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Ameliorative effect of dietary supplementation of kinnow peel powder on the health status of *Cyprinus carpio* (L.) fingerlings

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ABSTRACT

An outdoor feeding trial (120 days) was carried out in FRP pools (1.5 × 1.0 × 0.75 m) to evaluate the efficacy of dietary incorporation of kinnow peel powder (KPP) on the health status of common carp (*Cyprinus carpio* L.) fingerlings. Healthy fingerlings (av. length 9.19 cm, and av. weight 10.51 g) were stocked in five feeding treatments @ 15 nos./tank following a complete randomized design with three replicates for each treatment and daily fed with five iso-proteinous experimental feeds containing KPP at 0 (KPP0), 0.5 (KPP1), 1.0 (KPP2), 1.50 (KPP3) and 2.0% (KPP4) inclusion levels @ 2% of fish body weight. The results demonstrated that the water quality parameters remained within the optimum/permissible limit for freshwater carps in all the treatments. Further, KPP supplementation improved the haematological indices (total erythrocyte count, total leucocyte count, haemoglobin and haematocrit) and immunological parameters (serum total protein, Albumin (A), globulin (G) and A: G ratio) of fish at all the inclusion levels in respect to control, while the best ($P \leq 0.05$) findings observed at 1.5% KPP inclusion level. Additionally, in comparison with the control group, KPP supplementation decreased the glucose, cholesterol, triglycerides, alanine aminotransferase, aspartate aminotransferase, lipid peroxidation level, being lowest ($P \leq 0.05$) in KPP3, while increased the superoxide dismutase activity and recorded highest ($P \leq 0.05$) in KPP3 group. The results revealed that KPP can be potentially utilized as health promoting natural feed additive at a 1.5 % inclusion level to improve the overall health condition of *C. carpio* fingerlings.

Key words: *Carp, Cyprinus carpio, Fish health, Hemato-immunological response, Kinnow peel powder*

Introduction

Freshwater aquaculture in India has been primarily supported by the carps which account for more than 70% of the country's inland output. Among carps, the common carp (*Cyprinus carpio*) is a commercially important and robust freshwater fish that can be reared under diverse environmental conditions (Dawood *et al.*, 2020) and contributed around 8.6% of global aquaculture production during 2020 (FAO, 2022). The foremost objective of aquaculture is to improve productivity while maintaining fish health and sustainability (Acar *et al.*, 2015). To achieve these objectives several technological interventions such as water quality management, nutritional innovations, and health management are used in aquac-

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ulture practices (Srivastava, 2019). Further, the intensification of culture systems also leads to stress on fish making them vulnerable to a variety of diseases, thus adversely affecting fish health and production (Kumar *et al*., 2019). However, several chemical substances, therapeutics, antibiotics, and feed additives are commonly used for enhancing the growth performance, immunity, disease resistance, and overall health status of fish (Austin and Austin, 2007; Acar *et al*., 2015) but due to ecological as well as food safety concerns like environmental pollution, antibiotic resistance in bacteria and residual effect in fish etc., an eco-friendly and sustainable alternative is a need of the hour (Esiobu *et al*., 2002; Dawood *et al*., 2018).

Owing to the several physiological and pharmacological benefits, formulating fish feed using locally available, cost-effective, biodegradable and environmentally friendly agro-industrial by-products appears to be a viable option for reducing industrial pollution, improving fish survival, productivity and health status in aquaculture systems (Elferink *et al*., 2008, Singh and Srivastava, 2022). In India, large quantity of fruits is processed annually which produces significant amounts of agro-industrial by-products/waste materials such as peel, seeds, stone, pomace, bagasse, etc. These by-products are not only a potential source of energy and various nutrients but also contain a significant amount of several bioactive phenolic compounds with considerable antioxidant activity (Safdar *et al*., 2017; Rafiq *et al*., 2018). As of now, relatively little emphasis has been given to the use of these byproducts in supplementary fish feed but considering their potential health benefits, scientists are investigating potential routes for reusing these waste materials and reincorporating them into the circular economy (Campos *et al*., 2020).

Kinnow (family: Rutaceae), a mandarin hybrid of two citrus cultivars [king (*Citrus nobilis*) X willow leaf mandarin (*C. deliciosa)*], is one of the economically important citrus fruit crops and widely grown in northern India (Mahawar *et al*., 2020). Processing of kinnow fruit by the juice industries/juice vendors generates various by-products and among them, peel is a major processing by-product contributing around 30-40% of the fresh fruit weight (Safdar *et al*., 2017; Rafiq *et al*., 2018; Godara *et al*., 2020). Peel is a rich source of numerous beneficial biologically active compounds such as phenolic acids and flavonoids, essential oils, *d*- limonene, pectin, antioxidant compounds besides being a good source of ascorbic acid, β-carotene, and carotenoids (Sidhu *et al*., 2016; Safdar *et al*., 2017; Godara *et al*., 2020). Further, citrus peel is well known for its immunomodulatory, anti-inflammatory, anti-stress, antioxidant, antimicrobial, anticancer properties and holistic health management (Cook and Sammon, 1996; Manthey and Grohmann, 2001; Tripoli *et al*., 2007; Safdar *et al*., 2017; Mahawar *et al*., 2020). Therefore, it has been hypothesized that owing to several health ameliorative properties, supplementation of kinnow peel in the fish feed would be helpful in improving the overall health status of fish.

Studies on the use of kinnow peel powder (KPP) in fish feed are scanty. In one recent study, Singh and Srivastava (2022) documented that dietary supplementation of KPP improved the survival, growth performance and flesh quality of common carp (*C. carpio*). Besides, several studies have demonstrated the ameliorative effect of other citrus fruits (orange, lemon etc.) byproducts on the survival, antioxidant activity, haemato-immunological profile and health status of various fish species (Acar *et al*., 2015; Baba *et al*., 2016; Salem *et al*., 2019; Vicente *et al*., 2019; Hosseini *et al*., 2020; Mohamed *et al*., 2021; Chekani *et al*., 2021 and Gheytasi *et al*., 2021). To the best of our knowledge, no comprehensive study has been reported so far on the dietary utilization of kinnow waste (peel, pomace etc.) on the health status (haemato-immunological and blood metabolic profile) of fish. Hence, the aim of the present study was to assess the efficacy of kinnow peel powder, as a feed additive, for enhancing the health status of one of the highly demanded exotic carp, common carp (*C. carpio*) fingerlings.

Materials and Methods

The present experimental work was conducted at the Instructional cum Research Farm, College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana (30°90'49.44"N and 75°80'19.02" E) for 120 days.

Preparation of kinnow peel powder (KPP) and experimental fish feeds

For KPP preparation, fresh kinnow peels were collected from local juice vendors, cleaned manually and peels with fungus or any other contamination were discarded. The fresh peel fragments were dried in a tray oven @ 45-50 °C (24 hrs.) and ground to fine powder form for incorporation in experimental feeds. Five iso-proteinous experimental feeds (crude protein 26.29-26.73%) were prepared using locally available ingredients (rice bran, mustard meal) and commercial vitamin-mineral mixture (Agrimin-forte) and were supplemented with KPP @ 0% (KPP0/Control), 0.5 % (KPP1), 1.0% (KPP2), 1.5% (KPP3) and 2.0% (KPP4) levels (Table 1). All the ingredients were mixed thoroughly and sinking pellets were made by using a mechanical pelletizer. The feed pellets were dried overnight in the hot air oven at 40-45°C and stored in an airtight container in a cool and dry place for further use.

Proximate analysis of feed ingredients and formulated feeds

The proximate composition (crude protein, crude fiber, ether extract, moisture, ash and nitrogen free extract) of different feed ingredients and experimental feeds (Table 1) was estimated on a dry matter (DM) basis as per the standard methods (AOAC, 2005).

Experimental setup

The experiment consisted of five treatments, in triplicate, and was carried out in rectangular FRP pools (1.5x1.0x0.75m) for 120 days. All the FRP pools were cleaned properly and layered with 5 cm thick soil and liming was done with limestone ($CaCO_3$) at a rate of 250 kg ha^{-1} in order to maintain water pH and also to carry out disinfection. During the experiment, the water depth was kept constant at 65±2.0 cm and aeration was provided in all the FRP pools. After proper acclimatization, common carp (*C. carpio*) fingerlings (Av. length 9.19 cm, Av. weight 10.51 g), procured from the Instructional cum Research Farm, College of Fisheries, GADVASU, Ludhiana, were stocked @ 15 fingerlings FRP $pool^{-1}$ following a complete randomized design and experimental pools were covered with a fine mesh net to avoid any predation. Fish were fed with experimental feeds (KPP0-KPP4) @ 2 % of fish body weight (BW), two times a day after sunrise, for a period of 120 days. The amount of feed was adjusted at monthly intervals, after every sampling, in accordance with the average weight gain of fish.

Observation recorded

Water quality parameters

All the physico-chemical parameters of water viz. temperature, pH, dissolved oxygen (DO), total alkalinity (TA), total hardness (TH), ammonical nitrogen (NH_3-N), nitrate-nitrogen (NO_3-N), and orthophosphate (O-PO_4) were analyzed at monthly intervals using standard methods (APHA, 2005).

Haemato-immunological and blood metabolic profile parameters

The haemato-immunological and blood metabolic profile parameters were studied at the termination of the experiment.

Blood and serum collection

For the haematological study, blood samples were

Table 1. Experimental feeds and proximate composition (DM basis) of different feed ingredients and prepared (KPP0-KPP4) feeds

Parameters	Treatments					Feed ingredients		
	KPP0	KPP1	KPP2	KPP3	KPP4	Rice Bran	Mustard Meal	KPP
Physical Composition (%)								
[1]Basal Feed	100	99.5	99.0	98.5	98	-	-	-
[2]KPP	0	0.5	1.0	1.5	2.0	-	-	-
Chemical Composition (%)								
Crude Protein	26.73	26.63	26.56	26.44	26.29	13.86	39.90	5.25
Ether Extract	1.72	1.67	1.63	1.65	1.59	1.57	2.47	1.60
Crude Fiber	12.26	12.19	12.21	12.14	12.12	13.76	10.96	7.72
Ash	9.28	9.23	9.21	9.17	9.19	11.23	7.69	2.52
NFE	50.01	50.28	50.39	50.60	50.81	59.58	38.98	82.91

[1]Basal feed: 49 % Rice bran + 49 % Mustard meal + 1.5 % Vitamin-mineral mixture (Agrimin –forte) + 0.5% Common salt (KPP0)

[2]Kinnow peel powder (KPP) incorporated in KPP1, KPP2, KPP3 and KPP4 treatments @ 5g, 10g, 15 g and 20g Kg^{-1} feed, respectively.

collected by the caudal vein puncture, with the help of a sterilized 2 ml hypodermal syringe and 24 gauge needles, and pooled from a random sample of five fish in each experimental tank after anaesthetizing fish by clove oil @ 30-50 mg l^{-1} (Hajek *et al*., 2006) in blood collection vials containing EDTA. For serum collection, the blood was collected without any anticoagulant, transferred to a 2.0 ml eppendorf tube and allowed to clot and then centrifuged to obtain serum samples and stored at -20 °C until analysis.

Haematological parameters

Total erythrocyte count (TEC) and total leucocyte count (TLC) were estimated using a Neubauer haemocytometer (Mukherjee, 1988), while haemoglobin (Hb) was assessed by acid hematin method using Sahli's haemoglobinometer and hematocrit (Hct) value or packed cell volume (PCV) was estimated by micro-capillary method (Mukherjee, 1988).

Immunological Parameters

Serum samples were analyzed for total protein (TP) and albumin (A) content using Erba Diagnostic Mannheim GmbH kits by following the principles of Biuret reaction (Gornall *et al*., 1949) and BCG dye-binding method (Doumas *et al*., 1971), respectively while, globulin (G) content and albumin: globulin ratio (A: G ratio) were estimated by standard formulae as follows:

Globulin (g dl^{-1}) = Total protein (g dl^{-1}) – Albumin (g dl^{-1})

A: G ratio = Albumin level in serum (g dl^{-1})/Globulin level in serum (g dl^{-1})

Blood metabolic profile parameters

Glucose was estimated as per Trinder (1969) method (GOD-POD, end point method) using 'Erba Diagnostic Mannheim GmbH kit. Among lipid profile indices, cholesterol was estimated using CHOD-PAP method (Roeschlau *et al*., 1974), while triglycerides was estimated using GPO-Trinder, end point method (Fossati and Prencipe, 1982) using Erba Diagnostic Mannheim GmbH kits. Liver activity markers viz. alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were analyzed by the method of International Federation of Clinical Chemistry (IFCC) using Erba Diagnostic Mannheim GmbH kits. For the estimation of lipid peroxidation (LPO) and superoxide dismutase (SOD) activity, blood haemolysate was prepared and LPO was estimated as described by Placer *et al*. (1966) and SOD activity was estimated following the protocol of Nishikimi *et al*. (1972).

Statistical analysis

Analysis of data was done using one way ANOVA and Duncan's multiple range tests using statistical package SPSS 20.0 to study the significant differences ($P \leq 0.05$) among different treatments with respect to haemato-immunological and blood metabolic profile parameters of fish.

Results and Discussion

Water quality parameters

The mean value of water quality parameters viz. temperature, pH, DO, TA, TH, NH_3-N and NO_3-nitrogen and orthophosphate, ranged from 26.57 to 31.80 °C, 7.62 to 8.19, 7.33 to 8.87 mg l^{-1}, 135.33 to 221.33 mg l^{-1} as $CaCO_3$, 172.67 to 254.67 mg l^{-1} as $CaCO_3$, 0.012 to 0.031 mg l^{-1}, 0.03 to 0.20 mg l^{-1} and 0.016 to 0.035 mg l^{-1}, respectively in different treatments (KPP0-KPP4) during the experimental period. All of these water quality parameters were within the optimum range/permissible limit right through the experimental study in all the treatments (Boyd and Tucker, 1998; Mishra *et al*., 2003). The results indicated that dietary inclusion of KPP in fish feed had no detrimental effect on the water quality, which helped the animal to be stress free resulting in better physiological performance.

Haematological parameters

Haematological indices are the important diagnostic tool used to detect the physiological and pathological changes, physiology, stress, and overall health status of fish (Kumar, 2016) and varies with respect to various factors including nutraceutical quality of supplementary feed, among others (Acar *et al*., 2015). The present feeding trial demonstrated that dietary supplementation of KPP improved the haematological profile in all the treatments as compared to control (Table 2), though the best ($P \leq 0.05$) results were recorded at 1.5% inclusion level (KPP3). The TEC, TLC, Hb and Hct/PCV values increased significantly ($P \leq 0.05$) from 1.77x 10^6 mm^{-3}, 2.32 x 10^3 mm^{-3}, 6.20 g% and 25.33% in control (KRP0) to 2.27x 10^6 mm^{-3}, 2.69 x 10^3 mm^{-3}, 7.03 g % and 39.00 % in KPP3 treatment, respectively which showed the positive impact of KPP on the hematology, thus, it can serve as a potential feed additive in a supplementary feed of common carp to improve the haematological profile. This may be attributed to the presence of various biologically active phytochemicals in kinnow peel which may have activated the hematopoietic tissue of the fish (Samavat *et al*., 2019). Further, flavonoids present in kinnow peel may have prevented the phospholipid peroxidation on the erythrocyte membrane, preventing the lysis of red blood cells (Hrubec and Smith, 2010). However, further study is needed to demonstrate the mode of action. Report on the effect of KPP supplemented feed on the haematological profile of fish is lacking. However, significant (P≤0.05) improvement in the haematological profile by dietary inclusion of essential oil (citrus EO) extracted from sweet orange peel was reported in Mozambique tilapia, *Oreochromis mossambicus* (Acar *et al*., 2015). Similar observations were also recorded in Asian sea bass, *Lates calcarifer* fed with diet containing 1% fermented lemon peel (Zhuo *et al*., 2021). These studies support the findings of the present study. Further, several previous studies depicted the positive role of herbs that possess similar potent bioactive components (Citarasu, 2010; Chakraborty and Hancz, 2011). For instance, amla (*Phyllanthus emblica*), which contains very important phytochemicals including flavonoids, hydrolysable tannins, quercetin and vitamin C (Singh *et al*., 2011), significantly improved the haematological profile in rohu (*L. rohita*) fingerlings (Srivastava, 2019).

Immunological parameters of *C. carpio* fingerlings

The serum TP and albumin are considered as a key indicator of non-specific immune response of fish (Wiegertjes *et al*., 1996). The immune system defends the host against infectious agents found in the environment therefore, a well-functioning immune system is critical to the host's ability to fight infectious diseases (Calder, 2007). Thus, amelioration in the health status of fish is a prime requirement of aquaculture. The present study depicted that the dietary supplementation of KPP significantly (P≤0.05) improved the immunity with respect to serum TP and albumin at all the inclusion levels (0.5-2%) as compared to the control, with best observation recorded at 1.5% inclusion level (Table 3). However, globulin level showed a significant increase at 1-2% inclusion levels only. The increment recorded in total protein, albumin and globulin was 32.53, 18.88 and 44.89%, respectively at 1.5% KPP inclusion level (KPP3) as compared to control. Similarly, the A: G ratio was found to be best in KPP3 treatment as compared to other treatments and control. It illustrates that the incorporation of KPP in fish feeds resulted in better the immunity of common carp fingerlings.

Reports on the efficacy of KPP as a feed additive

Table 2. Hematological profile of *C. carpio* fingerlings in different treatments at the termination of the experiment

Parameters	Treatments				
	KPP0	KPP1	KPP2	KPP3	KPP4
TEC (No.x10^6 mm^{-3})	$1.77^c \pm 0.03$	$1.86^c \pm 0.04$	$2.02^b \pm 0.03$	$2.27^a \pm 0.06$	$2.12^b \pm 0.01$
TLC(No.x10^3 mm^{-3})	$2.32^c \pm 0.08$	$2.45^{bc} \pm 0.08$	$2.57^{ab} \pm 0.06$	$2.69^a \pm 0.03$	$2.52^{abc} \pm 0.03$
Hb (g %)	$6.20^c \pm 0.06$	$6.23^c \pm 0.12$	$6.53^b \pm 0.09$	$7.03^a \pm 0.09$	$6.63^b \pm 0.09$
Hct (%)	$25.33^c \pm 0.88$	$31.33^b \pm 1.20$	$36.00^a \pm 1.53$	$39.00^a \pm 0.58$	$38.00^a \pm 0.58$

*Values (mean ± S.E.) with same superscripts in a row do not differ significantly (P ≤ 0.05)

Table 3. Serum immunological parameters of *C. carpio* fingerlings in different treatments at the termination of the experiment

Parameters	Treatments				
	KPP0	KPP1	KPP2	KPP3	KPP4
Total Protein(g dl^{-1})	$2.89^d \pm 0.02$	$3.13^c \pm 0.04$	$3.20^c \pm 0.02$	$3.83^a \pm 0.04$	$3.65^b \pm 0.08$
Albumin (g dl^{-1})	$1.43^c \pm 0.01$	$1.50^b \pm 0.01$	$1.53^b \pm 0.03$	$1.70^a \pm 0.01$	$1.67^a \pm 0.03$
Globulin (g dl^{-1})	$1.47^c \pm 0.01$	$1.64^{bc} \pm 0.06$	$1.70^b \pm 0.03$	$2.13^a \pm 0.04$	$1.98^a \pm 0.10$
A: G ratio	$0.97^a \pm 0.01$	$0.92^{ab} \pm 0.04$	$0.90^{ab} \pm 0.03$	$0.80^b \pm 0.01$	$0.85^b \pm 0.05$

*Values (mean ± S.E.) with same superscripts in a row do not differ significantly (P ≤ 0.05) on the immunity status of *C. carpio* are inadequate. However, the positive influence of other citrus fruit's byproducts on the said immunological parameters of fish has been reported in various fishes viz. Mozambique tilapia (Acar *et al*., 2015; Baba *et al*., 2016), Nile tilapia, *O. niloticus* (Mohamed *et al*., 2021), and rainbow trout (Gheytasi *et al*., 2021) which support the findings of the present study.

Blood metabolic profile of *C. carpio* fingerlings

At the termination of the experiment, the findings demonstrated that KPP supplementation improved the blood metabolic profile with respect to glucose, cholesterol, triglycerides, ALT, AST, LPO and SOD level in *C. carpio* fingerlings at all the inclusion levels, but best ($P \leq 0.05$) results were recorded with 1.5% level (KPP3) (Table 4, Fig. 1-2)

To cope with the energy demand imposed by stressors, various stress hormones, in combination with cortisol, mobilize and elevate the glucose production in fish via gluconeogenesis and glycogenolysis pathways (Iwama *et al*., 1999). The results demonstrated that common carp fed with KPP supplemented feeds exhibited significant ($P \leq 0.05$) reduction in serum glucose level as compared to control group, with the lowest value being recorded in KPP3 treatment which reflects that there was no stress in fish fed with KPP supplemented feeds. The ability of plant oils to reduce the effects of stressors could be a possible reason for the decrease in glucose concentrations (Güleç *et al*., 2013) in KPP fed groups, as KPP is also rich in essential oils. Previous studies have demonstrated that EO extracted from other citrus fruits viz. sweet orange (*C. sinensis*) and bitter lemon (*C. limon*) peels has been shown to reduce the serum glucose level in *Labeo victorianus* (Ngugi *et al*., 2017) and O. *niloticus* (Mohamed *et al*., 2021).

With respect to lipid profile, serum cholesterol and triglyceride level was significantly ($P \leq 0.05$) lower at 1-2% inclusion levels as compared to control and decreased significantly from 151.0 mg dl^{-1} and 142.67 mg dl^{-1} in control (KPP0) to 125.0 mg dl^{-1} and 121.33 mg dl^{-1} in KPP3 treatment, respectively. It might be attributed to the presence of limonene in citrus peel, which has been shown to reduce the cholesterol and triglycerides in fish (Ngugi *et al*., 2017). Similar to our findings, serum cholesterol and triglyceride levels have also been decreased in various fishes fed with diets supplemented with graded levels of citrus (*C. sinensis*/*C.limon*) peel essential oil (EO) viz. Mozambique tilapia, *O. mossambicus* (Acar *et al*., 2015; Baba *et al*., 2016), *L. victorianus* (Ngugi *et al*., 2017), rainbow trout, *O. mykiss* (Gheytasi *et al*., 2021) and Nile tilapia, *O. niloticus* (Mohamed *et al*., 2021).

ALT and AST are known as a marker of liver health and their higher level beyond the normal range indicate liver damage (Uneo and Komatsu, 2017). At the termination of the trial, significant differences ($P \leq 0.05$) were recorded in the serum level of liver enzymes (ALT and AST) among the treatments. The ALT and AST level decreased significantly ($P \leq 0.05$) from 32.37 U l^{-1} and 101.40 U l^{-1} in control to 28.79 U l^{-1} and 81.52 U l^{-1}, respectively at 1.5 % inclusion level (KPP3). This reduction in ALT and AST values in KPP fed treatments may be due to the hepato-protective role of citrus byproducts in fish diet owing to the presence of several bioactive phytochemicals (Mohamed *et al*., 2021; Gheytasi *et al*., 2021). Moreover, ascorbic acid, present in citrus peel, also possesses hepato protective property (Abdulrazzaq *et al*., 2019). This observation is similar to that recorded in Nile tilapia (Mohamed *et al*., 2021) and rainbow trout (Gheytasi *et al*., 2021) fed with citrus essential oil (EO) supplemented diets.

The LPO level in fish that were fed with KPP supplemented feeds was significantly ($P \leq 0.05$) lower than the control group (Figure 1). The KPP3 treatment had the lowest (1.31 nmol MDA g Hb^{-1})

Table 4. Blood metabolic profile of *C. carpio* fingerlings in different treatments at the termination of the experiment

Parameters	Treatments				
	KPP0	KPP1	KPP2	KPP3	KPP4
Glucose(mg dl^{-1})	52.24^{a}±0.94	49.81^{b}±0.19	48.86^{bc}±0.11	48.01^{c}±0.30	48.85^{bc}±0.03
ALT(U l^{-1})	32.37^{ab}±1.74	33.91^{a}±0.89	30.50^{bc}±0.70	28.79^{c}±0.42	30.37^{bc}±0.46
AST(U l^{-1})	101.40^{a}±2.06	94.60^{b}±2.60	85.59^{c}±2.37	81.52^{c}±0.54	82.88^{c}±0.50
Cholesterol(mg dl^{-1})	151.00^{a}±1.53	143.67^{ab}±3.38	136.00^{b}±2.08	125.00^{c}±4.16	123.67^{c}±3.18
Triglycerides(mg dl^{-1})	142.67^{a}±2.91	137.67^{ab}±1.86	135.00^{bc}±2.08	121.33^{d}±1.45	130.00^{c}±1.15

*Values (mean ± S.E.) with same superscripts in a row do not differ significantly ($P \leq 0.05$)

LPO value, while it was recorded highest (1.98 nmol MDA g Hb^{-1}) in the control. Salem *et al*. (2019) documented that dietary orange peel decreased the malondialdehyde (MDA), a metabolic product of lipid peroxidase, in gilthead sea bream, *Sparus aurata*.

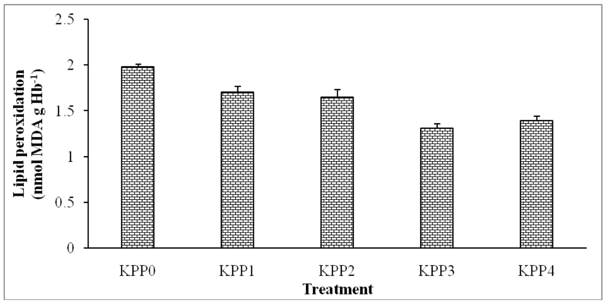

Fig. 1. LPO (nmol MDA g Hb^{-1}) level in blood haemolysate of *C. carpio* fingerlings in different treatments at the termination of the experiment

With respect to the antioxidant index, enhanced SOD activity was recorded in all the KPP fed treatments as compared to the control; though the increment was significant (P≤0.05) @ 1-2% inclusion levels (Figure 2), being highest (0.66 U mgHb^{-1}) at 1.5 % inclusion level (KPP3) and lowest (0.36U mgHb^{-1}) in control (KPP0). SOD is an antioxidant enzyme that degrades superoxide, a major cause of oxidative stress and plays a crucial role in overall defense mechanisms and strategies (Ighodaro and Akinloye 2018). Citrus peel flavonoids are known to be an excellent dietary source of antioxidants and protect cells by hydrogen transfer, free radical scavenging, and divalent metal ion chelation (Sarian *et al*., 2017). In the present trial, reduced stress and better health condition of tested fish may be corroborated with the increased anti-oxidant activity in KPP fed groups. Though, to the best of author's knowledge, no report is available on the effect of kinnow peel on stress response and antioxidant activity of common carp, but increased SOD activity through dietary supplementation of lemon/orange peel in various fish species viz. gilthead sea bream (Salem *et al*., 2019) and common carp (Sadeghi *et al*., 2021) supports the finding of the present study. Similarly enhanced SOD activity was also recorded in Nile tilapia under heat/dissolved oxygen-induced stress (Vicente *et al*., 2019) and rainbow trout under crowding stress (Chekani *et al*., 2021) fed with a diet containing orange peel and dehydrated lemon peel, respectively. Further, better LPO and SOD activities were recorded in rohu, *L. rohita* fed with diets supplemented with amla fruit powder (Srivastava, 2019), which also contains various biologically active compounds comparable with citrus peel. The overall findings of this study revealed that KPP can be used as a potential feed additive in the supplementary feed of common carp to improve the blood metabolic profile, which may be due to the presence of various biologically active ingredients like flavonoids, alkaloids, coumarins, limonoids, phenol acids and vitamin C in citrus fruit (Economos and Clay, 1999; Lv *et al*., 2015; Rafiq *et al*., 2018).

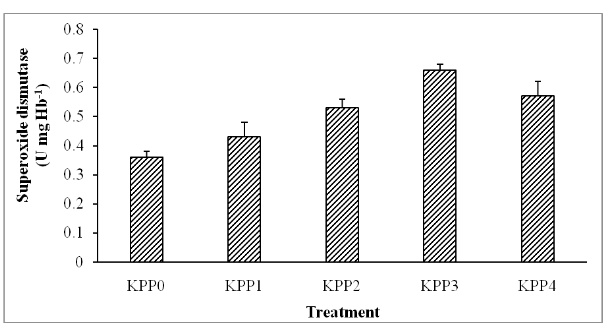

Fig. 2. SOD (U mg Hb^{-1}) level in blood haemolysate of C. carpio fingerlings in different treatments at the termination of the experiment

Conclusion

The overall results revealed that KPP can be potentially utilized as an organic health ameliorating feed additive in supplementary feed for potential health benefits in *C. carpio* fingerlings, with 1.50 % being the best. The findings of the present study are important from a farmer's point of view in order to improve fish productivity and also for better utilization and management of kinnow fruit processing waste for environmental health management.

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Conflict of interest

Authors declare no conflict of interest.

Ethics approval

The study protocol and all experimental procedures were approved by the competent authority of Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana.

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