Hematology and Plasma Chemistry Values for Production Tilapia (*Oreochromis* hybrid) Raised in a Recirculation System

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ABSTRACT

Tilapia are a frequently aquacultured fish, yet little is known about their normal physiology and response to disease. To assess specific diseases in mammals, blood constituents are routinely analyzed and compared to previously determined standardized values. Research to establish hematologic values for normal healthy fish is required before blood analysis can be used for diagnostic purposes in fishes. This study determined hematology and plasma chemistry values for production tilapia (*Oreochromis* hybrids) raised in a recirculation system. Using standard clinical techniques, the following hematologic parameters were determined: PCV (packed cell volume), plasma protein and MCV (mean cell volume) values; and erythrocyte, leukocyte, lymphocyte (small and large), neutrophil, monocyte, eosinophil, thrombocyte-like-cell, and thrombocyte numbers. Additionally, the following plasma chemistry values were determined: total protein, albumin, globulin, creatinine, total bilirubin, ALP (alkaline phosphatase), AST (aspartate aminotransferase), sodium, potassium, chloride, calcium, phosphorus, magnesium, glucose, cholesterol, ammonia and osmolality. Analysis of blood parameters can enhance production of hybrid tilapia by providing a means for the early detection of infectious diseases, and by assisting in the identification of sub-clinical conditions affecting production performance.

INTRODUCTION

Tilapia are the second most commonly cultured fish in the world, and are a food staple in many parts of Africa, Asia, and South America (CEAH 1995; Anonymous 1996). In the USA, tilapia consumption has increased and these fish are the fourth most commonly cultured fish. Intensive aquaculture of tilapia, as with other species of finfish, is adversely affected by production-related disorders and infectious diseases (CEAH 1995). This is particularly true of fish raised in recirculating systems, where high stocking densities promote the spread of infectious diseases. As the aquaculture industry expands, there is an increasing need for improved diagnostic methods to detect disease and to diagnose causes of poor production performance. Hematology and clinical chemistry analysis are not regularly used in fishes, as baseline values are not available for most species; however, blood analysis can provide significant diagnostic information once baseline values are established. Successful culture and maintenance of tilapia can be enhanced by developing such a clinical tool to monitor the health of production fish. Previously reported blood values for tilapia are from non-production or low intensity production tilapia (Terao and Ogawa 1984; Haniffa and Vijayarani 1989; Hussein et al. 1996). This present study determined a complete profile of hematologic and biochemical blood values for production hybrid tilapia raised in a commercial recirculating system. This profile can serve as a guide for comparisons in the analyses of blood values from other production hybrid tilapia.

MATERIALS AND METHODS

Hybrid tilapia were stocked into a 231,900 L recirculation system as fingerlings. At the time of sampling, the fish had an average weight of 551 g, a length of 26.7 cm and were stocked at a density of 70 g/L. The fish were fed a commercial tilapia feed (Tilapia Grower - floating, Southern States Cooperative, Richmond, VA, USA) at 2% of body weight per day. Water quality was monitored at the times of sampling for standard parameters and the values are shown in Table 1. Fish with any gross abnormalities were not included in the study.

The fish were rapidly netted, anesthetized in aerated buffered tricaine methanesulfonate (MS-222, Sigma Chemical Co. St. Louis, MO, USA)

Table 1. Mean water quality values for hybrid tilapia maintained in high density (70 g/L) recirculating systems.

PARAMETER	VALUE
Temperature (°C)	29
pH	7.2
NH_3 un-ionized (mg/L)	0.017
NO_2-N (mg/L)	0.39
NO_3-N (mg/L)	54
Alkalinity (mg/L)	188
Hardness (mg/L)	154
DO (mg/L)	8.0

and bled with a 23 gauge needle and a 3 mL syringe from the caudal vessels. The collected blood was placed in blood tubes containing either ethylenediamine tetra-acetic acid (EDTA) for hematological analysis, or lithium heparin for chemistry analysis. Any hemolyzed or clotted samples were discarded before analysis.

Blood from the EDTA tube was drawn into microhematocrit tubes (Fisher Scientific, Norcross, GA, USA) and the packed cell volume (PCV) determined after centrifugation at 10,000 x g for 5 min. Plasma protein was determined with a clinical refractometer using plasma from the microhematocrit tube. The total red cell count and total white cell counts were determined manually as previously described (Hrubec et al. 1996a) with a Neubauer hemacytometer using Natt-Herrick's solution (Natt and Herrick 1952) as a diluent stain. Briefly, as thrombocytes can not be reliably distinguished from leukocytes on the hemacytometer, both were counted to give a combined leukocyte/thrombocyte count. Thrombocytes were then enumerated and subtracted from the combined count during the differential. Manual determination of total red and white cell counts is recommended for use with fish blood, as the nucleated red cells prevent accurate enumeration using automated analysis (Huffman et al. 1997). Blood smears, using the EDTA treated blood, were stained with Wright's Geimsa stain and used for the differential count as described previously (Hrubec et al. 1996a).

Blood in the heparinized tubes was centrifuged (14,000 x g) for 5 min and the plasma collected and frozen at -10°C until analyzed

(approximately 3 weeks later). Plasma samples were analyzed using an automated dry chemistry system (Kodak Ektachem 700, Rochester, NY, USA) for total protein, albumin, creatinine, total bilirubin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), sodium, chloride, potassium, calcium, magnesium, phosphorus, glucose, cholesterol, and ammonia. Dry chemistry analysis systems are standardized and automated to reduce variation in results. They are used routinely in human and veterinary medicine, and have been documented to be accurate for fish samples (Warner et al. 1978; Warner et al. 1979; Smith and Ramos 1980). Globulin was calculated from the difference between the total protein and albumin. Osmolality was determined using an osmometer (Multiosmette Micro-Osmometer 2430, Precision Systems Inc., Natick, MA, USA).

Table 2. Hematologic values for production tilapia reared in recirculating systems.

Analyte	n[1]	Range	Mean	SEM[2]
PCV[3] (%)	35	23-35	28	0.5
Plasma Protein (mg/dl)	35	4.9-8.4	6.3	0.2
Erythrocytes (x 10^6/µL)	35	1.96-2.91	2.43	0.046
MCV[4] (fl)	35	90-143	114	2
Leukocytes (#/µL)	31	13,200-110,300	55,700	4111
Lymphocytes (#/µL)				
Small	31	9,300-78,900	44,200	3170
Large	31	700-11,700	4,000	469
Neutrophils (#/µL)	30	1000-9,300	3,200	348
Monocytes (#/µL)	30	0-3,300	900	148
Eosinophils (#/µL)	31	0-1,600	500	83
TLC[5] (#/µL)	31	500-5,700	1,800	235
Thrombocytes (#/µL)	31	18,800-80,300	39,500	2787

[1] *Number of fish,*
[2] *Standard error of the mean,*
[3] *Packed cell volume,*
[4] *Mean cell volume,*
[5] *Thrombocyte-like-cell*

RESULTS and DISCUSSION

Although tilapia are one of the most frequently cultured fish in the world, there are surprisingly few papers that present normal blood values (Terao and Ogawa 1984; Haniffa and Vijayarani 1989; Hussein et al. 1996). In these previous studies, only a few fish were used, or only a small number of analytes were determined. Additionally, these studies were conducted on pure species of non-production tilapia and not the more commonly cultured hybrids. The blood values of the pure strains may be quite different from the values for a production hybrid. The data presented in the present study should be relevant for production hybrids raised in recirculating systems under similar culture conditions.

The values for the hematologic parameters are given in Table 2. The plasma protein, white cell count and differential white cell count can provide valuable information about the immune and inflammatory responses of an individual. The packed cell volume, red cell count and MCV can provide information about anemias and red cell production and hydration status. The hematologic values determined in this study are comparable to those published previously with the exception of Haniffa and Vijayarani (1989) who reported lower PCV (10%), and erythrocyte counts (0.9 x10^6/μL) in *Oreochromis mossambicus*. Conversely, Terao and Ogawa (1984) reported higher PCV values (35%) in *Tilapia nilotica*. The leukocyte types observed in the fish from the present study: small lymphocytes, large lymphocytes, neutrophils, monocytes, and eosinophils, are similar to those described for *Oreochromis mossambicus* (Doggett et al. 1987) and other species of fish (Ellis 1977). The thrombocyte-like-cell (TLC) is a cell type that has been identified in different species, and superficially resembles a thrombocyte and probably represents a maturational stage of one of the leukocytes (Hrubec et al. 1996a).

The values for the plasma chemistry parameters are given in Table 3. Total protein, albumin and globulin can provide information about the immune status, liver function, hydration and osmoregulation. The blood chemistries: AST, ALP, total bilirubin, creatinine, and cholesterol can indicate changes in organ function; while the electrolytes and plasma osmolality are indicators of osmoregulatory ability. Electrolytes and glucose can also provide information about stress levels. Comparison of previously reported blood values with those determined in this study

Table 3. Serum biochemical values for production tilapia reared in recirculating systems.

Parameters	n[1]	Range	Mean	SEM[2]
Total Protein (g/dL)	40	3.9-8.6	5.8	0.3
Albumin (g/dL)	40	1.8-3.0	2.4	0.1
Globulin (g/dL)	40	2.1-5.6	3.4	0.2
Creatinine (mg/dL)	40	0.2-0.6	0.4	0.02
Total bilirubin (mg/dL)	40	0.1-0.5	0.2	0.02
ALP[3] (U/L)	37	12-48	28	1
AST[4] (U/L)	28	21-770	238	44
Sodium (mEq/L)	40	141-161	151	1
Potassium (mEq/L)	40	3.57-6.16	4.84	0.1
Chloride (mEq/L)	39	110-129	121	1
Calcium (mg/dL)	40	16.5-165.0	60.1	6.58
Phosphorus (mg/dL)	38	9.8-60.1	21.5	1.7
Magnesium (mg/dL)	38	2.8-5.0	3.7	0.1
Glucose (mg/dL)	39	49-120	78	3
Cholesterol (mg/dL)	40	126-313	208	8
Ammonia (µmol/L)	39	157-500	324	17
Osmolality (mOsm)	39	310-359	328	2

[1] *Number of fish,*
[2] *Standard error of the mean,*
[3] *Alkaline phosphatase*
[4] *Aspartate aminotransferase (SGOT)*

revealed similar values for most analytes. Terao and Ogawa (1984) did report higher levels of creatinine (4.3 mg/dL), chloride (192 mEq/dL), cholesterol (567 mg/dL), and glucose (408 mg/dL) and lower levels of calcium (11.8 mg/dL) in *Tilapia nilotica*. Hussein et al. (1996) reported slightly lower levels of total protein, albumin and globulin (3.4, 0.7, and 2.7 g/dL respectively). The blood values reported in this present study are generally consistent with those of other species of finfish (McDonald and Milligan 1992). The calcium and phosphorus levels are higher than usually reported for other species, but the reason for this is unknown.

Differences in hematologic and biochemical blood values may be due to a wide variety of factors. Blood values in fishes are affected by capture and sample collection technique, environmental factors, culture conditions, diet, and age and sex of the fish (McDonald and Milligan 1992; Lane 1979; Ram-Bhaskar and Srinivasa-Rao 1989; Hrubec et al. 1996a,b; Hrubec et al. 1997a,b). Additionally, as reported previously, different hybrids of striped bass have different normal blood values (Hrubec et al. 1996a). In the current study, the tilapia were hybrids of the parental species used in the previously published tilapia hematology studies. The water quality in the production tanks, although typical for intensively reared hybrid tilapia in recirculating systems, is different from the water quality of non-production tanks. These differences may account for some of the variance observed between studies.

In order to develop hematology and clinical chemistry as diagnostic tools for use with fishes, one must determine normal values and then determine whether environmental factors influence these values. Finally, one determines how the values change under pathologic conditions. In the early stages of this process, the range of normal blood values may appear unduly broad until the effects of external factors that influence the blood values (water quality, diet, age, culture conditions, etc.) are identified and mitigated. This is the most likely cause for the range of values observed in this study. Similar ranges of normal values were observed in hybrid striped bass from high density recirculating systems (Hrubec et al. 1996a,b).

Taken together, the analyses of hematologic and biochemical parameters provide direct information on the overall immune status and health of an individual, contributing information relevant to disease diagnosis. As the aquaculture industry expands, tools to monitor the health status of fishes using standardized non-lethal and inexpensive methods will be needed. This study provides baseline blood values for production hybrid tilapia in a recirculation system. Generating these values is the first step in developing hematology as a diagnostic tool for use in these fish.

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