"Effect of Chicken Waste Silage in the diet of Rainbow Trout *(Oncorhynchus mykiss)* fingerlings to ascertain growth and survival"

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(MSFY-2021-152)

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“Effect of Chicken Waste Silage in the diet of Rainbow Trout *(Oncorhynchus mykiss)* fingerlings to ascertain growth and survival”

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Thesis

Submitted to

Faculty of Fisheries
Sher-e-Kashmir
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in partial fulfilment of requirement for the award of the degree of

Master of Fisheries Science
(Fish Nutrition and Feed Technology)
2024

Dedicated to
My parents
and Teachers

Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
Division of Fish Nutrition and Biochemistry,
Faculty of Fisheries, Rangil, Ganderbal

Certificate – I

This is to certify that the thesis entitled, **"Effect of Chicken Waste Silage in the diet of Rainbow Trout *(Oncorhynchus mykiss)* fingerlings to ascertain growth and survival"** submitted in partial fulfilment of the requirements for the award of the degree of **Master of Fisheries Science (Fish Nutrition and Feed Technology)**, to the **Faculty of Fisheries, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir** is a record of bonafide research work carried out by **Mr. Ubaid Shakeel Wani (Reg. No. MSFY-2021-152)** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that information received during the course of investigation has duly been acknowledged.

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Certificate – II

We, the members of the Advisory Committee of **Mr. Ubaid Shakeel Wani (Reg. No. MSFY-2021-152)**, a candidate for the degree of **Master of Fisheries Science (Fish Nutrition and Feed Technology)** have gone through the manuscript of the thesis entitled, **"Effect of Chicken Waste Silage in the diet of Rainbow Trout *(Oncorhynchus mykiss)* fingerlings to ascertain growth and survival"** and recommend that it may be submitted by the student in partial fulfilment of the requirements for the award of the degree.

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ABSTRACT

A ninety (90) days experimental trial was conducted to evaluate the effect of diet with partial replacement of fish meal by chicken waste silage on rainbow trout fingerlings (*Oncorhynchus mykiss*). After an acclimatization period of seven (7) days, a total of one hundred and sixty fingerlings (160) were distributed to sixteen (16) fiber tanks of 100 litre capacity by following a completely randomized design. Four iso-nitrogenous (42% crude protein) diets were prepared to replace 0%, 10%, 20% and 30% of fish meal with chicken waste silage and in accordance with four treatments each with four replicates were designed as (Control) T_0, T_1, T_2 and T_3 diet respectively. Feeding was done twice a day @ 5%

of the body weight of the fish. Each Fiber tank/ experimental unit was observed daily for mortality of fish and removed immediately if any. Water quality parameters were analyzed, recorded weekly and maintained at optimal levels by using air pumps with partial replacements of fresh water. Sampling was done fortnightly for growth parameters. The results indicated that the growth performance in terms of total weight gain (WG), percentage weight gain (WG %), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and feed efficiency ratio (FER) depicted significant difference ($p<0.05$) between different treatment groups with best values in T_2 (@ 20% of inclusion level). However, the present study demonstrated that inclusion of chicken waste silage in the diet of rainbow trout (*Oncorhynchus mykiss*) fingerlings enhanced their growth and survival compared to the control diet.

The trend of growth of fish was observed as $T_2 > T_3 > T_1 > T_0$

Keywords: Fish, Growth, *Oncorhynchus mykiss*, Chicken Waste silage, Diet, Fingerlings.

Signature of Student **Signature of Major Advisor**

Dated: **Dated:**

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Place: Rangil, Ganderbal **_Ubaid Shakeel Wani_**

Dated:

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Chapter -1

INTRODUCTION

The world population has been drastically expanding since the 21st century. While the agriculture industry is working hard to meet the world's food demand, however the population growth has accelerated at a rate that is significantly faster than agricultural output. This has led to an imbalance in the amount of essential proteins, fats and calories in the diet consumed by the people (Pradhan *et al.*, 2020). According to United Nations statistics, more than 900 million people suffer globally from malnutrition. Children under the age of four or five make up one-fourth of this population and they are primarily affected by the negative effects of severe protein energy malnutrition (PEM).

Over the past few decades, fish has gradually become a substantial source of protein in the majority of developing countries (FAO, 2020). There is no denying fact that fish contributes to human health as a healthy source of food because of its high content of nutrients viz., high-quality proteins, minerals and lipids (high in omega-3 unsaturated fatty acids) which make it one of the most deserving foods for humans. Fish has more notable satiating effects than terrestrial sources of animal proteins as beef and chicken meats due to its high protein content and unique nutritional profile (Uhe *et al.*, 1992). Besides it offers a reasonable source of protein within a wide range of nutrients in different combinations. While over 80% of people in most wealthy nations get less than 20% fish provides them with animal protein, about 60% of people in poor countries primarily rely on fish for at least 30% of their animal protein sources (FAO, 2018). However, compared to the above, the consumption is significantly higher in other Asian nations (Delgado, 2003). Despite the fact that fish and its items associated to fishing provide the majority of the food for a significant section of the population and makes fish as a key commitment to the endurance and soundness of that population (Minar *et al.*, 2012; Chakraborty *et al.*, 2019; Gandotra *et al.*, 2017).

The fastest-growing food production sector is aquaculture, where in rapid expansion has pushed fisheries and aquaculture production worldwide to all-time high as aquatic foods become more important for 21st-century for food security and nutrition (FAO, 2022). According to the State of World Fisheries and Aquaculture (SOFIA) reports 2022, the growth of aquaculture during the year 2020, especially in Asia, reached to an all-time high of 214 million tonnes, out of this production 178 million tonnes of aquatic animals and 36 million tonnes of algae produced. Over the last 20 years it has been seen a growing recognition and vital role of fisheries and aquaculture at global level. In order to achieve sustainable, inclusive and equitable global fisheries and aquaculture, scaling up transformative changes in policy, management, innovation & investment is necessary for expanding the role for human nutrition, food security and uplifting the standards of living.

India is blessed within a wide range of resources including cold-water resources, pure water with different thermal regimes and priceless native fish germ-plasm. In cold water fisheries, the Himalayan states thus present a distinctive value proposition. The Indian government is concentrating on raising the country's cold-water fish production from 52,084 million tonnes to 90 thousand million tonnes by 2024–2025 and its productivity from 1 ton/ha to 3 tons/ha due to its potential. Inland Fisheries, 2020 states that cold-water fisheries have played a significant role in generating 40,000 job opportunities and doubling the engagement across the focus States/UTs by promoting omega-loaded trout in all Himalayan States and UTs with the goal of producing 10 million tonnes of trout is pushing cold water as a niche market (Department of Fisheries, 2020).

Jammu and Kashmir is Situated between the Karakoram Range in the North, the Pir Panjal Range in the South and West and the Zanskar Range in the East. It is a landlocked area with a moderate climate characterized by cool spring, mild summers, shivering autumn and severe winters. The climatic condition makes animal protein as a valuable food source with each person consuming 9 kg of fish annually. Jammu and

Kashmir currently produces 20.06 thousand tonnes of fish annually i.e., consumption of fish is higher than production (J&K Dept. of Fisheries, 2021). The main cause of the aquatic ecological pollution is encroachment, deforestation, urbanization and industrialization. As a result, declining naturally cultivable or culturable areas (Yousuf 1996; Bakhi 2007 and Bhat *et al*., 2010; 2020).

In order to increase the production of captive fish, Jammu and Kashmir Fisheries Department (2023) has established over 1144 trout and 1222 carp rearing units in the private sector. This has effectively turned the fisheries sector into private enterprise to reduce the gap between availability and demand. Proper management with efficient feed formulation, feeding and stocking regimes are necessary for maximizing the yield.

The cold-water fish Rainbow trout (*Oncorhynchus mykiss*) belongs to Salmonidae family. The name, mykiss originates from the far-east of Russia, where the species was initially documented in the 1700s (NPS,2015). The retail market of trout is becoming more popular in terms of quality and quantity. It's crucial to remember that J&K leads the trout production, producing over 1990 tons per year due to abundant water supply (J&K Dept. of Fisheries, 2022-23). Fish farmers require an increasing amount of seed and feed due to the growing trend of trout culture and provides major source of income for youth of Jammu and Kashmir. Rainbow trout is widely cultivated aquaculture species and it has fast growth rate, ease of acceptance of artificial feed and ability to be cultivated at high stocking densities make them ideal for captive breeding. Nowadays it has been widely used as domestic strains raised for intensive fish farming as a food fish, sold to restaurants, supermarkets and consumers fresh, fresh-frozen, or smoked. It is one of the highly valued salmonid species used in aquaculture (FAO, 2020).

Nutrients play a physiologically significant role in the cellular and metabolic processes of many animals including fish. Fish growth, reproduction, and overall health depend on nutrients. Among all the nutrients that fish needs and some are not able to be manufactured by the fish itself, as a result they must rely on outside sources which are provided through artificial diets. All of the micro- and macronutrients required for the fish growth and maintenance should be present in a fully balanced diet. Fish needs energy to maintain basic metabolic activities and to support growth, reproduction and health (the macronutrients) viz., proteins, fats and carbohydrates. It also contains a variety of other substances and elements known as micronutrients, such as vitamins and minerals. The primary expensive dietary segment and the first macronutrient that affects fish growth performance is protein (NRC, 2011; Zehra and Khan, 2012; Rahimejad and Lee, 2013; Kim *et al*., 2016; Sankian *et al*., 2017; Bennet *et al*., 2018; Ahmed and Ahmad, 2020; Bowen, 2021). Protein in the diet can either be insufficient or excessively beneficial for fish growth. Excessive consumption of protein in the diet can have three detrimental effects: first it can hinder growth, raise the cost of the diet unnecessarily and too much nitrogen can also pollute fish environment. Thus, in addition to lowering feed preparation costs, it is crucial to create a diet that meets the fish's dietary requirements for healthy growth (Sa *et al*., 2006). Amino acids are the building blocks of proteins and are essential to the structure and metabolism of fish. All living things including fish synthesis proteins, enzymes, hormones, and other metabolites (Meijer, 2003; Rahimnejad and Lee, 2013; Teles *et al*., 2020). Since fish cannot synthesize all the essential amino acids so supplement feed must contain an adequate quantity of essential amino acids (EAA) or a high-quality source of nitrogen to meet the requirements for growth, metabolism and overall health (Meijer, 2003; Teles *et al*., 2020).

The major problem which hits the trout farming is the cost of the fish meal. In an intensive culture system fish meal is the single most crucial component that determines the profitability of the system. Feed is the most expensive input used in trout farming which is done in raceways and it has a significant impact on the industrial profit margins. Fish meal is a crucial component of trout feed where comes from outside states, which raises the cost due to transportation. In trout farming, feed accounts for 76% of total variable costs. Therefore, it is required to focus on locating different sources of protein supplement in trout farming which is locally accessible and comparatively less expensive source for trout feed without affecting growth, survival and quality of trout.

Fish meal is considered as an essential source of diet for fish which is rich in essential proteins, unsaturated fats, vitamins and minerals. Fish meal has proven high protein quality, palatability and digestibility which made it a valuable protein source for fish diets. Traditionally small pelagic species like sardines, herring, anchovies and mackerel as well as industrial processing wastes were the main sources of fish meal used in aqua-feeds. Supply of fish meal is declining due to its high demand in feed industries. Cost of fish meal is increasing which makes it necessary for the aquaculture sector to reduce or remove fish meal from aqua-feeds. Moreover, lowering the amount of fish meal in diets is one way to promote reasonably priced formulations, according to Hardy (2008).

Millions of tonnes of wastes including heads, necks, legs, intestines, blood, feathers, and even entire inedible carcasses are produced by the expanding global poultry industry. Large amounts of intestines are produced as waste by poultry processing facilities. There are about 3430 million poultry populations in India, with a waste generation of 3.30 million tons per year (Kokilan *et al*., 2022). The waste that is produced can be further utilized for the production of fish feed because it contains valuable nutrients like proteins and minerals. The most widely used waste utilization techniques that involve making fertilizer and animal feed. These techniques help to reduce environmental pollution by paving the way for a zero-waste poultry industry.

By converting chicken silage, it is advantageous to upgrade chicken waste into fish feed. This process is safe, economical and environmentally beneficial (Hanafy and Ibrahim, 2004). A stable liquid stock feed made by directly adding acids to chicken waste and the availability of raw materials for fish feed usually determine how it is prepared (Hasan, 2003). Formic acid is the best option for making chemical silage because the silages prepared with formic acid are not overly acidic and they don't need to be neutralized before being used (Oetterer, 2002).

The purpose of the research was assess the impact of gradually substituting acid-fermented chicken silage for fish meal in the experimental diet of rainbow trout fingerlings. This method could provide an alternative and less expensive source of protein.

OBJECTIVES

The current study entitled **"Effect of Chicken Waste Silage in the diet of Rainbow Trout *(Oncorhynchus mykiss)* fingerlings to ascertain growth and survival"** were conducted with the following objectives:

1. Preparation of chicken waste silage from chicken waste using acid fermentation.
2. Partial replacement of fish meal by chicken waste silage.
3. To study the effect of chicken waste silage on growth and survival of Rainbow Trout *(Oncorhynchus mykiss).*

Chapter-2

REVIEW OF LITERATURE

Mbokane *et al.* (2022) examined acid-fermented chicken silage on growth, digestive enzyme activity and histology of the intestine and liver of juvenile Mozambique tilapia (*Oreochromis mossambicus*). Their findings revealed no detrimental changes in fish liver and intestines across diets. The study affirms the viability of acid-fermented chicken silage as a cost-effective protein source and complete diet substitute for *Oreochromis mossambicus*.

Safari *et al.* (2022) examined the effects of replacing fish meal with bio-silage from chicken intestine in rainbow trout diets. The study assessed serum indices, muscle quality and digestive system bacterial flora in Rainbow trout raised in two different farms. Diets with 50% and 100% bio-silage were compared with a control diet. In both farms, the 100% bio-silage treatment led to increased total protein, IgM, and lysozyme levels ($p<0.05$). Albumin levels were similar in the Karkandeh village fish but higher in the 100% bio-silage treatment for dam fish ($p<0.05$). Dam fish on the 100% treatment had higher free oxygen radical levels ($p<0.05$). Carcass analysis showed higher protein and ash content in the 50% bio-silage treatment in both farms ($p<0.05$), but lower fat content ($p<0.05$). The 50% bio-silage treatment led to higher populations of aerobic and lactic-acid bacteria in the fish gastrointestinal tract compared to the other treatments ($p<0.05$). Overall, using bio-silage as an alternative source of fish meal in rainbow trout diets was found to improve serum indices, muscle quality and bacterial flora in the gastrointestinal tract.

Kanungo *et al.* (2022) investigated the potential use of poultry waste to reduce fish feed costs. They investigated the effectiveness of using chicken visceral silage as a substitute for fish meal in fish feed formula. The researchers obtained broiler offal from multiple slaughterhouses, processed it by grinding and preserving it with an acid mixture (sulphuric and formic acid). The resulting fluid ensilage was neutralized and sun-dried to create dry silage. They conducted their study on common carp fry with a mean weight of

0.71g. Five types of feeds were formulated, each having different inclusion levels (0%, 5%, 10%, 15%, and 20%) of visceral silage replacing equal amounts of fish meal. The fish were divided into triplicate tanks for each feed group and were subjected to a 60-day feeding trial. At the end of the experimental trial, the researchers assessed growth parameters and conducted histopathological examinations. The results showed that treatment T_4 (20% inclusion of visceral silage) led to a significant increase in body weight Gain and Specific Growth Rate (SGR) ($p<0.05$). Additionally, a comparative histopathology study of the intestine and liver revealed no major signs of disease. In conclusion, the study suggests that broiler visceral silage has the potential to be a viable replacement for fish meal in feed formulations.

Safari *et al.* (2021) explored the use of bio-silage made from chicken waste as replacement for fish meal in rainbow trout diets. They aimed to assess the growth performance and feed efficiency of rainbow trout fed diets with 50% bio-silage - 50% fish meal and 100%- bio-silage, compared to a control diet with 100% fish meal. The study found that the 50% bio-silage - 50% fish meal treatment outperformed the other two treatments in terms of weight gain, condition factor and specific growth rate. Additionally, this treatment showed improved feed conversion ratio and protein efficiency ratio. The study concluded that chicken waste-derived bio-silage is a promising substitute for fish meal. Notably, the 50% bio-silage - 50% fish meal treatment showed superior growth and feed efficiency compared to both the 100% fish meal and 100% bio-silage treatments.

Poolsawat *et al.* (2021) conducted two trials to optimize enzymatic feather meal production and evaluate its utilization in tilapia diets. In the first trial, they varied enzymolysis parameters such as time (30, 60, and 90 minutes), temperature (25°C, 45°C, and 60°C), moisture content (500 g/kg, 1000 g/kg, and 1500 g/kg feather meal), and protease inclusion (0 g/kg, 5 g/kg, 10 g/kg, and 15 g/kg feather meal) to determine the optimal conditions. The findings identified the best conditions as 60 minutes enzymolysis time, 60°C temperature, 1000 g/kg moisture inclusion and 10 g/kg protease inclusion. In the second trial, they fed 300 tilapia (14.00 ± 0.03 g) with five experimental diets over a

9-week period. These diets included a control diet with 6% fish meal (FM), two diets with feather meal (FeM) substitution (50% and 100%), and two diets with enzymatic feather meal (EFeM) substitution (50% and 100%). The study found no significant differences ($P > 0.05$) in weight gain and feed conversion ratio among the control, FeM-50, EFeM-50, and EFeM-100 groups. However, apparent coefficient digestibility of dry matter and protein decreased in the two FeM groups and increased in the EFeM-100 group ($P < 0.05$).In conclusion, the research indicated that enzymatic feather meal can effectively replace fish meal in tilapia diets without adversely affecting the growth, nutrient retention, and digestibility.

Hong *et al.* (2019) undertook a feeding trial to explore the consequences of substituting dietary fishmeal (FM) with a blend of poultry by-product meal (PBM), fish silage (FS), and fish protein hydrolysate (FPH) on the growth performance of the Asian sea-bass species *Lates calcarifer*. The outcome indicated that the combination of PBM, FS, and FPH exhibited the potential to replace dietary fishmeal protein in the *Lates calcarifer* diet by up to either 25% or 50%, without causing any detrimental impacts on the fish's growth performances.

Boitai *et al.* (2018) conducted a study to examine the impact of including fish silage in the diet of broiler chickens. They investigated its effects on growth performance, serum biochemical parameters, and carcass characteristics. The findings revealed that when acid-treated fish silage was integrated into the diet, it could be added up to 10% without having an adverse effect on the broiler chicken’s ability to grow.

Cayen *et al.* (2016) conducted a 42-day experiment to assess the impact of replacing fish meal with chicken viscera meal in the experimental diet of *Clarias gariepinus* on growth, feed utilization, and production. The fish, initially weighing 8.0 ± 0.4g, were fed twice daily to apparent satiation with three diets of equal nitrogen content (43% crude protein) and caloric value (20 KJ/ggross energy). These diets contained varying levels of chicken viscera: 0% (diet A0), 30% (diet A30), and 50% (diet A50). A commercial diet (Coppens) developed for Catfish served as a reference, and diet A_0 acted as the control The high survival rates ranged from 94.3% to 97.5%. Fish consuming viscera meal exhibited notably lower total feed intake (ranging from 2117g to 2183g) compared to those fed Coppens (5195g) and viscera-free diets (5800g). Final weight (ranging from 39g to 111g), specific growth rate (ranging from 3.8%/day to 6.3%/day), food conversion ratio (ranging from 1.1 to 1.4), protein efficiency ratio (ranging from 1.7 to 2.2), and annual production (ranging from 14.7 Kg/m^3/year to 50.6 Kg/m^3/year) were significantly higher in fish fed Coppens and A_0 ($P<0.05$). Notably, the feed conversion ratio in fish fed chicken viscera remained relatively low (1.4 ± 0.1), indicating the value of this protein source for the experimental fish. The inclusion of chicken viscera resulted in a 58% reduction in financial expenses related to fish production. These Results are anticipated to be very important in promoting the rearing of *C. gariepinus* in rural areas.

Ekawati *et al.* (2016) carried out a study to explore the impact and optimal usage of chicken feather silage meal as a substitution for fish meal protein in the feed formulation for *Colossoma macropomum*. They employed an experimental approach based on the Completely Randomized Design (CRD), involving 5 treatments with 3 replications each. The results showed that the feed conversion ratio, protein efficiency ratio, specific growth rate, protein retention and survival rate were not significantly impacted by the treatments. However, there was an observable effect on protein digestibility. Based on these findings, the study concluded that fishmeal protein could be completely replaced with chicken feather silage meal in the feed formula for *Colossoma macropomum*, up to a 100% substitution level.

Bhaskar *et al.* (2014) conducted an investigation into the nutritional value of commonly employed fish feed components. The selection of these ingredients was guided by factors such as their high nutrient content, wide availability, and cost-effectiveness. The study aimed to determine the crude protein percentages of various ingredients, including poultry viscera, fishmeal, mustard oil cake, wheat bran, wheat flour, and rice bran. The results indicated that the crude protein percentages were 60.67%, 55.19%, 34.86%, 15.29%, 15.19%, and 11.37%, respectively. Additionally, the study found crude lipid percentages of 12.05% in poultry viscera, 7.77% in fish meal, 11.77% in mustard oil cake, 6.85% in wheat bran, 3.35% in wheat flour, and 10.05% in rice bran. The composition analysis highlighted that crude protein played a vital role, as feed cost constraints influenced the enhancement of fish farming practices. Fish meal has been traditionally a primary source of animal protein supplement in fish diets, but its in-sufficiency and high cost have led to the exploration of alternative options. Among these, poultry viscera emerged as a promising protein source derived from poultry waste, facilitating waste recycling and offering an alternative solution to supplementing fish diets.

Nandakumar *et al.* (2013) conducted a study to explore the consequences of replacing fish meal with processed chicken waste meal (CWM) in the diet of Asian sea-bass, *Lates calcarifer.* The results from this research suggest that (CWM) holds promise as a viable component in the diet of Asian sea-bass (*L. calcarifer)* with the potential to be included at levels ranging from 5% to 10% as a replacement for fish meal. However, additional research is needed to fine-tune the optimal incorporation level of CWM in the sea-bass diet.

Tabinda *et al.* (2013) investigated the potential utilization of chicken viscera as an alternative protein source in the diets of *Cirrhinus mrigala* fingerlings. Their study aimed to determine if chicken viscera could effectively replace fishmeal in *Cirrhinus mrigala* diets, even up to levels ranging from 75% to 100%, without necessitating the inclusion of extra amino acids.

Soltan and Tharwat (2006) conducted two growth trials aimed at investigating the impact of substituting fish meal (FM) with dried fermented fish silage (FFS) in the diets of Nile tilapia (*Oreochromis niloticus*) and African catfish (*Clarias gariepinus*). They formulated five different dry pelleted diets containing varying percentages (0, 10, 20, 30, and 40%) of FFS to replace FM at levels of 0, 25, 50, 75, and 100% based on protein content. These diets were designed to have equal nitrogen content (30% CP) and caloric value (2600 kcal ME/kg diet). Over a three-month period, they conducted two separate trials to assess the effects. The findings demonstrated that dried FFS could effectively replace up to 25% of FM in tilapia diets and up to 50% in catfish diets, without causing notable declines in growth performance or feed utilization. However, substituting FM with higher levels of FFS (50, 75, or 100% in tilapia diets, and 75 or 100% in catfish diets) led to significant reductions in growth performance and feed utilization. These higher replacement levels also had a significant impact on the body composition of tilapia and catfish. Evidently, FFS has the potential to serve as a valuable protein source in the diets of Nile tilapia and catfish, helping to reduce feed expenses for these species. From an economic standpoint, the study noted that replacing 25% of FM in tilapia diets or 50% in catfish diets with FFS did not result in significant adverse effects on the growth or feed utilization parameters of either fish species. This substitution also led to reductions in feed costs per kilogram of diet and feed costs per kilogram of weight gain, with reductions of 7.35% and 11.30% for tilapia, and 15.59% and 19.39% for catfish, respectively.

Giri *et al.* (2003) conducted a study where they incorporated fermented silkworm pupae (SWP) silage or untreated fresh SWP pastes into carp feed formulations, replacing fishmeal. These feed formulations were standardized to have equivalent protein content (30.2–30.9%) and caloric value (ME-2905–2935 kcal/kg). The feeding trial took place in a polyculture pond system, consisting of 30% catla *(Catla catla)*, 30% mrigal (*Cirrhinus mrigala*), 30% rohu (*Labeo rohita)*, and 10% silver carps *(Hypophthalmychthys molitrix*), to assess the nutritional value of SWP silage. The results indicated that fermented SWP silage outperformed both untreated SWP and fishmeal in terms of key growth parameters. The feed conversion ratio, specific growth rate and survival rate for fermented SWP silage were 84.2%, 2.10, and 2.39, respectively. In comparison, untreated SWP showed values of 65.8%, 2.98, and 2.26, while fishmeal demonstrated values of 67.5%, 3.16, and 2.20. These outcomes clearly indicated that fermented SWP silage exhibited superior nutritional qualities compared to untreated SWP or fishmeal.

Fagbenro and Jauncey (1994) Fish silage was produced from juvenile tilapia by fermenting the whole fish using *Lactobacillus plantarum* at 30°C. The fermentation process involved the use of alternative carbohydrate sources, including molasses, corn flour, and tapioca flour, at a ratio of 150 g kg−1 (w/w). This was carried out over a 7-day period, resulting in a pH level below 4.5 that remained stable. The choice of fermentation substrate did not impact the soluble nitrogen content or the overall composition of the liquid silage after it was stored for 30 days. To enhance the nutritional content of the silage, soybean meal was added as a filler material in varying ratios of 1:1, 2:1, 3:1, and 3:2 (w/w) before being oven-dried. This blending process led to improvements in the nutrient and dry matter (DM) composition of the silage after drying. Specifically, the 1:1 ratio of dried fish silage to soybean meal was used in formulating dry pelleted diets (at 650 g kg^{-1}) intended for tilapia (*Oreochromis niloticus*). These pellets demonstrated robustness and maintained their shape when immersed in water. The water stability of the dried fish silage pellets felt similar to that of a reference diet. In terms of digestibility, the apparent digestibility values for DM, protein, and energy in the silage-based diet were determined to be 82.8%, 84.5%, and 82.8%, respectively. As a result of these findings, it was concluded that co-dried fermented fish silage has potential as a valuable protein feed ingredient in fish diets.

Chapter-3

METERIALS AND METHODS

The current study was conducted to study the effect of chicken waste silage on growth parameters of Rainbow Trout (*Oncorhynchus mykiss*) fingerlings. The aim of the study was to investigate the use of diets containing various inclusion levels of acid-fermented chicken waste silage on growth performance of rainbow trout. Water quality parameters viz., water temperature, pH and dissolved oxygen were monitored on weekly basis to examine the effect of dosages on aquatic environment of fish.

3.1 Experimental procedure

3.1.1 Study period and site

The experimental study was conducted for a period of 90 days from 15th April to 14th July 2023, at hatchery unit, Division of Aquaculture, Faculty of Fisheries, Rangil, Ganderbal of Sher-e-Kashmir University of Agricultural sciences and Technology of Kashmir (34°.21', 74°.80').

3.1.2 Collection and acclimatization of experimental fishes

A total of 205 rainbow trout uniformly sized fingerlings were from Trout Farm Khag, Budgam. The obtained fingerlings were fed with the basal diet @ 5% body weight per day and allowed to acclimatize for a period of seven days in the tubs. A total of 160 uniformly weighted fingerlings that had successfully acclimated were split up into sixteen groups (10 fishes in each tank). These fingerlings were kept in 100-litre fiber tanks. The tanks were labelled with 4 treatments viz., T_0, T_1, T_2 & T_3 and 4 replications viz., R_1, R_2, R_3 and R_4 respectively. The fishes were given the experimental diets at 5% of their body weight. The tanks were thoroughly scrubbed with salt, cleaned with $KMnO_4$ solution (4 mg L^{-1}) and left overnight. The following day, clean water was used to completely wash the tanks and flush them out and then dried for hygienic purposes before being filled. A constant level of water was maintained in all of the tanks throughout the experimental period. Showers are used to set up a continuous water supply and aquarium air pumps were used to supply compressed air via air stones for maintaining dissolved oxygen (DO). The parameters of the water quality were measured and recorded on weekly basis. Fish excreta and feed residue were removed by siphoning with a portion of the water replaced. The dead fish if any were recorded on daily basis and removed from the tanks. Successively, the weight of the fingerlings was measured fortnightly and rations were adjusted accordingly. At the end of the experimental setup, the growth characteristics of the experimental fish and the water characteristics of the tanks were measured, recorded and the total amount of feed given was computed for each tank. After obtaining the final data, various growth parameters were computed and subjected to statistical analysis.

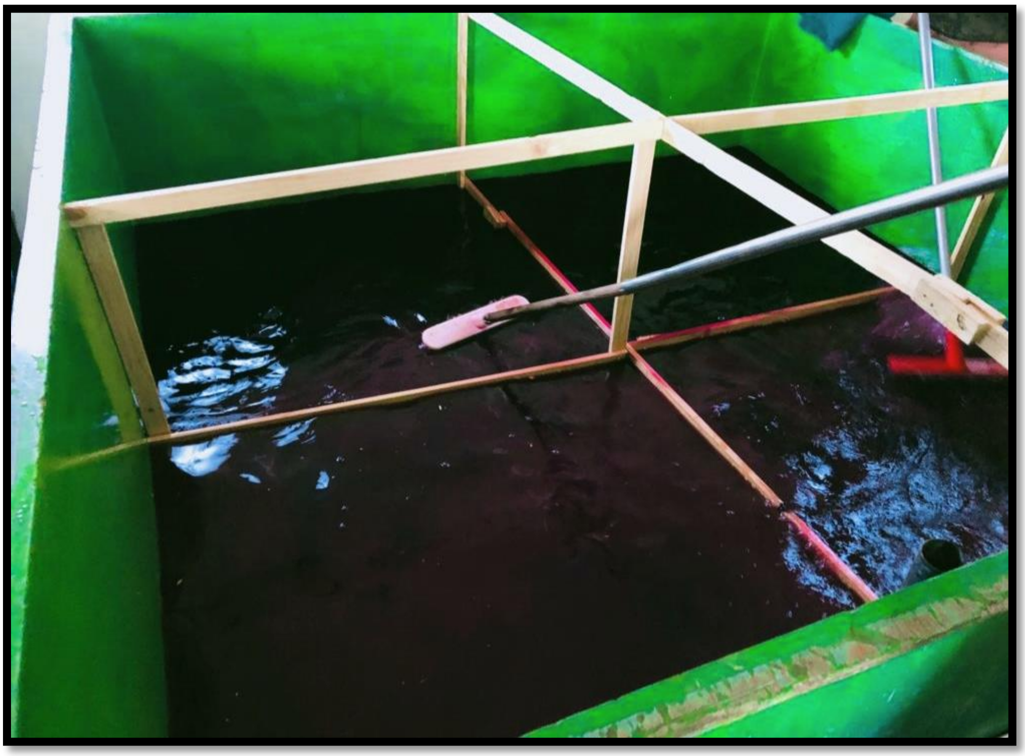

Plate 1: Cleaning of experimental tanks with common salt and $KMnO_4$

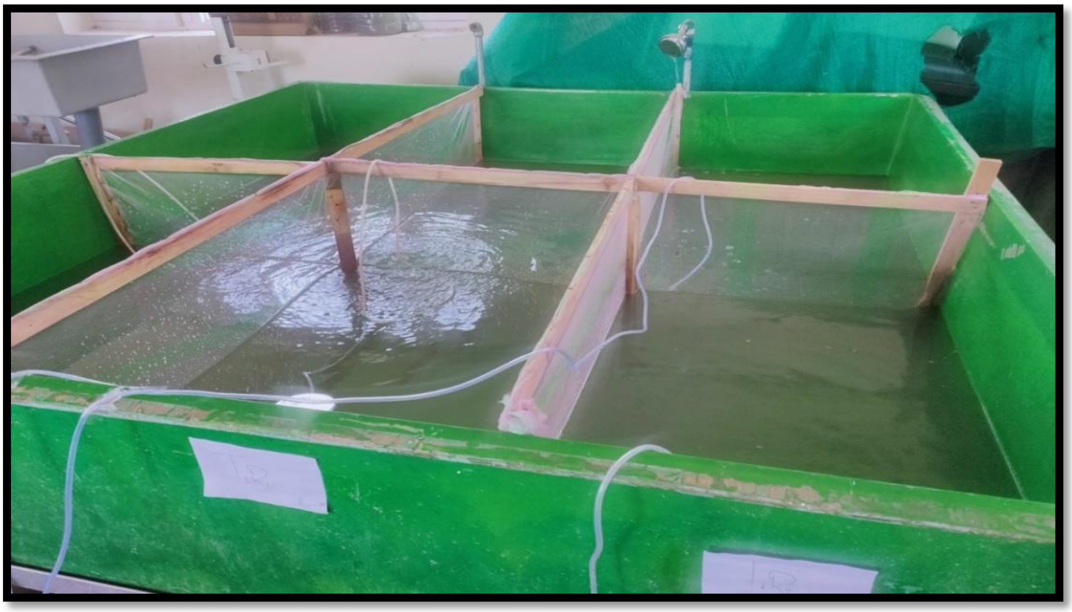

Plate 2: Placement of experimental tanks

Plate 3: Collection of experimental fishes Rainbow Trout (*Oncorhynchus mykiss*)

3.2 Procurement and processing of the experimental feed

3.2.1 Procurement of raw material

Chicken intestines were obtained from nearby market immediately after slaughtering the bird. The tissues were cleaned properly with tap water and stored in freezer until use.

3.2.2 Preparation of chicken waste silage

Chicken waste silage is a liquid material obtained from the enzymatic dissolution of chicken waste in the presence of acid. During the preparation of chicken silage, fresh chicken intestines were procured and washed with clean water over the intestines. After chopping the raw material, formic acid was added to preserve the waste. The amount of acid required to stop bacteria growth depends on the raw material. (Toppe *et al*., 2018) added 3% formic acid (w/w) to the chicken waste and the pH of the mixture was maintained at 4. The hydrolysis of the chicken waste's enzymes occurs at this pH, resulting in a highly nutritious liquid product. The silage was dried in a hot air oven at 30°C for 2-3 days to lower its moisture content.

3.2.3 Feed ingredients

In order to prepare the feed, the following basic ingredients were used viz., Mustard oil cake (MOC), Fish meal (FM), Soybean flour, Rice bran (RB), Wheat bran (WB), Maize, Vegetable oil and Vitamin-Mineral mixture. Using an electric grinder, the ingredients were separately ground into a fine powder form and then sieved through a 200mm standard mesh sieve. Diet preparation and proximate composition of the diets was carried out in Fish nutrition and Bio-chemistry Laboratory, Faculty of Fisheries, Rangil, SKUAST-K.

3.2.4 Diet Preparation

The basal/control diet (T_0) was formulated with fish meal, soybean flour, rice bran, wheat bran, vegetable oil, vitamins and minerals to have complete diet with crude protein level of 42%. A Total of four experimental diets, viz., T_0 (0%), T_1 (10%), T_2 (20%), and T_3 (30%) of the fish meal were replaced with chicken waste silage. To make dough balls, all the ingredients were evenly combined with the right amount of water and the balls were autoclaved at 121°C for 15 min. to cook and then allowed to cool. The dough balls were thoroughly mixed with oil, vitamin-mineral mixture and chicken silage to prepare the desired pellets through pelletizer. All the wet pellets were spread over papers in open air for drying. For later use, the dried pellets were kept in airtight plastic containers.

3.2.5 Feeding regime:

After completion of acclimatization period of 7 days for 24 hours prior to the commencement of the experiment, feeding was not done for 24 hours. With the commencement of experiment, the fingerlings were fed with their respective diets @ 5% of body weight twice a day. The daily ration was split into two equal portions and fed at nine o'clock in the morning and 5 o'clock in the evening. Left over feed if any and faecal matter was removed by siphoning and water exchange @ 50% was done each day with fresh water (Acar *et al.*, 2018).

Table 1: Diet composition of the experimental diets (g %) fed to Rainbow Trout fingerlings during the experimental period.

Ingredients (%)	Protein %	T0 (0%)	T1 (10%)	T2 (20%)	T3 (30%)
Fish Meal	65.00	23.71	21.34	18.97	16.6
Mustard Oil Cake	38.00	27.71	28.76	29.81	30.86
Soyabean flour	48.00	27.72	28.77	29.82	30.87
Wheat Bran	13.90	4.62	3.92	3.22	2.52
Rice Bran	12.80	4.62	3.92	3.22	2.52
Maize	9.00	4.62	3.92	3.22	2.52
Vegetable Oil	0.00	5.00	5.00	5.00	5.00
Vitamin Mineral Premix	0.00	2.00	2.00	2.00	2.00
Chicken Waste Silage	30.01	0.00	2.37	4.74	7.11
Total		100.00	100.00	100.00	100.00
CP		40.89624	40.72221	40.54818	40.37415

Plate 4: Cleaned Chicken intestines

Plate 5: Chicken waste silage

Plate 6: Sieving of feed ingredients

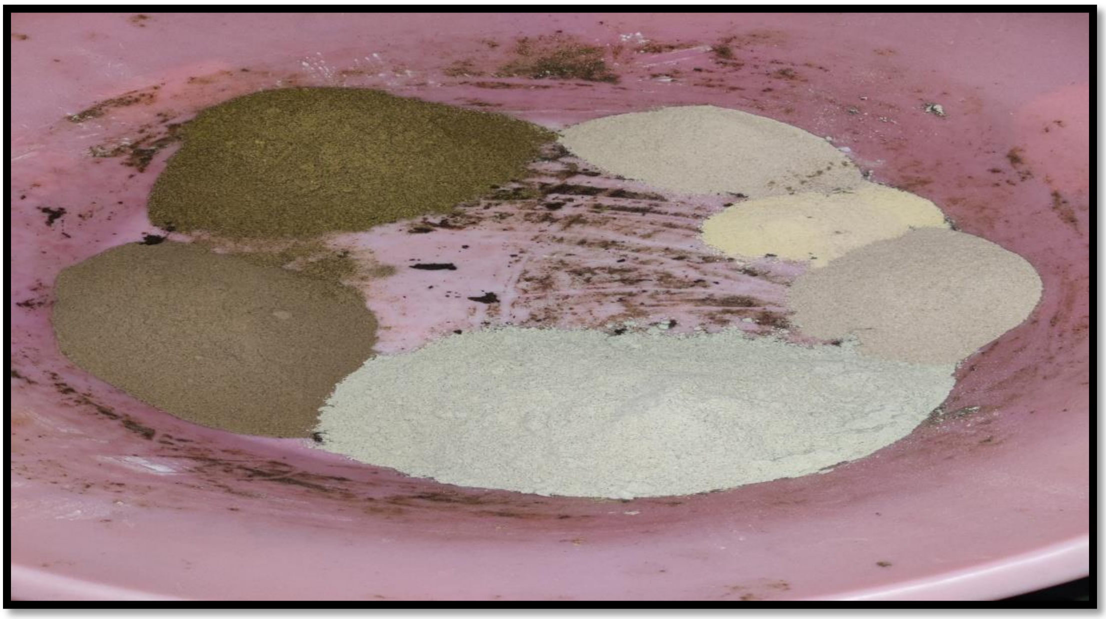

Plate 7: Mixing of feed ingredients

Plate 8: Dough preparation

Plate 9: Dough formation and Autoclaving

Plate 10: Preparation of Noodles

Plate 11: Drying of pellets

Plate 12: Experimental Diets

Plate 13: Final experimental feed in air tight containers.

3.3 Experimental set up

A total of sixteen (16) fiber tanks with hundred (100) litres capacity were installed at hatchery unit, Division of Aquaculture and each tank was stocked with ten (10) fingerlings of rainbow trout of uniform size. Each tank had a continuous supply of compressed air via air-stones using aquarium air pumps. Siphoning of tanks was done on alternate day to remove excreta and any left-over feed residue and water was partially replaced to a level of eighty (80) litres. Each tank was fed twice a day with the respective diet @ 5% of live body weight of fish for the experimental period.

3.4 Physio-chemical parameters of water

Water quality parameters viz., Temperature, pH and dissolved oxygen were recorded in all the tanks were analyzed at weekly intervals as per the standard method (APHA, 2012).

3.4.1 Water temperature

The ambient temperature of water was measured using a thermometer by submerging it in the tank for roughly two minutes. The results were expressed in degree Centigrade (ºC).

3.4.2 Hydrogen ion concentration (pH)

A digital pH meter (Eutech) was used to check the pH of the water in each of the experimental unit.

3.4.3 Dissolved oxygen (D.O)

Glass DO bottles with a 125 ml capacity were used to collect samples of waters from each experimental tank without any bubbling. Immediately, airtight stoppers were used to seal the glass bottles with following sample collection to reduce the possibility of air mixing. Using different pipettes, the collected samples were quickly fixed with 1ml of alkaline potassium iodide and 1ml of manganous sulphate solution. After the samples were brought to the Fish nutrition and Bio-chemistry (FNB) lab for additional examination. The precipitate was then dissolved by adding 1ml of concentrated H_2SO_4. This sample was titrated against 0.01N sodium thiosulphate in a known volume of 50 ml in volumetric flask until it turned pale straw in colour. This pale straw solution turned blue when a few drops of starch indicator were added. The titration procedure was continued until the solution's blue colour became colourless. Using the below mentioned standard formula, the amount of dissolved oxygen in the sample was determined and expressed in mg/l.

3.5 Periodic sampling

Sampling was done fortnightly (after every 15 days) i.e. on 15th, 30th, 45th, 60th, 75th and 90th days of the experiment.

Calculation

$$\text{Dissolved oxygen (mg/l)} = \frac{8 \times 1000 \times N \times v}{V}$$

Where,

V = volume of titrant used (ml)

v = volume of sample (ml)

N = normality of the titrant.

3.6 Proximate analysis of chicken waste silage

3.6.1 Moisture

By placing a known weight of the sample in the petri dish and drying it in a hot air oven at 100–105 °C until the weight remains constant was attained and the moisture content of chicken silage was calculated. The moisture content was determined using the following formula based on the difference in weight of the samples.

$$\text{Moisture (\%)} = \frac{\text{Wet weight of sample} - \text{Dried weight of sample}}{\text{Wet weight of sample}} \text{ x } 100$$

3.6.2 Crude protein (CP)

Using the Micro Kjeldahl method, we quantified the nitrogen content of the sample. This method employs titration to calculate the nitrogen percentage. Initially, the compound was broken down using concentrated sulfuric acid and a catalytic mixture of copper sulphate and potassium sulphate was added. This process converted the nitrogen in the compound to ammonium sulphate. Subsequently, alkalis were introduced to dissolve ammonium sulphate, and the generated ammonia was absorbed by boric acid. The absorbed ammonia was then titrated against sulphuric acid. The crude protein percentage was measured by multiplying the nitrogen percentage by a factor of 6.25.

$$\text{Crude Protein (\%)} = N_2(\%)\ x\ 6.25$$

3.6.3 Ash

A known weight of sample in a silica crucible was placed in a muffle furnace at around 600 °C for six hours to calculate the amount of ash present. The calculation was done as follows:

$$\text{Ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of the sample}} \text{ x } 100$$

Table 2: Proximate analysis of chicken waste silage (g/100g of dry weight)

Moisture	Crude Protein	Fat	Ash
12.3	30.01	13.2	3.8

3.7 Fish Growth Parameters

The fishes were sampled at 15-day intervals to determine their body weight. An electronic weighing balance was used to determine the weight.

i. Weight Gain

$$\text{Weight Gain} = W_f - W_i$$

Where,

W_f = Weight of Fish at the end of experiment

W_i = Weight of Fish at the beginning of experiment

ii. Feed conversion ratio (FCR)

$$FCR = \frac{\text{Feed given (dry weight)}}{\text{Body weight gain (wet weight)}}$$

iii. Specific growth rate (SGR)

The Specific Growth rate was calculated by the following formula

$$SGR\ (\%) = \frac{\text{Log}_e \text{ final weight} - \text{Log}_e \text{ initial weight}}{\text{Number of days}} \times 100$$

iv. Protein efficiency ratio (PER)

Protein efficiency ratio was calculated by us the following formula

$$PER = \frac{\text{Net weight gain (g)}}{\text{Protein fed (g)}}$$

v. Feed efficiency ratio (FER)

Feed efficiency ratio was calculated by the following formula

$$FER = \frac{\text{Wet weight gain (g)}}{\text{Feed intake (g)}}$$

vi. Survival rate (SR)

$$SR\ (\%) = \frac{\text{Final number of fish}}{\text{Initial number of fish}} \times 100$$

3.8 Statistical analysis

The statistical package SPSS version 23 was used to analyze the collected data. One-way ANOVA was applied to the data and Duncan's multiple range test was employed to identify significant differences between the means, $p<0.05$ was considered as statistically significant. The results were expressed as mean±standard error.

Chapter- 4

EXPERIMENTAL FINDINGS

4.1 Growth parameters

The findings of this study showed that replacement of the diet of rainbow trout fingerlings with chicken waste silage led to an improvement in the growth of the fishes. Different parameters related to growth such as weight gain, weight gain percentage, specific growth rate, feed conversion ratio, feed efficiency ratio and protein efficiency ratio were evaluated at various inclusion levels of chicken waste silage. The experimental findings are outlined below.

4.1.1 Weight Gain

The four treatments viz., T_0, T_1, T_2 and T_3 which contain 0, 2.37, 4.47 and 7.11 g of chicken waste silage respectively added in conventional fish feed showed significant increase in weight gain compared over control treatment (T_0) which is without supplementation of chicken waste silage (Figure 1). Highest weight gain of 576.25±5.03g was observed in T_2 treatment (conventional feed of 1 kg + 4.47 g chicken waste silage) as depicted in Table 3.

4.1.2 Weight Gain Percentage

The percentage increase in body weight gain provides a broader perspective on weight increments offering a comprehensive change in body weight. Considerable differences were found by statistical analysis in body weight gain percentages among different treatments ($p<0.05$) as depicted in Table 3. Treatment-T_2 exhibited the highest body weight gain percentage (260.25±2.2%), followed by treatment-T_3 (235.61±3.64%) and treatment-T_1 (231.04±3.56%). Whereas the control-T_0(206.27±1.94%) group showed the lowest body weight gain percentage (Figure 2).

The comparison between T_0, T_1, T_2 and T_3 by Duncan's multiple range test (DMRT) revealed statistical analysis showed notable differences expect for T_1-T_3, as depicted by superscripts assigned in Table 3.

4.1.3 Feed Conversion Ratio (FCR)

The Feed conversion ratio (FCR) is ability of a fish in order to increase body mass from feed mass. The mean FCR of the different experimental groups was noted as control-T_0 (2.18±0.02), treatment-T_1 (1.95±0.03), treatment-T_2 (1.73±0.01) and treatment-T_3 (1.91±0.03). However, the FCR in diets viz., T_1, T_2 and T_3 showed better and statistically significant differences in FCR values compared to T_0 but T_2 showed the best result with lowest FCR (Figure 3).

The comparison between T_0, T_1, T_2 and T_3 by Duncan's multiple range test (DMRT) revealed significant difference between treatments groups expect for T_1-T_3 as depicted by superscripts assigned in Table 3.

4.1.4 Specific Growth Rate (SGR)

The specific growth rate is a coefficient that measures the daily percentage rise in the weight of fish.

The mean SGR values of the different treatments recorded were control-T_0 (1.01±0.01), treatment-T_1 (1.08±0.01), treatment-T_2 (1.15±0.01) and treatment-T_3 (1.09±0.01). The highest specific growth rate (1.15±0.01) was shown by T_2 feeding group and the lowest (1.01±0.01) was shown by T_0 feeding group (Figure 4). SGR values among different treatment groups showed statistically significant differences with $p<0.05$, as depicted in Table 3. The comparison between T_0, T_1, T_2 and T_3 by Duncan's multiple range test (DMRT) revealed significant difference between treatment groups expect for T_1-T_3, as depicted by superscripts assigned in Table 3.

4.1.5 Feed Efficiency Ratio (FER)

FER is a crucial factor that measures an animal's ability to transform feed into body mass effectively. A higher FER suggests improved utilization of feed and greater efficiency in promoting growth.

FER was found to be highest in T_2 (57.83±0.49) whereas lowest FER value (45.84±0.43) was found in T_0. A statistically significant difference in feed efficiency ratio was calculated across the treatments from T_0 to T_3 feeding groups (p-value <0.05). However, T_1 and T_3 showed statistically non-significant difference (Figure 5).

4.1.6 Protein Efficiency Ratio (PER)

The protein efficiency ratio (PER) determines the quality, digestibility and bioavailability of protein in a diet. A feed with a higher PER indicates improved availability and digestibility of its protein content. The T_2 feeding group exhibited the highest protein efficiency ratio (1.41±0.01) whereas the T_0 feeding group was shown lowest PER value (1.12±0.01). There was a significant difference in protein efficiency ratio between treatments from T_0 to T_3 feeding groups (p-value$<$ 0.05). However, the difference between T_1 and T_3 was not statistically significant indicating that the results in both T_1 and T_3 treatments were statistically similar (Figure 6).

4.1.7 Survival Rate (SR)

Throughout the experimental period, no mortality was observed. All of the experimental groups, as well as the control group exhibited 100% survival rate.

Table 3: Growth parameters of different experimental groups fed different experimental diets.

Parameters	Percentage of fish meal replaced by chicken waste silage			
	Control-T_0 (0%)	Control-T_1 (10%)	Control-T_2 (20%)	Control-T_3 (30%)
Initial weight (g)	221.15±0.45	221.22±0.53	221.42±0.57	220.08±0.86
Final weight (g)	677.35±5.6^a	732.32±7.58^b	797.68±5.19^c	738.62±9.33^b
Body weight gain	456.2±5.17^a	511.1±7.63^b	576.25±5.03^c	518.55±8.86^b
Body weight gain percentage	206.27±1.94^a	231.04±3.56^b	260.25±2.2^c	235.61±3.64^b
SGR	1.01±0.01^a	1.08±0.01^b	1.15±0.01^c	1.09±0.01^b
FCR	2.18±0.02^c	1.95±0.03^b	1.73±0.01^a	1.91±0.03^b
PER	1.12±0.01^a	1.25±0.02^b	1.41±0.01^c	1.28±0.02^b
FER	45.84±0.43^a	51.34±0.79^b	57.83±0.49^c	52.36±0.81^b
SR	100	100	100	100

Data were presented as mean ±SE (n=4). Values within the same column having different superscripts are significantly different ($p<0.05$).

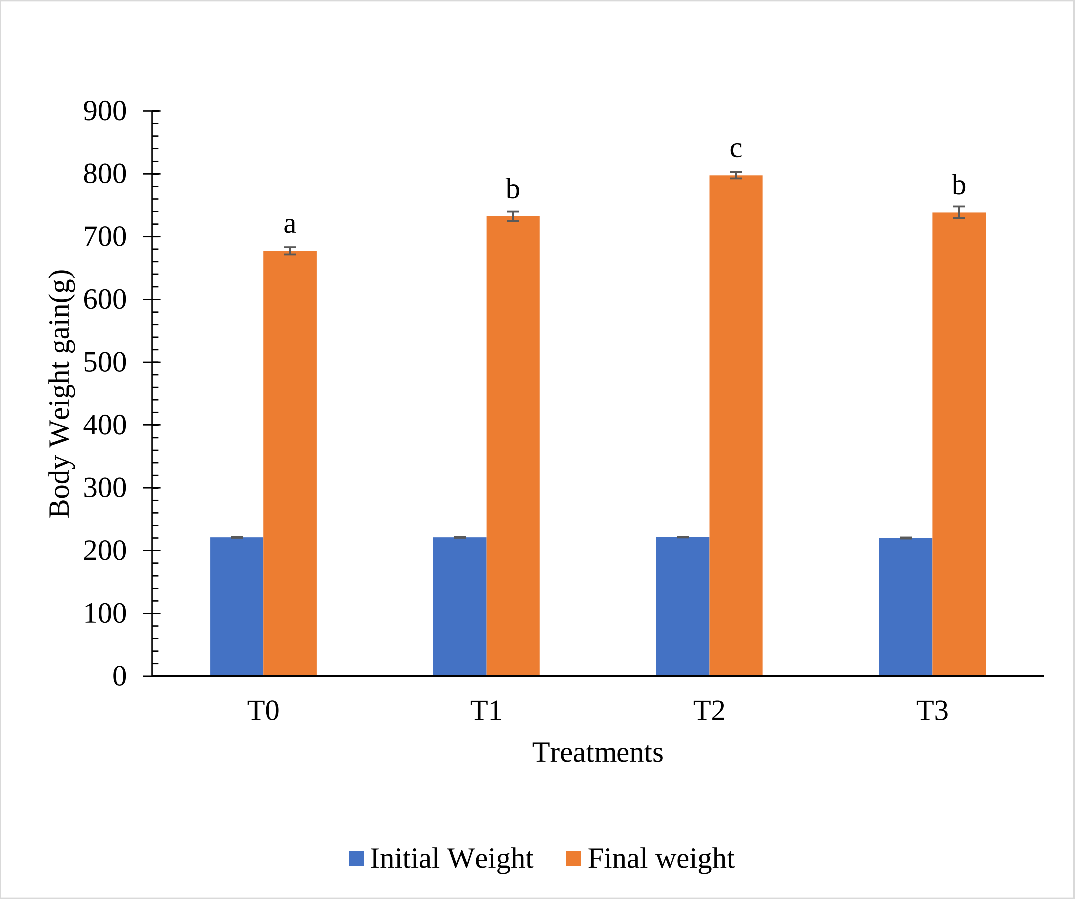

Figure 1: Body weight gain of *Oncorhynchus mykiss* fingerlings fed with different experimental diets.

T_0= Control feed without Chicken waste silage

T_1 = Feed supplemented with Chicken waste silage @ 10%

T_2 = Feed supplemented with Chicken waste silage @ 20%

T_3 = Feed supplemented with Chicken waste silage @ 30%

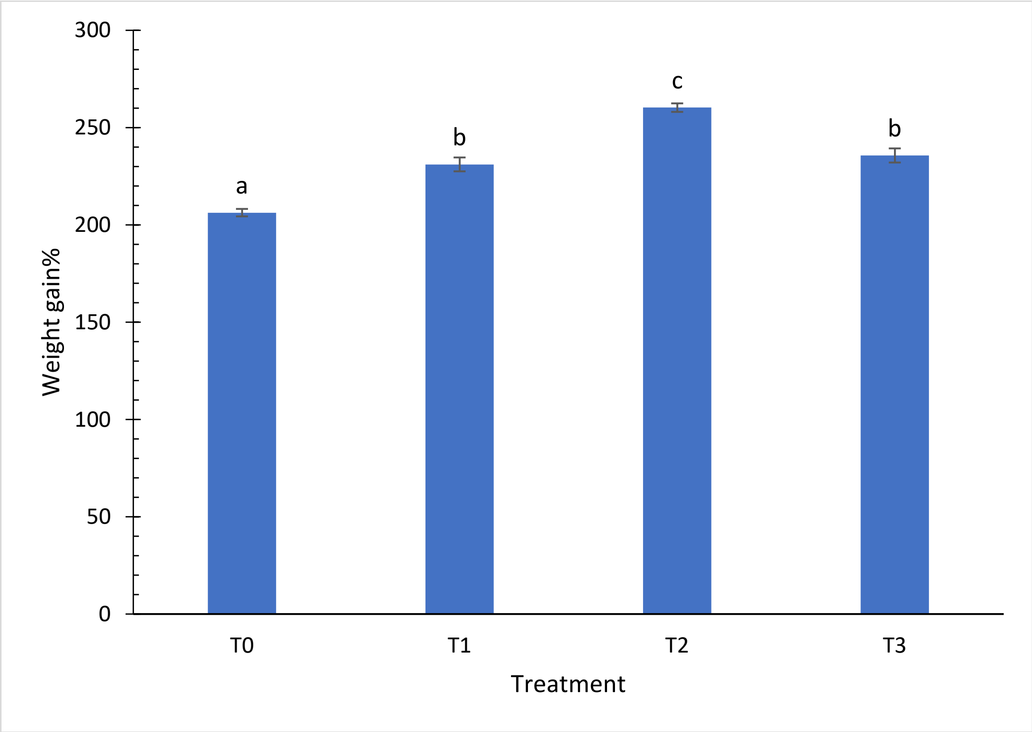

Figure 2: Body Weight gain percentage of *Oncorhynchus mykiss* fingerlings fed with different experimental diets.

T_0= Control feed without Chicken waste Silage

T_1 = Feed supplemented with Chicken waste silage @ 10%

T_2 = Feed supplemented with Chicken waste silage @ 20%

T_3 = Feed supplemented with Chicken waste silage @ 30%

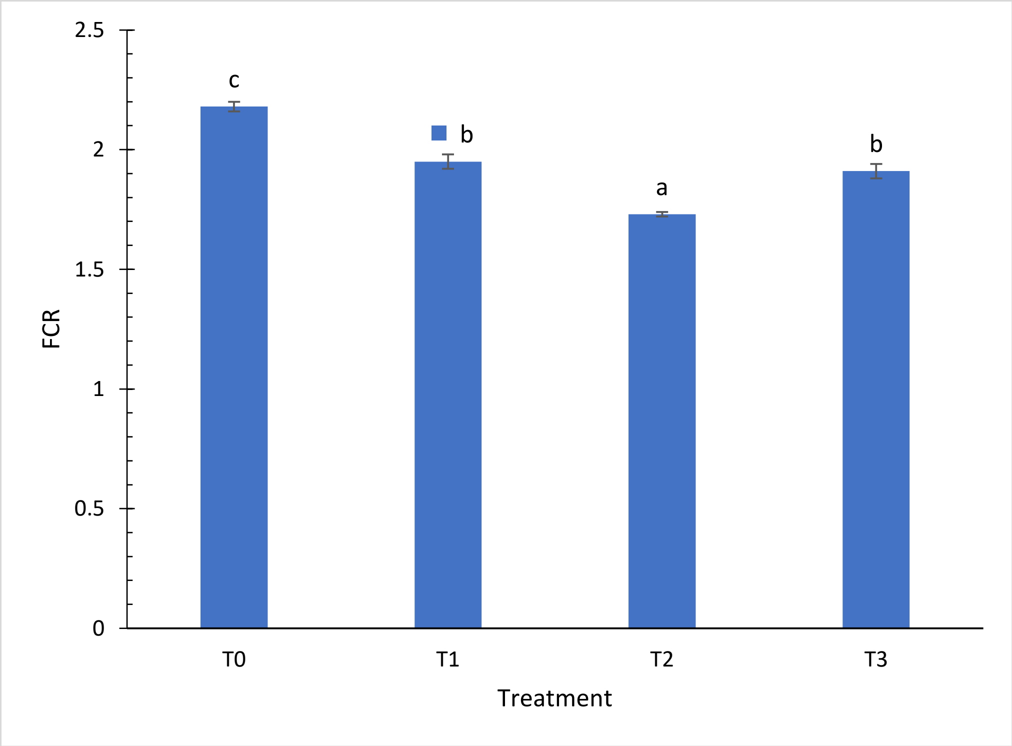

Figure 3: Feed conversion ratio of *Oncorhynchus mykiss* fingerlings fed With different experimental diets.

T_0= Control feed without Chicken waste Silage

T_1 = Feed supplemented with Chicken waste silage @ 10%

T_2 = Feed supplemented with Chicken waste silage @ 20%

T_3 = Feed supplemented with Chicken waste silage @ 30%

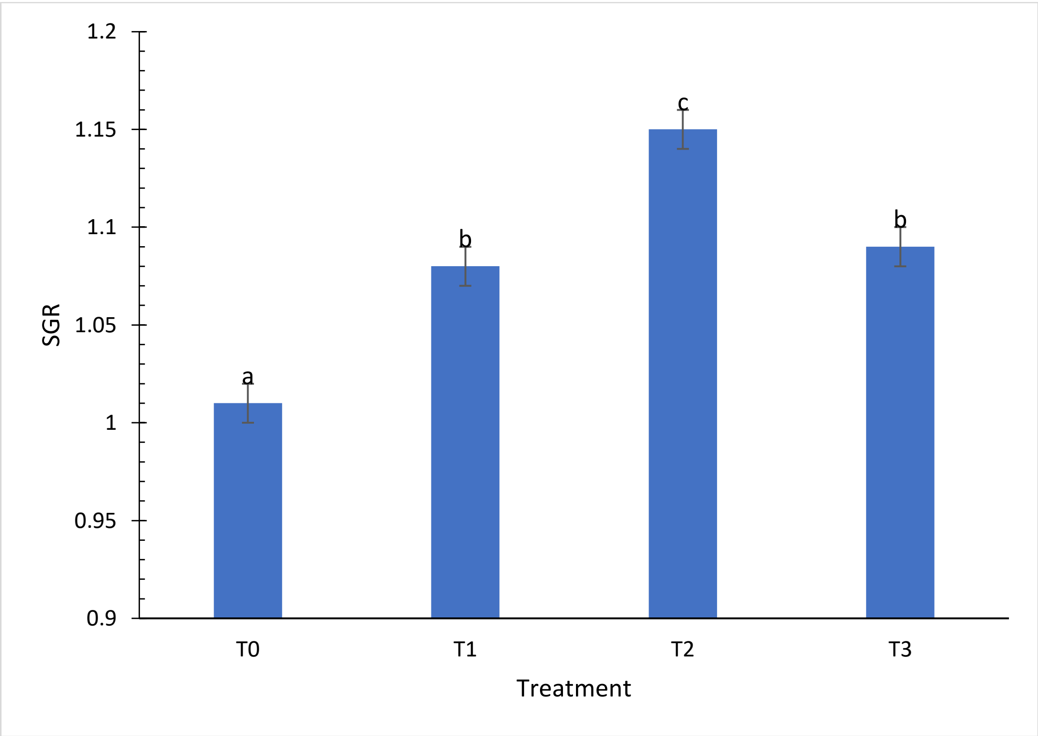

Figure 4: Specific Growth Rate of *Oncorhynchus mykiss* fingerlings fed with different experimental diets.

T_0= Control feed without Chicken waste Silage;

T_1 = Feed supplemented with Chicken waste silage @ 10%

T_2 = Feed supplemented with Chicken waste silage @ 20%

T_3 = Feed supplemented with Chicken waste silage @ 30%

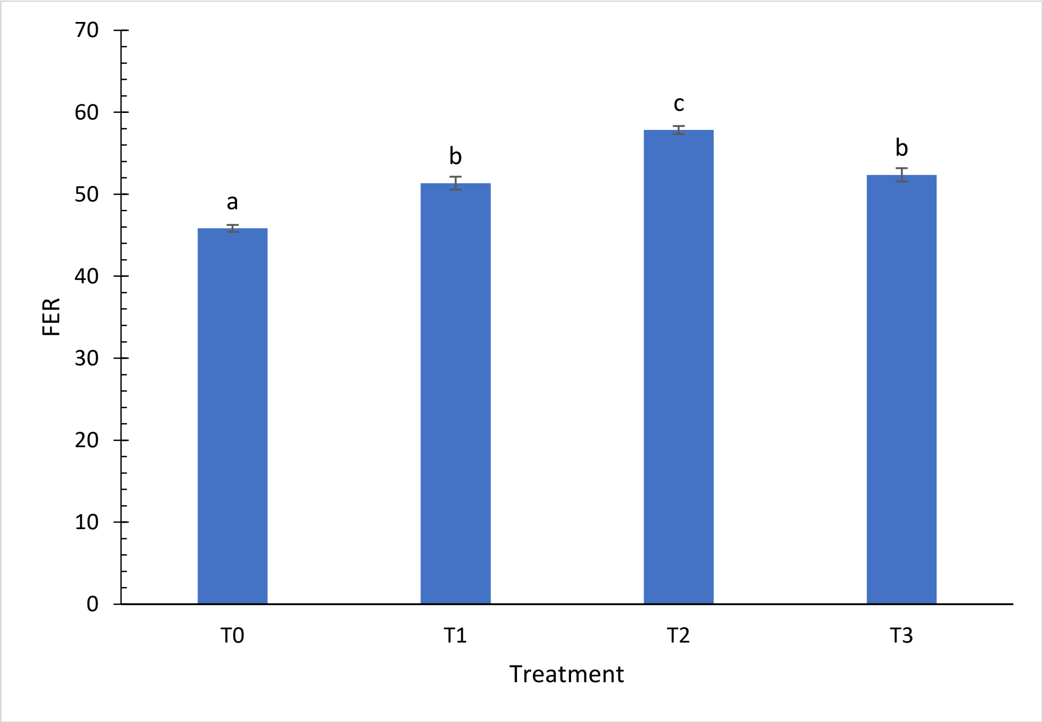

Figure 5: Feed Efficiency Ratio of *Oncorhynchus mykiss* fingerlings fed with different experimental diets.

T_0= Control feed without Chicken waste Silage;

T_1 = Feed supplemented with Chicken waste silage @ 10%

T_2 = Feed supplemented with Chicken waste silage @ 20%

T_3 = Feed supplemented with Chicken waste silage @ 30%

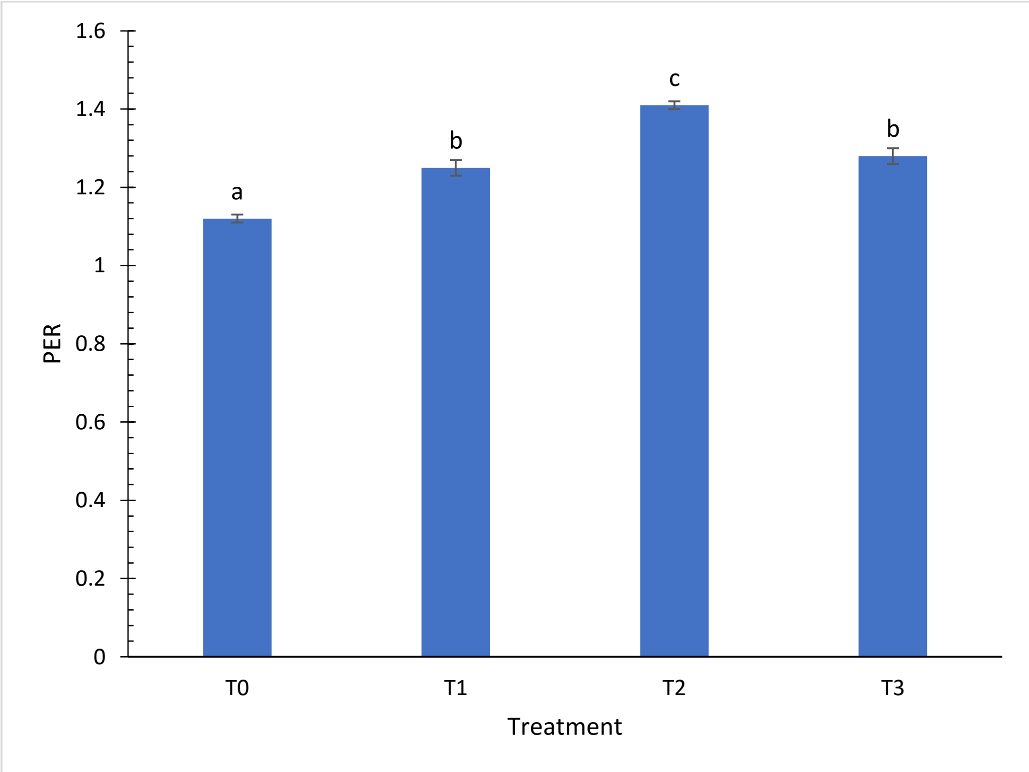

Figure 6: Protein Efficiency Ratio of *Oncorhynchus mykiss* fingerlings fed with different experimental diets.

T_0= Control feed without Chicken waste Silage;

T_1 = Feed supplemented with Chicken waste silage @ 10%

T_2 = Feed supplemented with Chicken waste silage @ 20%

T_3 = Feed supplemented with Chicken waste silage @ 30%

4.2 Water quality parameters

Ensuring the quality of water is crucial, as it plays a significant role in influencing the growth and survival of fish in an aquaculture system. Throughout the experimental period the physio-chemical factors such as water temperature (^{0}C), pH and dissolved oxygen (DO) was recorded on weekly basis and the average values for all treatment groups are presented in Table 4.

4.2.1 Water temperature (^{o}C)

The physiological and metabolic functions of fish and feeding are impacted by water temperature. Temperature plays an crucial role in the rate of chemical transformations in water including concentration and pressure of dissolved gases. Throughout the 90-days experimental period, the water temperature varied between 11.1 and 14.6°C among the different experimental units.

4.2.2 pH

The pH level of natural waters is a crucial environmental factor that shows significant impact on the health, body growth and feed utilization of fish. Fish culture generally benefits from slightly alkaline waters. Acidic waters reduce fish appetite, hamper their growth and increase susceptibility to various diseases. Throughout the experimental period, the pH values were within the range of 7.0-8.4 as depicted in Table 4.

4.2.3 Dissolved oxygen (DO)

Continuous monitoring of dissolved oxygen is crucial in aquacultural practices as fishes rely only on water for their metabolic oxygen demand. The amount of oxygen dissolved in water is influenced by factors such as temperature, partial pressure of atmospheric oxygen and the concentration of dissolved salts. Throughout the 90-days experimental period, the dissolved oxygen concentration in all experimental tanks remained within the range of 7.5 to 8.6 (mg/l).

Table 4: Physio-chemical parameters of water throughout the experimental period for different experimental groups.

Treatments	Temperature (°C)	pH (mg/l)	DO (mg/l)
	Min – Max (mean)	Min –Max (mean)	Min –Max (mean)
T_0	11.1-14.4	7.5-8.1	7.5-8.6
T_1	11.5-14.6	7.6-8.2	7.6-8.5
T_2	11.2-14.5	7.7-8.4	7.6-8.6
T_3	11.3-14.2	7.4-8.0	7.3-8.4

Chapter– 5

DISCUSSION

The present study was conducted to partially replace fish meal with chicken waste silage in order to bring down the cost of fish feed in aquaculture by incorporating animal-based waste products. In order to achieve this, the main costly ingredient present in fish feed i.e., fish meal was partially replaced by chicken waste (intestines). Chicken waste silage was prepared by the method described by (Toppe *et al*., 2018) with slight modifications i.e. the silage was dried in a hot air oven at 30°C for 2-3 days to lower its moisture content. Fish meal was replaced by chicken waste silage with inclusion levels of T_1 (10%), T_2 (20%), T_3 (30%) and T_0 (0%) in the control diet. Various workers have employed different methods for the preparation. Chicken silage can be manufactured from different raw materials as documented by various authors (S Mbokane *et al.,* 2022, Rachmawati *et al.,* 2019). Several studies have shown that acid-fermented chicken silage could serve as an alternative and cheaper protein source and can be used to replace fish meal with no negative implications on the overall health and nutritional status of fish (Kanugo *et al*., 2022, Eissa *et al.*, 2022).

5.1 Growth parameters

The science of feeding the fish to ensure its maintenance, health and development is known as nutrition. In all forms of animal husbandry, providing a supply of nutrients to match the requirements of cultured animal is fundamental in achieving optimal growth and efficiency and hence maximizing economic return (Anderson and De silva, 2003).

Fishery products, either in the form of low-value trash fish or rendered as fish meal are presently the major sources of protein in the grow-out culture of most fish species and constitutes up to 70% by weight of their diet (Tacon, 1995). As the demand for fish meal and fishery products for aquaculture increases while their availability decreases, the cost is expected to rise. A dependable supply of low-cost feed ingredients as an alternative source of protein must be provided to fish for profitable fish farming.

In the present study the growth performance of rainbow trout was determined by Weight Gain, Weight Gain Percentage (%), Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), Feed Efficiency Ratio (FER) and Protein Efficiency Ratio (PER).

5.1.1 Weight gain (g)

The treatment group T_2 (20%) exhibited the highest weight gain (576.25±5.03g). Conversely, the control group T_0 (0%) demonstrated the lowest weight gain (456.2±5.17g), and this disparity was found to be statistically significant ($P< 0.05$), as indicated in Table 2. This finding aligns with similar results reported by Sandra *et al.*, (2022) in her study on the replacement of fishmeal with poultry-based protein sources in feeds for pikeperch. In her research @ 20% inclusion levels of poultry-based protein demonstrated superior growth compared to the control group. Rachmawati *et al.* (2018) reported substitution of fish Meal with 25% chicken feather silage meal is the best dose for protein digestibility and growth of *Pangasius hypothalmus*. The present study is further supported by several authors who worked on trout fish (Gomes *et al*., 1995; Brown *et al.,* 2010; Kizak *et al*., 2011).

5.1.2 Feed Conversion Ratio (FCR)

FCR is a calculation of how efficient fish are able to convert diet into muscle. The FCR is an indicator of food conversion efficiency. Moreover, the less FCR the more efficient diet is (Oishi *et al.,* 2010). The current study showed better values of feed conversion ratio (FCR) in all treatments having fish meal replaced with chicken waste silage, when compared to control diet (T_0). Treatment T_2 showed the most improved FCR (1.73±0.01). The present experimental study showed similar results and backed up by the results and findings of (Farhangi and Carter, 2001; Yoshitomi *et al.*, 2006; Shafaeipour *et al.*, 2008; Gullu *et al.*, 2014) where partial or total fish meal was substituted with different inclusion levels of poultry materials in rainbow trout diets. Similar results were found by Diana Rachmawati and Istiyanto Samidjan, (2018) who reported substitution of fish Meal with 25% chicken feather silage meal (Treatment B) was the lowest FCR among other treatments of *Pangasius hypopthalmus*. Furthermore, similar results have been reported by (Hernandez *et al.*, 2014, Pares-Sierra *et al.*, 2014, Ma, Wang, Han & Lin, 2014).

5.1.3 Specific Growth Rate (SGR)

The specific growth rate of rainbow trout in the present study was found to be significantly increasing between control T_0 and the experimental diets T_1, T_2 & T_3. Highest SGR (1.15±0.01) was observed in T_2 which was significantly higher than control T_0, T_1 and T_3 diets. Similar results were reported by Tabinda *et al.* (2012). When using PBM meal (chicken intestine) in place of fish meal as a protein source in carp (*grass carp*) fry diet. Diana Rachmawati and Istiyanto Samidjan (2015) examined the effects of feeding chicken feather silage instead of fish meal on the growth of saline tilapia fingerlings (*Oreochromis niloticus*).

5.1.4 Feed Efficiency Ratio (FER)

FER is a crucial factor that measures an animal's ability to convert food into body mass effectively. A higher FER suggests improved utilization of feed and greater efficiency in promoting growth. The highest FER value was observed in treatment T_2 (57.83±0.49). According to the current study, the following studies viz., Feed utilization of olive flounder (Cho *et al*., 2006; Kim *et al*., 2014). (Hu *et al*., 2013) was affected by dietary supplementation of fish meal with different animal protein sources and Baboli *et al*. (2013) in his study reported that incorporated dietary poultry-based meal could positively improve the feed utilization for juvenile rainbow trout, findings of this study align with present study.

5.1.5 Protein Efficiency Ratio (PER)

PER is used to determine the quality of proteins in diet (Tacon, 1987). The value of protein digestibility was one of the markers of protein quality. Protein that was easily digested indicated better absorption of amino acids by the fish. The results of the experimental study showed that treatment T_2 @ 20% of chicken waste silage had the highest PER value (1.41±0.01) and the treatment T_0 (1.12±0.01) had the lowest PER value. The results of this study period are similar to those performed by Arunlertaree and Rakyuttithamkul (2006), indicating that the highest protein digestibility of catfish hybrid fish (*Clarias macrocephalus x Clarias gariepinus*) was fed 25% silage diet meal. Arunlertaree and Moolthongnoi (2008) stated that 25% chicken silage meal on diet showed the highest protein digestibility of *Oreochromis niloticus*. These results are further validated by studies of (Pfeffer *et al.,* 1994; Somsueb and Boonyaratpalin, 2001; Nwanna, 2004 and Chor *et al*., 2013).

5.1.6 Survival Rate (%)

At the end of the trial period, no appreciable impact on the survival rate of fingerlings was reported. The finding suggests that the chicken waste silage utilized in the diets underwent proper sterilization and acid fermentation processes, thereby mitigating any adverse effects on the fish immune system and preserving essential compounds crucial for optimal survival. According to the study conducted by Widodo *et al.* (2010), a survival rate exceeding 90% is considered within the favorable range for fish farming. The outcomes of our study align with the findings of Min *et al.* (2020) who reported no difference in survival rate between control and all other treatments in olive flounder (*Paralichthy solivaceus*) who was fed with chicken by-product meal as place of fish meal. According to the study, the outcome of Parchekolaei *et al.* (2021), who reported no effect of replacing fish meal with probiotic supplemented soybean diets on fish survival.

Thus, the results of the present study concur with those of the afore-mentioned researchers. Based on nutrient utilization indices, estimated economic benefits and overall acceptance, chicken waste silage can effectively replace fishmeal in rainbow trout diets till the level of T_2 @ 20% without affecting growth for a profitable and sustainable aquaculture venture.

5.2 Physio- Chemical Parameters of Water

The physio-chemical analysis is the prime consideration to evaluate the quality of water for its utilization purposes like drinking, irrigation, and domestic use, besides this, it helps to understand the complex interaction between the climatic and biological processes in the water (Kulkarni and Tapase, 2011).

Rainbow trout *(Oncorhynchus mykiss)* requires cold and crystal-clear running water with low temperature of the water, high dissolved oxygen, moderate free carbon dioxide, proper water velocity and balanced water discharge (Huet, 1975). All the physio-chemical parameters of water such as temperature, pH and dissolved oxygen were observed to be within the optimum range of requirements for trout as suggested by (Huet, 1975). In the current study, water quality was maintained at congenial level for the raising of rainbow trout fingerlings. The physio-chemical parameters were monitored on weekly basis, during the experimental period. No negative impact of chicken silage on water quality was recorded during this experimental trial.

5.2.1 Water Temperature

Water temperature is an important factor indicating the quality of water. It influences aquatic life and the concentration of dissolved gases like oxygen and carbon dioxide (Dar and Singh, 2020). Generally, as temperature increases, so does the metabolic activity of fish, leading to higher energy demands and growth rates. Throughout the experimental period, the water temperature ranged from 11.1 to 14.6°C. Similar results were reported by (Boyd and Tucker 1998), for growth of rainbow trout in temperatures between 10 and 16°C with the optimal conditions for promoting the highest growth rates.

5.2.2 pH

The pH value is a very important factor in the study of water chemistry (APHA, 1995). pH explains significant biotic and abiotic ecological characteristics of aquatic systems. Throughout the study period, the pH levels measured in all treatment groups was uniform and ranged from 7.5and 8.4. (Ye and Randal 1991), studied the potential effects of low pH on rainbow trout. The study suggests that low pH levels can alter the swimming behavior of rainbow trout. Such behavioral changes may serve as an early indicator of increasing ammonia toxicity, which can be harmful to fish health. According to (Huet 1975), the ideal range for salmonid culture is between 6.5-8.5. Consequently, the data collected during the experiment aligns with the recommended pH range for salmonid culture.

5.2.3 Dissolved oxygen

Dissolved oxygen is of paramount importance in all aquatic environments as it regulates most of the organism's metabolic processes and the community architecture as a whole (Hussain and Pandit, 2008).

The fluctuation in dissolved oxygen (D.O.) levels ranged from 7.3 to 8.6 mg/l across all treatments during the study. It is noteworthy that maintaining concentrations of dissolved oxygen above 3 mg/l is vital to prevent mortality in rainbow trout as indicated by (Summerfelt 2000). According to (Widmeyer 1996), the recommended dissolved oxygen concentration for rainbow trout culture varies between 5-9 mg/l. As a result, the dissolved oxygen levels in the current study fall within the optimal range promoting improved growth and survival of fish.

All of the experimental treatment results for the water quality metrics fell within the ranges recommended by various scientists, researchers and authors, proving that chicken waste silage inclusion in the trout diet had no negative impacts on the water quality.

Chapter- 6

SUMMARY AND CONCLUSION

The current study entitled **"Effect of Chicken Waste Silage in the diet of Rainbow Trout *(Oncorhynchus mykiss)* fingerlings to ascertain growth and survival"** was conducted for a period of 90 days at hatchery unit, Division of Aquaculture, Faculty of Fisheries, Rangil, Ganderbal of Sher-e-Kashmir University of Agricultural sciences and Technology of Kashmir.

The study addresses the need of affordable and high-quality alternative feed ingredients in aquaculture. This involves replacing of costly proteinous for fish meal, which raises concerns due to its availability, sustainability and continuous increasing prices. The aim of the research was to incorporate considerations of economic aspects, fish growth, consumers health and environmental concerns.

The study was conducted to assess the effect of replacement of fish meal with chicken waste silage at various concentration levels. The fingerlings were provided diets twice a day @ 5% body weight of the fish. During the experiment, chicken waste silage replaced fish meal where four treatments viz., T_0 (0%), T_1 (10%), T_2 (20%) and T_3 (30%) each having four replicates were taken. The growth of fish was assessed by growth parameters viz., weight gain, percentage weight gain, specific growth rate (SGR), feed conversion ratio (FCR), feed efficiency ratio (FER) and protein efficiency ratio (PER) fortnightly observed and recorded. Water quality parameters viz., water temperature, pH and carbon dioxide were recorded on weekly bases.

At the end of study period, significant difference ($p<0.05$) in mean weight gain, percentage weight gain, specific growth rate (SGR), and feed conversion ratio (FCR) was identified between the T_2 (20%) and T_0 (0%) groups. The incorporation of chicken waste silage @20% in the diets significantly enhanced the growth performance of *Oncorhynchus mykiss* fingerlings in relation of mean weight gain (797.68±5.19), specific growth rate (SGR) (1.15±0.01), feed efficiency ratio (FER) (57.83±0.49) and protein efficiency ratio (PER) (1.41±0.01) in contrast to the control group. The T_2 group also exhibited the lowest FCR (1.73±0.01). It was noted that beyond the @ 20% inclusion level of chicken waste silage, there was a declining trend in mean weight gain, FER, PER and SGR while FCR exhibited a gradual increase across treatment groups. Overall, there was no significant differences observed in the body composition of fingerlings of rainbow trout fed with different experimental diets as compared to the control diet.

Conclusion:

The current work showed that inclusion levels of chicken waste silage in the diet of rainbow trout (*Oncorhynchus mykiss*) fingerlings enhanced their growth and survival in comparison to the control diet.

The trend of growth of fish was observed as $T_2 > T_3 > T_1 > T_0$.

Moreover, the incorporation of @ 20% chicken waste silage proves to be cost-effective i.e., economically viable and easily available compared to fish meal. The digestibility of chicken waste silage is also good as per the nature of the rainbow trout diet. Here the findings suggest a potential reduction in fish feed costs by utilizing more affordable ingredients like chicken waste silage, thereby lowering overall production costs. To long-term benefits, it is recommended to conduct further research and monitoring to optimize the incorporation level of silage. The feeding of rainbow trout fingerlings with chicken waste silage holds promise as an alternative and sustainable feed source. Scaling up this practice could convert chicken waste into valuable fish flesh i.e. conversion of waste into wealth which protect our earth from pollution and may promote the growth of aquaculture and aquafeed industry.

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CERTIFICATE

Certified that all Jthe corrections/amendments as suggested by External Examiner **Dr. Romaisa Gul** H.O.D, Industrial Fish and Fishiries, Govt. Degree College, Baramulla, during viva voce examination held on 07-03-2024 have been incorporated in the manuscript entitled **"Effect of Chicken Waste Silage in the diet of Rainbow Trout *(Oncorhynchus mykiss)* fingerlings to ascertain growth and survival"** submitted by **Mr. Ubaid Shakeel Wani (Reg. No. MSFY-2021-152).**

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