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Effect of Methionine on Growth Performance and Microflora in Common Carp

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Abstract

This study was conducted to assess the effect of methionine and water quality in terms of growth performance, intestinal microflora in common carp (Cyprinus carpio). A total of 30 fish with an average initial weight of (65 ± 0.1 g) will feed on experimental diets containing 3g or 0.3% methionine for 6 weeks. The specific growth rate (SGR) and body length growth rate (BLGR) values and intestinal microflora in 2 experimental groups will compare to those in the control group in the trial period. With the supplement of methionine, Amino acid profiles and microflora will be examined. Further examination will reveal how considerably methionine supplement enhances the growth of Cyprinus carpio in saline and normal water. Methionine supplementation improved intestine weight, length, folds, height. Intestinal Aeromonas, Escherichia coli, Lacto bacillus, and Bacillus were changed by dietary Methionine supplementations. The changes in these physiological factors will allow for the content of supplemental methionine in feed to improve growth performance and intestinal microflora of Cyprinus Carpio. The samples will be collected from freshwater earthen ponds from the University of Agriculture Faisalabad. The experiments will be conducted in the Microbiology and Immunology Lab of the Department of Zoology, Wildlife and Quantitative results obtained from treatments will be compared with each other by the help of treatments by using one-sided analyses of variance (ANOVA)

Keywords: *Methionine, Microflora, SGR, BLGR, Cyprinus carpio*

Introduction

The study was to assess the effect of Methionine on Intestinal microflora and the growth performance of common carp.

Li et al. (2009) studied methionine supplementation improved the proximate chemical analysis of the whole-fish body, including total lipids and crude protein, but ash content decreased considerably. High dosages of methionine appear to promote protein synthesis and deposition in the fish body, according to these findings. A higher level of intestinal bacteria activity and its beneficial influence on digestion and absorption of protein material in the gut could explain the increased body protein content (Ye et al., 2011).

Kaminogawa., (2010) reported that mutualism is a concept used to explain the interaction between the intestine's local immunity and its bacteria. Beneficial bacteria (such as Lactobacillus, Bacillus, and Bifidobacterium) eat and ferment non digestible fibers and polysaccharides, resulting in a group of

fermented short- and medium-chain fatty acids that can lower gut pH and so suppress dangerous bacteria (Dawood and Koshio, 2019).

Alam et al. (2010) reported on a several study on the effects of methionine on adult and juvenile fish nutrition and digestion. In fish, methionine promotes digestion and absorption, increases protein utilisation, and regulates sulphur metabolism.

Tacon et al. (2011) Aquaculture is one of the world's fastest-growing food-producing businesses, according to reports. Aquaculture's growth has associated with a rise in the demand for protein sources for aqua-feed production. Fish meal (FM), which is largely acquired from wild fish catches, is the most important component of fish diets. As natural fisheries capture has stabilised, FM supply has remained stable, but demand has increased, leading in greater pricing (Tacon and Metian 2009).

NRC, (2011) methionine is an important amino acid that influences the growth, reproduction, and physiological activities of animals, including tilapia; methionine deficiency is linked to poor growth and survival.

Salze and Davis (2015) studied that some fishes can biosynthesize taurine from methionine and cysteine. The availability of taurine precursors such as methionine or cysteine in diets may play a successful role in taurine production in some (Wang et al, 2016) but not all fish species. The meals of the participants in this study were deficient in methionine and cysteine when compared to the requirements of common carp.

OlivaTeles et al. (2015) reported that a number of initiatives have been launched to replace FM in aquaculture feeds with less expensive and more readily available plant protein sources. Anti-nutritional components in these plant protein meals, on the other hand, impact feed intake, digestion, and/or nutrient absorption, and they have a lower protein concentration than FM. Depending on the plant source and different species to whom they are provided, plant-derived proteins may cause a deficiency in one or more necessary amino acids; they are also taurine-free.

Bostick and Zhou (2016) reported that the intestinal micro biota can affect immunity within the body by delivering signals through immune cells in the intestine to change immunological tissue maturation, antibody generation, T cell differentiation, and macrophage phagocytic response activation. As a result, the gut microbiota successfully regulates the physiological systems that control innate and adaptive immunity in the fish, avoiding outbreaks in the body (Nayak, 2010).

Aragona et al, (2017) studied that fish in the control group survived. It's possible that saline qualities helped mice withstand oxidative stress, both external and endogenous, by acting as a defensive mechanism in the gut. Lauriano et al., (2016)

Wangetal et al., (2017) studied that the microbiota of vertebrates' gastrointestinal tracts. This microbiome is essential for development, immunity, nutrition, and disease resistance. In recent decades,

study on mammalian G microbiota has intensified. Fish microbiome is a poorly understood topic. This fish microbiota study provides information on the sources, composition, and variables that influence GI bacteria in fish. By understanding more about the bacteria that live in the fish's stomachs, we can improve their health.

Roohi et al. (2017) reported that the goal of the Response To stress in Fish Evaluation is to see how feed supplements affect fish health in stressful situations.Various studies have employed a salinity stress challenge to measure fry quality Salze et al., (2008). Methionine supplementation had no influence on the survival of common carp stressed by thirteen ppt salinity.

Haygood and Jha (2018) reported that the activity of intestinal bacteria, as well as the GIT's ability to digest and absorb nutrients, can be affected by optimising nutritional requirements for aquatic animals. The amount of amino acids, fatty acids, vitamins, and minerals in aquaculture feed affects the activity of these microorganisms. The activity of these bacteria regulates metabolism within gut cells, which improves local physiology and immunity in the intestine and modulates the immune response of the entire organism, Egerton et al., (2018).

Asaduzzaman et al., (2018) studied that the transfer of digested nutrients via intestinal epithelial cells and the action of helpful bacteria in the intestine both require fatty acids produced by bacterial fermentation. Slow digestion, colon inflammation, and bacterial overgrowth are all signs of an imbalance in the activities of good and bad microorganisms in the stomach.

He et al., (2019) methionine (Met) is the principal limiting amino acid for aquatic animals as an essential amino acid (EAA), yet Met was lacking in plant-based meals. Excessive or insufficient Met levels in the diet have been shown in previous research to impair feed intake and growth performance in aquatic animals Fang et al., (2020). Low amounts of dietary Met can limit white muscle fibre hypertrophy, while high levels of Met can stimulate white myofiber hyperplasia and muscular growth, Alami-Durante et al. (2018)

Dawood et al., (2019) reported that the fish's ability to survive sickness and environmental stressors is associated to its intestinal biodiversity. The diet of the fish can affect the microbiotic content and variety of the fish. Beneficial microorganisms can break down food into amino acids, fatty acids, phenols, digestible carbohydrates, minerals, and vitamins, which epithelial cells can easily absorb.

Fang et al. (2020) studied that an optimum dose of Met boosted protein, lipid, and free amino acid content in grass carp muscle, improved fatty acid composition in muscle, and regulated muscle nutritional deposition and myofiber growth signal molecules connected to type I collagen formation. Met plays a critical function in the nutritional support of livestock, birds, fish, human, particularly young animals.

Methionine can improve intestinal development and enzyme function, influence intestinal microflora balance by stimulating the growth of beneficial bacteria while suppressing the growth of detrimental bacteria, and promote immunity by raising humoral immune factor content and activity. The amount of methionine in the diet is advised for maximum Carpio growth.

Objectives

1. To determine the microbiological contents in Common Carp by using Methionine.

2. To examine the impact of essential Amino Acid on the Growth Performance of cyprinus carpio.

Materials and Methods

Location

To manage the current research work, the Department of Zoology Wild Life and Fisheries was selected in the University of Agriculture, Faisalabad.

Investigational Species

Common carp (cyprinus carpio)

Sampling for Microbiological Work

The samples was taken at the freshwater earthen ponds of the University of Agriculture Faisalabad. The samples was moved to two aquariums, one with fresh water and the other with saline water, for further analysis. The study will take place in the Department of Zoology, Wildlife, and Fisheries' Microbiology and Immunology Lab, and will look into the effects of methionine on the growth performance and microbiota of Cyprinus carpio cultured in freshwater fish aquariums.

Apparatus/Chemicals

In microbiological research, the apparatus and chemicals indicated below was employed. Petri plates, cotton plugs, flasks, measuring cylinders, beakers Micropipette, Micropipette tips (100l, 1000l), Inoculating needles, Glass spreader, Eppendorf tubes, Slides, Coverslips, Compound microscope, Oven, flame, matchbox, distilled water, laminar air flame Media, and Ethanol

Cleaning and Disinfection

Before being used for microbiological investigations, all glassware, instruments, sampling bags, and working spaces shall be thoroughly cleaned and disinfected. Washing was done with groundwater, followed by rinsing with distilled water, and disinfection was done with ethanol on work surfaces, hands, and glassware.

Sterilization

The procedure of eradicating all forms of microbial life to generate sterile objects and a more conducive atmosphere for microbiological research

Many sterilization processes was employed for microbiological work.

• Dry sterilization

• Moist sterilization

For dry cleaning, culture medium and glassware was sanitized in an autoclave at 200°C for 15 minutes at 1210°C, while culture media and glassware was sanitized in an autoclave at 200°C for 15 minutes at 1210°C for wet disinfection. The needles used for inoculation was sterilized by being burned until they are red hot. About 30 samples from the Department of Zoology, Wildlife's earthen freshwater fish ponds was taken, and 15 samples from a nearby earthen pond was taken from the control group. Fish species was chosen for the aim of the study. Additional fishpond samples was collected, mostly in clear and disinfected polythene bags. These samples was delivered to the Aquarium right away for further investigation. Fish samples was collected with extreme caution using visual observation and random selection in sanitised polythene bags.

Growth and feed utilization parameters

At the conclusion of the experiment, fish were gathered from each aquarium, numbered, and weighed in groups.

The following factors for growth performance and feed utilisation were calculated:

/ Starting weight (g) = 100 [Final weight (g) / initial weight (g)]

SGR (% of weight gain per day) = 100 (Ln W2 Ln W1) / T, where W1 and W2 are the initial and end weights, respectively, and T is the trial period.

The overall amount of food consumed during the trial is equal to the total amount of diets offered. The following formula is used to compute the feed conversion ratio (FCR): Weight gain (kg) / feed intake (g).

Salinity stress tolerance

Aquaria were filled with tap water and salinized with sodium chloride at a concentration of 13 parts per million for this experiment. Fish from each treatment were collected and put to the challenge aquaria in duplicates at a rate of 15 fish per 17-L aquarium following the feeding trial. Each tank received compressed air from an aquarium air pump via an airstone. Each study meal was served to the fish for 60 days until they were satisfied at 8:00 and 15:00 h. Every day, half of the aquarium's water and settled fish excrement were evacuated and replaced with clean, aerated, and salinized water. The number of dead fish was counted and removed every day.

Analysis of water quality parameters

Weekly water samples were taken from each tank, and water quality measurements were recorded. An oxygen meter was used to assess the temperature and dissolved oxygen of each tank on the spot (970 portable DO meter, Jenway, London, UK). A Multi-Parameter Ion Analyzer was used to measure the unionized ammonia (HANNA Instruments, Woonsocket, Rhodes Island, USA). A pH meter was used to determine the pH (Digital Mini-pH Meter, model 55, Fisher Scientific, Denver, CO, USA.) The temperature was 28.3–29.7 °C, the dissolved oxygen concentration was 5.4–5.7 mg/L, the unionized ammonia concentration was 0.14–0.27 mg/L, and the pH was 7.6–7.8 in all treatments. All of the above requirements are within the range of allowable fish growth (Boyd and Tucker 2012).

Intestinal microbiota analysis

The levels of total viable heterotrophic aerobic bacteria (TAC) and lactic acid bacteria (LAB) was assessed at the beginning (n=10) and end (n=5 fish each tank) of the experiment. A culture-based analysis of the intestinal microflora was carried out using the method described earlier. To quantify total viable heterotrophic aerobic bacteria and lactic acid bacteria, 128 100 l of homogenised intestines (3 samples was pooled) was disseminated in triplicate over plate 129 count agar (PCA) (Merck, Germany) and deMan, Rogosa, and Sharpe (MRS) agar media (Merck, 130 Germany). Colony-forming units (CFU) g1 132 were determined from statistically viable plates after 5 days of incubation at room temperature (25oC) (i.e. plates containing colonies).

Preparation of Microbiological Media

Following the creation of solution, distilled water was utilized to make a microbiological culture medium, which was autoclaved for 15 minutes at roughly 120 degrees Celsius. All of the apparatus was properly cleaned and dried to avoid contamination and chemical reactions. The chemicals used in the research was of high quality and consistent. Bacterial contamination was tested using TSA, Eosin Methylene Blue

(EBM), Nutrient Agar (NA), RCV-glucose, and RCV-sucrose as culture media. These was used to calculate the total viable bacterial count.

CULTURING OF BACTERIA

Sample Preparation

To keep the samples taken from various parts of the fish's intestine separate, distinct Petri plates was used; each petri dish will contain roughly 20ml culture medium for microbe development. After being removed from the autoclave, the culture media solution was kept for a while to create a firm gel-like consistency. The preserved fish sample was dissected to remove its guts using sterilized dissection apparatus to avoid infection. The excised gut was scratched with a scratching apparatus, and the sloughed-off material was soaked in a 9% salt solution and shaken until all microbial components have been transferred to the liquid combination. Further thinning will separate the microbiological components for growth on the prepared media.

Heating will disinfect Eppendorf tubes with a capacity of 2 mL before filling with a 9 percent salt solution previously measured to 900 microliters. A small amount of gut fragment-containing solution (about 101 l) was transferred to the 900-l containing Eppendorf. The initial dilution was 100 times thinner than the original solution. To make the second dilution, 100 ml of the first dilution was blended with a 900L liquid combination in the Eppendorf. This is the second step of thinning, and it was 100 times more dilute than the first. A micropipette was used to acquire 15l of the first and second dilutions.

Using a micropipette, 15l of the first and second dilutions was transferred to the prepared culture media to establish colonies of gut microbial contents. Maintaining the culture media with shifting samples in the incubator for one to two days will provide the optimum temperature for microbiological content growth. After around 25 hours, the first colony was counted, and the second was counted after about 55 hours.

MICROBIAL COUNT

Total viable count

Melted nutrition media was placed uniformly on the floor of each petri dish containing distinct culture media for appropriate colony counting, and the liquid culture media was allowed to cool to achieve a solid gel-like appearance. A sterile rod was used to distribute liquid culture media evenly on the floor of the Petri dish. Three Petri dishes of each culture media was created independently for good colony counting results, and the average of the three was deemed an accurate colony count. To prevent condensation from mixing with the prepared media, each petri dish was kept inverted, reducing the risk

of moisture dropping on the culture media if condensation occurs. This method was performed for the various media that were employed during the research. The thinning feature will enhance the mean population in Petri dishes of a sufficient thinning, and the total viable count per ml of the fish sample was increased.

Dilution factor x Average number of colonies = Total Viable Count.

Determination of Physico-Chemical Parameters

HANNA HI-9146 electronic metres will measure dissolved oxygen and water temperature, while HANNA HI-8424 will calculate pH. An EC Meter is used to determine electrical conductivity. The total basicity and total amino acid content of water, i.e. Ca, Mg, Cl, Phosphates, Nitrogen, Carbonates, and Bicarbonates, was investigated using the A.P.H.A. method (2005).

Calculation and statistics

Weight gain (WG), specific growth rate (SGR), Fi, and feed conversion ratio (FCR) was calculated using data on initial weight, final weight, and feed intake (Fi). WG (g fish-1) = final weight (g fish-1) initial weight (g fish-1); SGR = ((ln mean final weight) - (ln mean initial weight)/no. of days) x 100; Fi (g fish-1) = (gross feed quantity - surplus feed quantity)/

All information was subjected to statistical analysis.

Conclusion

For common carp, dietary methionine levels had a substantial impact on plasma TIBC. With methionine supplementation, plasma TIBC steadily rose, peaking at 10 g kg)1 when methionine levels were at their greatest. There have been few studies on the effect of methionine on plasma TIBC to date. Iron is an essential nutrient for microorganism development; yet, siderophilin has a high affinity for iron, suggesting that it might hinder microorganism growth (Langston et al. 1998). Methionine was linked to microorganism growth inhibition, according to the findings.

In conclusion, methionine may enhance intestinal development and enzyme activity, alter intestinal microflora balance by stimulating the growth of helpful bacteria while suppressing the growth of harmful bacteria, and boost immunity by increasing the content and activity of humoral immune factor. For maximal growth of common carp, the ideal dietary methionine requirement is 12 g kg)1 dry diet in the presence of 3 g kg)1 cystine (equivalent to 33 g kg)1 dietary protein on dry matter).

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