How to Cite:
Qasim, M. A. A., & Mohsen, T. S. (2022). Effect of adding different levels of gel and coating dried Aloe vera leaves to the diets on Cyprinus carpio performance. *International Journal of Health Sciences*, *6*(S3), 3642–3651. https://doi.org/10.53730/ijhs.v6nS3.6589

Effect of adding different levels of gel and coating dried Aloe vera leaves to the diets on Cyprinus carpio performance

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***Abstract*---** This experiment was conducted in the Fish Laborator/ Department of Animal Production/College of Agricultural Engineering/University of Baghdad for 3 months from 10/21/2020, with the aim of evaluating the use of dried Aloe vera leaf and gel powder in the diets of common carp Cyprinus carpio L. In the experiment, 18 glass aquariums with dimensions 40 x 30 x 30 cm (capacity 36 liters) were used, in which 90 fish with an initial weight of 2 ± 45 g/fish were distributed randomly over nine treatments with two replicates, for each treatment, nine similar rations were made of protein and energy content and different In percentages added to the plant gel powder and aloe vera leaves, which were 0.5, 1, 1.5, 2% for the gel, as well as for the leaves of the treatments T2, T3, T4, T5, T6, T7, T8 and T9 respectively, in addition to the T1 control treatment that was devoid of the powder of the plant gel and aloe vera leaves, the fish were fed on rations. Experimental by 4% of its weight. The biochemical characteristics (AST, ALP, and ALT), lysozyme and ACH50 were studied, and the chemical composition of fish and forage was analyzed. The results showed that treatments T8, T4, T6 and T2 were among the best experimental treatments, and they gave the highest values for most of the studied traits. The results of the statistical analysis showed a significant decrease (P≤0.01) for liver enzymes AST, ALP and ALT, as the treatment T4 and T2 recorded the least significant, which amounted to 222.75 and 223.23 IU/L, respectively, in the concentration of AST enzyme, while treatment T9 and T7 recorded the lowest concentration of ALT enzyme, which reached 29.95 and 36.82 IU/L, respectively, and treatment T2 and T1 recorded the least significant in ALP concentration, which reached 24.72 and

32.35. IU/L respectively. As for the activity of Lysozyme, the superiority of treatment T8 was noted, which amounted to 31.13, as well as the superiority of gel treatments for ACH50 in terms of immunity, where the treatment T8 recorded the best (280.67). We conclude from this study that there is an important role for aloe vera gel powder mixed with feed and to improve growth parameters and blood characteristics, which is reflected on the productive and hematopoietic characteristics of common carp fish, and improve their health status.

***Keywords*---**aloe vera, cyprinus carpio, biochemical characteristics, lysozyme, ACH50.

Introduction

Fish wealth in the world is receiving increasing attention from scientists and researchers in order to develop its exploitation and maintain its productivity as part of the requirements for providing animal protein as a result of the huge increase in population numbers (Mohsen, 1988), and fish is one of the sources of good protein at a low cost, as studies and statistics have shown that The percentage of protein in fish meat ranges between 80 and 90% of the dry weight and 18.5% of the wet weight, which is more than the percentage of protein in beef 16.18%, eggs 13.6% and milk 3.8%. Also, this protein has a high nutritional value because it contains essential amino acids as well as fats contain high amounts of vitamin A, D and a number of important minerals such as calcium, phosphorous, iodine and iron (Hadid, 1986; Arab Organization for Agricultural Development, 1996). The global annual consumption rate of fish meat per capita during the past four decades has increased from about 19.7 kg in 2013 to 20.0 kg in 2015 and this indicates a significant increase in fish consumption globally and aquatic production in the world depends on natural fisheries that lead to stock depletion Fish in seas and rivers (Pauly and Zeller, 2017; Esilaba et al., 2017). Since fish farming is the fastest growing sector in fish production for more than two decades due to its growth of 10% per year since 1984, it is a major source for future supplies of fish instead of commercial fisheries and it is a reliable solution in providing the population's need for fish meat (Garcia) and others, 2005).

Fish farming in ponds and rivers is not free from health problems represented in infecting fish with some parasitic and bacterial diseases, and the use of chemical pesticides to eliminate diseases cannot be relied upon because of their negative effects on the aquatic environment and the elimination of plant and animal plankton as well as the deposition of these substances In the body of the fish and thus its impact on human health and the occurrence of health problems, so the modern world began to find means of defense against parasites and diseases, as well as to have a positive effect on the weight gain of fish as well as being palatable by the fish itself, and among these natural materials, aloe vera, which is One of the most valuable plants in the family Liliaceae, native to the tropics. Aloe vera contains more than 75 nutrients, 200 active compounds, 20 minerals, 18 amino acids, 12 vitamins and compounds such as Aloin, famodin, Antrokinon and barbaloin (Mandrioli et al., 2011). This plant has a variety of pharmacological

effects for skin lesions and wound healing. Antiviral, antibacterial and other effects have been attributed to this plant. However the effects of immunostimulation and growth in warm-blooded animals have not been demonstrated. As well as a little information on the immunomodulatory effects, antitoxicities, and effects of growth performance by A. vera in different fish species. (Wang et al., 2011; Alishahi and Abdi, 2013; Haghighi et al., 2014). Therefore, this study was conducted to find out the effect of adding different levels of aloe vera gel and dried leaves to the diets of common carp (Cyprinus carpio L).

Materials and Methods

On 9/1/2020, the preparation for preparing and cleaning the glass basins began. In the experiment, 14 glass basins with dimensions of 20 x 15 x 15 cm and a capacity of 18 liters were used. The walls of the basins were cleaned with water and sterilized with coarse salt and left for a whole week to get rid of fungi and bacteria. Salt completely. The tanks were filled with 30 liters/basin of chlorine-free liquefaction water. The basins are prepared with water through four tanks with a capacity of 1000 liters for each tank outside the laboratory, made of polyethylene, to be pumped into stainless steel tanks, open from the top, inside the laboratory, with a capacity of 500 liters. The tanks were equipped with oxygen by means of Chinese-origin Super Pump type air compressors. The water temperature of the experimental ponds was maintained by using split air conditioners (split) type Kraft in the summer, the ponds were equipped with thermal heaters (200 watts) of Italian origin Reco type equipped with a thermostat when it drops in winter in order to keep the water temperature of the ponds between 22 and 28 degrees Celsius. The basins are cleaned daily by withdrawing waste and uneaten food, if any, from the bottom of the basins .

The feed materials were brought from the local market to the fish laboratory and ground by a laboratory mill of Chinese origin. The protein and energy of the experimental diets were calculated based on the chemical composition of the materials included in the composition of the forages, to calculate the proportions of the components of seven experimental diets close to the protein and energy content. Aloe vera leaf powder was added to four of them. At rates of 0.5%, 1%, 1.5%, and 2% for treatments T2, T4, T6 and T8 respectively, and the leaf gel powder was added to four treatments, including at rates of 0.5%, 1%, 1.5 and 2% for treatments T3, T5, T7 and T9, respectively, while the control treatment T1 was free of powder. Leaves and gel of the aloe vera plant. Mix the materials of each ration separately by hand until the mixture is homogeneous. The addition of water ranged between 350 and 400 ml of water per kilogram of the mixture of components of each ration, to form a cohesive and homogeneous dough, then it was placed in a meat mincer machine (Chinese origin with 4.5 mm holes) to form cohesive fingers for the ration by re-mincing twice to increase the consistency of the ration and the process of mincing is done Each ration individually after cleaning the chopping machine after chopping the previous ration. The sticks of the bush were dried in the open air for 48 hours, then they were placed in the shade at a temperature of 43°C, with continuous stirring of the sticks to ensure the removal of excess moisture as well as avoiding the growth of fungi on them. After drying completely, the sticks of the ration were cut into small pieces commensurate with the size of the mouth of the experimental fish, then kept in

bags made of nylon. Each ration was placed in a bag with the treatment number attached, and it was placed in the freezer at a temperature of -18°C until it was presented to the experimental fish. Common carp (Cyprinus carpio L.) was brought in the early morning from Babylon Central Hatchery in Al-Mahaweel District, south of Baghdad, on October 21, 2020. The fish were transported in a vehicle designated for transporting fish, containing the water of the hatchery tank itself, and equipped with a pump to circulate the water and aerate when transporting.

The weight of the fish ranged between 40 g and 45 g. It was transferred to the Fish Laboratory / College of Agricultural Engineering Sciences / University of Baghdad / Fish Laboratory on 1/11/2020. It was isolated and sorted according to close weights to be distributed over the experiment ponds and placed in a saline solution with a concentration of 0.5% and until signs appeared. Stress on fish to get rid of external parasites, if any, before placing them in glass aquariums.120 fish of close weight were selected and distributed randomly and equally to 18 glass tanks, with a starting weight of 45 ± 2/gm and an average live mass of 222gm/duplicate. The experiment ponds were divided into nine treatments, with two replicates for each treatment and five fish for each replicate. Feed to saturation, then the remaining feed was withdrawn and dried, then weighed to be subtracted from the provided amount. The given quantity was calculated and was 4% (Jobling and Koskela, 1996) of the weight of the live mass in each tank at the rate of three meals per day (meal every four hours) starting at seven in the morning. The experiment began on 11/15/2020 when the fish were fed on experimental diets The nine (treatments) and for a period of 90 days (the duration of the experiment), the fish were individually weighed every 15 days with a Chinese-origin electronic sensitive scale, and the live mass was calculated for each replicate to adjust the amount of feed provided to the fish.

The blood samples placed in tubes containing gel-tube were placed in a centrifuge at a speed of 3000 dad for a period of 15 minutes. The serum produced from the centrifugation process was placed in sterile tubes for biochemical analyzes. The enzymes included aspartate amino transferase and ALT alanine amino acid. Transferase) and alkaline phosphatase (ALP Phosphatase) Alkaline) and the tests were conducted using the German-origin Reflotron Plus device. ACH50 was evaluated as reported by Yano (1992) and lysozyme turbidity assay was performed according to Sahoo et al. (2008) with slight modifications, thus plasma (50 µl) was added to 2 ml of Micrococcus lysodeikticus (0.2 mg/mL) in 0.05 M sodium phosphate solution (pH 6.2). Statistical analysis of the data using the statistical program (SAS, 2012) according to a complete random design (CRD) complete random design \, The significant differences between the means of the studied traits were tested using Duncans polynomial test Multiple Range TEST (Duncan, 1955) at the level of significance (0.05).

Results and Discussion

Liver enzymes (ALT, AST, ALP)

The results of the statistical analysis of AST tests showed that treatments T4 and T2 were the lowest on the rest of the experimental treatments, which amounted to

222,753 and 223.23 IU/L, respectively, while there were no significant differences for T6 and T1 that recorded 240.21 and 246.52 IU/L, respectively. Both treatments T4 and T2, followed by treatments T8 and T5, which amounted to 251.96 and 253.93 IU/L, respectively, which did not differ significantly with treatment with both treatments T3 and T9, which scored 255.38 and 264.72 respectively, and T7, which recorded 281.57 units. International/liter is higher than the rest of the experiment coefficients.

As for the ALT enzyme, the results of the statistical analysis showed that treatment T9 recorded the lowest on the other experimental treatments, which recorded 29.95 IU/L, followed by treatments T7, which recorded 36.82 IU/L, which did not differ significantly with treatment T5, which recorded 38.24 IU/L, then followed by T8, T3, T6, T4 and T2 treatments which recorded 43.47, 44.21, 44.63, 45.46 and 45.93 IU/L, respectively, which did not differ significantly with treatment T1 which recorded 52.27 IU/L. The results of the statistical analysis also showed that the T2 treatment, which recorded 24.72 IU/L, was the lowest among the experimental treatments in the concentration of ALP enzyme, followed by the T1 treatment, which recorded 32.35 IU/L, which did not differ significantly with the T7 treatment, which recorded 35.35 IU/L. , then followed by treatment T3, T4, T6, T8 and T5, which recorded 41.45, 44.89, 45.41, 49.55 and 49.55 IU/L, respectively, while the T9 treatment recorded the highest concentration of ALP enzyme, which recorded 51.640 IU/L.

The results of the statistical analysis of enzyme assays were within the normal range of all experiment treatments, and this is an indication of their safe and health effects, and the absence of diseases, damage or breakage in liver cells and in other cells as a result of the use of the aloe vera plant in the diet, and that the occurrence of high AST concentrations in the cytoplasm in acute cases Then it is followed by ALT, which is found in mitochondria and cytoplasm, which is more high in cases of chronic diseases and myocardial infarction, and AST percentage increases as well in cases of stress and muscle atrophy. The natural breakdown of body cells occurs due to continuous metabolic processes, so there are normal concentrations of AST and ALT to compensate for the normal damage, But when there is significant damage or damage to a large percentage of cells, especially liver cells (the center of nutrient metabolism), the concentrations of enzymes are high and abnormal due to their role in the process of forming non-essential amino acids that the body needs to compensate cells that have been damaged by stress or excessive consumption. Eating foodstuffs that may stress the liver, and any increase of these enzymes than the normal limit in the blood serum is a result of the breakdown of these now. History due to physiological, pathological, or nutritional conditions (Brett, 2009; Ebeid et al., 2005).

Table 1
Concentrations of ALT, AST, and ALP for serum of common carp fish fed on rations containing gel powder and olive leaf for a period of 90 days (mean ± standard error)

Treatments	AST	ALT	ALP
T1	4.02 ±246.52	4.72 ±52.27	1.23±32.35

	bc	a	d
T2	6.93±223.23 c	1.38±45.93 ab	0.77±24.72 e
T3	10.78±255.38 ab	1.43±44.21 bc	1.98 ±41.45 bc
T4	3.75±222.75 c	1.79±45.46 ab	1.18±44.89 ab
T5	13.79 ±253.93 b	0.91±38.24 cd	2.60±49.55 a
T6	7.71 ±240.21 bc	0.69±44.63 bc	0.65 ±45.41 ab
T7	8.57±281.57 a	2.32±36.82 d	4.20±35.35 dc
T8	5.81±251.96 b	1.31±43.47 bcd	1.830±49.55 ab
T9	3.72 ±264.72 ab	0.18±29.95 e	3.640±51.640 a
Means with different letters within the same column differ significantly (P ≤ 0.01) between them.			

Lysozyme analysis

It is clear from Table (2) the Lysozyme activity of the experimental fish that there are differences at a significant level (P ≤ 0.01), as the T8 treatment recorded the best in the values of Lysozyme activity in the serum of fish over the rest of the treatments. Compared with the rest of the treatments in the experiment (aloe vera leaves) and compared with the control group, the control treatment and the T9 treatment recorded the lowest values for Lysozyme activity, which confirms the results of the current study, an increase in the number of defense units after eating aloe vera gel, and it can be related to beta-glucan, which is able to identify Specific receptors in white blood cells, and when these receptors are occupied by glucans, the surrounding white blood cells are activated, further killing and digesting pathogenic bacteria improving the host immune system (Andrews et al., 2009). Lysozyme is an important component of non-specific immunity that causes bacterial wall destruction, enhances phagocytic activity and activates both complementary pathways involved in stimulating the fish immune response. The level of Lysozyme in the blood shows an improvement in the immune status of the fish and an increase in the immune system of the fish to improve the fight against infectious agents and reduce stress, and the activity of Lysozyme increases the immunostimulants after some vaccines and some prebiotics in fish (Alishahi et al., 2010). The results of this study showed that adding different levels of aloe vera powder affects the activity of lysozyme, as it had significant effects ($p < 0.05$) and the highest lysozyme activity was observed with 1.5% A. vera, and the study agrees with several reports that indicate the role of herbal immunostimulants in enhancing Lysozyme activity (Rao et al., 2006; Choi et al., 2008; Haghighi et al., 2014). The lysozyme is a humoral component of a nonspecific defense mechanism that has the ability to inhibit bacterial growth by splitting β-1,4 glycosidic bonds in the peptidoglycan of bacterial cell walls (Secombes, 1996; Choi et al., 2008).

Table 2
Shows the results of Lysozyme analysis

Treatments	Lysozyme
T1	11±0.58 a
T2	23.54±0.55 b
T3	21.33±0.67 b
T4	28.35±0.52 cd
T5	25.33±0.88 c
T6	30.81±0.53 cd
T7	28±0.58 bd
T8	31.13±0.62 d
T9	30.2±0.55 d
Means with different letters within the same column differ significantly (P ≤ 0.05) between them.	

ACH50 analysis

It is clear from Table (19) that there were significant ($p < 0.05$) differences in the ACH50 analysis, as treatment T8 recorded the best significant (31.13 ± 0.62), while treatment T8 had no significant differences with treatments T6 and T4 (30.81 ± 0.53, 28.35 ± 0.52) on the respectively, compared to the T1 control group, which scored 191.67 ± 1.45. Then came treatment T2 in the second degree, which scored 271 ± 1, and then treatment T7 (224 ± 2.3), which did not differ significantly with each of the treatment T9 and T3, respectively. This may be attributed to the significant increase in supplement activity by using aloe vera powder, which led to a marked increase in supplement activity and thus an increase in the activity of a strong non-specific defense mechanism to protect fish from a wide range of pathogenic organisms such as bacteria, fungi, viruses and parasites (Chiu et al., 2010). These results are in line with what was found by Alishahi and Abdi (2013), who reported that the use of aloe vera extract at 1.5% and 0.5% in the diet of common carp was able to significantly increase the amounts of ACH50 compared to the control group.

Table 3
Shows the results of the analysis of ACH50

Treatments	ACH50
T1	191.67±1.45 a
T2	271±1 c
T3	217.67±1.2 ab
T4	275±0.58 cd
T5	220±1 a
T6	277.67±0.88 cd
T7	224±2.3 b
T8	280.67±0.88 d
T9	219.51±1.3 ab
Means with different letters within the same column differ significantly ($P \leq 0.05$) between them.	

As a result, the use of 2% of aloe vera gel in Cyprinus carpio fish diets leads to higher growth rates and a contribution to fish immunity against bacteria and fungi.

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