observed at intervals of one minute. The extract's antioxidant activity was measured as a percent inhibition of the DPPH radicals.

Percent inhibition = [(A0 – As) /A0] ×100

The absorbance of the sample after 0 to 5 minutes is represented in this equation by the letters A0 and As, respectively.

Statistical analysis: One-way analysis of variance (ANOVA) was applied on the data (nutrient digestibility, carcass composition, antioxidant activity and growth) (Steel *et al*., 1996). Tukey's Honestly Significant Difference test was performed to compare variances in means (Snedecor and Cochran, 1991). The CoStat computer programme was used to carry out the statistical analysis.

RESULTS

Parameters of growth: Tables 2 and 3 indicate the different parameters of growth performance of *O. niloticus* and *C. carpio* juveniles fed on CQSM based test diets of different levels. CQSM based diet enhanced the feed quality and increased the weight of fish. Fingerlings showed non-significant differences (P>0.05) in feed intake against graded levels of CQSM based diets. The highest WG of *O. niloticus* (13.65) and *C. carpio* (14.12) juveniles was observed in CQSM based test diets having 20% level of FM replacement. After 20% CQSM level, less weight gain of fish was observed. The minimum WG of *O. niloticus* (11.95) and *C. carpio* (12.30) was noted at 0% level. At 10%, 30% and 40% levels, the WG was increased as compared to control diet but 20% level was the best level in terms of growth parameters. The FCR of juveniles of *O. niloticus* and *C. carpio* was also significantly improved by CQSM based diets. The value of FCR was 0.98 of *O. niloticus* at 20% level and was 0.92 in case of *C. carpio*.

Nutrient digestibility: The findings of the feces analysis demonstrated that feeding *O. niloticus* and *C. carpio* juveniles with 20% CQSM had a significant effect on nutrient digestibility ($P<0.05$). Both fishes showed lesser amount of nutrients in feces at 20% level of replacement as compared to other levels and control. It shows that the maximum nutrients were utilized and absorbed in fish body showing a satisfactory sign. Large amount of nutrients in feces were seen at the levels of 30% and 50% CQSM based diet, demonstrating that the majority of nutrients were excreted with feces and the least amount were absorbed by the fish's body.

The investigated percent composition of nutritional digestibility (CF, CP, and GE) in defecated components in *O. niloticus* and *C. carpio* juveniles is shown in Tables 4 and 5. According to the current study's findings in *O. niloticus* (68.59) and *C. carpio* (66.30), CP had the greatest rate of digestibility at 20% level, respectively and was significantly different from all other test diets ($P<0.05$). Additionally, it was noted that the (CF) and (GE) had the greatest values at the 20% replacement level of CQSM, which were substantially ($P<0.05$) different from those of the other test diets. When compared to control diet, nutrient digestibility was lower at replacement levels of 30%, 40%, and 50%.

Carcass composition: In the current study, fish fed 20% CQSM had the greatest CP value of 15.21 and 16.12 for *O. niloticus* and *C. carpio*, respectively while fish fed the control diet had the lowest values 13.71 and 13.49, respectively as shown in Figure 1 and 2. It indicates that CQSM-based diets are a good alternative source of protein. Similar results were reported for the CF%. The highest value CF % (6.36) was found when *O. niloticus* was fed with 50% CQSM and lowest value (5.73) for CQSM-based diets with 20%. In the case of *C. carpio* the highest (6.55) and lowest (5.10) CF values were observed at control and 20% CQSM based diet, respectively. It showed that 20% CQSM based diet was better than control diet. On the other hand, ash content for 20% CQSM replacement level was also higher than other diets. When the replacement levels of CQSM were increased in *O. niloticus* and *C. carpio* up to 50%, the values for CF increased and CP decreased. The findings show that the maximum ash content in *O. niloticus* (0.98) and in *C. carpio* (2.02) were found at 20% CQSM replacement level.

Evaluation of Antioxidant Activity: Percentage oxidation was utilized as a measure to assess the impact of quinoa on each diet in order to acquire the results. Figure 3 displays that experimental diet-III having 20% of CQSM was found to be the best because it shows lowest percentage of oxidation: 6.56 for *O. niloticus* and 7.13 for *C. carpio* while the maximum oxidation was obtained at 0% replacement level: 98.73 for *O. niloticus* and 96.69 for *C. carpio* juveniles.

Table 1. Ingredients and nutrient composition (%) of test diets

	Test Diet-I (control)	Test Diet-II	Test Diet-III	Test Diet-IV	Test Diet-V	Test Diet-VI
***Chenopodium quinoa* seed meal (CQSM)**	0	10	20	30	40	50
Fish meal	50	40	30	20	10	0
Wheat flour	17.1	17.1	17.1	17.1	17.1	17.1
Corn gluten (60%)	24	24	24	24	24	24
Fish oil	4.9	4.9	4.9	4.9	4.9	4.9
***Vitamin Premix**	1.0	1.0	1.0	1.0	1.0	1.0
****Mineral Premix**	1.0	1.0	1.0	1.0	1.0	1.0
Ascorbic acid	1.0	1.0	1.0	1.0	1.0	1.0
Chromic oxide	1.0	1.0	1.0	1.0	1.0	1.0
Total	100.0	100.0	100.0	100.0	100.0	100
Analyzed Nutrient Composition of diets (%)						
Crude protein	28.30	28.33	28.43	28.40	28.38	28.39
Crude fat	4.16	4.38	4.01	4.13	4.33	4.34
Gross energy (kcal g^{-1})	4.17	4.14	4.23	4.21	4.16	4.19

*Vitamin (Vit.) premix kg-1: Vit. A: 15,000,000 IU, Vit. B12: 40 mg, Vit. C: 15,000 mg, Vit. D3: 3,000,000 IU, B2: 7000 mg, Vit. E:30000 IU, Vit. B6: 4000 mg, Vit. Ca pantothenate: 12,000 mg, Vit. K3: 8000 mg, Folic acid: 1500 mg, Nicotinic acid: 60,000 mg, **Mineral premix kg-1: Fe: 1000 mg, Ca: 155 g, P: 135 g, Se: 3 mg, Na: 45 g, Co: 40 mg, Zn:3000mg, Cu: 600 mg, Mn: 2000 mg, I: 40 mg, Mg: 55 g

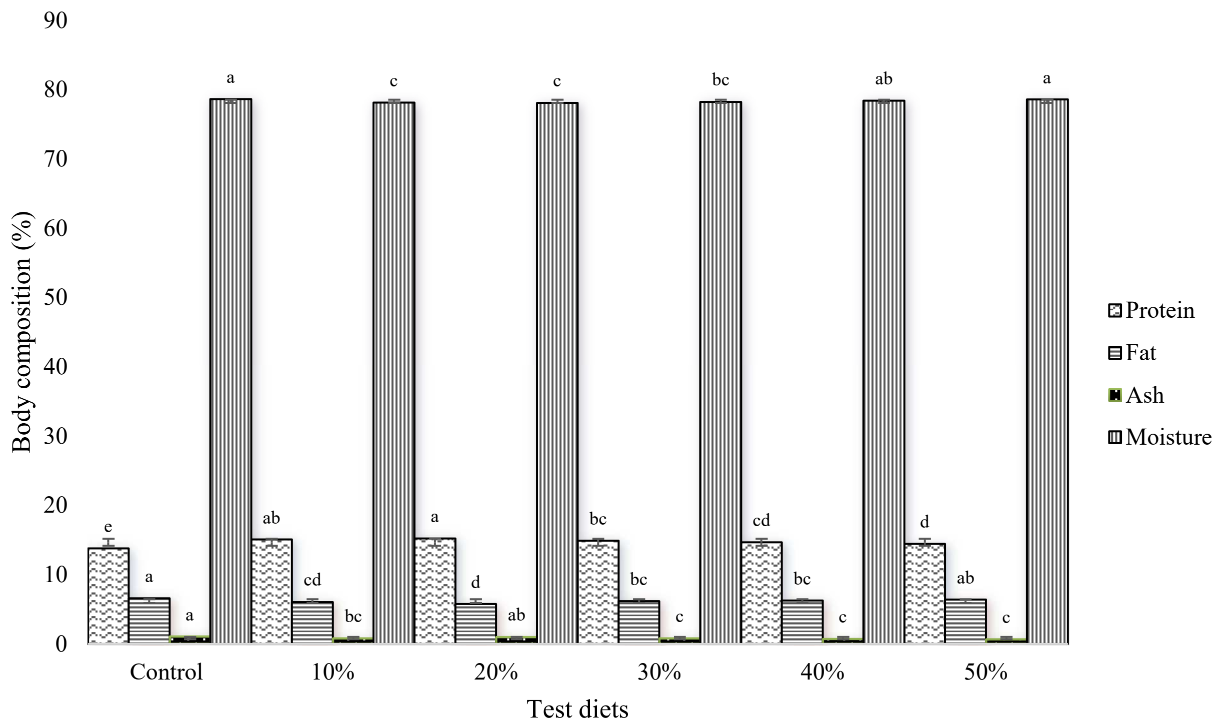

Figure 1. Protein, fat, ash and moisture content % of *O. niloticus* juveniles fed on *Chenopodium quinoa* seed meal based diets.

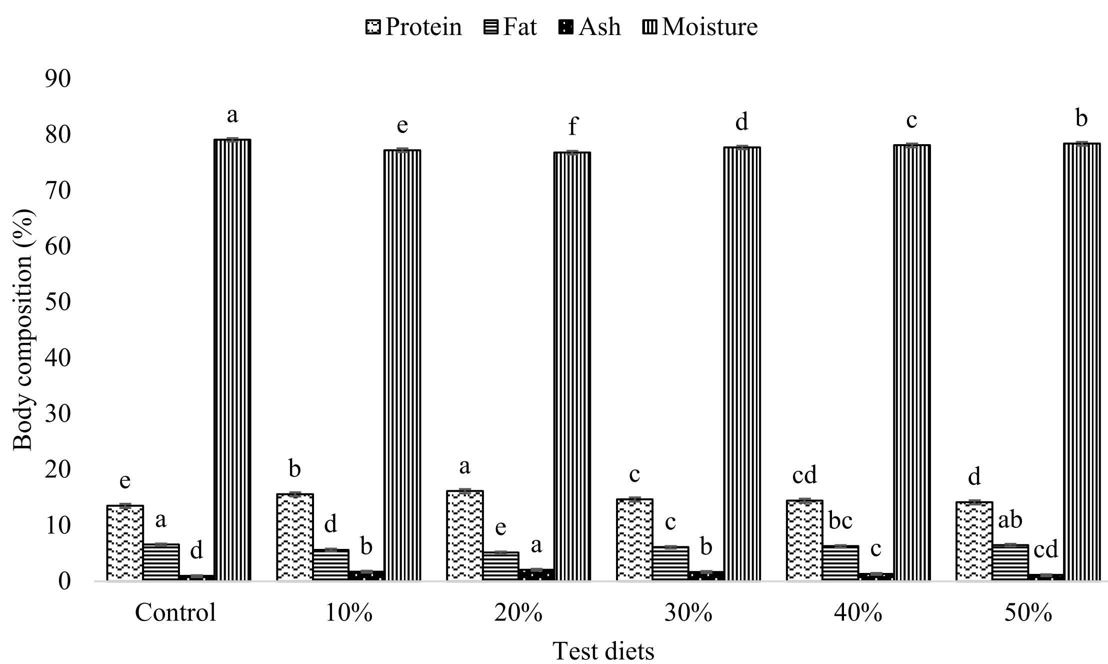

Figure 2. Protein, fat, ash and moisture content % of *C. carpio* juveniles fed on *Chenopodium quinoa* seed meal based diets

Table 2. Growth performance of *O. niloticus* fingerlings fed *Chenopodium quinoa* seed meal based diets

Experimental diets**	CQSM* Levels (%)	Initial weight (g)	Final weight (g)	WG (g)	WG %	Weight gain ($Fish^{-1}Day^{-1}$) (g)	FCR (%)	SGR (%)
Test diet-I (Control diet)	0	8.15±0.04	20.10±0.06^{c}	11.95±0.07^{c}	146.71±1.52^{c}	0.19±0.00^{c}	1.19±0.00^{a}	1.50±0.01^{e}
Test diet-II	10	8.09±0.02	20.91±0.04^{b}	12.82±0.03^{b}	158.44±0.66^{b}	0.21±0.00^{b}	1.10±0.00^{c}	1.58±0.00^{c}
Test diet-III	20	8.08±0.05	21.73±0.09^{a}	13.65±0.11^{a}	168.80±2.30^{a}	0.22±0.00^{a}	0.98±0.01^{d}	1.63±0.00^{b}
Test diet-IV	30	8.07±0.05	21.06±0.05^{b}	12.99±0.08^{b}	160.90±2.01^{b}	0.21±0.00^{b}	1.08±0.01^{c}	1.55±0.01^{d}
Test diet-V	40	8.09±0.05	20.86±0.13^{b}	12.77±0.16^{b}	157.94±2.72^{b}	0.21±0.00^{b}	1.09±0.00^{c}	1.67±0.01^{a}
Test diet-VI	50	8.06±0.04	20.33±0.13^{c}	12.27±0.17^{c}	152.22±2.95^{c}	0.20±0.00^{c}	1.15±0.01^{b}	1.60±0.00^{bc}

$^{a-e}$In columns the means having different superscripts differ significantly (*P<0.05*)
*CQSM = *Chenopodium quinoa* seed meal
**Test diet-I (Control diet) = 0% CQSM, Test diet-II = 10% CQSM, Test diet-III = 20% CQSM, Test diet-IV = 30% CQSM, Test diet-V = 40% CQSM, Test diet-VI = 50% CQSM
*

Table 3. Growth performance of *C. carpio* fingerlings fed *Chenopodium quinoa* seed meal based diets

Experimental diets**	CQSM* Levels (%)	Initial Weight (g)	Final Weight (g)	Weight Gain (g)	Weight Gain (%)	Weight gain ($Fish^{-1}Day^{-1}$) (g)	SGR (%)	(FCR)
Test diet-I (Control diet)	0	7.13±0.03	19.43±0.08^{cd}	12.30±0.08^{cd}	172.60±1.42^{cd}	0.20±0.001	1.11±0.00^{cd}	1.11±0.01^{b}
Test diet-II	10	7.09±0.04	20.61±0.20^{b}	13.52±0.23^{b}	190.52±4.28^{b}	0.22±0.00	1.18±0.01^{b}	1.02±0.02^{c}
Test diet-III	20	7.09±0.02	21.22±0.14^{a}	14.12±0.14^{a}	199.15±2.10^{a}	0.23±0.00	121±0.00^{a}	0.92±0.00^{e}
Test diet-IV	30	7.08±0.04	20.09±0.05^{ab}	13.82±0.03^{ab}	195.20±1.40^{ab}	0.23±0.00	1.20±0.00^{ab}	0.97±0.01^{d}
Test diet-V	40	7.09±0.02	19.73±0.13^{c}	12.64±0.12^{c}	178.24±1.73^{c}	0.21±0.00	1.13±0.00^{c}	1.09±0.00^{b}
Test diet-VI	50	7.06±0.00	19.11±0.02^{d}	12.04±0.02^{d}	170.55±0.48^{d}	0.20±0.00	1.10±0.00^{d}	1.21±0.02^{a}

$^{a-e}$In columns the means having different superscripts differ significantly (*P*<0.05)
*CQSM = *Chenopodium quinoa* seed meal
**Test diet-I (Control diet) = 0% CQSM, Test diet-II = 10% CQSM, Test diet-III = 20% CQSM, Test diet-IV = 30% CQSM, Test diet-V = 40% CQSM, Test diet-VI = 50% CQSM

Table 4. Apparent nutrient digestibility of *O. niloticus* juveniles fed on *Chenopodium quinoa* seed meal based test diets.

Experimental Diets**	CQSM* Levels (%)	Crude Protein (%)	Crude Fat (%)	Gross Energy (%)
Test Diet-I Control	0	42.52 ± 0.33^{f}	46.31 ± 1.18^{c}	46.92 ± 0.77^{d}
Test Diet-II	10	48.11 ± 0.39^{e}	54.54 ± 0.86^{b}	51.73 ± 2.66^{d}
Test Diet-III	20	68.59 ± 0.70^{a}	65.82 ± 2.70^{a}	72.36 ± 1.25^{a}
Test Diet-IV	30	50.71 ± 0.72^{d}	64.88 ± 1.96^{a}	55.94 ± 0.88^{b}
Test Diet-V	40	62.27 ± 0.39^{b}	65.94 ± 2.09^{a}	65.96 ± 2.80^{b}
Test Diet-VI	50	56.84 ± 0.99^{c}	57.44 ± 0.22^{b}	58.48 ± 2.06^{c}

$^{a\text{-}f}$In columns the means having different various superscripts differ significantly (P<0.05)
*CQSM = *Chenopodium quinoa* seed meal
**Test diet-I (Control diet) = 0% CQSM, Test diet-II = 10% CQSM, Test diet-III = 20% CQSM, Test diet-IV = 30% CQSM, Test diet-V = 40% CQSM, Test diet-VI = 50% CQSM

Table 5. Apparent Nutrient Digestibility of *C. carpio* juveniles fed on *Chenopodium quinoa* seed meal based diets

Experimental Diets**	CQSM* Levels (%)	Crude Protein (%)	Crude Fat (%)	Gross Energy (%)
Test Diet-I Control	0	41.90 ± 0.43^{f}	45.20 ± 1.08^{c}	55.86 ± 1.30^{d}
Test Diet-II	10	48.41 ± 0.46^{e}	51.44 ± 1.04^{b}	52.81 ± 1.61^{e}
Test Diet-III	20	66.30 ± 0.60^{a}	65.65 ± 1.51^{a}	65.60 ± 2.12^{a}
Test Diet-IV	30	53.18 ± 0.42^{d}	65.86 ± 1.70^{a}	65.54 ± 2.39^{b}
Test Diet-V	40	62.42 ± 0.41^{b}	67.33 ± 1.51^{a}	65.43 ± 1.81^{c}
Test Diet-VI	50	55.10 ± 0.17^{c}	55.32 ± 3.39^{b}	57.18 ± 2.00^{d}

$^{a\text{-}f}$In columns the means having different various superscripts differ significantly (P<0.05)
*CQSM = *Chenopodium quinoa* seed meal
**Test diet-I (Control diet) = 0% CQSM, Test diet-II = 10% CQSM, Test diet-III = 20% CQSM, Test diet-IV = 30% CQSM, Test diet-V = 40% CQSM, Test diet-VI = 50% CQSM

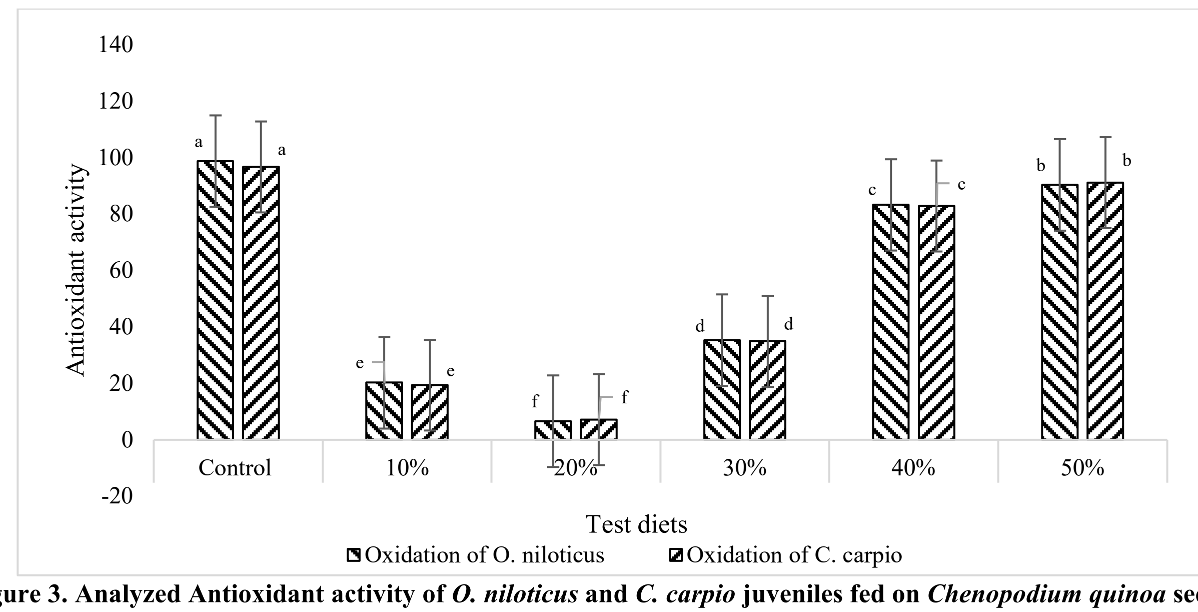

Figure 3. Analyzed Antioxidant activity of *O. niloticus* and *C. carpio* juveniles fed on *Chenopodium quinoa* seed meal based diets

DISCUSSION

This research work was carried out to check the growth efficiency, nutrient digestibility, carcass composition and antioxidant activity of *O. niloticus* and *C. carpio* juveniles fed on diets having different replacement levels of CQSM. Quinoa seeds are extremely popular all over the world due to their great nutritional content. Since the seeds are gluten-free, have a low glycemic index, and include all the essential amino acids, carbs, fats, minerals, vitamins, and dietary fibres, they are considered as a superfood (Vega-Galvez *et al*., 2010). Mean weight gain in all groups showed that all the experimental diets are nutritionally good. The phenolic compounds, saponins, and protein in quinoa are attributed to be the functional ingredients responsible for having positive effect on growth (Simnadis *et al*., 2015; Ali, 2020). Fish fed at test diet level-III (20% CQSM replacement) had given maximum WG% in *O. niloticus* and *C. carpio* when compared to the control diet (0% CQSM).

Dry quinoa seeds contain variable protein contents between 13.8 to 16.5% and on average it is about 15%. The protein of quinoa has high nutritional value is due to higher lysine levels (Navruz-Varli and Sanlier, 2016). In recent finding highest feed intake at level I&VI (0% & 50% CQSM) were observed in both *O. niloticus* and *C. carpio* but minimum feed intake was observed at level-III (20% CQSM) indicated that at maximum replacement level-VI (50% CQSM) FCR value was increased. Almost similar results were obtained by Molina-Poveda *et al*. (2017) replaced the shrimps diet with quinoa diet at levels 0%, 15%, 25%, 35% and 45% levels. Replacement with quinoa meal shows better growth performance. Quinoa replacement shows a better average of FCR. The findings of current study show that at level replacement level-III 20% CQSM based diet optimum growth performance was obtained. Results obtained from the study by Kiran *et al*. (2022) showed that *O. niloticus* fed on different inclusion levels of *C. quinoa* in feed exhibited effective antioxidant activity and immune response. Among the four inclusion levels studied, T2 and T3 produced the best results, demonstrating that substituting 30% and 45% of the FM in fish feed with quinoa seed meal is a superior alternative. This study's findings support the idea that using this super plant as an immune stimulant in *O. niloticus* culture systems is a more affordable option than using FM. The inclusion of 20% quinoa seeds in fish increased their immune response and resistance to several illnesses, according to another study by Mohamed Ahmed *et al*. (2021).

Natural antioxidants like tocopherols, flavonoids, phenolic acids, and carotenoids are present in plant tissues. These compounds delay the autoxidation by different mechanisms (Brewer, 2011). Researchers performed experiments on plant materials (many fruits and parts of olive plant) and numerous substances like spices, herbs, plant extracts (e.g. wheat, grapes, tea and coffee) (Brewer, 2011; Taghvaei and Jafari, 2015). Quinoa has flavonol glycosides which serve as good antioxidant (Zhu *et al*., 2001). The quinoa seeds have the ability to trap free radical and show higher antioxidant activity as compared to other cereal crops (Gorinstein *et al*., 2008). Some plant materials also contain high concentration of phenolic acid compounds which provide anti oxidation protection by hydrogen donation mechanism similar to quinoa seeds, so could be used as alternative to quinoa seeds. In our results antioxidant activity was increased in level-III (20%) replacement of CQSM based test diet.

Conclusion: In both *O. niloticus* and *C. carpio*, a diet supplemented with 20% CQSM showed improved growth performance, enhanced nutrient digestibility, and increased antioxidant activity, without any adverse effects. The results suggested that CQSM-based diets may be a viable substitute for fish diets, offering a promising alternative for the aquaculture industry to reduce costs while maintaining sustainability.

Conflicts of interest: According to all authors, there is no conflict of interest with the publication of this paper.

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Data availability statement: The associated authors can provide you with all the data from this experiment upon reasonable request.

Ethical statement: This study does not require ethical approval.

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