Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 26(1): 257 – 272 (2022)
www.ejabf.journals.ekb.eg

An alternative diet from agriculture wastes as feed for the grass carp (*Ctenopharyngodon idella*) in an integrated agri-aquaculture system in the Eastern Desert and its influences on haematological parameters, blood biochemistry, and immune status

Emad El-Din I. A. Mursy[1], Faiza M. Soliman[1], Hamdy A. M. Soliman[1] , Ahmed E. A. Badrey[2*] , Alaa G. M. Osman[2]

1- Department of Zoology, Faculty of Science, Sohag University, 8562 Sohag, Egypt

2- Department of Zoology, Faculty of Science, Al- Azhar University (Assiut Branch), 71524 Assiut, Egypt

***Corresponding Author: gmal_ahmed77@yahoo.com**

ARTICLE INFO

Article History:
Received: Sept. 16, 2021
Accepted: Nov. 28, 2021
Online: Jan. 11, 2022

Keywords:
Integrated agri-aquaculture,
Grass carp,
Egyptian desert,
Alfalfa,
Groundwater

ABSTRACT

The present work was conducted to evaluate the influence of replacing fish meal with agricultural wastes on the haematological, serum biochemical, and immunological parameters. A total of 400 grass carp (*Ctenopharyngodon idella*), with an average body weight of 100.1±1 g, were used in the present study. The fish were randomly divided into 4 groups (100 fish/pond). A basal control diet was formulated to fulfil the nutrient requirements of the fish that contained 25% crude protein (CP). The other 3 diets (treatment diets) were alfalfa diet, peanut leaves diet, and a mixture of alfalfa and peanut leaves diet. The fish were fed six days per week for 90 days at a daily feeding of 3% of the estimated fish-weight of the total biomass of the fish, with twice daily feeding at 8.00 am and 4.00 pm. Haematological analysis revealed that red blood cell (RBC) counts, haemoglobin levels (Hb), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) white blood cell (WBCs) counts, monocytes, and eosinophils were insignificantly different in the treated groups compared to the control group. While, Hematocrit (Hct), mean corpuscular volume (MCV) neutrophils, and platelets counts were significantly different from fish fed the plants feed diets compared to fish fed the control diet. Serum glucose, cholesterol, triacylglycerol, urea, and creatinine levels, as well as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were significantly increased in alfalfa + peanut leaves meal compared to the different feeding diets. Immunological parameters (IgM levels and lysozyme activity) were significantly improved by plants feed compared to the control group. These results revealed that the alfalfa induced the least side effects on the haematological and biochemical parameters in grass crap, while the mixture (alfalfa+peanut) highly improved the immune status, but induced the highest side effects on the haematological and biochemical parameters in grass crap.

INTRODUCTION

Over the last 30 years, aquaculture has grown faster worldwide than any other animal production sector **(Francesco *et al.*, 2004; FAO, 2007)**. Notably, its average

annual growth has been 10% compared with 3% in the cattle industry and 1.6% in capture of aquatic species from natural environments. Aquaculture's strong growth has generated a consequent 30% annual growth in the production of aquatic species feeds **(Francis *et al.*, 2001)**, and has made raw material supply a continuous challenge in this industry.

To attaina sustainable growth in the aquaculture industry, a progressive decrease should take place in the use of marine protein and lipids in feeds for farmed fish **(Francis *et al.*, 2001; Francesco *et al.*, 2004)**. Another industry goal is to decrease the levels of phosphorus and nitrogen in aquaculture farm effluents to lower aquacultures environmental impact **(Francesco *et al.*, 2004)**. Feed is one of the major inputs in aquaculture production, and fish feed technology is one of the least developmental sectors of aquaculture, particularly in Africa and other developing countries of the world **(Gabriel *et al.*, 2007)**. One of the problems hampering aquacultural development in Egypt is the high cost of fish feed **(Badrey *et al.*, 2019)**. Expensive feeds will marginalize or nullify the profitability of fish farming, thereby incapacitating the expansion of farms to increase production. Consequently, low fish yield, with respect to quality and , result in the scarcity of the commodity (fish), and eventually high cost of the few available ones would lead to the disadvantage of the populace **(Adikwu, 1992)**. Fish feed accounts for at least 60% of the total cost of production **(Gabriel *et al.*, 2007)**. This has motivated the research for local, cheap, and unsuitable for direct human consumption to act as an alternative energy feed for fishes that aim to reduce the cost of production without compromising fish quality **(Bichi & Ahmad, 2010)**. Animal- and vegetable-origin protein sources have been tested as alternative feed stuffs in fish feed production that recorded varying degrees of success **(Kaushik *et al.*, 2004; Li *et al.*, 2004; Fasakin *et al.*, 2005)**. Since vegetable products are found in great natural abundance, they have won a researchers' interest as ingredients for fish feed production **(El-Sayed, 1999; Ogunji, 2004)**. Among plant-derived raw materials, legumes have received special attention due to their high protein content. In this context, legumes have replaced fish meal in the tilapia feeds that reached a 100% substitution in the case of soybean meal, or at lower levels when using novel sources such as *Leucaena leucocephala* leaf meal **(Jackson *et al.*, 1982; Santiago *et al.*, 1988)**, protein concentrates **(Olvera-Novoa *et al.*, 1990; Borgeson *et al.*, 2006)** and alfalfa meal (*Medicago sativa*) **(Ali *et al.*, 2003)**. Remarkably, peanut or groundnut (*Arachis hypogaea*) is important for human nutrition due to its high protein and energy content. Annual worldwide production of peanuts in the shell surpasses 33 million tons in 20 countries of the highest recorded production **(FAO, 2006)**. In addition to the seed, peanut plants produce high protein forage that has long been used as swine and ruminant feed, and its flowers are nectar source in apiculture. Peanut stem and leaf production (excluding seed production) can be as high as 6 tons of dry matter per hectare. *Medicago sativa* or alfalfa is a flowering plant grown throughout the world mostly presented as forage for cattle due to its high protein content,

balanced amino acid profile, vitamins and carotenoids **(Olvera-Novoa *et al.*, 1990)**. **Olvera-Novoa *et al.* (1990)** reported that, inclusion of alfalfa leaf protein up to 35% can be used in feeds of the tilapia without compromising the growth or the survival of the fish. An enhancencement in the growth and survival of common carp and mrigal has been witnessed aligned with an increase in protein and lipid content upon adding alfalfa leaf meals to fish diet with percentages of 40 and 30, respectively **(Chatzifotis *et. al.*, 2006; Vhanalakar, 2009)**. Moreover, the use of dehydrated alfalfa leaves in the diet of the tilapia was determined (**Yosif *et al.*, 1994)**. Most researchers recommended low substitution levels. **Ali *et al.* (2003)** suggested an iclusion level of alfalfa leaves that does not exceed 5%. **Sklan *et al.* (2004)** reported that complete replacement may clog the growth performance. Blood characteristics are very important tools used as effective indices of water balance, nutritional status, and overall health condition of fish **(Nwani *et al.*, 2015; Zaahkook *et al.*, 2016)**. Numerous dietary supplements have measurable effects on blood constituents **(Animashahun *et al.*, 2006; Bhatti *et al.*, 2009)**. Therefore, haematological and blood biochemical variables have been used as indicators of health status in fish of many species fed different kinds of food. Diet composition and metabolic adaptation are the main factors responsible for changes in haematological and blood biochemical variables in fish **(Ighwela *et al.*, 2012)**. Such parameters are reliable indicators of fish physiological status and usually used to evaluate fish health and immune potential **(Kondera *et al.*, 2017)**. The grass carp is a rapid growing, phytophagous, cyprinid fish indigenous to the large rivers of China and Siberia **(Lin, 1935)**. The ability to turn large quantities of a wide variety of plant material into good quality protein has made this species an important aquaculture candidate worldwide. The objective of the present study was to determine the effect of fed plant-based feed diets (agriculture wastes) and formulated feed on haematology and biochemical parameters, as well as immune status, in grass carp.

MATERIALS AND METHODS

Experimental Design

The present study was carried out on a private agriculture farm in the desert of Qena valley, Qena Governorate, Egypt (Fig. 1). It was supported by water pond (reservoir) to secure the water needs for plants. This pond (2*2*1 meters) was lined with plastic layer of 1.0 mm thickness, provided by water from an underground water well supplied by machine. A number of about 400 fingerlings of fish grass carp (*Ctenopharyngodon idella*) was bought from the private hatchery Aihwa in Sohag and transported under suitable conditions at night to secure its transportation during the long distance with the consideration of 10-20% loss. In the farm, the fish fingerlings were checked again and acclimated for three weeks before being released to fishpond water. The specimens were divided into 4 groups. The control group was fed on the commercial extruded floating fish diet (25% crude protein), the second group was fed on alfalfa, the

third group was fed on peanut leaves, whereas the fourth group was fed on a mixture of alfalfa and peanut leaves. Fish groups were fed twice daily (at 8.00 am and 4.00 pm) for six days per week during the 90- day study period on a daily feeding basis of 3% of the estimated fish-weight of the total biomass of the fish **(Siddiqui *et al.*, 1997)**. Feed quantity was adjusted according to the average body weight of the samples. Hence, the amount of feed was changed to the new fish biomass.

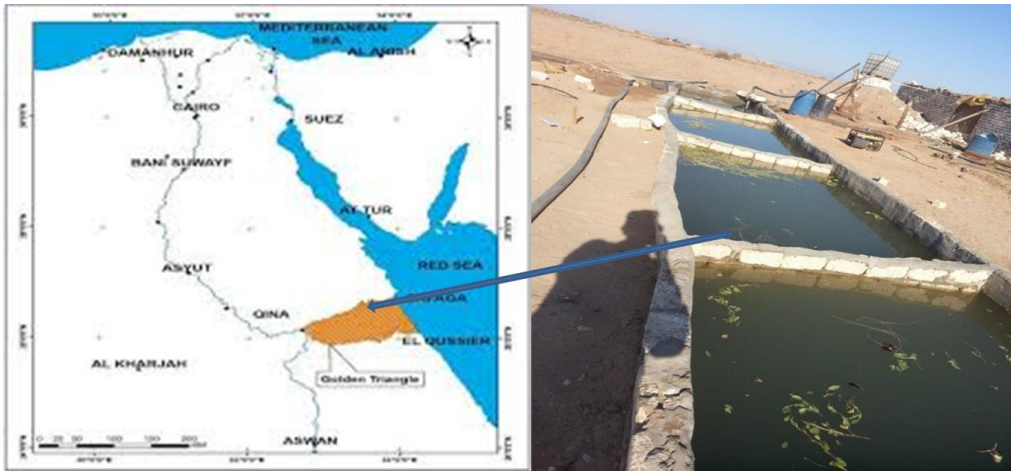

Fig. 1. A private agriculture farm in the desert of Qena valley, Qena Governorate, Egypt

Experimental analyses

Haematological analysis

For haematological measurements, the blood samples of fish individuals were collected twice during the study period; after 45 days and at the end of the experiment (at day 90). The blood samples were collected from the cardiac puncture as described in the study of **Osman *et al.* (2011)**. The haematological parameters, including haemoglobin concentration (Hb), haematocrit value (Hct), red blood cells count (RBCs), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were calculated following the methods of **Dacie and Lewis (2002)**.

Biochemical analysis

The blood biochemistry was performed using spectrophotometer (Jasco-V530) with the absorbency at wave length ranging from 340 to 546 nm; total protein (g/dl), creatinine, and urea (mg/dl) levels were estimated according to method of **Henry (1964)**. Cholesterol (mg/dl) and triglycerides (mg/dl) levels were analysed based on the steps of **Thomas (1992)** and **Friedewald *et al.* (1972)**, respectively. Glucose (mg/dl) level was evaluated in accordance to the study of **Trinder (1969)**. While, aspartate aminotransferase (AST, U/I) and alanine aminotransferase (ALT, U/I) activates were determined calorimetrically stepping the method of **Reitman and Frankel (1957).**

Immunological responses

Lysozyme activity was measured spectrophotometrically according to the method of **Ellis (1990)**. Immunoglobulin M (IgM) levels were determined using an ELISA kit (Catalogue No. CSB-E12045Fh, 96k test, Cusabio Biotech Co., Ltd.).

Statistical Analysis

The collected data were subjected to statistical analysis using general linear model's procedure adapted by **SPSS (1997)** Versions 16, with a one-way ANOVA. Means were statistically compared to detect the level of the significance ($p \leq 0.05$) using multiple range test SPSS 16.

RESULTS

Haematological parameters

The average values of RBCs, Hb, Hct, MCV, MCH, MCHC, WBCs, lymphocytes, neutrophils, monocytes, eosinophils, and platelets for the control group and alfalfa, peanut leaves and a mixture of alfalfa and peanut leaves treated groups are presented in Table (1). In a 45- day treatment, the Hct (peanut leaves treated group) and the MCV (a mixture of alfalfa and peanut leaves treated group) showed a significant decrease compared to the control group, while WBCs count (alfalfa, peanut leaves, and a mixture of alfalfa and peanut leaves treated groups) showed a significant increase compared to the control group. In addition, RBCs count, Hb content, MCH, MCHC, lymphocytes (%), neutrophils (%), monocytes (%), eosinophils (%), and platelets count (alfalfa, peanut leaves, and a mixture of alfalfa and peanut leaves treated groups) showed insignificant changes compared to the control group. In the 90- day treatment, Hct (alfalfa, peanut leaves, and a mixture of alfalfa and peanut leaves treated groups), and percentage of lymphocytes (peanut leaves treated group) showed significant decerease compared to the control group. While, MCV (peanut leaves treated group) , and platelets count (alfalfa, peanut leaves, and a mixture of alfalfa and peanut leaves treated groups) showed significant increase compared to the control group. Furthermore, RBCs count, Hb content, MCH, MCHC, and percentage of neutrophils, monocytes, and eosinophils (alfalfa, peanut leaves, and a mixture of alfalfa and peanut leaves treated groups) showed insignificant changes compared to the control group.

Blood biochemistry

The average values of total protein, glucose, cholesterol, triacylglycerol, creatinine, urea, ALT, and AST of the control group and alfalfa, peanut leaves, and the mixture of alfalfa and peanut leaves treated groups are presented in Table (2).

In the 45- day treatment, the level of total protein (a mixture of alfalfa and peanut leaves treated group), glucose (peanut leaves and a mixture of alfalfa and peanut leaves treated

groups), cholesterol (peanut leaves and a mixture of alfalfa and peanut leaves treated groups), triacylglycerol (alfalfa, peanut leaves, and a mixture of alfalfa and peanut leaves treated groups), and urea (a mixture of alfalfa and peanut leaves treated group), as well as the activity of ALT (peanut leaves and a mixture of alfalfa and peanut leaves treated groups) and AST (a mixture of alfalfa and peanut leaves treated groups) showed a significant increase compared to the control group. While, an insignificant change was detected in creatinine level (alfalfa, peanut leaves, and a mixture of alfalfa and peanut leaves treated groups) compared to the control group. On the other hand, in the 90- day treatment, a significant increase was recorded in the level of glucose (alfalfa, peanut leaves, and a mixture of alfalfa and peanut leaves treated groups), cholesterol (peanut leaves and a mixture of alfalfa and peanut leaves treated groups), triacylglycerol (peanut leaves and a mixture of alfalfa and peanut leaves treated groups), creatinine (a mixture of alfalfa and peanut leaves treated groups), and urea (alfalfa and a mixture of alfalfa and peanut leaves treated groups), as well as the activity of ALT (a mixture of alfalfa and peanut leaves treated groups) and AST (a mixture of alfalfa and peanut leaves treated groups) compared to the control group. While, compared to the control group, an insignificant change was defined in total protein (alfalfa, peanut leaves, and a mixture of alfalfa and peanut leaves treated groups).

Table 1. Haematological parameters (mean ±SD) of grass carp under plant-based feed and formulated feed for 45 and 90 days

Items / Treatment	45 Days				90 Days			
	Control	Alfalfa	Peanut leaves	Alfalfa + Peanut leaves	Control	Alfalfa	Peanut leaves	Alfalfa + Peanut leaves
RBCs ($\times 10^6$ µl)	2.9±0.1^{ab}	3.2±0.2^{b}	2.6±0.1^{ab}	2.5±0.1^{a}	3.2±0.1^{ab}	3.8±0.1^{b}	2.8±0.1^{a}	2.7±0.2^{a}
Hb (g/dl)	7.6±0.1^{ab}	8.4±0.3^{b}	7.4±0.3^{ab}	7.1±0.1^{a}	9.4±0.3^{a}	9.1±0.2^{a}	8.9±0.1^{a}	8.3±0.3^{a}
Hct (%)	28.5±0.2^{b}	29.6±0.3^{b}	26.6±0.5^{a}	28.5±0.2^{b}	31.4±0.4^{c}	29.5±0.2^{b}	29.6±0.4^{b}	26.8±0.5^{a}
MCV (fL)	109.2±1.3^{b}	102.8±0.3^{ab}	98.3±6.4^{ab}	96.1±0.4^{a}	101.1±1.4^{a}	101.2±0.5^{a}	106.9±1.2^{b}	100.3±1.3^{a}
MCH (pg)	36.9±0.4^{ab}	38.9±0.3^{b}	37.1±1.6^{ab}	34.6±1.08^{a}	38.3±0.2^{ab}	40.0±0.7^{b}	39.5±0.3^{b}	36.3±1.2^{a}
MCHC (g/dl)	38.6±0.4^{a}	40.1±1.1^{a}	40±1^{a}	37.4±0.6^{a}	41.2±0.5^{ab}	41.2±0.8^{ab}	43.3±0.9^{b}	39.7±0.4^{a}
WBCs ($\times 10^3$ µl)	27.4±0.6^{a}	30.4±0.6^{b}	30.3±0.5^{b}	30.3±1^{b}	28.6±0.2^{a}	31.0±0.4^{a}	30.3±0.2^{a}	30.4±1.3^{a}
Lymphocytes (%)	80.6±0.7^{a}	88±1.3^{a}	81±3.8^{a}	84±1.3^{a}	85.3±1.2^{b}	83.3±1.2^{ab}	78.6±2.6^{a}	87.6±1.2^{b}
Neutrophils (%)	11.6±0.2^{a}	6.3±2.3^{a}	13.6±3.6^{a}	9.3±1.2^{a}	11.6±1.9^{a}	11.0±1.0^{a}	18±1.7^{b}	8.6±1.2^{a}
Monocytes (%)	5±0.5^{a}	4.6±0.7^{a}	3.6±0.5^{a}	5±0.5^{a}	4±1.3^{a}	3.6±0.5^{a}	2.3±0.7^{a}	2.6±0.2^{a}
Eosinophils (%)	1.6±0.2^{a}	1.6±0.2^{a}	1.6±0.2^{a}	1.6±0.2^{a}	0.3±0.2^{a}	1.3±0.2^{a}	1.3±0.2^{a}	1.0±00^{a}
Platelets($\times 10^3$ µl)	146±6.9^{a}	138.6±53.5^{a}	182.6±2.8^{a}	232.3±15.4^{a}	179.6±5.1^{a}	205±1.3^{b}	195.3±2.8^{b}	276.6±5.0^{c}

Means in the same row with different superscript letters are significantly different ($P<0.05$).

Table 2. Biochemical parameters (mean ±SD) of grass carp under plant-based feed and formulated feed for 45 and 90 days

Treatment	45 Days				90 Days			
	Control	Alfalfa	Peanut leaves	Alfalfa + Peanut leaves	Control	Alfalfa	Peanut leaves	Alfalfa + Peanut leaves
Total Protein (mg/dl)	4.6±0.3^{a}	5.2±0.2^{ab}	4.2±0.3^{a}	6.3±0.2^{b}	6.4±1.1^{a}	6.6±0.2^{a}	4.6±0.2^{a}	6.9±0.3^{a}
Glucose (mg/dl)	110.3±1.2^{a}	114.6±2.8^{a}	128.3±3.3^{b}	177.6±1^{c}	122.6±3.3^{a}	216±1.8^{c}	185.3±6.1^{b}	292.3±3.0^{d}
Cholesterol (mg/dl)	154.6±5^{a}	158.6±2.6^{a}	223.3±5.3^{b}	211.6±5.9^{b}	179±0.8^{a}	186.6±5.3^{a}	259.3±0.5^{b}	295.6±3.1^{c}
Triacylglycerol (mg/dl)	147.6±4.7^{b}	109±3.1^{a}	207.6±5.6^{c}	200.6±2.5^{c}	148.6±4.0^{a}	122.6±6.7^{a}	192±8.5^{b}	213.6±7.0^{b}
Creatinine (mg/dl)	1.2±0.1^{ab}	0.8±0.2^{a}	1.2±0.2^{ab}	1.8±0.1^{b}	0.6±0.1^{a}	0.9±0.1^{a}	1.0±0.07^{a}	2.1±0.2^{b}
Urea (mg/dl)	26.3±1.8^{a}	30.3±1.5^{a}	28.3±1.2^{a}	39±1.3^{b}	22.6±0.5^{a}	30.3±0.7^{b}	26±1.8^{ab}	39.3±1.9^{c}
ALT activity (U/L)	38±2.3^{a}	45.3±2.5^{ab}	54.3±2^{b}	71.3±3.1^{c}	54.3±3.1^{a}	54±2.3^{a}	63±2.3^{a}	89.6±5.6^{b}
AST activity (U/L)	52.6±4.2^{a}	53.6±2.9^{a}	60±2.6^{a}	72.3±2.5^{b}	65±3.3^{a}	61±2.8^{a}	70.3±4.1^{ab}	82.3±3.1^{b}

Means in the same row with different superscript letters are significantly different (P<0.05).

Immunological responses

Figs. (1, 2) show the effect of alternative diets of plants on selected immunological parameters in the blood of grass carp (*Ctenopharyngodon idella*) fed a basal diet or alternative diets of plants. IgM concentration, compare to the control group, were in-significantly different in alfalfa, peanut leaves, and a mixture of alfalfa and peanut leaves treated groups compared to the control group. Lysozyme activity were found to be significantly increase in (a mixture of alfalfa and peanut leaves treated group).

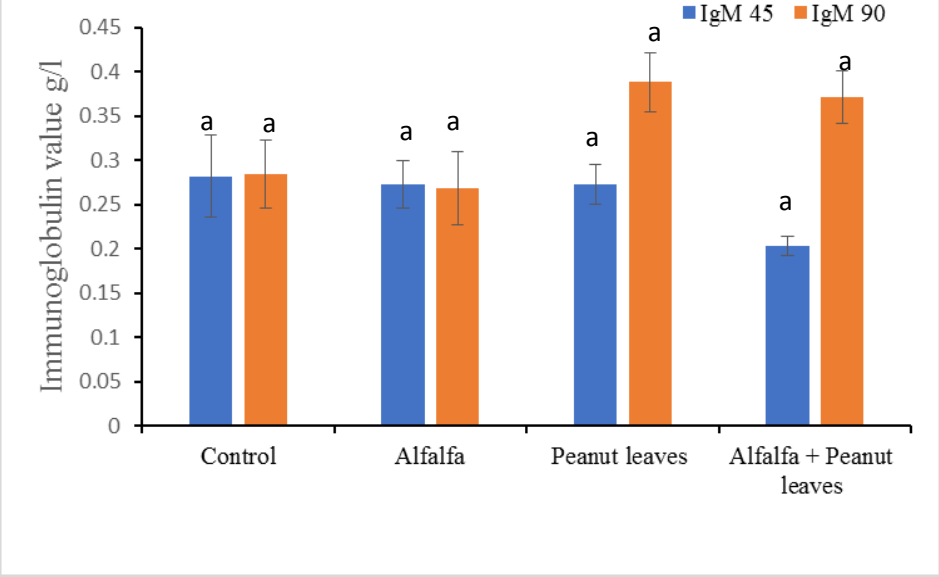

Fig. 2. Immunoglobulin value of grass carp under plant-based feed and formulated feed for 45 and 90 days. (Bars with different superscript letters are significantly different (P<0.05).

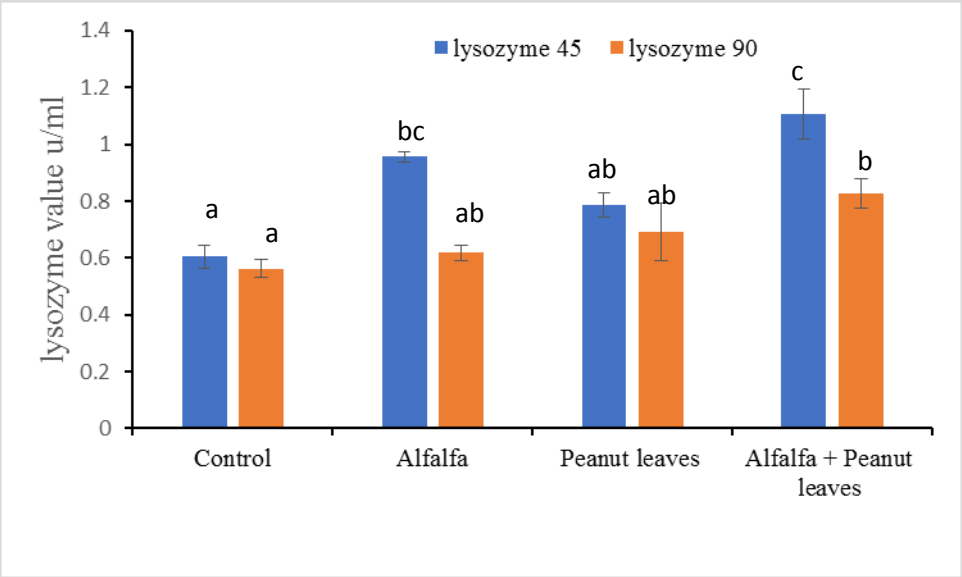

Fig. 3. Lysozyme value of grass carp under plant-based feed and formulated feed for 45 and 90 days.

Bars with different superscript letters are significantly different (P<0.05).

DISCUSSION

Blood monitoring is important in aquaculture given that cultivation conditions affect the basic physiological functions of farmed fish **(Tavares-Dias & Moraes, 2007)**. Thus, feed-induced changes in blood indices in fish have been reported by many authors **(Kelly, 1979; Kilgour, 1987)**. In the current study, specifically at the end of the experiments (90 days), the Hct (alfalfa, peanut leaves, and a mixture of alfalfa and peanut leaves treated groups) and the percentage of lymphocytes (peanut leaves treated group) recorded a significant decerease, while MCV (peanut leaves treated group) and platelets count (alfalfa, peanut leaves, and a mixture of alfalfa and peanut leaves treated groups) showed a significant increase compared to the control group. The highest hemoglobin, hematocrit and MCV values were obtained in alfalfa treatment in grass carp. Whereas, no significantly different value was recorded in WBC, RBC and MCHC between treatments **(Nekoubin & Sudagar, 2013)**. **Zheng *et al.* (2012)** stated that, values of red RBC, Ht and Hb were affected by dietary levels of cottonseed meal (CSM) in juvenile grass carp. No significant differences were observed with respect to the values of RBC as the replacement of SBM with CSM increased from 0 to 68%, but a significant decrease was recorded when the replacement level was up to 100%. Ht values increased significantly as the replacement level of SBM with CSM increased from 0 to 35%. However, they significantly decreased as the replacement level increased from 35 to 100% . Fish fed diets containing 16.64 and 32.73% of CSM as replacements of 35 and 68% of SBM improved Hb compared to fish in other treatments, but total replacement of SBM by CSM significantly decreased the Hb. **Anwer *et al.* (2018)** mentioned values of Hb and RBC showing significant differences in all experimental groups of grass carp

after feeding on onion powder; whereas, WBC and PLT values were higher with respect to control. Other parameters, such as neutrophils, lymphocytes, monocytes and eosinophils showed no significant differences between the experimental groups. **Sangeetha and Rajendran (2018)** determined the presence of a significant variation in red blood corpuscles (RBC), white blood corpuscles (WBC), haemoglobin (Hb), MCH, MCHC and PCV in the blood of grass carp fed *Cynodon dactylon* mixed diet.

Blood biochemical indices are useful for determining fish health status following different feeding trials **(Yılmaz & Ergun, 2012)**. In the present study, grass carp feeding on plants feed exhibited the same blood total protein levels of the control fish after 90 days of feeding, confirming good growth performance during this experiment. The maximum value of total protein was obtained in alfalfa treatment in grass carp **(Nekoubin & Sudagar, 2013)**. **Chen *et al.* (2020)** observed that, plasma total protein was remarkably increased in grass carp with dietary multi-strain probiotic (MP at >1.68 g kg^{-1}).

Blood glucose levels have been used as indicators of environmental stress, as they reflect changes in carbohydrate metabolism under stress conditions **(Kamal & Omar, 2011)**. Glucose levels increased significantly with plants-based feed over the 90-day feeding trial. Increased levels of glucose have previously been recorded in the blood of stressed fish **(Levesque *et al.* 2002; Poléo & Hytteørd, 2003; Adedeji *et al.* 2009; Mekkawy *et al.* 2010; Osman *et al.* 2010)** due to changes in the activity of glucose-6-phosphate dehydrogenase and lactate dehydrogenase, as previously detected in the study of **Osman *et al.* (2018)**. Additionally, **Vo *et al.* (2020)** found that under DO reduction stress, the fish fed germinated, fermented and untreated peanut meals resulted in the significantly four-fold increase in plasma glucose concentration. On the other hand, the serum levels of glucose value recorded no significant differences between Alfalfa treatment and artificial diet in grass carp **(Nekoubin & Sudagar, 2013)**.

Changes in blood cholesterol and triacylglycerol concentrations are sensitive indicators of liver dysfunction owing to the fact that lipid homeostasis is one of the principal functions of the liver **(Sayed *et al.* 2007)**. In the present study, cholesterol and triacylglycerol (peanut leaves and a mixture of alfalfa and peanut leaves treated groups), demonstrated significantly higher levels after the 90-day feeding trial compared to the control fish. These results disagree with those of **Yue *et al.* (2012)** who stated that, the plasma total cholesterol and triglyceride levels decreased with increasing the peanut meal inclusion levels in juvenile white shrimp. On the other hand, the serum levels of cholesterol value had not any significant differences between alfalfa treatment and artificial diet in grass carp **(Nekoubin & Sudagar, 2013)**. **Sangeetha and Rajendran (2018)** postulated that, a slight variation was monitored in the total serum protein, globulin, albumin, glucose, cholesterol and the triglycerides in the blood of grass carp fed *Cynodon dactylon* mixed diet. **Zhang *et al.* (2020)** mentioned that, the plasma

triglyceride content was significantly lower in grass carp fed dietary with 0.05 % and 0.1 % xylooligosaccharide (XOS).

The present results revealed that feeding on plant-based feed did not significantly change serum creatinine except in the case of a mixture of alfalfa and peanut leaves treated group, as well as urea except in the case of alfalfa and a mixture of alfalfa and peanut leaves treated groups. In the 90- day feeding trial, feeding on plant-based diet did not significantly change serum ALT or AST levels (except a mixture of alfalfa and peanut leaves) compared to those of the control fish.

The non-specific immune system of fish is considered the first line defence against invading pathogens. IgM levels and lysozyme activities are important indices of non-specific immunity in fishes. Lysozyme activity is an important parameter in the immune defence of both invertebrates and vertebrates. In the current study, IgM concentration were in-significantly different in the alfalfa, peanut leaves, and a mixture of alfalfa and peanut leaves treated groups compared to the control group. Lysozyme activity increased significantly in the mixture of alfalfa and peanut leaves treated group compared to the control group.

Furthermore, dietary phospholipids (PL) increased the lysozyme activity of the gill immune in juvenile grass carp (*Ctenopharyngodon idella*) (**Feng, *et al.*, 2016**). **Mo *et al.* (2015)** stated that, grass carp showed significant improvement in total serum immunoglobin after feeding with prebiotic fibers (inulin and mannanoligosaccharide). Moreover, the lysozyme activity was significantly higher in grass carp fed dietary with 0.05 % and 0.1 % xylooligosaccharide (XOS) **(Zhang *et al.*, 2020)**. **Ahmadifar *et al.* (2020)** noted a significant increase in the total immunoglobulin level and lysozyme activity of the grass carp after feeding on Purslane (*Portulaca oleracea* L.)

CONCLUSION

The upper- mentioned results indicate that the alfalfa induces the least side effects on the haematological and biochemical parameters in grass crap. On the other hand, the mixture supplemented diet (alfalfa+peanut) was proved to highly improve the immune status. Nevertheless, the latter diet induces the highest side effects on the haematological and biochemical parameters in grass crap. Thus, the former plant-based diet is recommended.

REFERENCES

Adedeji, O.B.; Adeyemo, O.K. and Agbede, S.A. (2009). Effects of diazinon on blood parameters in the African catfish (*Clarias gariepinus*). Afr. J. Biotechnol., 8(16): 3940-3946.

Adikwu, I. (1992). Dietary carbohydrate utilization in Tilapia (*Oreochromis niloticus*). J. Agric. Sci. Technol., 2(1): 33-37.

Ahmadifar, E.; Hoseinifar, S.H.; Adineh, H.; Moghadam, M. S. and Dawood, M. A. (2020). Assessing the impact of purslane (*Portulaca oleracea L.*) on growth performance, anti-oxidative, and immune activities in grass carp (*Ctenopharyngodon idella*). Ann. Anim. Sci., 20(4): 1427-1440.

Anwer, I., Hafeez-ur-Rehman, M., Abbas, F., Javid, A., Hussain, A., Khan, N. and Azmat, H. (2018). Effect of Onion Powder as Feed Additive on the Growth, Hematology and Biochemical Profile of Grass Carp (*Ctenopharyngodon idella*). Pak. J. Zool., (13): 58-64

Badrey, A.E.A.; Osman, A.G.M.; Farrag, M.M.S.; Toutou, M.M. and Moustafa, M.A. (2019). Influences of diets supplemented with pomegranate peel on haematology, blood biochemistry and immune status in monosex Nile tilapia, *Oreochromis niloticus*, Egypt. J. Aquat. Biol. Fish., 23(2): 133 – 144

Ali, A.; Al-Asgah, N. A.; Al-Ogaily, S. M. and Ali, S. (2003). Effect of feeding different levels of alfalfa meal on the growth performance and body composition of Nile tilapia (*Oreochromis niloticus*) fingerlings. Asian Fish. Sci., 16(1/2): 59-68.

Animashahun, R.A.; Omoikhoje, S.O. and Bamgbose, A.M. (2006). Hematological and biochemical indices of weaner rabbits fed concentrates and Syndrella nodiflora forage supplement. Proc. 11th Annual Conference of Animal Science Association of Nigeria. Institute of Agricultural Research and training, Nigeria, 29-32.

Bhatti, J.; Younas, M.; Abdullah, M. and Babar, M. (2009). Feed intake, weight gain and haematology in Nili -Ravi buffalo heifers fed on mott grass and Berseem fodder substituted with saltbush (*Atriplex amnicola*). Pak. Vet. J., 29(3): 133-137.

Bichi, A. and Ahmad, M. (2010). Growth performance and nutrient utilization of African catfish (*Clarias gariepinus*) fed varying dietary levels of processed cassava leaves. BAJOPAS., 3(1).

Borgeson, T. L.; Racz, V. J.; Wilkie, D. C.; White, L. J. and Drew, M. D. (2006). Effect of replacing fishmeal and oil with simple or complex mixtures of vegetable ingredients in diets fed to Nile tilapia (*Oreochromis niloticus*). Aquac. Nutr., 12(2): 141-149.

Chatzifotis, S.; Martin-Prat, A. V.; Limberis, N.; Papandroulakis, N. and Divanach, P. (2006). First data on growth of cultured brown meagre Sciaena umbra using diets with different protein and fat contents. Fish Sci., 72(1): 83-88.

Chen, X., Xie, J., Liu, Z., Yin, P., Chen, M., Liu, Y. and Niu, J. (2020). Modulation of growth performance, non-specific immunity, intestinal morphology, the response to hypoxia stress and resistance to Aeromonas hydrophila of grass carp (*Ctenopharyngodon idella*) by dietary supplementation of a multi-strain probiotic. Comp. Biochem. Physiol., 231, 108724.

Dacie, S. J. V. and Lewis, S. M. (2002). Practical haematology 11th edition. UK, Churchill Livingstone.

de Francesco, M.; Parisi, G.; Lupi, P.; Zampacavallo, G.; Giorgi, G.; Medale, F.; Kaushik, S. and Poli, B.M. (2004). The effect of total replacement of fish meal by plant proteins on sensory, physical and chemical traits during shelf-life in rainbow trout (*Oncorhynchus mykiss*). In Aquaculture Europe 04. European Aquaculture Society Meeting.

Ellis, A.E. (1990). Lysozyme assays. Techniques in Fish Immunology, 1: 101-103.

El-Sayed, A. F. M. (1999). Alternative dietary protein sources for farmed tilapia, Oreochromis spp. Aquaculture, 179(1-4): 149-168.

FAO, (2006). Fisheries Topics. Fisheries and Aquaculture Resources: FAO, Roma, Italy.

FAO, (2007). The State of World Fisheries and Aquaculture 2006. FAO Fisheries and Aquaculture Department, FAO. Rome. P. 162.

Fasakin, E. A.; Serwata, R. D. and Davies, S. J. (2005). Comparative utilization of rendered animal derived products with or without composite mixture of soybean meal in hybrid tilapia (*Oreochromis niloticus* × *Oreochromis mossambicus*) diets. Aquacult., 249(1-4): 329-338.

Feng, L.; Chen, Y.P.; Jiang, W. D.; Liu, Y.; Jiang, J.; Wu, P. and Zhou, X. Q. (2016). Modulation of immune response, physical barrier and related signaling factors in the gills of juvenile grass carp (*Ctenopharyngodon idella*) fed supplemented diet with phospholipids. Fish Shellfish Immunol., 48, 79-93.

Francis, G.; Makkar, H. P. S. and Becker, K. (2001). Effects of Quillaja saponins on growth, metabolism, egg production and muscle cholesterol in individually reared Nile tilapia (*Oreochromis niloticus*). Comp. Biochem. Physiol. Part - C: Toxicol. Pharmacol., 129(2):105-114.

Friedewald, W. T.; Levy, R. I. and Fredrickson, D. S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin. Chem., 18(6): 499-502.

Gabriel, W. N.; McNuff, R.; Patel, S. K.; Gregory, T. R.; Jeck, W. R.; Jones, C. D. and Goldstein, B. (2007). The tardigrade Hypsibiusdujardini, a new model for studying the evolution of development. Dev. Biol., 312(2): 545-559.

Henry, R.J. (1964). Clinical chemistry, New York and Row Publish.

Ighwela, K.A.; Ahmad, A.B. and Abol-Munafi, A.B. (2012). Haematological changes in nile tilapia (*Oreochromis niloticus*) fed with varying dietary maltose levels. W. J. F. M. S., 4(4): 376-381.

Jackson, A. J.; Capper, B. S. and Matty, A. J. (1982). Evaluation of some plant proteins in complete diets for the tilapia *Sarotherodon mossambicus*. Aquacult., 27(2): 97-109.

Kamal, S.M. and Omar. W.A. (2011). Effect of Different Stocking Densities on Hematological and Biochemical Parameters of Silver Carp, *Hypophthalmichthys molitrix* Fingerlings. Life. Sci., 8(4): 580-586.

Kaushik, S. J.; Coves, D.; Dutto, G. and Blanc, D. (2004). Almost total replacement of fish meal by plant protein sources in the diet of a marine teleost, the European seabass, *Dicentrarchus labrax*. Aquacult., 230(1-4): 391-404.

Kelly, D. (1979). Motion and vision. II. Stabilized spatio-temporal threshold surface. Josa, 69(10): 1340-1349.

Kilgour, O.F.G. (1987). Mastering Nutrition. Macmillan Education Limited, London.

Kondera, E.; Kościuszko, A.; Dmowska, A. and Witeska, M. (2017). Haematological and haematopoietic effects of feeding different diets and starvation in common carp *Cyprinus carpio* L. J. APPL. ANIM. RES., 45(1): 623-628.

Levesque, H.M.; Moon, T.W.; Campbell, P.G.C. and Hontela, A. (2002). Seasonal variation in carbohydrate and lipid metabolism of yellow perch (*Perca flavescens*) chronically exposed to metals in the field. Aquat. Toxicol., 60(3-4): 257-267.

Li, P.; Wang, X.; Hardy, R. W. and Gatlin III, D. M. (2004). Nutritional value of fisheries by-catch and by-product meals in the diet of red drum (*Sciaenops ocellatus*). Aquacult., 236(1-4): 485-496.

Lin, S.Y. (1935). Life history of Waan Ue Ctenopharyngodon idella. Lingsan Sci.J., 14,129.

Mekkawy, I.A.A.; Mahmoud, U.M.; Wassif, E.T. and Naguib, M. (2010). Effects of cadmium on some haematological and biochemical characteristics of *Oreochromis niloticus* (Linnaeus, 1758) dietary supplemented with tomato paste and vitamin E. Fish. Physiol. Biochem., 37(1): 71-84.

Mo, W.Y., Cheng, Z., Choi, W. M., Lun, C. H., Man, Y. B., Wong, J. T. and Wong, M. H. (2015). Use of food waste as fish feeds: effects of prebiotic fibers (inulin and mannanoligosaccharide) on growth and non-specific immunity of grass carp (*Ctenopharyngodon idella*). Environ. Sci. Pollut. Res., 22(22): 17663-17671.

Nekoubin, H. and Sudagar, M. (2013). Effect of Different Types of Plants (Lemna Sp., Azolla filiculoides and Alfalfa) and Artificial Diet (With Two Protein Levels) on Growth

Performance, Survival Rate, Biochemical Parameters and Body Composition of Grass Carp (*Ctenopharyngodon idella*). J. Aquac. Res. Dev., 4(2).

Nwani, C.D.; Ifo, C.T.; Nwamba, H.O.; Ejere, V.C.; Onyishi, G.C.; Oluah, S.N. and Odo, G.E. (2015). Oxidative stress and biochemical responses in the tissues of African catfish *Clarias gariepinus* juvenile following exposure to primextra herbicide. Drug Chem. Toxicol., 38(3): 278-285.

Ogunji, J. O. (2004). Alternative protein sources in diets for farmed tilapia. In Nutrition Abstracts and Reviews. Series B, Livestock Feeds and Feeding (Vol. 74, No. 9). CAB International.

Olvera-Novoa, M. A.; Campos, S. G.; Sabido, M. G. and Palacios, C. A. M. (1990). The use of alfalfa leaf protein concentrates as a protein source in diets for tilapia (*Oreochromis mossambicus*). Aquacult., 90(3-4): 291-302.

Osman, A.G.; Abd El Reheema, A.E.B.M.; Moustafa, M.A. and Mahmoud, U.M. (2011). In situ evaluation of the genotoxic potential of the river Nile: I. Micronucleus and nuclear lesion tests of erythrocytes of *Oreochromis niloticus* niloticus (Linnaeus, 1758) and Clarias gariepinus (Burchell, 1822). Toxicol. Environ. Chem., 93(5): 1002-1017.

Osman, A.G.; AbouelFadl, K.Y.; Abd El Baset, M.; Mahmoud, U.M.; Kloas, W. and Moustafa, M.A. (2018). Blood Biomarkers in Nile tilapia *Oreochromis niloticus* niloticus and African Catfish Clarias gariepinus to Evaluate Water Quality of the River Nile. Journal of Fisheries Sciences. Com, 12(1): 1-15.

Osman, A.G.; Koutb, M. and Sayed, A.H. (2010). Use of hematological parameters to assess the efficiency of quince (*Cydonia oblonga* Miller) leaf extract in alleviation of the effect of ultraviolet--A radiation on African catfish *Clarias gariepinus* (Burchell, 1822). J. Photochem. Photobiol. B., 99(1): 1-8.

Poléo, A. and Hytteørd, S. (2003). The effect of aluminium in Atlantic salmon (*Salmo salar*) with special emphasis on alkaline water. J. Inorg. Biochem., 97(1): 89-96.

Reitman, S. and Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin., 28(1):56-63.

Sangeetha, S. and Rajendran, K. (2018). Effect of Cynodon dactylon on the haematological parameters and haematocrit of grass carp *Ctenopharyngodon idella* (Val.). J. drug deliv. ther., 8(6-s): 192-194.

Santiago, C. B.; Aldaba, M. B.; Laron, M. A. and Reyes, O. S. (1988). Reproductive performance and growth of Nile tilapia (*Oreochromis niloticus*) brood stock fed diets containing Leucaena leucocephala leaf meal. Aquacult., 70(1-2): 53-61.

Sayed, A.H.; Ibrahim, A.T.; Mekkawy, I.A.A. and Mahmoud, U.M. (2007). Acute effects of Ultraviolet-A radiation on African Catfish *Clarias gariepinus* (Burchell, 1822). J. Photochem. Photobiol., 89(2-3): 170-174.

Siddiqui, M. S.; Amy, G. L. and Murphy, B. D. (1997). Ozone enhanced removal of natural organic matter from drinking water sources. Water Res., 31(12): 3098-3106.

Sklan, D.; Prag, T. and Lupatsch, I. (2004). Apparent digestibility coefficients of feed ingredients and their prediction in diets for tilapia *Oreochromis niloticus*× *Oreochromis aureus* (Teleostei, Cichlidae). Aquacult. Res., 35(4): 358-364.

SPSS, (1997). Statistical package for the social sciences, Versions16, SPSS in Ch, Chi-USA.

Tavares-Dias, M. and Moraes, F.R. (2007). Leukocyte and thrombocyte reference values for channel catfish (*Ictalurus punctatus* Raf), with an assessment of morphologic, cytochemical, and ultrastructural features. Vet. Clin. Pathol., 36(1): 49-54.

Thomas, K.W. (1992). Conflict and conflict management: Reflections and update. J. Organ. Behav., 13(3): 265-274.

Trinder, P. (**1969).** Determination of Glucose in Blood Using Glucose Oxidase with an Alternative Oxygen Acceptor. Clin. Biochem., 6(1): 24-27.

Vhanalakar, S. A. (2009). Growth response of *Cirrhinamrigala* and *Cyprinus Carpio* to plants formulated diets as protein source.

Yılmaz, S. and Ergün, S. (2012). Effects of garlic and ginger oils on hematological and biochemical variables of sea bass *Dicentrarchus labrax*. J. Aquat. Anim. Health., 24(4): 219-224.

Yue, Y. R.; Liu, Y. J.; Tian, L. X.; Gan, L.; Yang, H. J. and Liang, G. Y. (2012). Effects of replacing fish meal with soybean meal and peanut meal on growth, feed utilization and haemolymph indexes for juvenile white shrimp L itopenaeus vannamei, B oone. Aquac. Res., 43(11): 1687-1696.

Vo, B. V.; Siddik, M. A.; Chaklader, M. R.; Fotedar, R.; Nahar, A.; Foysal, M. J. and Nguyen, H. Q. (2020). Growth and health of juvenile barramundi (*Lates calcarifer*) challenged with DO hypoxia after feeding various inclusions of germinated, fermented and untreated peanut meals. PloS one., 15(4): e0232278.

Zaahkook, S.A.M.; Hesham, G.; Mohamed, H.G. and Salah, M.A. (2016). Physiological and oxidative Stress biomarkers in the freshwater catfish (*Clarias gariepinus*) exposed to pendimethalin-based herbicide and recovery with edta. Int. J. Adv. Res., 4(10): 243-264.

Zhang, Z. H.; Chen, M.; Xie, S. W.; Chen, X. Q.; Liu, Y. J.; Tian, L. X. and Niu, J. (2020). Effects of dietary xylooligosaccharide on growth performance, enzyme activity and immunity of juvenile grass carp, *Ctenopharyngodon idellus.* Aquac. Rep., 18: 00519.

Zheng, Q.; Wen, X.; Han, C.; Li, H. and Xie, X. (2012). Effect of replacing soybean meal with cottonseed meal on growth, hematology, antioxidant enzymes activity and expression for juvenile grass carp, *Ctenopharyngodon idellus*. Fish Physiol. Biochem., 38(4): 1059-1069.