World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

Check for updates

Analysis of the effects of dietary crude protein on growth performance and antibiotic susceptibility of microflora isolated from all-male tilapia (*Oreochromis niloticus*)

Hina Wahab Khan [1], Umbreen Rashid [1], Rehana Kauser [2], Kashif Abbasi [3], Anisa Wahab [4], Khadija Iqbal [5], Anita Zaib [6] and Muhammad Arsalan Iqbal [7,*]

[1] *Abasyn University Islamabad Campus, Department of life sciences, Islamabad, Pakistan.*
[2] *National Agriculture Research Centre, Aquaculture and Fisheries Program, Islamabad Pakistan.*
[3] *University of Chester, Health Services Management, Chester CH1 4BJ, United Kingdom.*
[4] *Pakistan Agricultural Research Council (PARC), Islamabad, Pakistan.*
[5] *Gomal University, Faculty of Pharmacy, Dera Ismail Khan, Pakistan.*
[6] *Quaid-i-Azam University, National Centre for Bioinformatics, Islamabad, Pakistan.*
[7] *Research Institute for Farm Animal Biology, Institute of Genome Biology, Wilhelm-Stahl-Allee 2, 18196, Dummerstorf, Germany.*

World Journal of Advanced Research and Reviews, 2023, 17(01), 790–804

Publication history: Received on 05 December 2022; revised on 19 January 2023; accepted on 21 January 2023

Article DOI: https://doi.org/10.30574/wjarr.2023.17.1.0085

Abstract

The aquaculture industry is one of the fastest-growing sectors in the food industry. The Nile tilapia, *Oreochromis niloticus*, is an important and inexpensive species of fish. A study was conducted to determine the effects of divergent dietary crude proteins on fish growth performance and antibiotic susceptibility of bacterial pathogens infecting all-male tilapia (*Oreochromis niloticus*). Forty-eight fish samples were grouped into three groups based on crude protein percentages, including 15%, 30%, and 45%, and the fish's body weight and length were measured on days 15, 30, 45, and 60. Ordinary one-way ANOVA (Tukey's multiple comparison test) was applied to three divergent dietary crude protein groups for the examination of growth performance at different time points. Using the same fish samples, bacterial strains were isolated from fish skin and gills and identified based on their morphological characteristics and biochemical testing. Kirby Bauer's disc diffusion method was used to determine the antibiotic susceptibility of identified bacterial isolates. The ANOVA (Tukey's multiple comparison test) results showed that 45% crude protein level in fish feed led to a greater growth rate than 15% and 30% under high-performance conditions. The antibiotic susceptibility findings indicate that *Staphylococcus aureus* showed the highest sensitivity (50%) toward antibiotics while *Pseudomonas aeruginosa* showed the least susceptibility (10%). The study provides a new perspective on the impact of crude protein on fish growth performance, as well as baseline information for the management of fish diseases based on antibiotic sensitivity.

Keywords: Antibiotic susceptibility; Crude protein; Nile-tilapia; Aquaculture; Microflora

1. Introduction

Aquaculture is an emerging field of interest because it is the fastest-growing food production sector globally (1). In addition, fish is the most economical and easily digestible animal protein obtained from natural sources for consumption. Still, due to over-profiteering and pollution, the fish in the wild waters have declined noticeably, compelling scientists to implement different genetically modified approaches to enhance production (2, 3). According to the aquaculture review in 2017, the top 75% of aquaculture production contributed by these notable species included tilapia, seaweeds, carp, bivalves, and catfish (4).

* Corresponding author: Muhammad Arsalan Iqbal

Tilapia is the generic name of approximately a hundred species of cichlid fish, including *coelotilapine, coptodonine, heterotilapine, oreochromine, pelmatolapiine*, and *tilapiine* tribes (5). Tilapia originated exclusively from Africa and the Middle East. These extensively farmed fish species dominate the global aquaculture industry and are only stamped out by the leading carp and salmon (6, 7). Moreover, tilapia is considered food fish for the 21st century and is famous as an aquatic chicken. The aquaculture production of tilapia is about 4.0 million tonnes, with an expenditure of almost three billion dollars. Previously, many studies used tilapia as model fish because this species has many advantages: fast-growing, prefer all kinds of supplementary feed, high disease resistance ability, and is cultured in saline water (8). Tilapia contains various species, but the most common and significant species are the Nile, blue, and Mozambique.

Nile tilapia (*Oreochromis niloticus)* is the major fish in the 21st century. Researchers focus on the Nile tilapia in their studies due to its ability to breed and produce new generations expeditiously, high disease resistance ability, and flexibility for culture under various farming systems (9, 10). However, propelled by insufficient production in tilapia farms, the world fish organization conducted surveys on world tilapia genetic resources from 1980 to 1987. They interpret that inadequate seed supply and deteriorating performance in several aquaculture systems are primary concerns (11). As a result, world fish and its partners from Norway and the Philippines established the Genetically Improved Farmed Tilapia (GIFT) project in 1988. GIFT aimed to develop a rapid-growing strain of Nile tilapia fit for both small-scale and commercial aquaculture (12-14).

The primary concern is that the mixed-sex culture of tilapias in ponds results in a high level of uncontrolled breeding and precocious sexual maturity controlled by raising the monosexual population (15, 16). Monosexual tilapia production is attained through sex reversal methods (17) by applying diverse approaches, such as hormonal sex reversal, intra, and interspecific hybridization, and genetically all-male tilapia from YY x XX crosses (16, 18-20). One of the most common techniques for the commercial production of all-male tilapia is a direct application of 17α-methyltestosterone (17α-MT) hormones (21, 22). Nonetheless, a primary limitation of this method is the limited availability, affordability, and restriction in many countries because of its unfriendliness and adverse human health effects (23). In addition, males are preferred due to their high growth ability than females; males grow almost twice as fast as females (15).

Protein is the nutrient that produces the greatest energy, but it is also the most expensive and crucial for disease resistance, optimal physiological performance, and motility in fish (24). Increasing the protein content in fish feeds can increase production, but too much dietary protein will be converted to harmful nitrogenous chemicals when it is metabolized as an energy source, which could be bad for fish growth (25). Fish often eat protein to receive both essential and non-essential amino acids, which are required for the development of muscles and the activity of enzymes and, to a lesser extent, supply energy for maintenance (26). Previously, a study on *Siniperca scherzeri* evaluated the effects of dietary protein and lipid content on fish growth, feed utilization efficiency, and muscle-proximate composition. A diet containing three proteins (35, 45, and 55%) and two dietary lipid levels (7 and 14%) was fed to triplicate groups of fish for eight weeks. Compared to the diet with a protein value of 35% and 45%, the diet with a 55% protein value resulted in drastically increased Weight Gain (WG) and Specific Growth Rate (SGR), but lipid levels remained stable.

Globally bacterial pathogens are a colossal threat to fish production due to the tremendous economic significance of the disease they cause (27, 28). Previously, several bacterial pathogens involved in fish diseases worldwide were well documented (29-31). Awareness of the antibiotic susceptibility of the bacteria is crucial for the proper management of the diseases caused by these bacteria. Earlier, the global utilization of antibiotics in aquaculture and the probable transmission of resistant bacteria among earthbound and aquatic species were well documented (32, 33). Nonetheless, numerous studies demonstrated antimicrobial resistance (AMR) transmission between humans and terrestrial food animals with less consideration of the aquatic ecosystem (34, 35). Thus, inappropriate information regarding antimicrobial drug susceptibility of the marine ecosystem provides a significant aspect of understanding AMR's epidemiology (36).

This study consisted of two trials: in the first trial, the effects of divergent dietary Crude Protein (CP) levels on fish growth and development were investigated, while in the second trial, antibiotic sensitivity of isolated bacteria from fish skin and gills was examined. The findings of the first trial of this study recommend that fish diets be enriched in crude protein, paving the way for further research regarding fish feed formulations to promote fish growth. Additionally, the second trial of the study provides baseline information for future reference and disease management.

2. Material and methods

2.1. Experimental design

The experiment was conducted at the aquaculture and fisheries department, National Agriculture Research Centre (NARC), Islamabad, Pakistan, collaborating with Abasyn University, Islamabad campus. Initially, *Oreochromis niloticus* fish were sexually transformed into monosexual tilapia (all-male tilapia) by using the sex reversal method of direct application of 17α-methyltestosterone (17α-MT) hormone which has been well documented (21, 22). The duration of the trial was two months, and the fish was acclimatized for one week. After acclimatization, in total 48 uniformed-sized fingerlings were selected. Additionally, three aquaria were utilized, and each aquarium housed 16 fish and contained 100 liters of water, and attached a suitable aerator to ensure the water is adequately oxygenated. Water quality parameters will be maintained by changing 20% of water daily (37). The control diet of 30% CP was formulated as (soya bean, rice polish, wheat bran, canola meal, gluten 30 to 60%, sunflower, sodium carboxymethyl cellulose 1%, di-calcium phosphate 2%, soya bean oil, and vitamin premix). There were three divergent dietary CP experimental groups in this study, including the control group ($CP_{control}$ = 30%), a reduction group (CP_{low} = $CP_{control}$ -15%), and an increased group (CP_{high} = $CP_{control}$ +15%), and each dietary group contained 16 fish. Finally, the isolated bacteria from the fish's gills and skin were tested for antibiotic susceptibility.

2.2. Measurement of Body Weight (BW) and Length (L)

Fish were fed at the rate of 5% body weight, having $CP_{control}$ = 30%, CP_{low} = 15%, and CP_{high} = 45%. Before handling fish, wet hands protect the mucous layer and minimize handling to avoid unnecessary stress and/or mortality. Take out the fish from the aquarium and placed it on the measuring board. Afterward, in centimeters, measure the length (L) of the entire fish from the tip of its snout to the end of its caudal fin. Subsequently, a weight scale was used to measure the body weight (BW) of individual fish. Primarily, it was important to place the weight scale in a stable location, start the scale at zero, and remove excess water from individual fish as much as possible. Additionally, fish was placed on the scale and measured the BW was in grams. After every 15 days, fish (BW) and (L) were measured for all 48 samples and recorded in excel sheets. Furthermore, we calculated the mean weight and length of the population by using this equation as shown.

$$\mu(BW) = \frac{\Sigma X}{N} \quad \text{and} \quad \mu(L) = \frac{\Sigma X}{N}$$

[Where μ= Population means, ΣX = sum of all individuals (BW) and (L), and N = total numbers of individuals].

2.3. Bacterial Isolation and identification

A total of 48 fresh all-male tilapia fish were selected for bacterial sample collection. Different samples were randomly isolated/collected from the fish skin and gills with the help of a sterile cotton swab. The samples were inoculated on Blood agar (tryptones, soya bean protein digest, agar, and 5% sheep blood) and MacConkey agar (gelatin peptone, HMC peptone (peptones meat and casein), lactose monohydrate, sodium chloride, bile salts, neutral red, crystal violet, and agar) and placed the plates in an incubator at 37°C for 24 hours.

We distinguished the colonies based on their morphological traits after a 24-hour incubation period. Furthermore, we perform biochemical tests by using Oxidase, indole, catalase, coagulase, DNase, citrate test, and Gram staining for the prediction of bacterial strains (38).

2.4. Media Preparation

Three different media were prepared, Blood agar, MacConkey agar, and Muller Hinton agar. Bacterial isolation was done using Blood and MacConkey agars, and antibiotic susceptibility testing was done on identified and confirmed bacteria using Muller Hinton agar. Blood agar composition includes the mixing of 1 liter of distilled water with 28 grams of nutrient agar powder; boil the mixture while constantly stirring until the ingredients are completely dissolved; then place the liquid in an autoclave set at 121 ° C for 15 minutes. The mixture should then be allowed to cool to between 45 and 50 ° C, 5% sheep blood added, and then poured into sterile glass plates to allow the material to harden. (39). MacConkey agar composition includes 51.5g of nutrient agar that must be suspended in 1 liter of distilled water. After thoroughly blending the powder, autoclave for 15 minutes at 121 ° C. Last but not least, pour the mixture into sterile glass plates and let it set (40). Mueller Hinton agar preparation includes suspending 38 g of Mueller Hinton agar in one liter of distilled water. For the medium to fully dissolve, heat it while stirring often and bring it to a boil for one minute. Autoclave it for 15 minutes at 121 °C. Mueller Hinton Agar that has cooled down to room temperature should be added to sterile Petri dishes on a level, horizontal surface to ensure equal depth. Keep the plates between 2 and 8°C (41).

2.5. Antibiotic susceptibility testing

The disc diffusion method was employed to conduct the antibiotic sensitivity test. A total of ten different antibiotic disc types were used: Ampicillin (10µg), Oxacillin (1µg), Florfenicol (30µg), Kanamycin (30µg), Gentamycin (10µg), Erythromycin (15µg), Nitrofurantoin (300µg), Nalidixic acid (30µg), Neomycin (10µg), and Amoxicillin (10µg). By using a swab, the identified bacteria were streaked into a plate of Muller-Hinton agar. Using sterile forceps, the antibiotic discs were then kept on the agar and incubated for 24 hours at the 37°C methodology adopted (42). The Kirby-Bauer disc diffusion's zones of inhibition were measured from the disk's center to the periphery of the bacterial growth and all tests were performed twice, methods were well-documented (43).

2.6. Statistical Analysis

Initially, normality and lognormality tests were performed on the individual group, including $CP_{control}$, CP_{low}, and CP_{high} with D'Agostino & Pearson normal distribution test within GraphPad Prism (version.8.0.1) environment. The passing threshold for the normality test was established by using an alpha score ($\alpha \leq 0.05$). Furthermore, ordinary one-way ANOVA was performed with Tukey's multiple comparisons test on three comparison groups: ($CP_{control}$ *vs*. CP_{low}), ($CP_{control}$ *vs*. CP_{high}), and (CP_{low} *vs*. CP_{high}) for the examination of growth performance (BW and L) within different time points, including day 15, 30, 45 and 60, respectively. The comparison group was considered significant with $P \leq 0.05$.

3. Results

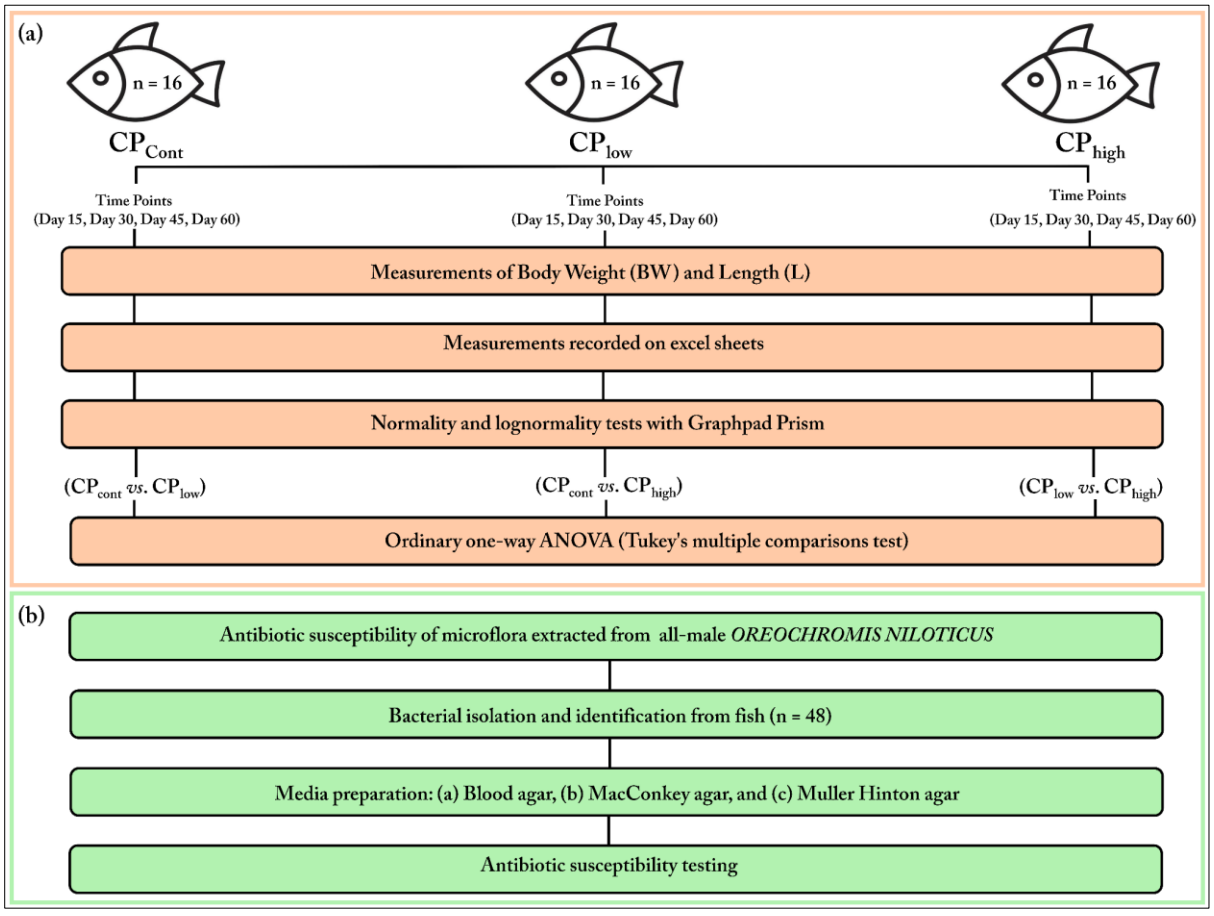

Figure 1 Schematic representation of the experimental design. (a) In the first part of the study, Body Weights (BW) and Lengths (L) were measured for three different fish groups: CPcontrol, CPlow, and CPhigh, each containing 16 individuals within different periods (day 15, day 30, day45, and day60). Each orange block represents the study workflow for divergent dietary crude protein trails. (b) A second part of the study examined the antibiotic susceptibility of selected bacteria isolated from the skin and gills of 48 fish. The green block depicts each step followed for antibiotics susceptibility trials

This study revealed the impact of dietary crude protein on fish growth and development. In total, 48 fish were classified into three groups, each containing 16 fish, based on divergent crude protein proportions, such as 15%, 30%, and 45% as shown in Figure 1a. Interestingly, in the pre-processing step, all the datasets computed in this study passed the normality and lognormality test D'Agostino & Pearson normal distribution with a threshold of the alpha score ($\alpha \leq 0.05$). Afterward, comparing three groups, including (CPcontrol vs. CPlow), (CPcontrol vs. CPhigh), and (CPlow vs. CPhigh) with one-way ANOVA (Tukey's multiple comparisons test) indicated the significant differences in weight and length of the fish between the groups within different time point, including day 15, 30, 45 and 60, respectively. The comparison groups with adjusted $P \leq 0.05$ were considered significant. Initially, seven bacteria were isolated from the skin and gills of the 48 fish samples for investigation of antibiotic susceptibility analysis, as shown in Figure 1b. Three out of seven (3/7) bacteria, including (Streptococcus agalactiae, Enterococcus, and Proteus) indicated contamination on the media plates and were excluded from the experiment. Finally, four out of seven (4/7) bacteria, including (Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, and Pseudomonas aeruginosa) were used to examine the sensitivity with response to these ten antibiotics, including Erythromycin (E), Gentamycin (CN), Oxacillin (OX), Kanamycin (K), Florfenicol (FFC), Ampicillin (AMP), Amoxycillin (AML), Nitrofurantoin (F), Neomycin (N), and Nalidixic acid (NA).

3.1. Pairwise comparison of fish Lengths (L) between divergent crude protein groups within different time points

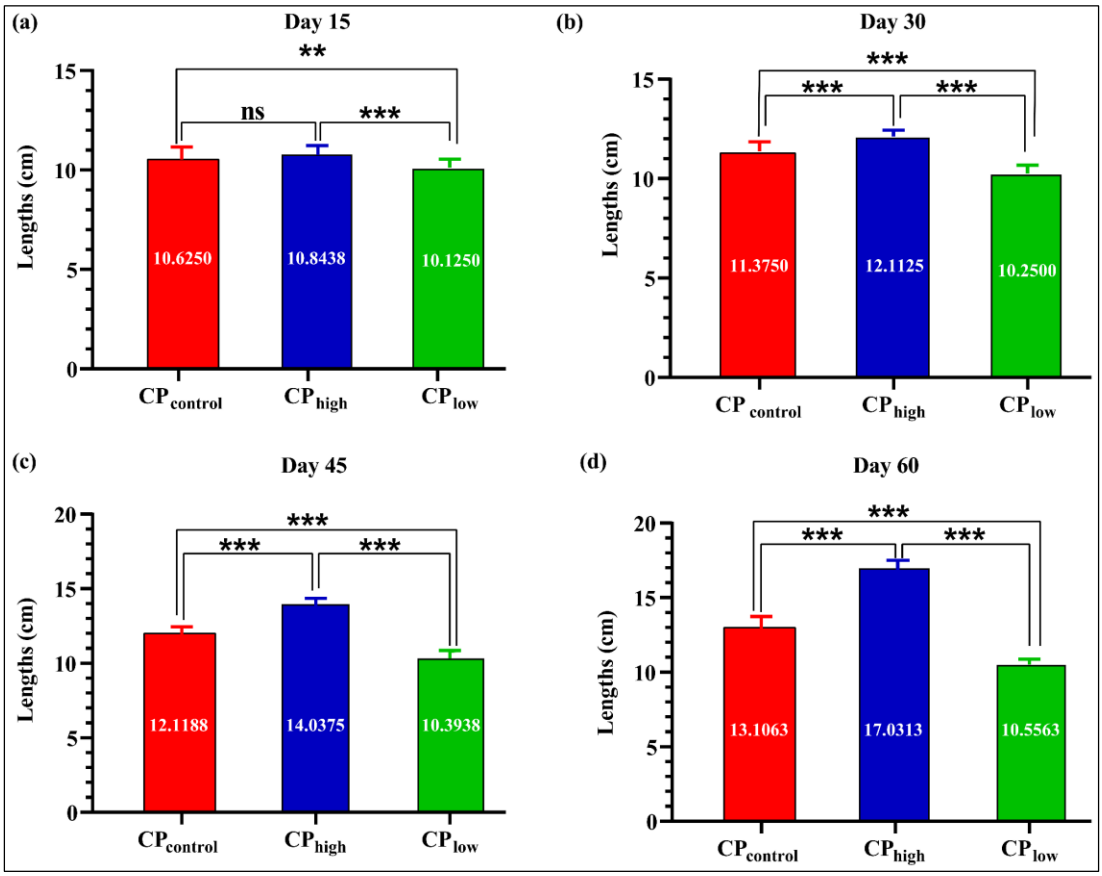

Figure 2 Comparison of fish length between $CP_{control}$, CP_{high}, and CP_{low} at different time intervals. A bar chart depicts the average length of fish between $CP_{control}$, CP_{high}, and CP_{low} at (a) day 15 (b) day 30 (c) day 45, and (d) day 60. The red, blue, and green bars represent the mean length values for the $CP_{control}$, CP_{high}, and CP_{low}, respectively. The black lines illustrate the pairwise comparisons, including ($CP_{control}$ *vs.* CP_{high}), ($CP_{control}$ *vs.* CP_{low}), and (CP_{high} *vs.* CP_{low}), while the asterisk symbol represents the significance between the groups. ns: no significance ($P > 0.05$), **: $P \leq 0.01$, and ***: $P \leq 0.001$

Multiple pairwise comparisons on divergent crude protein groups with one-way ANOVA using Tukey's multiple comparisons test within different time points, including days 15, 30, 45, and 60). A significant difference in fish lengths was not found when $CP_{control}$ was compared to CP_{high} on day 15 and the mean difference between comparison groups was (-0.2188) with a P-value of (0.33). Conversely, when $CP_{control}$ was compared to CP_{low} a visible difference in fish length was observed with a mean difference of (0.5) and P- the value of (0.006). Similarly, in CP_{high} *vs.* CP_{low} comparison, a significant difference in fish lengths was found with a mean difference of (0.7188) and $P < 0.001$ (Figure 2a).

Furthermore, on day 30 a significant difference in fish lengths was found between all three groups. Interestingly, $CP_{control}$, $CP_{high,}$ and CP_{low} passed the cut-off criteria $P < 0.05$ and showed substantial differences in fish length.

In the comparison of CP_{high} and CP_{low}, CP_{high} displayed the largest difference in fish length with a mean difference of (1.86), as shown in Figure 2b. Likewise, the results of day 45 and day 60 were in line with day 30 and showed the same trend in the length differences of the fish. The CP_{high} and $CP_{control}$ group indicated a substantial difference in fish length when compared to the CP_{low} group, while the CP_{high} group also indicated significant differences in length when compared to the $CP_{control}$ group, as indicated in Figures 2c & d.

3.2. Pairwise comparison of fish Body Weights (BW) between divergent crude protein groups within different time points

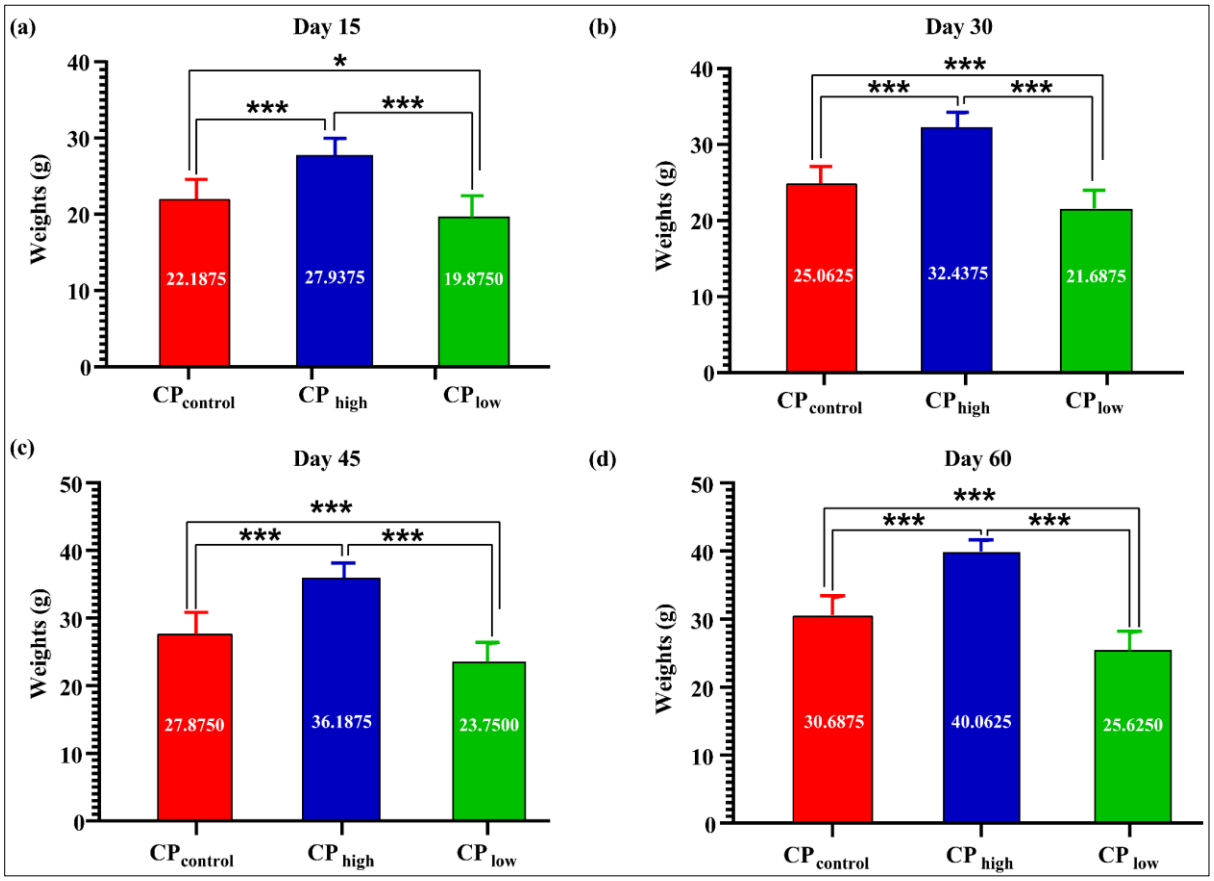

Figure 3 Comparison of fish weight between $CP_{control}$, CP_{high}, and CP_{low} at different time intervals. A bar chart depicts the average weight of fish between $CP_{control}$, CP_{high}, and CP_{low} at (a) day 15 (b) day 30 (c) day 45, and (d) day 60. The red, blue, and green bars represent the mean weight values for the $CP_{control}$, CP_{high}, and CP_{low}, respectively. The black lines illustrate the pairwise comparisons, including ($CP_{control}$ *vs.* CP_{high}), ($CP_{control}$ *vs.* CP_{low}), and (CP_{high} *vs.* CP_{low}), while the asterisk symbol represents the significance between the groups. *: $P \leq 0.05$, and ***: $P \leq 0.001$

Tukey's multiple comparisons test was applied to multiple pairwise comparisons on divergent crude protein groups with one-way ANOVA at various time points, including days 15, 30, 45, and 60. A significant difference in fish weights was found when $CP_{control}$ was compared to CP_{high} on day 15 and the mean difference between comparison groups was (-5.75) with a P-value of (<0.001). Similarly, when $CP_{control}$ was compared to CP_{low} a visible difference in fish length was observed with a mean difference of (2.313) and P-value of (0.0324). Additionally, in CP_{high} *vs.* CP_{low} comparison, a significant difference in fish weights was found with a mean difference of (8.063) and a P<0.001 (Figure 3a). Moreover, on day 30 a significant difference in fish weights was also found between all three groups. A significant difference in fish weights was found when $CP_{control}$ was compared to CP_{high} on day 30 and the mean difference between comparison groups was (-7.375) with a P-value of (<0.001). Likely, when $CP_{control}$ was compared to CP_{low} a visible difference in fish weight was observed with a mean difference of (3.375) and P-value of (0.005). However, in CP_{high} *vs.* CP_{low} comparison, a significant difference in fish weights was observed with a mean difference of (10.75) and a P <0.001 as shown in Figure 3b. Likewise, the results of day 45 and day 60 showed a significant difference in fish weights between all three groups.

Interestingly, $CP_{control}$, $CP_{high,}$ and CP_{low} passed the cut-off criteria $P < 0.05$ and showed substantial differences in fish weights. The CP_{high} and $CP_{control}$ group indicated a substantial difference in fish weights when compared to the CP_{low} group, while the CP_{high} group also indicated significant differences in weight when compared to the $CP_{control}$ group, as indicated in Figures 3c & d.

3.3. The susceptibility of *Staphylococcus aureus* to antibiotics

An antimicrobial susceptibility test was performed on *Staphylococcus aureus* using ten different antibiotics. Remarkably, the finding indicated that 5/10 (50%) of the antibiotics tested were susceptible to *Staphylococcus aureus*, including Florfenicol (FFC), Erythromycin (E), Nalidixic acid (NA), Gentamycin (CN), and Neomycin (N), demonstrated in Figure 4a & 4b. In addition, 4/10 (40%) of the antibiotics tested were resistant to *Staphylococcus aureus*, including Nitrofurantoin (F), Ampicillin (AMP), Amoxycillin (AML), and Oxacillin (OX) while only Kanamycin (K) antibiotic test results was specified as intermediate to *Staphylococcus aureus* (Figure 4a & 4b). In the zone of inhibition analysis, Florfenicol (FFC) antibiotic indicated the highest zone inhibition of 33 millimeters (mm), followed by Erythromycin (E) 25mm, Nalidixic acid (NA) 21mm, Gentamycin (CN) 20mm, and Neomycin (N) 18 mm, respectively according to their reference value ranges mentioned in Figure 4c. Kanamycin (K) antibiotic showed a zone of inhibition of 16mm and was categorized as intermediate. Moreover, Nitrofurantoin (F), and Oxacillin (OX) antibiotics indicated a zone of inhibition of 14mm and 4 mm, respectively while Ampicillin (AMP), and Amoxycillin (AML) showed no zone of inhibition (Figure 4c).

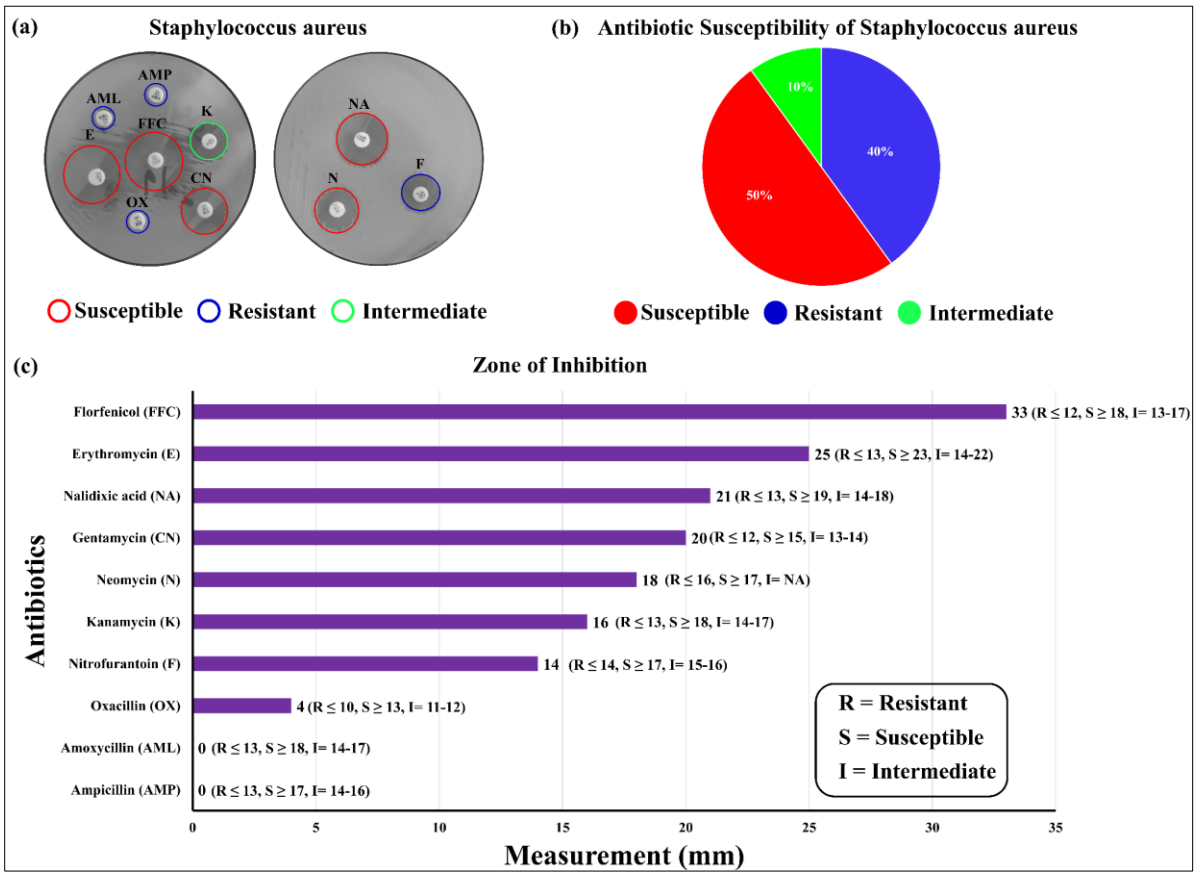

Figure 4 Antibiotic sensitivity of *Staphylococcus aureus*. (a) Media plates indicate that the ten different antibiotics were tested against *Staphylococcus aureus*. The red stroked circle depicts a susceptible, the blue stroked circle illustrates resistant, and the green stroked circle indicates intermediate. (b) The pie chart indicates the proportion of antibiotics that were susceptible, resistant, and intermediate. The red, blue, and green specify susceptible, resistant, and intermediate, respectively. (c) The bar chart illustrates the zone of inhibition of antibiotics. The x-axis shows the measurement in millimeters (mm), while the y-axis represents the name of antibiotics. The values in the bracket denote the reference score for Resistant (R), Susceptible (S), and Intermediate (I)

3.4. The susceptibility of *Klebsiella Pneumoniae* to antibiotics

An antibiotic susceptibility test was performed on *Klebsiella Pneumoniae* using ten different antibiotics. Interestingly, results indicated that 4/10 (40%) of the antibiotics tested were sensitive to *Klebsiella Pneumoniae*, including Florfenicol (FFC), Nitrofurantoin (F), Nalidixic acid (NA), and Kanamycin (K), as shown in Figure 5a & 5b. Whereas, 6/10 (60%) of the antibiotics tested were resistant to *Klebsiella Pneumoniae*, including Erythromycin (E), Neomycin (N), Gentamycin (CN), Ampicillin (AMP), Amoxycillin (AML), and Oxacillin (OX) (Figure 5a & 5b). The results of the zone of inhibition revealed that the Nalidixic acid (NA) antibiotic indicated the highest zone of inhibition of 25 millimeters (mm), followed by Florfenicol (FFC) 24mm, Nitrofurantoin (F) 20mm, and Kanamycin (K) 20mm, respectively according to their reference value ranges mentioned in Figure 5c. Furthermore, Neomycin (N) antibiotic showed a zone of inhibition of 13 mm while Erythromycin (E) was 10mm, Gentamycin (CN) 9mm, and Oxacillin (OX) 5mm. Moreover, both Ampicillin (AMP), and Amoxycillin (AML) showed the same zone of inhibition of 2mm. (Figure 5c).

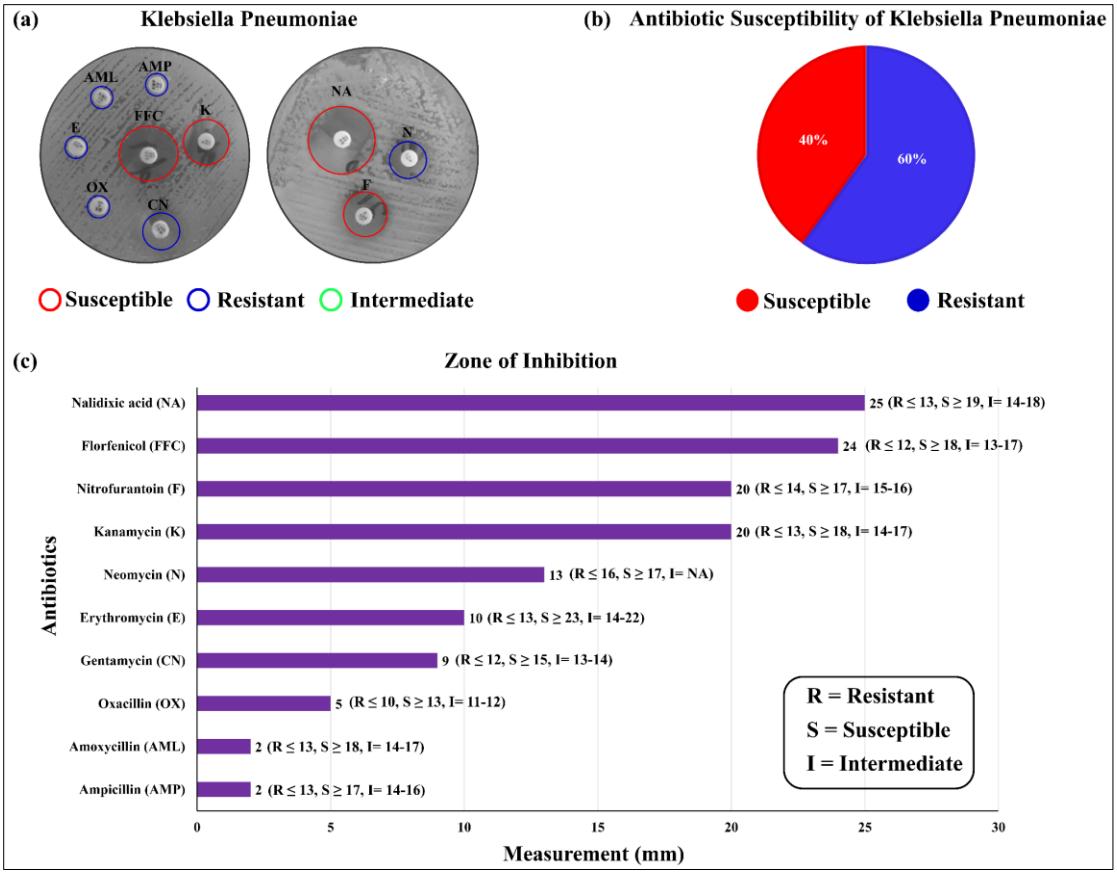

Figure 5 Antibiotic sensitivity of *Klebsiella pneumoniae*. (a) Media plates show that the ten different antibiotics were tested against *Klebsiella Pneumoniae*. The red stroked circle illustrates sensitivity, the blue stroked circle shows resistant, and the green stroked circle represents intermediate. (b) The pie chart depicts the percentages of antibiotics that were susceptible, and resistant. The red, and blue specifies susceptible, and resistant respectively. (c) The bar chart demonstrates the zone of inhibition of antibiotics. The x-axis shows the measurement in millimeters (mm), while the y-axis represents the name of antibiotics. The values in the bracket denote the reference score for Resistant (R), Susceptible (S), and Intermediate (I)

3.5. The susceptibility of *Pseudomonas aeruginosa* to antibiotics

Pseudomonas aeruginosa was tested for antibiotic susceptibility to 10 different antibiotics. It was revealed that Gentamycin (CN), 1/10 (10%) of the antibiotic, was susceptible to *Pseudomonas aeruginosa*. Furthermore, 9/10 (90%) of the antibiotics including Florfenicol (FFC), Nitrofurantoin (F), Nalidixic acid (NA), Kanamycin (K), Erythromycin (E), Neomycin (N), Ampicillin (AMP), Amoxycillin (AML), and Oxacillin (OX) were indicating resistance towards *Pseudomonas aeruginosa* as demonstrated in Figure 6a & 6b. Zone of inhibition analysis's findings showed that the greatest zone of inhibition 18mm was formed by an antibiotic, Gentamycin (CN) according to its reference value range mentioned in Figure 6c. Moreover, both Neomycin (N), and Kanamycin (K) showed the same zone of 9mm, followed by

Florfenicol (FFC) 6mm, Oxacillin (OX) 5mm, Amoxycillin (AML) 4mm, and Erythromycin (E) 4mm. Additionally, Ampicillin (AMP), Nalidixic acid (NA), and Nitrofurantoin (F) showed no zone of inhibition. (Figure 6c).

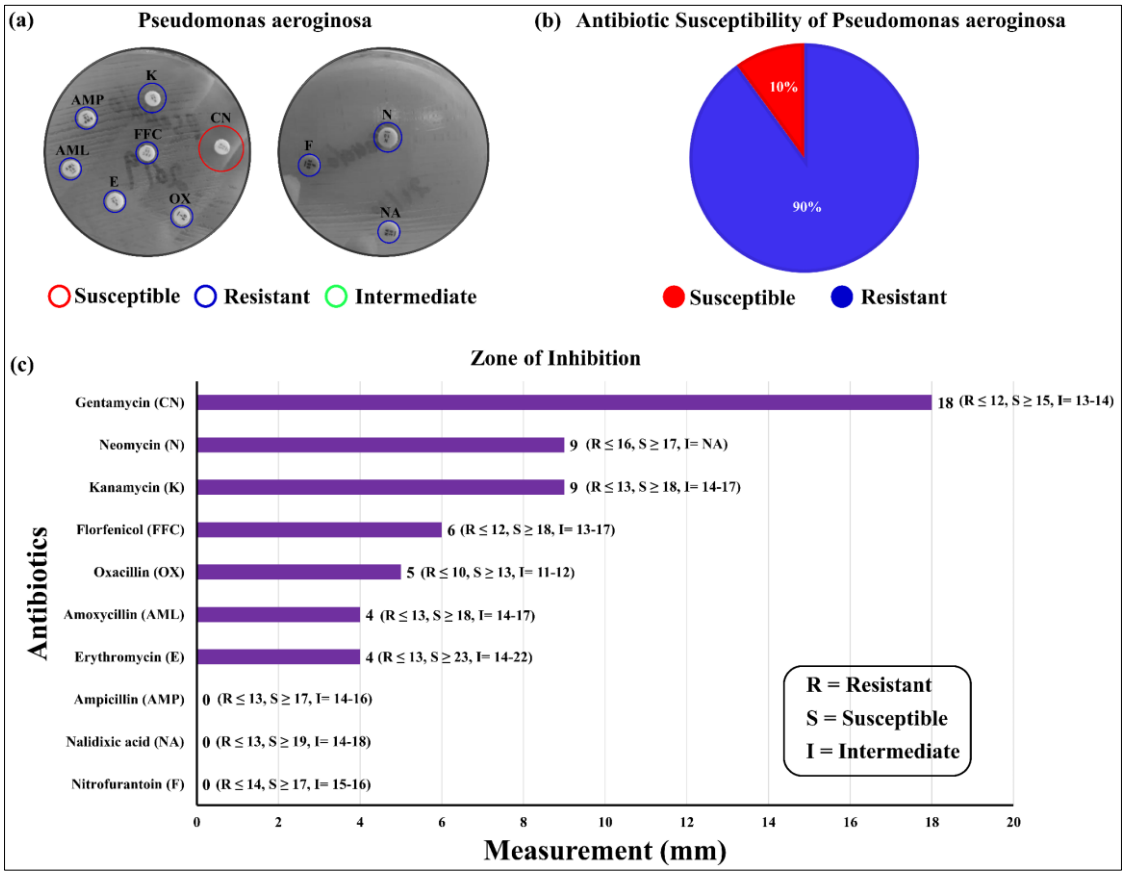

Figure 6 Antibiotic sensitivity of *Pseudomonas aeruginosa*. (a) Media plate results display that the ten different antibiotics were tested against *Pseudomonas aeruginosa*. The red stroked circle demonstrates sensitivity, the blue stroked circle shows resistant, and the green stroked circle denotes intermediate. (b) The pie chart represents the percentages of antibiotics that were susceptible, and resistant. The red, and blue depicts susceptibility, and resistance respectively. (c) The bar chart determines the zone of inhibition of antibiotics. The x-axis shows the measurement in millimeters (mm), while the y-axis represents the name of antibiotics. The values in the bracket denote the reference score for Resistant (R), Susceptible (S), and Intermediate (I)

3.6. The susceptibility of *Escherichia Coli* to antibiotics

Antibiotic susceptibility testing was performed on *Escherichia Coli* using 10 different antibiotics. *Escherichia Coli* was found to be susceptible to Florfenicol (FFC), and Nitrofurantoin (F), indicating a percentage of 2/10 (20%) of antibiotics as illustrated in Figures 7a & 7b. Also, 8/10 (80%) of the antibiotics showed resistance towards Gentamycin (CN), Nalidixic acid (NA), Kanamycin (K), Erythromycin (E), Neomycin (N), Ampicillin (AMP), Amoxycillin (AML), and Oxacillin (OX) according to their reference value ranges mentioned in Figure 7a & 7b. The results for the zone of inhibition are shown in Figure 7c. It was revealed that an antibiotic, Florfenicol (FFC) formed the largest zone of inhibition 25mm, followed by Nitrofurantoin (F) 21mm. interestingly, the zone of inhibition formed by Neomycin (N), and Oxacillin (OX) was 9mm, and 5mm respectively. Likewise, Gentamycin (CN), and Ampicillin (AMP) showed the same zone of inhibition of 4mm. Furthermore, the zone of inhibition of Amoxycillin (AML) was 3mm, and Erythromycin (E) was 2mm. Additionally, Nalidixic acid (NA), and Kanamycin (K) showed no zone of inhibition. (Figure 7c).

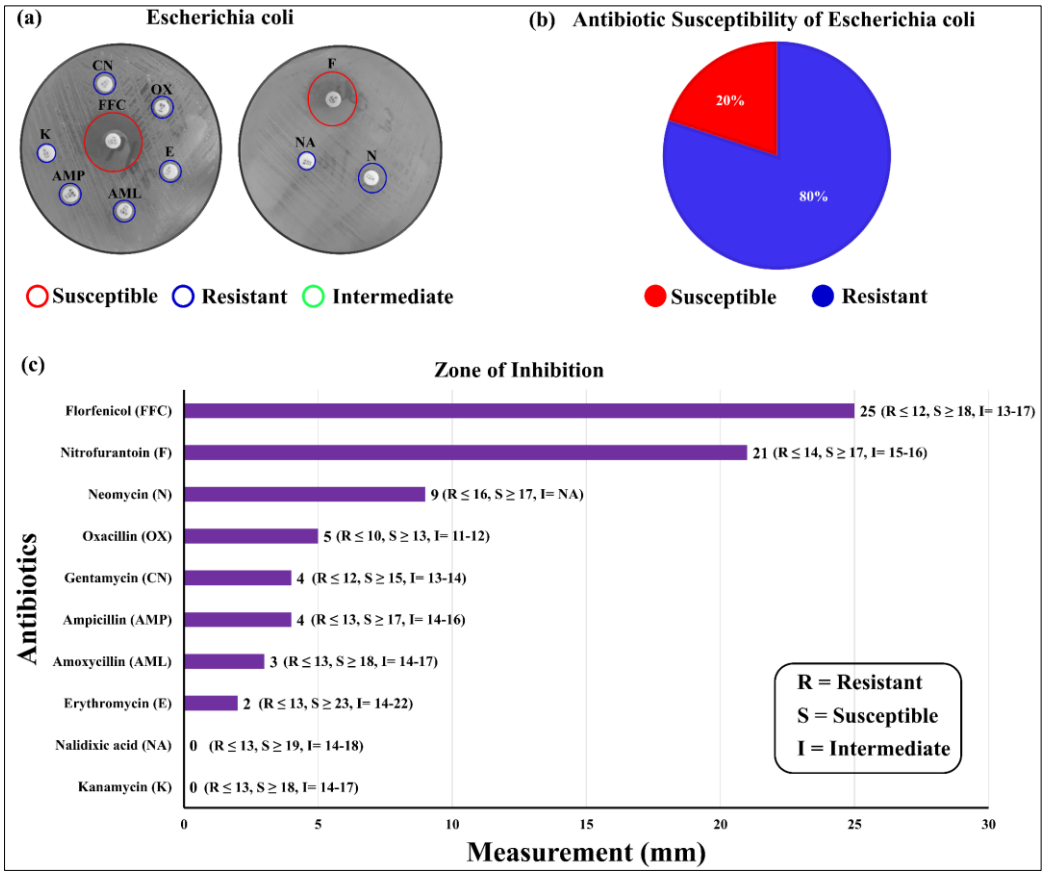

Figure 7 Antibiotic sensitivity of *Escherichia coli*. (a) Media plates depict that the ten different antibiotics were tested against *Escherichia Coli*. The red stroked circle reveals sensitivity, the blue stroked circle illustrates resistant, and the green stroked circle represents intermediate. (b) The pie chart denotes the percentages of antibiotics that were susceptible, and resistant. The red, and blue color represents sensitivity, and resistance respectively. (c) The bar chart determines the zone of inhibition of antibiotics. The x-axis shows the measurement in millimeters (mm), while the y-axis represents the name of antibiotics. The values in the bracket denote the reference score for Resistant (R), Susceptible (S), and Intermediate (I)

4. Discussion

This Study's objectives included evaluating the effectiveness of dietary crude protein on fish growth and development, and examining the antibiotic susceptibility of fish pathogenic bacteria for disease control.

For this trial, fish were fed with divergent crude protein (CP) proportions, including 15%, 30%, and 45% to estimate the effect on fish weight and length (Figure 1a). Likewise, a study conducted by Tawwab *et al.,* (2010), examined the effects of dietary protein content on Nile tilapia (*Oreochromis niloticus*) growth, feed consumption, and physiological changes. To each fish weight, duplicate diets containing 25, 35, or 45% crude protein (CP) were given. The advanced juvenile tilapia fed the 25%-CP diet had the lowest Protein Growth Rate (PGR), while fry tilapia fed the 45%-CP diet had the greatest (44).

Likewise, in our study, dietary protein intake considerably improved the Body weight (BW) and Length (L) of *Oreochromis niloticus* (Figures 2 and 3). The highest values were seen in fish fed the highest dietary protein level, which was 45%. Our results fall within the range of other exclusively carnivorous fish species, like yellow snapper (*Lutjanus argentiventris*), which has been the subject of prior investigations (45). In addition, it can be inferred that *Oreochromis niloticus* need dietary protein of at least 45% to sustain its rapid growth because growth performance did not rise over the examined protein levels in our study and fish-fed diets containing 45% protein showed faster growth rates than fish-fed diets containing dietary protein of 15 and 30%. Researchers have discovered a similar tendency for other freshwater carnivorous fish species, such as *pikeperch* and *Sander lucioperca* (46). Similarly, (44) found that a high dietary protein level (45%) rather than 15% or 30% protein was attained to achieve the optimal growth of Nile tilapia fry.

To effectively manage and control disease in a susceptible population, one must know the most common microbial pathogens and available treatments. In this study, *Oreochromis niloticus* (Nile tilapia) microflora was examined for antibiotic susceptibility. The isolates, including *Staphylococcus aureus, Klebsiella Pneumoniae*, *Pseudomonas aeruginosa,* and *Escherichia Coli* belonged to normal flora, and in some conditions, these microbes become pathogenic and lead to severe disease conditions in fish.

Previously, studies reported 15 species of bacteria isolated from *Oreochromis niloticus* (Nile tilapia), and *Clarias gariepinus* (African catfish) in Uganda for antimicrobial testing (47) with only two genera that were similar to our study. In addition, specified seven bacterial strains were obtained from retail fish and shrimp in Tanzania (48) with four species common to our study. Some of the bacteria discovered in our study could be responsible for food poisoning and gastroenteritis. Another study revealed that organisms such as *Staphylococcus aureus, Salmonella, Shigella,* and *Pseudomonas* are most likely to cause gastrointestinal illnesses when present in food. While *Shigella, Salmonella,* and *E. coli* infections in fish were the result of feces and the surroundings (49). *E. coli* are aberrant bacteria that are frequently encountered in fish whenever there has been a fecal invasion from warm-blooded mammals (50). *S. aureus* is one of the most effective and adaptable human infections because of its aptitude for gaining antibiotic-resistant pathways and pathogenic characteristics, which allowed it to emerge in both nosocomial and community-based settings (51).

Antibiotics were chosen depending on their level of application and marketability. In our study, ten antibiotics were used namely, Gentamycin, Nalidixic acid, Kanamycin, Erythromycin, Neomycin, Ampicillin, Amoxycillin, Oxacillin, Florfenicol, and Nitrofurantoin. Remarkably, the findings of our study indicated that 50% of the antibiotics tested were susceptible to *Staphylococcus aureus*, including Florfenicol, Erythromycin, Nalidixic acid, Gentamycin, and Neomycin. Also, Mwape *et al*., (2021) results were similar to our study. They presented in their report that Chloramphenicol, cefoxitin, ciprofloxacin, amikacin, and gentamicin showed the greatest susceptibility to the isolates of *S. aureus* (52). Similarly, a recent study showed that the three antibiotics that worked the best were gentamicin, trimethoprim-sulfamethoxazole, and linezolid. Likewise, Tetracycline, erythromycin, clindamycin, and ciprofloxacin all had a high susceptibility rate (53).

Furthermore, in our study the antibiotics that were resistant to *Staphylococcus aureus*, including Nitrofurantoin, Ampicillin, Amoxycillin, and Oxacillin. Previously, a study reported that the resistance of *S. aureus* towards penicillin G and ampicillin was the highest, followed by erythromycin, clindamycin, and rifampicin (54). Similarly, Kitara *et al*., (2011) used eight antibiotics to check the antibiotic susceptibility testing of *Staphylococcus aureus* and they showed that Ampicillin, one of eight studied antibiotics, demonstrated the greatest overall resistance, followed by cotrimoxazole, tetracycline, chloramphenicol, and erythromycin, in that order (55). In our findings, only the Kanamycin (K) antibiotic out of all was specified as intermediate to *Staphylococcus aureus.*

Our study concluded that *Klebsiella pneumoniae* showed sensitivity towards Florfenicol, Nitrofurantoin, Nalidixic acid, and Kanamycin. However, our results contradicted the previous study conducted by Manjula *et al*., (2014) which demonstrated that *K. pneumoniae* were resistant to ampicillin, followed by nitrofurantoin and cefuroxime (56). Moreover, our results indicated that *Klebsiella pneumoniae* showed resistance to Erythromycin, Neomycin, Gentamycin, Ampicillin, Amoxycillin, and Oxacillin. Correspondingly, study findings demonstrated the resistance of *Klebsiella pneumoniae* towards Amoxicillin/clavulanic acid, gentamicin, kanamycin, streptomycin, tetracycline, and sulfamethoxazole-trimethoprim (57).

Additionally, study results revealed that gentamicin might be more effective towards *Pseudomonas* strains of animal origin (58). These findings were similar to our study which revealed the sensitivity of *Pseudomonas aeruginosa* to Gentamycin. In another study, it was shown that a significant number of *Pseudomonas* strains were resistant to amoxicillin, and ampicillin. Also, the resistance of Oxolinic acid and florfenicol was found to be very high (59). In our study, it was proved that *Pseudomonas aeruginosa* exhibited resistance to 90% of the antibiotics including Florfenicol, Nitrofurantoin, Nalidixic acid, Kanamycin, Erythromycin, Neomycin, Ampicillin, Amoxycillin, and Oxacillin.

The results of our study stated that *E. coli* isolates showed resistance to various antibiotics, including Gentamycin, Nalidixic acid, Kanamycin, Erythromycin, Neomycin, Ampicillin, Amoxycillin, and Oxacillin. However, the results of Kibret and Abera contradicted our study, which found that erythromycin and tetracycline were both extremely resistant to *E. coli* isolates (60). Furthermore, a recent finding reported on *E. coli* that ciprofloxacin, gentamycin, tetracycline, and nalidixic acid performed very well in their attempts to stop the growth of the isolate, in comparison to antibiotics like Streptomycin and ampicillin (61). Our results showed that *E. coli* exhibited sensitivity towards Florfenicol and Nitrofurantoin. Likely, a study presented the sensitivity of *E. coli* to imipenem, nitrofurantoin, amikacin, chloramphenicol, piperacillin-tazobactam, gentamicin, aztreonam, and norfloxacin (62).

The results of our study suggest that feeds containing 45% to 50% crude protein might be sufficient to promote optimum growth and development in fish under high-performance conditions. Additionally, our study recommends that fish diets be enriched with crude protein to promote fish growth, paving the way for future research on fish feed formulation. Based on the antibiotic sensitivity results of selected bacteria, these results can serve as a basis for future reference and management of the fish disease.

5. Conclusion

As a result of this study, it was shown that dietary crude protein and host microbiota are related because adequate diet composition contributes to healthy microbiota, and both of these factors are important in shaping host physiological and immune characteristics. To conclude, our findings provide a foundation for further investigation of how the microbiota of fish is influenced by diet crude protein.

Compliance with ethical standards

Acknowledgments

In this study, we acknowledge and appreciate the assistance and support provided by the National Agriculture Research Centre, aquaculture and fisheries program, as well as Abasyn University Islamabad Campus, Department of Life Sciences, Islamabad, Pakistan.

Disclosure of conflict of interest

No conflict of interest.

Statement of ethical approval

Approved by Institutional ethical approval.

Funding

No funding sources.

References

[1] Pillay TVR. Aquaculture and the Environment: John Wiley & Sons; 2008.

[2] Schlag AK. Aquaculture: an emerging issue for public concern. Journal of Risk Research. 2010;13(7):829-44.

[3] Naylor RL, Goldburg RJ, Primavera JH, Kautsky N, Beveridge MC, Clay J, et al. Effect of aquaculture on world fish supplies. Nature. 2000;405(6790):1017-24.

[4] Naylor RL, Hardy RW, Buschmann AH, Bush SR, Cao L, Klinger DH, et al. A 20-year retrospective review of global aquaculture. Nature. 2021;591(7851):551-63.

[5] Dunz AR, Schliewen UK. Molecular phylogeny and revised classification of the haplotilapiine cichlid fishes formerly referred to as "Tilapia". Molecular Phylogenetics and Evolution. 2013;68(1):64-80.

[6] Fessehaye Y. Natural mating in Nile tilapia (Oreochromis niloticus L.): implications for reproductive success, inbreeding and cannibalism2006.

[7] FAO. The state of world fisheries and aquaculture satatistics 2014. Opportunities and challenges. 2014.

[8] Stickney RR, Davis JT. Tilapia culture. Leaflet/Texas Agricultural Extension Service; no 1863. 1981.

[9] Yosef S. Farming the aquatic chicken: improve tilapia in the Philippines. Millions fed: proven successes in agricultural development. 2009:125-30.

[10] Soto-Zarazúa GM, Rico-García E, Ocampo R, Guevara-González R, Herrera-Ruiz G. Fuzzy-logic-based feeder system for intensive tilapia production (Oreochromis niloticus). Aquaculture International. 2010;18(3):379-91.

[11] Brown E. World fish farming: cultivation and economics: Springer Science & Business Media; 2012.

[12] Ponzoni RW, Nguyen NH, Khaw HL, Hamzah A, Bakar KRA, Yee HY. Genetic improvement of Nile tilapia (Oreochromis niloticus) with special reference to the work conducted by the WorldFish Center with the GIFT strain. Reviews in Aquaculture. 2011;3(1):27-41.

[13] Lal MM, Waqairatu SS, Zenger KR, Nayfa MG, Pickering TD, Singh A, et al. The GIFT that keeps on giving? A genetic audit of the Fijian Genetically Improved Farmed Tilapia (GIFT) broodstock nucleus 20 years after introduction. Aquaculture. 2021;537:736524.

[14] De-Silva M. Genetic diversity of genetically improved farmed tilapia (GIFT) broodstocks in Sri Lanka. International Journal of Scientific Research and Innovative Technology. 2015;2:66-76.

[15] Fuentes-Silva C, Soto-Zarazúa GM, Torres-Pacheco I, Flores-Rangel A. Male tilapia production techniques: a mini-review. African journal of Biotechnology. 2013;12(36).

[16] Mair G, Little D. Population control in farmed tilapias. Naga, the ICLARM Quarterly. 1991;14(3):8-13.

[17] Wohlfarth GW, Hulata GI. Applied genetics of tilapias. 1981.

[18] Wohlfarth GW, Wedekind H. The heredity of sex determination in tilapias. Aquaculture. 1991;92:143-56.

[19] Cnaani A, Lee B-Y, Zilberman N, Ozouf-Costaz C, Hulata G, Ron M, et al. Genetics of sex determination in tilapiine species. Sexual development. 2008;2(1):43-54.

[20] Volff J-N, Schartl M. Variability of genetic sex determination in poeciliid fishes. Genetica. 2001;111(1):101-10.

[21] Wahbi O, Shalaby S. Oral administration of testosterone in fish diet affect sex differentiation and testis development in tilapia. Research Journal of Agriculture and Biological Sciences. 2010;6(6):946-52.

[22] Celik I, Guner Y, Celİk P. Effect of orally-administered 17α-Methyltestosterone at different doses on the sex reversal of the Nile tilapia (Oreochromis niloticus, Linneaus 1758). Journal of Animal and Veterinary Advances. 2011;10(7):853-7.

[23] Kefi A, Kang'ombe J, Kassam D, Katongo C. Growth, Reproduction and Sex Ratios in Oreochromis Andersonii (Castelnau, 1861) Fed with Varying Levels of 17α-Methyl Testosterone. Journal of Aquaculture & Research Development. 2012;3(8).

[24] Kiriratnikom S, Kiriratnikom AJSJoS, Technology. Growth, feed utilization, survival and body composition of fingerlings of Slender walking catfish, Clarias nieuhofii, fed diets containing different protein levels. 2012;34(1).

[25] Hu L, Yun B, Xue M, Wang J, Wu X, Zheng Y, et al. Effects of fish meal quality and fish meal substitution by animal protein blend on growth performance, flesh quality and liver histology of Japanese seabass (Lateolabrax japonicus). 2013;372:52-61.

[26] Yang S-D, Liou C-H, Liu F-GJA. Effects of dietary protein level on growth performance, carcass composition and ammonia excretion in juvenile silver perch (Bidyanus bidyanus). 2002;213(1-4):363-72.

[27] Bondad-Reantaso MG, Subasinghe RP, Arthur JR, Ogawa K, Chinabut S, Adlard R, et al. Disease and health management in Asian aquaculture. Veterinary parasitology. 2005;132(3-4):249-72.

[28] Subasinghe R, Bondad-Reantaso M, McGladdery S. Aquaculture development, health and wealth. 2001.

[29] Cipriano RC, Bullock GL, Pyle S. Aeromonas hydrophila and motile aeromonad septicemias of fish. 1984.

[30] Meyer F, Bullock G. Edwardsiella tarda, a new pathogen of channel catfish (Ictalurus punctatus). Applied microbiology. 1973;25(1):155.

[31] Abowei J, Briyai O. A review of some bacteria diseases in Africa culture fisheries. Asian Journal of Medical Sciences. 2011;3(5):206-17.

[32] Cabello FC. Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. Environmental microbiology. 2006;8(7):1137-44.

[33] Sørum H, Sunde M. Resistance to antibiotics in the normal flora of animals. Veterinary research. 2001;32(3-4):227-41.

[34] Baya A, Toranzo A, Lupiani B, Santos Y, Hetrick F. Serratia marcescens: a potential pathogen for fish. Journal of Fish Diseases. 1992;15(1):15-26.

[35] van den Bogaard AE, Stobberingh EE. Epidemiology of resistance to antibiotics: links between animals and humans. International journal of antimicrobial agents. 2000;14(4):327-35.

[36] Biyela P, Lin J, Bezuidenhout C. The role of aquatic ecosystems as reservoirs of antibiotic resistant bacteria and antibiotic resistance genes. Water Science and Technology. 2004;50(1):45-50.

[37] Salama A. ECONOMICAL EVALUATION OF NILE TILAPIA (OREOCHROMIS NILOTICUS) CULTURE IN RICE FILEDS RECEIVING VARYING FEEDING INPUTS. Egyptian Journal of Aquatic Biology and Fisheries. 2003;7(4):125-40.

[38] Ali HHJBJoVR. Isolation and identification of Staphylococcus bacteria from fish of fresh water and its antibiotics sensitivity in mosul city. 2014;1(1):33-42.

[39] Pasnik D, Evans J, Klesius PJB-EAOFP. Nile tilapia, Oreochromis niloticus, blood agar and the culture of fish bacterial pathogens. 2005;25(5):221.

[40] Sanaa OYJAJoBR. Isolation of Enterobacteriaceae and Pseudomonas spp. from raw fish sold in fish market in Khartoum state. 2009;1(7):085-8.

[41] Dalsgaard IJA. Selection of media for antimicrobial susceptibility testing of fish pathogenic bacteria. 2001;196(3-4):267-75.

[42] Bekele B, Workagegn KB, Natarajan PJIJoA, Sciences F. Prevalence and antimicrobial susceptibility of pathogenic bacteria in Nile Tilapia, Oreochromis niloticus L. 2019;5(4):022-6.

[43] Qin X, Weissman SJ, Chesnut MF, Zhang B, Shen LJAocM, Antimicrobials. Kirby-Bauer disc approximation to detect inducible third-generation cephalosporin resistance in Enterobacteriaceae. 2004;3(1):1-6.

[44] Abdel-Tawwab M, Ahmad MH, Khattab YA, Shalaby AMJA. Effect of dietary protein level, initial body weight, and their interaction on the growth, feed utilization, and physiological alterations of Nile tilapia, Oreochromis niloticus (L.). 2010;298(3-4):267-74.

[45] Maldonado-García M, Rodríguez-Romero J, Reyes-Becerril M, Álvarez-González CA, Civera-Cerecedo R, Spanopoulos MJLAJoAR. Effect of varying dietary protein levels on growth, feeding efficiency, and proximate composition of yellow snapper Lutjanus argentiventris (Peters, 1869). 2012;40(4):1017-25.

[46] Nyina-wamwiza L, Xu XL, Blanchard G, Kestemont PJAR. Effect of dietary protein, lipid and carbohydrate ratio on growth, feed efficiency and body composition of pikeperch Sander lucioperca fingerlings. 2005;36(5):486-92.

[47] Wamala SP, Mugimba KK, Mutoloki S, Evensen Ø, Mdegela R, Byarugaba DK, et al. Occurrence and antibiotic susceptibility of fish bacteria isolated from Oreochromis niloticus (Nile tilapia) and Clarias gariepinus (African catfish) in Uganda. 2018;21(1):1-10.

[48] Marijani EJIJoM. Prevalence and Antimicrobial Resistance of Bacteria Isolated from Marine and Freshwater Fish in Tanzania. 2022;2022.

[49] Sichewo PR, Gono RK, Sizanobuhle JJIjoS, Research. Isolation and identification of pathogenic bacteria in edible fish: A case study of Fletcher Dam in Gweru, Zimbabwe. 2013;2(9):269-73.

[50] Chao K-K, Chao C-C, Chao W-LJJom, immunology,, zhi iWmygrz. Suitability of the traditional microbial indicators and their enumerating methods in the assessment of fecal pollution of subtropical freshwater environments. 2003;36(4):288-93.

[51] Zetola N, Francis JS, Nuermberger EL, Bishai WRJTLid. Community-acquired meticillin-resistant Staphylococcus aureus: an emerging threat. 2005;5(5):275-86.

[52] Mwape L, Samutela M, Yamba K, Kalonda AJUoZJoA, Sciences B. Antimicrobial Susceptibility of Staphylococcus aureus Isolated from Recreational and Natural Water Bodies in Lusaka, Zambia. 2021;5(3):50-9.

[53] Derakhshan S, Navidinia M, Haghi FJBid. Antibiotic susceptibility of human-associated Staphylococcus aureus and its relation to agr typing, virulence genes, and biofilm formation. 2021;21(1):1-10.

[54] Akanbi OE, Njom HA, Fri J, Otigbu AC, Clarke AMJIjoer, health p. Antimicrobial susceptibility of Staphylococcus aureus isolated from recreational waters and beach sand in Eastern Cape Province of South Africa. 2017;14(9):1001.

[55] Kitara L, Anywar A, Acullu D, Odongo-Aginya E, Aloyo J, Fendu MJAhs. Antibiotic susceptibility of Staphylococcus aureus in suppurative lesions in Lacor Hospital, Uganda. 2011;11:34-9.

[56] Manjula N, Math GC, Nagshetty K, Patil SA, Gaddad SM, Shivannavar CTJJoC, et al. Antibiotic susceptibility pattern of ESβL producing Klebsiella pneumoniae isolated from urine samples of pregnant women in Karnataka. 2014;8(10):DC08.

[57] Barati A, Ghaderpour A, Chew LL, Bong CW, Thong KL, Chong VC, et al. Isolation and characterization of aquatic-borne Klebsiella pneumoniae from tropical estuaries in Malaysia. 2016;13(4):426.

[58] Dégi J, Moţco O-A, Dégi DM, Suici T, Mareş M, Imre K, et al. Antibiotic susceptibility profile of Pseudomonas aeruginosa canine isolates from a multicentric study in Romania. 2021;10(7):846.

[59] Thomassen GMB, Reiche T, Tennfjord CE, Mehli LJM. Antibiotic Resistance Properties among Pseudomonas spp. Associated with Salmon Processing Environments. 2022;10(7):1420.

[60] Kibret M, Abera BJAhs. Antimicrobial susceptibility patterns of E. coli from clinical sources in northeast Ethiopia. 2011;11:40-5.

[61] Assefa A, Regassa F, Ayana D, Amenu K, Abunna FJH. Prevalence and antibiotic susceptibility pattern of Escherichia coli O157: H7 isolated from harvested fish at Lake Hayq and Tekeze dam, Northern Ethiopia. 2019;5(12):e02996.

[62] Kulkarni SR, Peerapur BV, Sailesh KSJJons, biology,, medicine. Isolation and antibiotic susceptibility pattern of Escherichia coli from urinary tract infections in a tertiary care hospital of North Eastern Karnataka. 2017;8(2):176.