DETERMINATION OF THE BLOOD HEALTH STATUS OF CATFISH FED DIETS SUPPLEMENTED WITH DIFFERENT LEVELS OF OYSTER MUSHROOM (*PLEUROTUS OSTREATUS).*

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BENIN CITY

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A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF AQUACULTURE AND FISHERIES MANAGEMENT, FACULTY OF AGRICULTURE, UNIVERSITY OF BENIN, BENIN CITY, NIGERIA IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF AGRICULTURE (B. AGRIC) DEGREE IN FISHERIES

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CERTIFICATION

This is to certify that this project was carried out by Amarachi Linda ALOZIE in the Department of Aquaculture and Fisheries Management, Faculty of Agriculture, University of Benin, Benin City.

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DEDICATION

This project work is dedicated to God Almighty for His providence throughout my academic journey. To my parents Mr& Mrs. Vincent Alozie and my siblings for their prayers, motivation and support and to my fiance Francis Ijezie for his love, encouragement and prayers.

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ABSTRACT

This study was carried out to determine the Hematology performance of *Clarias gariepinus* fed diets supplemented with oyster mushroom *(Pleurotus ostreatus)* at different inclusion levels. 300 post juveniles were stocked randomly into five tanks at a density of 60 fish per tank; (T1 0%, T2 1.0%, T3 1.5%, T4 2.0%, T5 2.5%) and were fed twice daily to satiation with the prepared diet. Each treatment was replicated 3 times. The experiment lasted for a period of 9 weeks after which blood samples were randomly collected three per treatment for haematological studies and data was subjected to analysis of variance. The result of this study showed that WBC had the highest value of 25.17×10^3uL at T4 and the lowest value at T5 (16.37×10^3uL), RBC performed better at T3 (2.23×10^6uL) and lowest at T5 (1.70×10^6uL) and HCT values were found to be in the normal range for catfish, T3 (1.5%) being the highest and T5 recorded the lowest 2.5% inclusion level of mushroom.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of Study

The African catfish *(Clarias gariepinus)* is omnivorous, grows fast and tolerate relatively poor water quality, therefore it is a excellent species for aquaculture (Rad *et al.,* 2003).

The Fish is important as a valuable source of animal protein in human diets. Its demand in developing countries is higher than the supply (Ashley-Dejo and Adelaja, 2022). Fish has continued to be the most easily affordable source of animal protein to millions of people in both developed and developing nation (FAO, 2020). It is a good source of thiamine, riboflavin, vitamins A and D, phosphorus, calcium and iron (FAO, 2020). In meeting the supply of fish most especially in developing countries there is need to venture into more productive fish production enterprise (Ashley-Dejo and Adelaja, 2022). Fish production in Nigeria is adversely affected by spontaneous rise in cost of production especially fish feed which account for over 65% of cost of production (Ashley-Dejo *et al.,* 2017). In order to intensify fish production through aquaculture, adequate nutrition would be required for attaining high fish yields. Okoye and Sule (2001) stated that the rapid expansion and success of commercial fish culture depends largely on availability of good quality and cheap feed. To fish farmers, in order to minimize running cost, it is important to use cheaper alternative feed ingredients that are locally available to produce good quality, suitable and palatable fish feeds (Omojowo *et al.,* 2010; Ashley-Dejo *et al.,* 2014).

For the purpose of nutritional and economic benefits, researchers have made attempts at increasing the use of non-conventional plant and animal materials to replace conventional feed ingredients such as maize and fish meal in fish feed (Falaye, 1988;

Fagbenro, 1992; Olatunde, 1996; Baruah *et al.,* 2003; Eyo, 2003). The use of cereal products, especially maize in fish feeds is becoming increasingly unjustified in economic terms, because of its ever-increasing cost. There is therefore the need to exploit cheaper energy sources to replace expensive cereals. Also, to relieve the food competition between man and animal and for-profit maximization.

Two major components (protein and energy) are important in fish feed for growth and reproduction. They are needed in adequate supply both in quality and quantity to enable fish reach their full growth potential. Conventionally, fishmeal and cereals have been used as protein and energy sources respectively (El-Sayed, 1999; Gatlin *et al.,* 2007).

Supplementary feed has been known to increase the carrying capacity of culture systems and also enhance fish production (Hepher, 1975; Devaraj *et al.,* 1986), among natural products with high potential are mushrooms, a viable alternative in preparing foods that can provide high nutritional value and immunostimulant properties for fish's good development and growth within the culture (Ahmed *et al.,* 2017).

The mushroom *Pleurotus* species, is most cultivated globally for being edible and nutritious (Singhal *et al.,* 2019). Studies have shown that *Pleurotus* species*,*contains polypeptides and has potential in functional foods as a natural and anti-virus agent with antioxidant and immunostimulatory activities(Sun *et al.,* 2017). The physiological and metabolic conditions in culture condition can be improved by mushroom (*Pleurotus ostreatus*) as it improves the growth of the fish. (Khalafalla and El-Sayed, 2015).

Mushrooms are the fruiting body of micro-organisms, also known as fungi. Like other plants, mushrooms lack chlorophyll and do not produce through the process of photosynthesis. It has medicinal values, aside from being good source of carbohydrate, vitamins, minerals, antimicrobial and therapeutic qualities (Batool *et al.,* 2020). The wellbeing of fish is reflected significantly on its blood profile, this have made haematology major monitoring indicator in fish culture (Jan and Ahmed, 2021)

Blood in fish transports a variety of constituents such as nutrients, hormones, minerals, immune components, microorganisms, water, gases, toxins, and waste products (Ciesla, 2007). The most important functions of blood are the supply of oxygen and nutrients (including glucose, amino acids, and fatty acids) to cell tissues, removal of wastes (such as carbon dioxide, urea, and lactic acid), immunological functions, coagulation, and messenger functions (Ciesla, 2007). Given the diverse critical roles of blood, measuring blood parameters may provide a more reliable picture of fish metabolism and health status.

1.2 Justification

Pleurotus ostreatus, along with other species of mushroom, has been confirmed to have medicinal value. The biological functionality of these mushrooms ranges from antioxidative and immuno-stimulating to antiviral, anti-carcinogenic, anti-hypercholesterolaemic, and the ability to regulate blood lipid and glucose levels (Gordon *et al.,* 1998; Wasser and Weis, 1999; Lakhanpal and Rana, 2005).

Hematological studies of cultured fish important in order to monitor the health of fish during culture. Such studies are particularly useful in assessing fish physiological and physio-pathological status since morphological and biometric do not always give a complete picture (Adakole, 2012; Travares-Dias and Morases, 2007).

In recent years, hematological parameters have been used to evaluate the response to applying additives and products of natural origin and determine tilapia's health status (Reda *et al.,* 2016)

It was found that the supplementation of a mushroom meal in a proportion of 15 to 25% in the diet of the fish can improve the health status, growth, and survival of Nile tilapia, which is in accordance with what has been reported for the response of tilapia to the inclusion in the diet of other species of *Pleurotus* spp. (Srichanun *et al.,* 2017, Safari & Sarkheil 2018).

In general, the inclusion of mushroom in the diet has increased the hematology and physiological response, high growth rate and has functioned as immunostimulant (Hleap-Zapata *et al.,* 2021). However, studies of the effect of mushroom on the blood response and serum biochemical parameters in *Clarias gariepinus* are limited.

This study is aimed at evaluating the effects of supplementing experimental fish diets with graded levels of *Pleurotus ostreatus* in the culture of *Clarias gariepinus* hematological parameters.

1.3 Aim and Objectives of Study

The aim of this study is to determine the blood health status of African catfish *(Clarias gariepinus)* fed diet supplemented with different levels of oyster mushroom (*Pleurotus ostreatus*).

Specific objectives of the study are to determine the:

1. white blood cells (WBC) of fish fed different levels of mushroom supplemented diets
2. hemoglobin estimation (Hb) of fish fed different levels of mushroom supplemented diets
3. hematocrit (Ht) of fish fed different levels of mushroom supplemented diets
4. platelets

5. red blood cells (RBC) and its indices

CHAPTER TWO

2.0 LITERATURE REVIEW

Mushroom is a macro fungus which has distinctive fruiting bodies that can either be epigeous (above ground) or hypogenous (below ground) large enough to be seen with the naked eye and to be picked by hand (Chang, 1992). Due to lack of chlorophyll on mushroom it cannot synthesis it’s own food so it depends on dead and decay matters, therefore they are saprophytic fungi. And it has the potential to solve many growing global problems like food demand, unemployment, environmental pollution amongst others.

Among the different types of mushroom, *Pleurotus ostreatus* is one of the most cultivated species. *Pleurotus ostreatus* belongs to the family *Agaricaceae* and class *Basidiomycetes* commonly known as “Dhingri” in India. Oyster mushroom is known for rich content in Vitamin C and Vitamin B complex and its protein content varies from 16 to 25 percent along with mineral salt which is essential for human body (Randive, 2012).

Oyster mushroom is also known for its medicinal value in fighting diabetics and cancer (Sivrikaya *et al.,* 2002). Due to its high potassium to sodium ratio its is ideal for people suffering from heart disease and hypertension (Sharma *et al.,* 2013).

Mushroom cultivation depends on many factors such as temperature, humidity and sterility of the substrate (Bellettini *et al.,*2019)

2.1 Common Cultivated Mushrooms

Although there are over 300 genera of mushroom only few of the species are cultivated commercially. Some of the commonly cultivated mushroom are given below in Table 1.

Table 1: Different species of cultivated mushroom (Nongthombam *et al.,* 2021)

Common name	Scientific name
Button mushroom	*Agaricus bisporus*
White jelly fungi	*Tremella fuciformis*
Oyster mushroom	*Pleurotus ostreatus*
Paddy straw mushroom	*Volvariella volvacea*
Velvet stem mushroom	*Tuber aestivum*
Ear fungus	*Auricularia auricila*
Reishi mushroom	*Ganoderma lucidum*
Nameko mushroom	*Pholiota nameka*
Shiitake mushroom	*Lentinus edodes*
Truffle velvet stem mushroom	*Flammulina velutipes*

Plate 1: Button mushroom

Plate 2: White jelly fungi

Plate 3: Oyster mushroom

Plate 4: Paddy straw mushroom

Plate 5: Velvet stem mushroom

Plate 6: Ear fungus

Plate 7: Reishi mushroom Plate 8: Nemako mushroom

Plate 9: Shiitake mushroom Plate 10: Truffle velvet stem mushroom

2.2 Production of Oyster Mushroom

Mushroom are grown mostly on waste materials like sawdust, composting materials, straw and garbage (Gbolagade 2006). It is reported that mushroom cultivation started in France around 1630, to monitor different growth parameters of mushroom a series of experiments and studies have been conducted since then (Miles *et al.,* 2004)

2.3 Climatic Requirements

Bano and Rajarathnam (2017) observed maximum yield of oyster mushroom during season, when the temperature are nearly 20-26°C and relative humidity 70-90%. A fairly good yield can be obtained up to 30°C.

2.4 Steps in The Production of Oyster Mushroom

2.4.1 Substrate Preparation

Mostly used substrates are organic materials like lingo-cellulosic farm waste on which growth of mycelium takes place to produce mushroom, substrate used include sawdust, rice bran, rice straw, wheat bran and wheat straw (Khare *et al.,* 2018).

Oseni *et al.* (2012) while working on substrate preparation and processing used two locally available substrates namely: sugarcane base and horse manure compost. To each of the substrates, 20% of wheat bran supplement was added, thoroughly mixed and soaked in a clean water for 8-10 hours and allot imbibe the water. The substrates were separately subjected to short composting procedure using the method of Sinden and Hauser (1980). The substrates were divided into batches of 500g each and packed into heat resistant autoclavable bags of 18×14 cm inside, which were fastened at the end using plastic rings. The substrates were then pre-treated using the following methods- steam sterilization in autoclave at 121°C for 4 hours, hot water dipping in steel drum at 60°C (pasteurization) for 2 hours and hot water dipping in steel drum at 60°C (pasteurization) for 3 hours and allowed to cool.

2.4.2 Spawning of Substrate

Spawning can be prepared from different carriers of grain such as wheat, sorghum, barley and rice (Khonga *et al.,*2013). It is reported that as compared to grains of wheat and barley, grains of sorghum are better mycelium carrier. The yield and biological efficiency can be increased by colonization of the spawn and spawning running time gets reduced (Khare et al., 2004).

The quality of carriers and moisture (60-70%) greatly affect the quality of spawn as it determine the growth and colonization of the mycelium on the substrate. The spawned substrate requires a certain temperature ranging from 25- 30% and a dark room which satisfy the mycelia for proper growth and to colonise the substrate

2.4 3 Source of Substrate

Common substrates for growing mushroom involve straw or hardwood sawdust.

The mushroom substrate needs to be properly prepared by adding water, potentially adding additional nutrient and further processed by sterilization or pasteurization. When ready it can be inoculated wherein broken up pieces of mycelium- covered grain also known as grain spawn are evenly mixed into the substrate.

2.4.4 Incubation and Fruiting

During the incubation period temperature is optimised to 20-25°C in order to get best results and kept in the incubation room without disturbance for 15-25days also depends on size and conditions of the bags (Saurab Dulal, 2019). At the time of fruiting, the relative humidity should be maintained at 80-85% by spraying and sprinkling water to the gunny bags or in the sand spread on the floor with a nozzled bottle 2-3 times daily.

2.4.5 Harvesting and Yielding

The procedure for mushroom harvesting involves grasping each mushroom stalk individually and twisting the mushroom until it pull out of the substrate. As it begins fruiting it is important to keep humidity high and temperature and constant spray of water it may be harvested in numbers of flushes (cropping cycle) (Asmama *et al.,* 2015).

2.5 Blood Parameters

2.5.1 White blood cells (WBC) Leukocyte count

Leukocyte count (WBC) is an important parameter in the assessment of the immune status in vertebrates. As previously reported by different authors, a manual method is commonly used to determine the number of white blood cells (Svobodova *et al.,* 1991; Svobodova *et al.,* 2012; Lugowska *et al.,* 2017; Bojarski *et al.,* 2018a). Fish white blood cells are counted in diluted blood using a hemocytometer and microscope, similarly as erythrocytes. According to Witeska *et al.* (2016), Natt-Herrick's, Prochazka-Skrobak's or Dacie's solutions were most often applied by various authors to assess the number of WBC. Sometimes an indirect method is applied that involves calculation of WBC using RBC value and the ratio of leukocytes to erythrocytes in the blood smear (Tavares-Dias *et al.,* 2002). Identification of leukocytes in a stained smear may be easier than in hemocytometer but lymphocytes are sometimes similar to round forms of thrombocytes. Lugowska *et al.* (2017) result showed that the leukocyte count was significantly affected by the rater (the influence of the person who performed the procedure) and the type of method (direct vs. indirect) – WBC values obtained using the indirect method were higher compared to direct hemocytometer counting. Faggio *et al.* (2013) revealed that there was no significant difference between manually and automatically obtained WBC, although automatic analysers are not often used for fish leukocyte determination. WBC values in fish show wide ranges and are very variable even within the same species. According to Svobodova *et al.* (2012) WBC in *C. carpio* was 10–80 × 10^3/µL. Review of WBC values in the blood of juvenile *C. carpio* obtained by various authors was 1.4 to 197.3 × 10^3/µL (Witeska *et al.,* 2016). Fazio *et al.* (2019) reported that WBC determined automatically in different fish species ranged from 9.41 to 829.33 × 10^3/µL. Moreover, many intrinsic and environmental factors may affect leukocyte count in fish such as sex, season, feeding habits, stress, aquatic pollution and diseases (Ahmed *et al.,* 2020).

2. 5.2 Erythrocyte Count (Red Blood Cell)

According to Fange (1994), erythrocytes are the most abundant blood cells in fish. They usually constitute 98–99% of all blood cells in these animals. Erythrocyte count (RBC), an important diagnostic parameter, depends on various environmental factors like water temperature (Paul *et al.,* 2019). It can also be modulated by many biological factors, such as fish activity, age, sex, nutritional state, and reproductive status, and can differ among various populations of the same species. RBC usually ranges from 0.5–1.5 × 10^6/µL in less active species to 3.0–4.2 × 10^6/µL in more active ones (Witeska, 2013). According to Fazio *et al.* (2019), RBC determined automatically in different fish species ranged between 0.81 to 3.73 × 10^6/µL. RBC measured in the blood of *C. carpio* was found to be 0.33 to 2.95 × 10^6/µL (Witeska *et al.,* 2016). It is also worth mentioning that erythrocyte count can show seasonal variability, even if fish are kept under laboratory conditions (Kondera *et al.,* 2019). For RBC determination both manual and automatic counting methods can be used

(Faggio *et al.,* 2013), although in the case of fish, majority of researchers choose manual analyses (Bojarski *et al.,* 2015, Witeska *et al.,* 2017a, Witeska *et al.*, 2017b, Bojarski *et al.,* 2018a, Kondera *et al.,* 2020). Faggio *et al.* (2013) revealed that manually obtained RBC was similar to the automatically measured values. The manual determination of RBC values usually uses the Bürker, Neubauer or Thoma haemocytometer (Acar *et al.,* 2018; Fazio, 2019). For manual calculation of erythrocyte count blood should be diluted 1:100 with a diluent in a plastic Eppendorf tube and gently mixed. The use of glass blood diluting pipettes (Malassez-Potain) for fish blood may result in formation of micro clots and thus in considerable calculation error. Immediately before erythrocyte counting in a hemocytometer diluted blood must be thoroughly and gently mixed (very vigorous mixing may result in bubble formation and cell damage). Transfer of diluted blood into a hemocytometer chamber covered with a cover glass may be done using hematocrit capillary tube or a micropipette. First drops should be rejected and then capillary or pipette opening should touch the edge of the cover glass so as diluted blood could fill the hemocytometer chamber by itself, using fluid capillary force, without air bubbles. Cell counting should be done under × 400 magnification, after the cells settle on the chamber bottom. Correct counting of cells in a hemocytometer grid involves “the rule of two edges”: cells that are on or touching the top and left lines are counted, but the ones on or touching the right or bottom lines are ignored. Calculation of RBC is performed using the number of counted cells, number of squares in which they were counted, square volume and blood dilution.

2.5 2.1 Erythrocytes indices (MCV, MCH and MCHC)

The hematological examination also includes the analysis of additional erythrocyte indices which describe the morphology and properties of red blood cells like the

MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin) and MCHC (mean corpuscular hemoglobin concentration). They can be calculated using Ht, RBC and Hb values, according to the formulas: MCV = (Ht × 10)/RBC, MCH=Hb/RBC, and MCHC = (Hb × 100)/Ht or determined automatically. Faggio *et al.* (2013) showed that manually obtained MCV, MCH and MCHC did not differ from automatically obtained results. The data summarized by Witeska *et al.* (2016) indicate that MCV for *C. carpio* was in the range of 130.9–367.3 fL, MCH 31.8–139.0 and MCHC 150–446 g/L. The analysis of these parameters may be useful in diagnostics of some fish diseases, like anemia. The increase in MCV is a "low cost" response to anaemic state in some fish species. However, fish suffering with anemia can exhibit decreased values of all red blood cell parameters (Witeska, 2015). Lay and Baldwin (1999) analysed MCV values in many fish species and demonstrated that erythrocyte volume is highly correlated with oxygen delivery, however, many of the analysed MCV changes could not be explained. Increased values of MCV and MCH were observed in fish suffering from ichthyophthiriasis (Witeska *et al.,* 2010a).

2.5.3 Hematocrit (Ht) or Packed cell volume (PCV)

Hematocrit (or packed cell volume) is the simplest measure of erythrocyte content in blood as a percentage of erythrocytes in blood volume. For hematocrit (Ht) determination in fish blood analysis, microhematocrit method is a standard, although automated analyzers to determine this parameter are also used (e.g. Harabawy and Ibrahim, 2014; Velmurugan *et al.,* 2016; Ghayyur *et al.,* 2019). According to Gebretsadkan *et al.* (2015), in the case of human blood analysis, the manual Ht and automated Ht were significantly different. Furthermore, the results showed higher coefficient of variation in manual method as compared to automated procedure, which indicated that the precision was higher in the case of automated method. However, the results obtained by Faggio *et al.* (2013) showed no statistically significant difference between Ht values obtained for fish blood using manual and automated methods. Nevertheless, hematocrit value determined by microhematocrit method can be obtained with different centrifugation parameters, for example 3000 rpm for 30 min (David *et al.,* 2015), 10,000 rpm for 5 min (Girón-Perez *et al.,* 2008), 12,000 rpm for 5 min (Witeska *et al.,* 2017a), 16,000 g for 5 min (Gul *et al.,* 2012), which may probably affect the results. According to data summarized by Grant (2015), Ht values for different fish species (including various teleosts and elasmobranchs) range from 9.4 to 33.53%. According to Fazio *et al.* (2019) Ht measured automatically in different fish species was from 17.80 to 53.33%. In the case of *C. carpio* the range obtained by different authors was 14.0–44.0% as reviewed by Witeska *et al.* (2016). Hematocrit value depends on erythrocyte number and size and can be affected by various factors, such as water quality parameters, drugs and some infectious diseases (e.g. McBeath *et al.,* 2015; Witeska *et al.,* 2015).

2.5.4 Hemoglobin concentration (Hb)

Measurement of haemoglobin concentration in fish blood is routinely performed using spectrophotometric cyanmethemoglobin method. It involves mixing the whole blood with Drabkin reagent to convert blood hemoglobin to stable methemoglobin and next reading extinction at 540 nm wavelength. Hemoglobin concentration can be calculated on the basis of the relationship between standard hemoglobin solutions and their extinction. The results obtained with manual spectrophotometric method and the values obtained with automatic method did not differ statistically (Faggio *et al.,* 2013). According to Fazio *et al.* (2019) Hb determined automatically in different fish species was from 4.70 to 16.6 g/dL. The study performed by Witeska *et al.* (2016) exhibited that blood Hb level in clinically healthy 5–8 month old *Cyprunis carpio* was 62.4–

69.6 g/L. The literature data revised by Witeska *et al.* (2016) show that Hb in *C. carpio* is in the range of 34.1–114.3 g/L. Suljevic *et al.* (2016) showed that hemoglobin concentration in *C. carpio* (56.80– 87.50 g/L) was significantly lower than *Carassius carassius* Hb level (60.42–97.66 g/L). Hemoglobin concentration in the blood of *Solea senegalensis* juveniles kept under intensive aquaculture conditions was 26–63 g/L (Peres *et al.,* 2015).

2.5.5 Thrombocyte count (TC) / Platelets

Thrombocytes are described as the most abundant blood cells after erythrocytes, is frequently used as an indicator of health status because they are mainly involved in forming defensive barriers (Magnadóttir, 2006).

According to Stosik *et al.* (2002) and Stosik *et al.* (2019), fish thrombocytes participate in haemostatic processes and in the immune response. Thrombocytes count may change in relation to various biotic factors (age, sex, season, maturity status) and abiotic conditions (water temperature, pH, dissolved oxygen level) and stress (Tavares-Dias and Oliveira, 2009), feeding habits (Ahmed and Sheikh, 2020) or infection (Parrino *et al.,* 2018). Thrombocythemia was observed in fish suffering from acute form of branchiomycosis and erythrodermatitis while thrombocytosis was noted in fish diagnosed with chronic form of these diseases (Stosik *et al.,* 2001). Martins *et al.* (2008) reported an increase in thrombocyte count in *O. niloticus* infected with *Enterococcus* sp.

According to various authors, Thrombocyte count values show high inter- and intraspecific variability, e.g. in *C. carpio* 0.9–115 × 10^3/μL (Witeska *et al.*, 2016), in *C. gariepinus* 106–458 × 10^3/μL and in *Chrysichthys nigrodigitatus* 106–362 × 10^3/μL (Adedeji and Adegbile, 2011), in *Sorubim lima* 9.0–19.9 × 10^3/μL (Bianchi *et al.,* 2014), in *Rhamdia quelen*, *O. niloticus*, *Brycon orbignyanus* and *P. mesopotamicus* 2 1.9–75.9, 10.3–66.8, 22.8–54.1 and 16.1–41.6 × 10^3/µL, respectively (Dal'Bo *et al.,* 2015), in *Schizopyge plagiostomus* 22.8–34.4 × 10^3/µL or in *Schizopyge niger* 24.67–36.54 × 10^3/µL (Ahmed and Sheikh, 2020), and in various fish species 2.0–78.9 × 10^3/µL (Tavares-Dias and Oliveira, 2009).

2.6 Factors that may affect hematological values.

2 .6.1 Stress

Stress in fish may be caused by various factors, like chasing and harvesting (Fagundes and Urbinati, 2008; Aguirre-Guzman *et al.,* 2016), crowding (Barcellos *et al.,* 2004), transportation (Dobsikova *et al.,* 2009; Pilinkovskij *et al.,* 2017; Oliveira *et al.,* 2018), hypoxia (Pollock *et al.,* 2007; McBryan *et al.,* 2013), change in water temperature (Del Rio Zaragoza *et al.*, 2008; Ji *et al.,* 2016; Simide *et al.,* 2016), air exposure (Grzelak *et al.,* 2017), disease (Fazio, 2019), aquatic pollution (Zutshi *et al.,* 2010; Bojarski and Witeska, 2020) or in case of experimental conditions like starvation (Hernandez *et al.,* 2019).

Stress may change the values of red blood parameters (Ht, Hb, RBC, MCV) and various biochemical indices (glucose, catecholamine and cortisol levels). Increase in the values of red blood parameters often occurs due to stress since stress reaction involves higher energy expenditure (fight or flight) and increase in oxygen transport is one of stress-related adaptive mechanisms. In fish it may involve erythrocyte swelling (increase in MCV and Ht), release of splenic erythrocyte reserve or in a longer period of time increased erythropoiesis (increase in Ht, Hb and RBC). Stress-induced increase in red blood indices were reported by Dobsikova *et al.* (2009), Fazio *et al.* (2015) and Aguir re-Guzman *et al.* (2016). Stress may also affect white blood parameters. Short-term stress sometimes results in an increase in WBC but chronic and/or strong stress usually causes leukopenia (Tort, 2011).

2.6.2 Anaesthesia

Blood collection is a fast and little invasive procedure if the fish are big enough to make a puncture. In such a case, if the procedure is performed by a skilled and experienced person, anaesthesia usually is not necessary and may be even more stressful to fish than blood collection itself (Witeska *et al.,* 2017b; Soldatov, 2021). On the other hand, if the fish are very small so as blood sampling involves severance of the caudal peduncle or other invasive technique to access blood vessels, anaesthesia must be applied. Also, in case of blood sampling from very big fish or species susceptible to management, anaesthesia may be necessary to handle them.

Common anaesthesia used are MS-222, benzocaine, quinaldine, eugenol (clove oil), etomidate or 2-phenoxyethanol, or the fish are anesthetized with ice, stunned or killed before blood sampling. However, one should be aware that anaesthetics may affect hematological parameters. Rożyński *et al.* (2018) observed transient but significant hematological and biochemical alterations in *Perca fluviatilis* anesthetized with etomidate compared to the unanesthetized fish.

Alterations in blood biochemical values were also reported by Lepic et al. (2014) in *Vimba vimba* treated with 2-phenoxyethanol and etomidate, while clove oil showed no effect. According to Witeska *et al.* (2015) and Witeska *et al.* (2017b), 2-phenoxyethanol, etomidate and tricaine induced hematological changes in *Cyprinus* *carpio*. Therefore, anaesthesia should be used only when necessary and pilot trials should be performed to choose an anaesthetic, dose and time appropriate for a particular fish species and size.

2.6.3 Blood sampling

A review by Lawrence *et al.* (2020) provides recommendations of caudal puncture optimization to make it quick, easy and relatively non-invasive method for obtaining a blood sample. Method and site of blood collection may affect the results of analyses (Bojarski *et al.,* 2018a; Bojarski *et al.,* 2021). Bojarski *et al.* (2021) demonstrated that RBC count was higher in caudal vein blood, while Ht value, MCV and MCH were higher in cardiac blood samples.

In the case of very small fish such as *Danio rerio*, most methods of blood collection are lethal, such as lateral incision, decapitation or tail ablation. Therefore, alternative methods were developed to obtain blood from live fish, as puncture of dorsal aorta or posterior cardinal vein using a needle or glass capillary tube (Zang *et al.,* 2013; Zang *et al., 2014;* Argungu *et al.,* 2017).

Sampling should be done using a heparinized needle into a chilled heparinized plastic Eppendorf tube. Blood should be gently mixed immediately after sampling to prevent clot formation and then kept refrigerated (for later analyses) or placed in a blood roller mixer if analyses are performed immediately (Svobodova *et al.,* 1991; Svobodova *et al.,* 2012; Witeska *et al.,* 2016).

2 6.4 Anticoagulant

Blood coagulation in fish follows a similar pattern as observed in other vertebrates (Jiang and Doolittle, 2003). However, clot formation in fish is faster than in mammals and, according to Tavares-Dias and Oliveira (2009) lasts from 10.8 to 530 seconds in various species. Rapid decline of whole blood clotting time takes place when the number of thrombocytes increases (Casillas and Smith, 1977). Li *et al.* (2006) found that water initiated blood clotting in *C. carpio*.

Hattingh (1975) observed that EDTA caused haemolysis of blood from 5 fish species (*C. carpio*, *Labeo umbratus*, *L. capensis*, *Clarias gariepinus* and *Barbus holubi*) within 1–48hours and always increased cell volume. Similar observation was reported by Korcock *et al.* (1988) for *Oncorhynchus mykiss*. Increases in Ht, MCV and haemolysis in EDTA stored blood of *Piaractus mesopotamicus* were observed by Vaz Farias *et al.* (2016). However, Tavares-Dias and Silva Sandrim (1998) found that EDTA reduced Ht and Hb values in blood of *Colossoma macropomum* compared to heparin.

According to Adeyemo *et al.* (2009), both heparin and EDTA can be used for hematological evaluation of *C. gariepinus* blood.

2.6.5 Blood storage

Korcock *et al.* (1988) reported an increase in MCV and changes in plasma and cell Na and K content in *Salmo gairdneri* blood stored for 24 hours at room temperature, while no alterations were observed in refrigerated samples. According to Tavares-Dias and Silva Sandrim (1998), 10 hours storage at room temperature did not affect Ht, Hb and MCHC values in *C. macropomum*. The results obtained by Witeska *et al.* (2017a) showed that storage of *C. carpio* blood for 2 hours at 22 °C resulted in a decrease in RBC, WBC and TC, while at 4 °C an increase in MCH and MCHC, and WBC decrease occurred after 24 hours.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of Study Area

The study was carried out at the Department of Fisheries and Aquaculture, University of Benin, Ugbowo, Benin City and analysis of blood samples was carried out at the University of Benin Teaching hospital, Ugbowo, Benin City.

3.2 Experimental Design

The experiment was laid out as a Randomised Complete Block Design (RCBD).

3.3 Experimental Fish Culture and Management

Before commencement of this experiment, 300 post juveniles of *Clarias gariepinus* was procured from a reputable hatchery in Benin city and then acclimatised before transferring to the experimental tanks.

The experiment made use of 5 tanks with 3 replicates, making a total of 15 tanks. Each tank will be properly washed and disinfected, after which water was impounded before use and labelled appropriately according to the different experimental diet used. Each tank was stocked with 20 post juveniles of *Clarias gariepinus.* The experiment lasted for a period of 9weeks.++

3.4 Preparation of Diets Supplemented with *Pleurotus ostreatus*

Freshly harvested oyster mushroom (*Pleurotus ostreatus*) to be used in the feed formulation was obtained from MYCO Farm, rinsed severally before been oven dried at 60°C for 48 hours in the laboratory, subsequently grounded to obtain a smooth mushroom powder (MP), to be used in the preparation of ration. The proximate composition of the mushroom was determined and was used to supplement the regular diet at different inclusion levels on a dry matter based (0%, 1.0%, 1.5%, 2.0% and 2.5%)

Five Iso-nitrogenous diets of 40% crude protein was compounded, formulation of fish feed was calculated using Pearson square method (Hardy, 2000). The calculation was based on protein and energy requirements at different stages of growth. Protein source include fish meal, soybean, energy source include maize, sorghum. Other ingredients such as palm oil, bone meal, vitamin premix and starch which served as binder before being extruded into pellets. These ingredients as procured from a local market in Benin city. The formulation was done based on 40% crude protein for post juvenile and 30% crude protein for semi adult size. Fish was fed to satiation 2 times (8am, and 5pm) daily.

The mushroom was then used to supplement the feed at 0%, 1.0%, 1.5%, 2.0% and 2 5% as shown in Table 2. The feed was pelletized into three sizes; 2mm, 3mm and 4mm to fit the fish gape as they grow throughout the culture period.

Table 2: Ingredients and their different inclusion levels in percentages

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Fish meal	20.0	19.8	19.7	19.6	19.5
Rice bran	7.0	6.92	6.88	6.84	6.8
Maize	20.0	19.76	16.64	19.54	19.4
Groundnut cake	25.5	25.26	25.14	25.02	24.6
Soybean meal	20.0	19 84	19.76	19.68	19.6
Blood meal	5.0	4.92	4.88	4.84	4.8
Oyster mushroom	0.0	1.0	1.5	2.0	2.5
Starch	1.2	1.2	1.2	1.2	1.2
Palm oil	1.0	1.0	1.0	1.0	1.0
Vitamin premix	0.3	0.3	0 3	0.3	0.3
Total	100	100	100	100	100

3.5 Hematological and serum protein evaluation of *Clarias gariepinus.*

Hematological evaluation of fishes includes:

- determination of total erythrocyte count (RBC),
- hematocrit (PCV),
- hemoglobin concentration (Hb),
- erythrocyte indexes (Mean corpuscular volume MCV, Mean corpuscular haemoglobin MCH, Mean corpuscular hemoglobin concentration MCHC),
- white blood cell count (WBC)
- platelets concentration

3.5.1 Blood sample collection

Blood sample for haematology studies was collected from fifteen randomly selected fish per treatment at the end of the experiment.

The fish was sacrificed and 2ml of blood samples was collected with a sterile syringe and kept in a bottle containing ethylenediamine tetra acetate (EDTA) as anticoagulant.

3.6 Blood Analysis

3.6.1 PCV/ Hematocrit

Anticoagulated blood was taken in a wintrobe tube using the wintrobe method. The wintrobe tube was filled from the bottom up to 100 mark using a pasteur pipette. After which the blood sample was centrifuged using an hematocrit centrifuge.

3.6 2 Red blood cell count

Fish blood was diluted with Neubauer pipette with citrate buffer fluid at 1:200. The diluted blood will be introduced into a Neubauer counter and white blood cells was counted under the microscope (Shah and Altindag, 2004).

3.6.3 Red blood cell indices

3.6.3.1 Mean corpuscular volume (MCV)

MCV was calculated from the hematocrit values (PCV and the RBC count)

$$\text{MVC} = \frac{\text{Hematocrit } (\%) \times 10}{\text{RBC (million/L blood)}}$$

3.6.3.2 Mean corpuscular hemoglobin concentration (MCHC)

MCHC was calculated using Heamoglobin concentration and hematocrit

$$\text{MCHC } (\%) = \frac{\text{Hb concentration (g/dl)}}{\text{Ht}(\%)} \times 100$$

3.6.4 White blood cell count

Fish blood was diluted with Neubauer pipette with citrate buffer fluid at 1:20. The diluted blood was introduced into a Neubauer counter and white blood cells was counted under the microscope at a magnification of ×40 (Shah and Altindag, 2004).

3.6.5 Platelets concentration

Platelet counts are usually performed using blood collected with EDTA as the anticoagulant. Stained blood films will be examined each time platelet counts are done to verify that low platelet counts determined manually or by machine are valid (John and Harvey, 2012).

$$\text{Number of platelet} = \frac{\text{Number of cells counted} \times \text{dilution}}{\text{Area counted} \times \text{Depth of fluid.}}$$

3.8 Statistical analysis

Data collected was subjected to analysis of variance (ANOVA). Comparison of difference among individual means was carried out using the New Duncan Multiple Range Test (DMRT) at 5% probability level (Snedecor and Cochran, 1988)

CHAPTER FOUR

4.0 HEMATOLOGY

The result of the mean hematological parameters of *Clarias gariepinus* post juveniles fed oyster mushroom based diets at different inclusion levels is as presented in Table 3.

4.1 White blood cell (10^3/uL)

The values obtained for WBC is shown in Table 3. T1 and T2 was not significantly different ($p>0.05$) but increased gradually with T4 having the highest value and T5 having the lowest value.

4.2 Red blood cell (10^6/uL)

The RBC highest recorded value was T3 and this was significantly different ($p<0.05$) from other treatments. T1, T2 and T4 were not significantly different ($p>0.05$), while T5 recorded the lowest.

4.3 Hematocrit (%)

The values obtained for HCT in T1-T4 were not significantly ($p>0.05$), only T5(18.67%) was significantly different ($p<0.05$) from all the other treatments with T3 having the highest value of 26.90% and T5 had the lowest value (18.67%).

4.4 Mean corpuscular hemoglobin (pg)

The result of MCH value between the control (T1) and T2 were not significantly different ($p>0.05$) but were significantly different ($p<0.05$) from the others. These other treatments did not differ significantly amongst themselves, although T2 (36.83pg) at 1% inclusion mushroom level resulted to higher MCH.

4.5 Mean corpuscular hemoglobin concentration (g/dL)

The MCHC value did not differ significantly amongst the treatments but T5(29.07g/dL) ranked lowest during the period of this study.

4.6 Mean corpuscular volume (fL)

T3 recorded the highest value (120.90fL) and this was significantly different ($p<0.05$) from T1(118.10fL) and T2(117.53fL) which showed no significant differences ($p>0.05$), T4 (106.03fL) and T5 (110.17fL) recorded the lowest with no significant differences.

4.7 Hemoglobin (g/dL)

The hemoglobin value during the period of this study showed no significant differences ($p>0.05$) T1, T2 and T4, but T3 recorded the highest value (8.57g/dL) and T5 recorded the lowest value at (5.43g/dL).

4.8 Platelets (10^3/uL)

The platelet value recorded in the period of study showed that T2 the highest value, followed by T3 and T1 which had no significant differences ($p>0.05$) among themselves while T4 and T5 recorded the lowest with no significant differences ($p>0.05$)

Table 3: Hematological profile of *Clarias gariepinus* fed experimental diets supplemented with graded levels of mushroom.

Parameters	**T0 (0%)**	**T1 (1.0%)**	**T2 (1.5%)**	**T3 (2.0%)**	**T4 (2.5%)**	**F**	**p**
WBC (10^3/uL)	20.53±1.53^{a}	20.13±2.31^{a}	24.37±4.90^{a}	25.17±14.82^{a}	16.37±2.56^{a}	0.737	0.588
RBC (10^6/uL)	2.03±0.04^{a}	2.02±0.18^{a}	2.23±0.20^{a}	2.05±0.60^{a}	1.70±0.12^{a}	1.221	0.361
HCT (%)	23.90±2.01^{a}	23.77±2.87^{a}	26.90±2.52^{a}	21.30±4.68^{a}	18.67±1.06^{a}	3.439	0.051
MCH(pg)	35.97±2.15^{a}	36.83±2.86^{a}	31.63±15.46^{a}	32.30±1.40^{a}	31.93±0.70^{a}	0.358	0.833
MCHC(g/dL)	30.57±1.12^{a}	31.50±3.12^{a}	31.67±2.06^{a}	30.67±2.00^{a}	29.07±1.03^{a}	0.788	0.559
MCV(fL)	118.10±11.11^{a}	117.53±7.90^{a}	120.90±10.69^{a}	106.03±9.66^{a}	110.17±3.93^{a}	1.408	0.300
HGB(g/dL)	7.30±0.36^{abc}	7.43±0.25^{bc}	8.57±1.36^{c}	6.57±1.66^{ab}	5.43±0.29^{a}	4.122	0.032
PLT (10^3/uL)	63.00±19.97^{a}	74.33±16.50^{a}	63.67±17.04^{a}	38.00±11.53^{a}	36.67±15.04^{a}	3.230	0.060
LYM (%)	97.47±0.55^{a}	97.00±0.72^{a}	96.07±1.70^{a}	92.90±7.80^{a}	97.37±0.51^{a}	0.839	0.531
MID (%)	1.73±0.32^{a}	2.07±0.55^{a}	3.00±1.56^{a}	3.83±3.79^{a}	1.67±0.40^{a}	0.753	0.578
GRAN (%)	0.80±0.26^{a}	0.93±0.21^{a}	0.93±0.21^{a}	3.27±4.01^{a}	0.97±0.12^{a}	1.029	0.439
LYM (10^3/uL)	20.00±1.39^{a}	19.53±2.20^{a}	23.37±4.35^{a}	22.67±11.23^{a}	15.93±2.41^{a}	0.826	0.538
MID (10^3/uL)	0.37±0.12^{a}	0.43±0.15^{a}	0.77±0.55^{a}	1.37±1.85^{a}	0.30±0.10^{a}	0.772	0.568
GRAN (10^3/uL)	0.17±0.06^{a}	0.17±0.06^{a}	0.23±0.06^{a}	1.13±1.79^{a}	0.13±0.06^{a}	0.863	0.518
RDW-SD(fL)	57.73±2.15^{a}	59.17±9.62^{a}	53.43±9.78^{a}	60.60±5.38^{a}	59.17±7.53^{a}	0.410	0.798
RDW-CV (%)	14.43±1.27^{a}	14.83±2.53^{a}	13.03±2.42^{a}	16.83±0.72^{a}	15.80±2.31^{a}	1.561	0.258
MPV (fL)	9.43±0.15^{a}	9.47±0.32^{a}	9.17±0.60^{a}	9.87±0.84^{a}	8.80±0.72^{a}	1.365	0.313
PDW (%)	7.83±0.81^{a}	9.03±0.76^{a}	7.93±0.68^{a}	9.03±2.94^{a}	7.07±0.57^{a}	1.011	0.446
PCT (%)	0.05±0 02^{a}	0.07±0.02^{a}	0.06±0.02^{a}	0.03±0.02^{a}	0.03±0.02^{a}	2.593	0.101

NB: Means with same superscript along rows are not significantly different ($p > 0.05$).

WBC- white blood cell, RBC- red blood cell, HCT- Hematocrit, MCV- mean corpuscular volume, MCH- mean corpuscular hemoglobin, MCHC- mean corpuscular hemoglobin concentration, PLT- platelets, HGB- hemoglobin, RDR-SD- red cell distribution width (standard deviation), RDW-CV- red cell distribution width (co-efficient of variance), LYM- lymphocytes, GRAN- granulocytes

CHAPTER FIVE

5.0 DISCUSSION

5.1 White blood cell

The result showed that there was an increase in value from T1 to T4 as the inclusion level of mushroom increases, but experienced a decrease at the highest inclusion level T5. This is in agreement with Eyiwunwi *et al* (2018) who used moringa leaf meal as a replacement of vitamin premix. This indicates that the higher the inclusion level the higher the WBC resulting to a higher survival rate.

The decreased value of WBC may be due to stress stimulus (Rehulka 2002, Chen *et al.,*2004) or food composition (Secombes *et al.,* 1994).

5.2 Red blood cell

RBC was not significantly different (p>0.05) at T1 to T4 but recorded the highest value at 1.5% (T3) inclusion level of mushroom meal and the lowest value at 2.5% (T5) inclusion level. The high value of RBC in this study is as a result of sufficient oxygen molecules required in the production of more red blood cells. This was also observed by Doncan *et al* (2010) who recorded the highest RBC count of 21.2×10^6uL in increasing density of European catfish *Silrus glanis.*

The decrease in RBC count may be as a result of the increased rate of erythrocyte destruction in the hematopoietic organ or due to the inhibition of erythropoietic, haemosynthesis or osmoregulatory dysfunction (Vani *et al* 2011).

5.3 Hematocrit

The result of Hematocrit shows that there was decrease in value in T5 (18.67%) while other treatments did better as they fell within the ideal range of hematocrit (20-30%) as reported by Esmaeili 2021.

5.4 Red blood cell indice: (MCHC, MCH, MCV)

The MCHC value in this study were insignificant ($p>0.05$). Higher result of 31.67g/dL was observed in fish fed 1.5% (T3) inclusion level of mushroom compared to lower of 29.07g/dL (T5). The reduction in MCHC is attributable to the loss of HGB and damaged RBC (Bojarski and Witeska 2020).

The increase of the MCH value in this study is an indication of an increase of regularly shaped erythrocyte in circulation and swelling (Bojarski and Witeska 2020).

The mean corpuscular volume MCV in this study showed that T3 (120.90fL) had the highest value This increase might indicate that the fish under study had high immunity or resistance to disease. Akinwande *et al*. (2004) opined that a measurable increase in white blood count of fish or any animal is a function of immunity and animals' resistance to some vulnerable illness or disease.

5.5 Hemoglobin

The HGB values obtained in this study showed that 1.5% inclusion of mushroom meal had the highest value of 8.57g/dL and 2.5% inclusion of mushroom meal had the lowest at 5.43g/dL.This is not in agreement with Yanuhar et al., (2021) who reported that the normal range of HGB in catfish is 10-14g/dL).

This decrease in HGB value in this indicates abnormality in fish health.

5.6 Platelets

The result of PLT in this study shows that there was an increase from T1 to T3 with T2 ranking the highest at 74.33×10^3/uL. T4 and T5 recorded the lowest value. This change could be in relation to various biotic factors (age, sex, season, maturity status) and abiotic conditions (water temperature, pH, dissolved oxygen level) and stress (Tavares-Dias and Oliveira, 2009).

5.7 White blood cell subtypes (LYM, MID and GRAN)

The increased Lymphocytes (LYM) value aa observed in this study may be associated with enhanced release of LYM from Lymphoid stress. This could be an adaptive mechanism to boost the immune system of the fish and give it a positive survival value needed in the sub-lethal toxic environment pr possibility of leukaemia due to prolonged toxic assault (Akinrotimi *et al.,* 2015).

The monocytes (MID) value in this study showed significant differences. Witeska *et al.,* 2016 reported that monocytes values ranges from 0.1 – 13.6% in *C. Carpio.* This study agrees to this statement as values obtained are within this range.

Increased granulocytes (GRAN) as observed in this study may be associated with invasion of the system by pathogenic microorganisms, viruses and debris which may have been occasioned by tissue and organ damage (Bouck and Ball 2006)

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The study has shown that mushroom can be used as a supplement in the diet of *Clarias gariepinus* without having any negative effect on the hematological parameters. Hematological parameters are tools used in monitoring health status in farmed fishes in order to prevent the outbreak of disease.

The results obtained in this study supports the statement that Hematology can be used as an indicator of fish health in catfish. It has been proven that mushroom aids immunostimulatory activities and helps to fight diseases, this was also confirmed in this study. Treatment 4 at 2.0% inclusion level of mushroom had an increase in WBC which suggests that higher inclusion level of mushroom helps in the survival rate of fish. RBC and HCT produced better results at 1.5% inclusion level. The HCT value in this study had good performances as it was found to be in the range recorded by previous authors.

6.2 Recommendations

I Therefore recommend that:

1. Oyster mushroom should be added to fish diets for its immunostimulatory properties and the ability to fight disease.
2. Further studies should be done on mushroom supplemented diets on *Clarias gariepinus* as there are limited studies on this topic.

REFRENCES

Acar, U., Parrino, V., Kesbic, O.S., Lo Paro, G., Saoca, C., Abbate, F. and Fazio, F. (2018). Effects of different levels of pomegranate seed oil on some blood parameters and disease resistance against *Yersinia ruckeri* in rainbow trout. *Frontiers in Physiology.* **9**: 1-6.

Adakole, J. A. (2012). Change on some hematological parameters of the African catfish *(Clarias gariepinus)* exposed to a metal fishing company effluent. *Indian Journal of science and technology.* **4**: 2510-2514.

Adedeji, O.B. and Adegbile, A.F. (2011). Comparative haematological parameters of the bagrid catfish (*Chrysichthys nigrodigitatus*) and the African catfish (*Clarias gariepinus*) from Asejire Dam in south-western Nigeria *Journal of Applied science Research.* **7 (7)**: 1042-1046.

Adeyemo, O.K., Naigaga, I. and Alli, R.D. (2009). Effect of handling and transportation on haematology of African catfish (*Clarias gariepinus*). *Journal of Fisheries Sciences.* **3** (**4**)**:** 333-341.

Aguirre-Guzman, G., Carvajal-de-la-Fuente, V., Neri-Coronado, M., Loredo-Osti, J. and Rábago-Castro, J.L. (2016). Hematological and clinical chemistry changes induced by acute stress during handling and capture of catfish (Ictalurus punctatus). *MVZ Córdoba Magazine.* **21** (**2**): 5345-5354.

Ahmed, M., Abdullah, N., Yusof, H.M., Shuib, A.S. and Razak, S.A. (2017). Improvement of growth and antioxidant status in Nile tilapia, *Oreochromis niloticus*, fed diets supplemented with mushroom stalk waste hot water extract. *Aquaculture Research*. **48**: 1146-1157.

Ahmed, I. and Sheikh, Z.A. (2020). Comparative study of hematological parameters of snow trout *Schizopyge plagiostomus* and *Schizopyge niger* inhabiting two different habitats. *European Zoological. Journal*. **87 (1)**: 12-19.

Ahmed, I., Reshi, Q.M, and Fazio, F. (2020). The influence of the endogenous and exogenous factors on hematological parameters in different fish species: a review. *Aquaculture International.* **28:** 869-899.

Akinwande, A.A., Moody, F.O., Sogbesan, O A., Ugwumba, A.A.A. and Ovie, S.O. (2004). Haematological response of *Heterobranchus longifilis* fed varying dietary protein levels. Proceedings of the 19th Annual Conference of the Fisheries Society of Nigeria, November 29-December 3, 2004, Ilorin, Nigeria, pp: 715-718.

Argungu, L.A., Siraj, S.S., Christianus, A., Amin, M.S.N., Daud, S.K., Abubakar, S.A. and Aliyu-Paiko, M. (2017). A simple and rapid method for blood collection from walking catfish, *Clarias batrachus* (Linneaus, 1758). *Journal of Fisheries Science.* **16** (**3**)**:** 935-944.

Akinrotimi O.A., Gabriel, U.U. and Edun, O.M. (2015). The efficacy of clove seed and extracts as an anesthetic agent and its effect on hematological parameters of African catfish (*Clarias gariepinus). International Journal of Aquatic Fishery Science.* **1(2): 0**42- 047.

Ashley-Dejo, S.S., Olaoye, O. J., Adelaja, O. A. and Abdulraheem, I. (2014**).** Effects of feeding levels on growth performance, feed utilization and body composition of African catfish (*Clarias gariepinus*, Burchell 1822). *International Journal of Biology and Biological Sciences.* **3(2**); 12-16.

Ashley-Dejo, S. S., Olaoye, O. J and Adelaja, O. A. (2017**).** Analysis of profitability of small-scale catfish farmers in Oyo State, Nigeria. Malaysia *Journal of Animal Science.* **20**(**2**): 11-24.

Ashley-Dejo, S.S. and Adelaja, O. A. (2022**).** Economics of catfish hatchery farmers and its contribution to household poverty alleviation in Nigeria. *Tropical and Subtropical Agriculture.* **55:** 19–29.

Asmama, W.T., Abebe, T. and Gebre, K. (2015). Optimisation of oyster mushroom *(Pleurotus ostreatus)* cultivation using locally available substances and materials in Debra Berhan, Ethiopia. *Journal of Applied Biology and Biotechnology.* **3 (1):** 5-20.

Barcellos, L.J.G., Kreutz, L.C., Quevedo, R.M., Fioreze, I., Cericato, L., Soso, A.B. and Ritter, F. (2004). Nursery rearing of jundia, *Rhamdia quelen* (Quoy and Gaimard) in cages: cage type, stocking density and stress response to confinement. *Aquaculture*. **232 (1-4):** 383-394.

Batool, S., Khan, S. and Basra, S.M.A. (2020). Foliar application of moringa leaf extract improves the growth of moringa seedlings in winter. *South African Journal of Botany.* **129**: 347-353.

Bellettini, MB., Fernanda, A.F., Helayno, A.M., Gerson, L. T., Suelen, A., Polyanna, S.H., Agenor, M.J. and Rosemary, H.R. (2019). Factors affecting mushroom *Pleurotus* spp. *Saudi Journal* of Biological Science. **6**(**4**): 633-646.

Bianchi, M.B., Jeronimo, G.T., Padua, S.B., Satake, F., Ishikawa, M.M., Tavares-Dias, M. and Martins, M.L. (2014). The haematological profile of farmed *Sorubim lima*: reference intervals, cell morphology and cytochemistry. *Veterinary Achieve*. **84** (**6**): 677-690.

Bona. Z. and Rajarathnam, S. (2011). Production of Oyser Mushroom in Different Seasonal Condition of Bangladesh. *Journal of Scientific Research.* **3 (1):** 161-167.

Bojarski, B., Socha, M., Drąg-Kozak, E., Rombel-Bryzek, A., Kapinos, S., Szała, L. and Witeska, M., (2021). Does the site of blood collection in fish affect haematological and blood biochemical results? *Folia Biology.* **69** (**2**)**:** 51-56.

Bojarski, B., Ludwikowska, A., Kurek, A., Pawlak, K., Tombarkiewicz, B. and Lutnica, H. (2015). Hematological alterations in common carp (*Cyprinus carpio* L.) exposed to herbicides: pendimethalin and ethofumesate tested separately and in mixture. *Biological Foil.* **63** (**3**)**:** 167-174.

Bojarski, B., Kondera, E., Witeska, M. and Ługowska, K. (2018). Differences in hematological values of common carp between cardiac and venous blood. *Bulletin of European Association of Fish Pathologists.* **38** (**6**)**:** 234-238.

Bojarski, B. and Witeska, M. (2020). Blood biomarkers of herbicide, insecticide, and fungicide toxicity to fish—a review. *Environmental Science and Pollution Research*. **27:** 19236-19250.

Bouck, G.R and Ball, R.C (2006). Influence of capture methods on blood characteristics and mortality in the rainbow trout *(Salmon gairdneri). Translantic American Fisheries Society* **95(2):** 170-176.

Casillas, E. and Smith, L.S. (1977). Effect of stress on blood coagulation and haematology in rainbow trout (*Salmo gairdneri*). *Journal of Fish Biology*. **10** (**5**)**:** . 481-491.

Chang, S.T. (1992). Miles PG. Mushroom biology- a new discipline. Mycologist. **6** (**2**): 64-65.

Ciesla, B. (2007). Hematology in Practice; FA Davis Company: Philadelphia, PA, USA. 2nd edition. 230pp.

David, M., Sangeetha, J., Shrinivas, J., Harish, E.R. and Naik, V.R. (2015). Effects of deltamethrin on haematological indices of indian major carp, *Cirrhinus mrigala* (Hamilton). *International Journal on Pure and Applied Zoology.* **3** (**1**); 37-43.

Dal'Bo, G.A., Garcia Sampaio, F., Eliseu Losekann, M., Ferraz de Queiroz, F., Barreto Luiz, A.J., Gyarfas Wolf, V.H. and Carra, M.L. (2015). Hematological and morphometric blood value of four cultured species of economically important tropical food fish. *Neotrophical Ichthyology.* **13** (**2**): 439-446.

Del Rio Zaragoza, O., Hernandez Rodriguez, M. and Buckle Ramirez, L.F. (2008). Thermal stress effect on tilapia *Oreochromis mossambicus* (Pisces: Cichlidae) blood parameters. *Marine and Freshwater Behaviour and Physiology.* 41 (**2**)**:** 135-145.

Devaraj, K.V., Keshavappa, G.Y., and Manissery, J.K. (1986). Growth of grass carp, *Ctenopharyngodon idella* (val.) fed on two terrestrial fodder plants. Aquaculture Research and Fisheries Management **17** **(2)**: 123-128.

Dobsikova, R., Svobodova, Z., Blahova, J., Modra, H. and Velisek, J. (2009). The effect of transport on biochemical and haematological indices of common carp (*Cyprinus carpio* L.). *Czech Journal of Animal Science.* **54 (11):** 510-518.

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

Eyo, A. A. (2003**).** Fundamentals of fish nutrition and diet development. An overview. *National Workshop of Fish Feed Development of Feeding Practices in Aquaculture Organised by Fisheries Society of Nigeria (FISON) in Collaboration with National Institute of Freshwater Fisheries Research (NIFER) and FAO National Special Programme for Food Security (FAO-NSPES).* 1-33.

Fagbenro, O. A. (1992**).** Utilization of cocoa pod husk in low cost diets by *Clarias isheriensis*, (Syhenham). *Aquaculture* **4:** 175-182.

Falaye, A. E. (1992**).**Utilization of agro-industrial wastes as feed stuffs in Nigeria. *Nigerian Journal of Animal Production.* **10**: 47-57.

Fange, R. (1994). Blood cells, haemopoiesis and lymphomyeloid tissues in fish. *Fish and Shellfish Immunology*. **4** (**6)** 405-411.

Faggio, C., Casella, S., Arfuso, F., Marafioti, S., Piccione, G. and Fazio. F. (2013) Effect of storage time on haematological parameters in mullet (*Mugil cephalus*). *Cell Biochemistry and function*. **31** (**5):** 412-416.

Fagundes, M. and Urbinati, E.C. (2008). Stress in pintado (*Pseudoplatystoma corruscans*) during farming procedures. *Aquaculture*. **276** (1-4**):** 112-119.

Fazio, F., Saoca, C., Costa, G., Zumbo, A., Piccione, G. and Parrino. V. (2019). Flow cytometry and automatic blood cell analysis in striped bass *Morone saxatilis* (Walbaum, 1792): a new haematological approach. *Aquaculture*, 513: 734398.

Fazio, F. (2019). Fish hematology analysis as an important tool of aquaculture: a review. *Aquaculture*, **500:** 237-242.

Fazio, F., Ferrantelli, V., Fortino, G., Arfuso, F., Giangrosso, G. and Faggio, C. (2015). The influence of acute handling stress on some blood parameters in cultured sea bream (*Sparus aurata* Linnaeus, 1758). *Italian Journal of Food Safety* **4** (**1):** 4174.

Food and Agriculture Organization (2020). The State of World Fisheries and Aquaculture. Sustainability in action. Rome 224.

Gatlin, D. M., Barrows, F. T., Braown, P., Dabrowski, K., Gaylord, T.G., Hardy, R.W., Herman, E., Krogdahl, A., Nelson, R., Overtuf, K., Rust, M., Sealy, W., Skonberg, D., Souza, E J., Stone, D., Wilson, R., and Wurtele, E. (2007**).**

Expanding the utilization of sustainable plant products in aquafeeds: a review on *Aquaculture Research.* **38:** 5551-579.

Gebretsadkan, G., Tessema, K., Ambachew, H. and Birhaneselassie, M. (2015). The comparison between and automated methods for hematocrit determination. *International Journal on Blood Research Disorder.* **2 (1):** 1-3.

Gbolagade, J.S. (2006). Bacteria associated with compost used for cultivation of Nigeria edible mushroom *Pleurotus tuber-regium* (Fr.) Singer and *Lentinus squarrosulus* (Berk). *African Journal of Biotechnology.* **5 (4)**: 338-342.b

Ghayyur, S., Tabassum, S., Ahmad, M.S., Akhtar, N. and Khan, M.F. (2019). Effect of chlorpyrifos on hematological and seral biochemical components of fish *Oreochromis mossambicus* Pakistan. *Journal on Zoology.* **1** (**3**)**:** 1047-1052.

Girón-Perez, M.I., Montes-Lopez, M., Garcia-Ramirez, L.G., Romero-Banuelos, C.A. and Robledo-Marenco, M.L. (2008). Effect of sub-lethal concentrations of endosulfan on phagocytic and hematological parameters in Nile tilapia (*Oreochromis niloticus*) *Bulletin of Environmental Contamination and Toxicology.* **80:** 266-269.

Gordon, M., Bihari, B., Goosby, E., Gorter, R., Greco, M., Guralnik, M., Mimura, T., Rudinicki, V., Wong, R. and Kaneko, Y. (1998). A place of controlled trial of the immune modulator, lentinan, in HIV-positive patients: A phase I/II trial. *Journal of Medicinal Chemistry*. **28:** 305–330.

Gornall, A.G., Bardawill, C.J. and David, M.M (1949). Determination of serum protein by means of the Biureto reaction. *Journal on Biology Chemistry.* **177:** 751-766.

Gul, A., Benli, C.K., Ayhan, A., Memmi, B.K., Selvi, M., Sepici-Dincel, A. and Erkoc, F. (2012). Sublethal propoxur toxicity to juvenile common carp (*Cyprinus carpio* L., 1758): biochemical, hematological, histopathological, and genotoxicity effects. *Environmental Toxicology and Chemistry.* **31 (9):** 2085-2092.

Grant, K.R. (2015). Fish hematology and associated disorders Veterinary Clinic Exotic Animals. *Veterinary Clinics of North America Exotic Animal Practice.* **18 (1):** 83-103.

Grzelak, A.K., Davis, D.J., Caraker, S.M., Crim, M.J., Spitsbergen, J.M. and Wiedmeyer, C.E. (2017). Stress leukogram induced by acute and chronic stress in zebrafish (*Danio rerio*). *Comparative Medicine.* **67** **(3):** 263-269

Harabawy, A.S.A. and Ibrahim, A.T A. (2014). Sublethal toxicity of carbofuran pesticide on the African catfish *Clarias gariepinus* (Burchell,1822): hematological, biochemical and cytogenetic response. *Ecotoxicology and Environmental Safety*. **103:** 61-67.

Hattingh, J. (1975). Heparin and ethylenediamine tetra-acetate as anticoagulants for fish blood. *Pfluger's Achieve*. **355:** 347-352.

Hepher, B. (1975). Supplementary feeding in fish culture. In: Proceedings of the 9th International Congress on Fish Nutrition and Feeding, held at Mexico in 1972. **3:** 183-198.

Hernandez, C., Hurtado-Oliva, M.A. and Pena, E. (2019). Effect of short-term starvation on hematological and blood biochemical parameters in juvenile spotted rose snapper *Lutjanus guttatus* (Steindachner, 1869). *Latin American Journal on Aquatic Research.* **47 (1):** 9-17.

Hleap-Zapata, J.I., and Rodríguez-de-la-Pava, G.C. (2021). Evaluation of oyster mushroom powder (*Pleurotus ostreatus*) as a partial substitute for phosphates in red tilapia fillet sausage. *British Food Journal.* **123:** 2107-2122.

https://learn.freshcap.com/growing/understanding-mushrooms-substrates. 2:11pm, 4th April 2023.

https://www.agricultureinindia.net/cultivation/mushroom-cultivation/mushroom-cultivation-in-india-cost-procedure-profit-and-methods/20717. 9:56am, 17th April.

Jha, A.K., Pal, A., Sahu, N., Kumar, S. and Mukherjee, S. (2007). Haemato-immunological responses to dietary yeast RNA, ω-3 fatty acid and β-carotene in *Catla catla* juveniles. *Fish Shellfish Immunology.* **23:** 917–927.

Jamiu, D. M and Ayinla, O.A. (2003**).** Potential for the development of aquaculture in Africa. *Journal of Research in Bioscience.* **26** (**3**): 9-13.

Jan, K., and Ahmed, I. (2021). The influence of sex and season on some hematological and biochemical parameters of snow trout *Schizothorax labiatus* in the Indian Himalayan Region. *Fish Science.* **87:** 39–54.

Jiang, Y. and Doolittle, R.F. (2003). The evolution of vertebrate blood coagulation as viewed from a comparison of puffer fish and sea squirt genomes. *Proceedings of the National Academy of Sciences.* **100** (**13**)**:** 7527-7532.

Ji, L., Jiang, K., Liu, M., Wang, B., Han, L., Zhang, M. and Wang, L. (2016). Low temperature stress on the hematological parameters and HSP gene expression in the turbot *Scophthalmus maximus*. *Chinese Journal of Oceanography and Limnology.* **34:** 430-440.

John, W., and Harvey DVM. (2012). Introduction to Veterinary Hematology. *A Diagnostic Guide and Colour Atlas.***1:** 1-10.

Khalafalla, M.M. and El-Sayed, B. (2015). Biological treatment of broad bean hulls and its evaluation through tilapia fingerlings (*Oreochromis niloticus*) feeding. *Journal Aquaculture Research and Development*. **6**: 1-5.

Khare, K.B., Krishna, B., Mosimanegape, J. and Daniel, L. (2018). Oyster mushroom cultivation at different production systems. *European Journal of Biomedical and Pharmaceutical Sciences.* **5 (5):** 72-79.

Khare, K.B., Jongman, M. and Khonga, E.B. (2004). Effect of different grain spawns and substrates sterilization methods on yield of oyster mushroom. *International Journal of Bioassays.***2 (10):** 1308-1311.

Khonga, E.B., Khara, K.B. and Jongman, M. (2013). Highlights of oyster mushroom production research in Botswana. *UNISWA Journal of Agriculture.* **12(1):** 45-52.

Kondera, E., Witeska, M. and Ługowska, K. (2019). Annual changes in hematological parameters of common carp juveniles under laboratory conditions Annals of Warsaw University of Life Science. *Animal Science.* **58 (2):** 143-151.

Kondera, E., Bojarski, B., Ługowska, K., Kot, B., Witeska, M. (2020). Effects of oxytetracycline and gentamicin therapeutic doses on hematological, biochemical and hematopoietic parameters in *Cyprinus carpio* juveniles. Animals. **10 (2278):** 1-12.

Korcock, D E., Houston, A.H. and Gray, J.D. (1988). Effects of sampling conditions on selected blood variables of rainbow trout, *Salmo gairdneri* Richardson. *Journal of Fish Biology.* **33** (**2):** 319-330.

Lakhanpal, T.N. and Rana, M. (2005). Medicinal and nutraceutical genetic resources of mushrooms. *Plant Genetic Resource, Characterization and utilization.* **3:** 288–303.

Lawrence, M.J., Raby, G.D., Teffer, A K., Jeffries, K.M , Danylchuk, A J., Eliason, E.J. and Cooke, S.J. (2020). Best practices for non-lethal blood sampling of fish *via* the caudal vasculature. *Journal on fish Biology.* **97** (**1):** 4-15.

Lay, P.A. and Baldwin, J. (1999). What determines the size of teleost erythrocytes? Correlations with oxygen transport and nuclear volume. *Fish Physiological Biochemistry*. **20:** 31-35.

Lepic, P., Stara, A., Turek, J., Kozak, P. and Velisek, J. (2014). The effects of four anaesthetics on haematological and blood biochemical profiles in vimba bream, Vimba vimba. *Veterinary Medicine*. **59** (**2):** 81-87.

Li, S., Liu, Y., Zhao, G., Li, J., Hou, J., Gao, M. and Li, Y. (2006). Mechanism of blood coagulation in common carp (*Cyprinus carpio*). *Integrative Zoology.***1** (**3):** 117-121.

Lugowska, K., Kondera, E. and Witeska, M. (2017). Leukocyte count in fish – possible sources of discrepancy. *Bulletin of European Association of Fish Pathologists*. **33** (**3):** 94-99.

Magnadóttir, B. (2006). Innate immunity of fish (overview) *Fish Shellfish Immunology.***20** (**2**)**:** 137-151.

Martins, M.L., Mourino, J.L.P., Amaral, G.V., Vieira, F.N., Dotta, G., Jatoba, A.M.B. and Pereira-Jr, G. (2008). Haematological changes in Nile tilapia experimentally infected with *Enterococcus* sp. *Brazilian Journal of Biology.* **68 (3):** 657-661.

McBeath, A., Aamelfot, M., Christiansen, D.H., Matejusova, I., Markussen, T., Kald husdal, M. and Falk, K. (2015). Immersion challenge with low and highly virulent infectious salmon anaemia virus reveals different pathogenesis in Atlantic salmon, *Salmo salar* L *Journal on Fish Disease.* **38** (**1**)**:** 3-15.

McBryan, T L., Anttila, K., Healy, T.M. and Schulte, P.M. (2013). Responses to temperature and hypoxia as interacting stressors in fish: implications for adaptation to environmental change. *Integrative and Comparative Biology.* **53** (**4**)**:** 648-659.

Miles. P.G. and Shu- Ting, C. (2004). Mushroom cultivation, nutritional value, medicinal effect and environmental impact. CRC press. 2nd edition: 319pp

Nongthombam, J., Kumar, A., Ladli, Manikanta, B., Madhushekhan, M. and Patidar, S. (2021). A review on the study of growth and cultivation of oyster mushroom. *Plant cell Biotechnology and Molecular Biology.* **22** (**5 and 6**)**:** 55-65.

Omojowo, T.M, Omojowo, F.S and Alatise, P. S. (2010). Growth response and nutritional evaluation of mango peel-based diets on *Oreochromis niloticus* fingerlings. *The Egyptian Journal of Aquatic Research*. **45** (**3**), 265-271.

Oseni, O.T., Sikhumbuzo, O.D., Diana, M. E. and Masarirambi, M. (2012). Effect of substrate pre treatment methods on oyster mushroom *(Pleurotus ostreatus)* production. *International Journal of Agriculture and Biology.* ISSN print: 1560-8530. **14 (2).**

Okoye, F.C and Sule, O.D. (2001). Utilizaiton and composition of conventional and non-conventional Fish Feedstuffs in Arid Zone of Nigeria. *Journal of Arid Zone Fisheries.* **1:** 23-32.

Oliveira, B.L., Souza, R.M., Silva Filho, J.P., Gomes, L.C. and Loureiro Fernandes, L.F. (2018). Hematological and biochemical characteristics during the transport of dog snapper *Lutjanus jocu* (Perciformes: Lutjanidae). *Neotropical Ichthyology*. **16 (1):** 170140.

Paul, T., Shukla, S.P., Kumar, K., Poojary, N. and Kumar, S. (2019). Effect of temperature on triclosan toxicity in *Pangasianodon hypophthalmus* (Sauvage, 1878): Hematology, biochemistry and genotoxicity evaluation. *Science of The Total Environment.* **668:** 104-114.

Parrino, V., Cappello, T., Costa, G., Cannava, C., Sanfilippo, M., Fazio, F. and Fasulo, S. (2018). Comparative study of haematology of two teleost fish (*Mugil cephalus* and *Carassius auratus*) from different environments and feeding habits. *European Zoological Journal.* **85 (1):** 193-199.

Peres, H., Costas, B., Perez-Jimenez, A., Guerreiro, I. and Oliva-Teles, A. (2015). Reference values for selected hematological and serum biochemical parameters of Senegalese sole (*Solea senegalensis* Kaup, 1858) juveniles under intensive aquaculture conditions. *Journal on Applied Ichthyology.* **31 (1):**65-71.

Pilinkovskij, A., Vosyliene, M.Z., Kazlauskiene, N. and Jakubauskaite, J. (2017). Hematological effects of transportation stress on Atlantic sturgeon *Acipenser oxyrinchus* Mitchill L. 1815. *Journal on Applied Ichthyology.* **33 (5):** 1021-1023.

Pollock, M.S., Clarke, L.M.J. and Dube, M G.(2007). The effects of hypoxia on fishes: from ecological relevance to physiological effects. *Environmental Reviews*. **15:** 1-14.

Rad, F., Kurt, GI. and Ankabozaoulu, A.S. (2003). Effects of spatially localised and dispersed patterns of feed on the growth, size dispersion and feed conversion ratio of the African catfish *(Clarias gariepinus)*. *Turkish Journal on Veterinary Animal science*. **28:** 855-856.

Randive, S.D. (2012). Cultivation and study of growth of oyster mushroom on different agricultural waste substrate and its nutrient analysis. *Advances in Applied Science Research.* **4**: 1938-1949.

Reda, R.M., Mahmoud, R., Selim, K.M. and El-Araby, I.E. (2016). Effects of dietary acidifiers on growth, hematology, immune response and disease resistance of Nile tilapia, *Oreochromis niloticus*. *Fish and Shellfish Immunology.* **50:** 255-262.

Rożyński, M., Demska-Zakęś, K., Sikora, A. and Zakęś, Z. (2018). Impact of inducing general anaesthesia with Propiscin (etomidate) on the physiology and health of European perch (*Perca fluviatilis* L.). *Fish Physiology and Biochemistry.* **44:** 927-937.

Safari, O. and Sarkheil, M. (2018). Dietary administration of eryngii mushroom (*Pleurotus eryngii*) powder on haemato-immunological responses, bactericidal activity of skin mucus and growth performance of koi carp fingerlings (*Cyprinus carpio koi*). *Fish and Shellfish Immunology.* **80**: 505-513.

Saurab, D. (2019). An overview of mushroom farming. *Journal of Global Ecology and Environment.* **11 (1)**: 1-6.

Sharma, S., Ram, K.P.V. and Chandra, P.P. (2013). Growth and yield of oyster mushroom *(Pleurotus ostreatus)* on different substrates. *Journal on New Biology Reports.* **2** (**1**): 03- 08.

Simide, R., Richard, S., Prevot-D'Alvise, N., Miard, T. and Gaillard, S. (2016). Assessment of the accuracy of physiological blood indicators for the evaluation of stress, health status and welfare in Siberian sturgeon (*Acipenser baerii*) subject to chronic heat stress and dietary supplementation. *International Aquatic Research*. **8:** 121-135.

Singhal, S., Rasane, P., Kaur, S., Garba, U., Singh, J., Raj, N. and Gupta, N. (2019). Mushroom cultivation, processing, and value-added products: a patent based review. *Recent Patents on Food, Nutrition & Agriculture.* **10**: 3-19.

Sivrikaya, H., Latife, B., Akin , S., Ihsan, T. and Hudaverdi, E. (2002). Trace elements in *Pleurotus sajorcaju* cultivated on chemithermomechanical pulp for bio- bleaching. *Food chemistry.* **79** (**2**): 173-179.

Shah, S.L. and Altindag, A. (2004). Hematological parameters of Tench (Tincatinca L.) after acute and chronic exposure to lethal and sub lethal mercury treatments. *Bulletin of Environmental Contaminated Toxicology*. **73:** 911-918.

Snedecor, G.W. and Cochran, W.G. (1989). The Lowa State University Press. 8th edition Statistical Methods. Applied to experiments in Agriculture and Biology.

Soldatov, A.A. (2021). Functional effects of the use of anaesthetics on teleostean fishes. A Review. *Inland Water Biology.* **14:** 67-77.

Stosik, M., Deptuła, W., Travnicek, M. and Baldy-Chudzik, K. (2002). Phagocytic and bactericidal activity of blood thrombocytes in carps (*Cyprinus carpio). Veterinary Medicine Czech.* **47:** 21-25.

Stosik, M., Tokarz-Deptuła, B., Deptuła, W. (2019). Characterisation of thrombocytes in Osteichthyes. *Journal Veterinary Research.* **63** (**1**)**:** 123-131.

Stosik, M., Deptula, W. and Travnicek, M. (2001). Studies on the number and ingesting ability of thrombocytes in sick carps (*Cyprinus carpio* L.) *Veterinary Medicine Czech*. **46 (1):** 12-16.

Srichanun, M., Nganwisuthiphan, T., Wanlem, S. and Rakkamon, T. (2017). Effects of different mushroom by-product types and levels on growth performance and survival rate in dietary of Nile tilapia (*Oreochromis niloticus*). *International Journal of Agricultural Technology***. 13**: 1093-1101.

Sun, Y., Hu, X. and Li, W. (2017). Antioxidant, antitumor, and immunostimulatory activities of the polypeptide from *Pleurotus eryngii* mycelium. *International Journal of Biological Macromolecules*. **97:** 323-330.

Suljevic, D., Alijagic, A., Mitrasinovic-Brulic, M., Focak, M. and Islamagic, E. (2016). Comparative physiological assessment of common carp (*Cyprinus carpio*) and crucian carp (*Carassius carassius*) based on electrolyte and hematological analysis. *Macedonian Journal of Animal Science.* **6** (**2**)**:** 95-100.

Svobodova, Z., Pravda, D. and Palackova, J. (1991). Unified methods of Haematological Examination of Fish. *Research Institute of Fish Culture and Hydrobiology, Vodnany, Czech Republic.* **31.**

Svobodova, Z., Pravda, D. and H. (2012). Faculty of Fisheries and Water Protection. *Methodology of Hematological Examination of Fish.* **122**: 38.

Tavares-Dias, M., Mataqueiro, M.I. and Perecin, D. (2002). Total leukocyte counts in fishes by direct or indirect methods. Fisheries Institute Bulletin. **28** **(2):** 155-161.

Tavares-Dias, M. and Silva Sandrim, E.F. (1998). Influence of anticoagulants and blood storage on hematological values in tambaqui, *Colossoma macropomum*. *Journal of Sciences.* **20**: 151-155.

Travares- Dias, M. and Moraes, F.R. (2007). Hematological and biochemical reference intervals for farmed channel catfish. *Journal of Fish Biology*. **71 (2)**: 383-388.

Tavares-Dias, M. and Oliveira, S.R. (2009). A review of the blood coagulation system in fish. *Brazilian Journal of Biosciences.* **(2):** 205-224.

Tort, L. (2011). Stress and immune modulation in fish. *Developmental and Comparative Immunology***. 35 (12):** 1366-1375.

Vaz Farias, T.H., Levy Pereira, N., Benites de Padua, S., de Oliveira Alves, L., Sakabe, R., de Andrade Belo, M.A. and Pilarski, F. (2016). Na_2EDTA anticoagulant impaired blood samples from the teleost *Piaractus mesopotamicus*. *Brazilian Veterinary Research.* **36** **(5):** 431-435.

Velmurugan. B., Cengiz, E.I., Senthilkumaar, P., Uysal, B. and Satar, P. (2016). Hematological parameters of freshwater fish *Anabas testudineus* after sublethal exposure to cypermethrin. *Environmental Pollution and Protection.* **1** **(1):** 32-39.

Wasser, S.P. and eis, A.L. (1999). Therapeutic effects of substances occurring in higher Basidiomycetes mushrooms: A modern perspective. Critical review on Immunology. *International Journal of Medicinal Mushrooms.* **19**: 65–96.

Witeska, M., Kondera, E. and Ługowska, K. (2010). The effects of ichthyophthiriasis on some haematological parameters in common carp. *Turkish Journal on Veterinary Animal Science.* **34.** **(3):** 267-271.

Witeska, M. (2013). Erythrocytes in teleost fishes: a review. *Zoology and Ecology.* **23** **(4):** 275-281.

Witeska, M. (2015). Anemia in teleost fishes. *Bulletin of European Association of Fish Pathologists*. **35** **(4):** 148-160.

Witeska, M., Dudyk, J. and Jarkiewicz, N. (2015). Haematological effects of 2-phenoxyethanol and etomidate in carp (*Cyprinus carpio* L.) *Veterinary Anaesthesia and Analgesia.* **42** **(5):** 537-546.

Witeska, M., Lugowska, K. and Kondera, E. (2016). Reference values of hematological parameters for juvenile *Cyrpinus carpio*. *Bulletin of European Association of Fish Pathologists.* **36** (**4**)**:** 169-180.

Witeska, M., Biardzka, J. and Kniaz, J. (2017). The effects of heparin concentration, storage time, and temperature on the values of hematological parameters in *Cyprinus carpio. Turkish Journal on Veterinary Animal science.* **41:** 351-356.

Witeska, M., Teodorczuk, B. and Lugowska, K. (2017). Hematological effects of etomidate and tricaine in common carp. *Turkish Journal on Veterinary Animal Science.* ***41:*** *93-98.*

Yanuhar, U., Raharjo, D.K W.P., Caeser, N.R. and Janirahma, N.S. (2021). The 3rd International Conference on Fisheries and Marine Science. **718**

Zang, L., Shimada, Y., Nishimura, Y., Tanaka, T. and Nishimura, N. (2014). Repeated blood collection for blood tests in adult zebrafish. *Journal of Visualised Experiments.* **10:** 499- 510.

Zang, L., Shimada, Y., Nishimura, Y., Tanaka, T. and Nishimura, N. (2013). A novel, reliable method for repeated blood collection from aquarium fish. *Zebrafish.* **10 (3);** 425-432.

Zheng, K., Wu, L., He, Z., Yang, B. and Yang, Y. (2017). Measurement of the total protein in serum by biuret method with uncertainty evaluation. Measurement. **112:** 16–21.

Zutshi, B., Raghu Prasad, S.G. and Nagaraja, R. (2010). Alteration in hematology of *Labeo rohita* under stress of pollution from Lakes of Bangalore, Karnataka, India. *Environmental Monitoring and Assessment*. **168:** 11-19.