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Haematological Changes in Nile Tilapia *(Oreochromis niloticus)* Fed with Varying Dietary Maltose Levels

[1]Keri Alhadi Ighwela, [2]Aziz Bin Ahmad and [1]A.B. Abol-Munafi

[1]Faculty of Fisheries and Aqua-Industry, Universiti Malaysia Terengganu, Malaysia.
[2]Department of Biology, Faculty of Science, Universiti Malaysia Terengganu, Malaysia

Abstract: The aim of the present study was to investigate the haematological parameters for Nile tilapia; *(Oreochromis niloticus),* feed with various levels of dietary maltose diets (0.0, 20, 25, 30 and 35%) for 14 weeks. The results showed no significant ($P < 0.05$) differences in packed cell volume (PCV), hemoglobin (Hb), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV), with exception of the red blood count (RBCs). Fish fed the control diet had the highest value of PCV, RBCs, Hb and MCHC, while fish fed the 35% maltose diet had the least. The MCH and MCV were shown the highest values in fish fed the 25% maltose diet while fish fed the 30% maltose diet had the least. In addition, there was a significant reduction in the number of leukocytes (WBCs) in fish feed on 20% maltose ($P < 0.05$) compare with control. While the percentage of lymphocytes, Neutrophils, basophiles and eosinophils in fish feed on 35% maltose in diet was significantly ($P < 0.05$) lower than that of the other treatments, but the monocytes were similar in all groups. These blood parameters results of tilapia could be used as reference for comparison to evaluate the health status of fish fed on maltose. The assessment of these parameters might be also used as quick tool for diagnosing diseases, stress and malnutrition in fish.

Key words: Nile tilapia % *Oreochromis niloticus* % Maltose % Haematological parameters

INTRODUCTION

The total world production of tilapias and other cichlids reached 2.6 million ton in 2004 and continued to rise to up to 3.6 million ton in 2008 [1]. Nile tilapia, *Oreochromis niloticus* is a highly favored culture fish with acceptance and among leading farmed species around the world [2]. The dietary requirements of the Nile tilapia are well documented [3, 4]. However, an alternative feed are very crucial for sustainable production with affordable sale prices.

The most viable option appears to be the exploitation of low costly energy source, which abound in the sub tropics, such as Cereal grains serve as inexpensive sources of carbohydrates for warm-water fish, but their use in cold-water fish feeds is limited. Barley is one of Cereal grains that recommend it for use as a substitute for fishmeal in fish feed. It is readily available, which can be converted to maltose by germination process [5]. Addition, dietary carbohydrates in fish diets provide inexpensive energy and also allow for pellet expansion during extrusion [6]. On the other hand, the relative use of dietary carbohydrates by fish varies and appears to be associated with the complexity of the carbohydrate [2]. However, there are instances of nutritional problems when excessive carbohydrates are fed to some species of fish [7].

The haematological characteristics of fishes are an integral part of evaluating their health status [8, 9]. However, the diet composition, metabolic adaptation and variation in fish activity are the main factors responsible for the change in haematological parameters of fish [10]. There are studies reported on the changes in blood parameters indices of fish as result of feed [11, 12]. Thus, determining the basal parameters of blood are importance in order to monitor the health status for commercial purpose, for example, PCV is a useful indicator of anemia, hypoproteinemia and leukocytosis of fish.

Therefore, the objective of the present study was to evaluate the changes of haematological parameters in *O. niloticus* fed with different levels of maltose in diets.

Corresponding Author: Keri Alhadi Ighwela, Faculty of Fisheries and Aqua-Industry, Universiti Malaysia Terengganu, Malaysia.

Table 1: Proportions of different ingredients in the formulated feeds

Ingredient	Feed A (0.0% Malt)	Feed B (20% Malt)	Feed C (25% Malt)	Feed D (30% Malt)	Feed E (35% Malt)
Fish meal	12	12	12	12	12
Soya bean	38	38	38	38	38
Wheat flour	10	10	10	10	10
Maltose	0	20	25	30	35
Cellulose	35	15	10	5	0
Palm oil	3	3	3	3	3
Mineral premix	0.5	0.5	0.5	0.5	0.5
Vitamin premix	0.5	0.5	0.5	0.5	0.5
Vitamin C	0.4	0.4	0.4	0.4	0.4
Binder (CMC)	0.5	0.5	0.5	0.5	0.5
Chromic oxide	0.1	0.1	0.1	0.1	0.1
Total (%)	100	100	100	100	100

Table 2: Mean ±S.E. proximate composition and gross energy of the test feeds (% dry matter)

Ingredient	Feed A (0.0% Malt)	Feed B (20% Malt)	Feed C (25% Malt)	Feed D (30% Malt)	Feed E (35% Malt)
Moisture	8.86±0.93	8.39±0.82	9.22±0.49	9.82±0.63	9.62±0.03
Protein	33.27±0.87	33.70±0.43	33.85±0.29	33.56±0.73	33.27±0.44
Lipid	4.67±0.04	4.83±0.04	4.68±0.08	4.83±0.17	4.67±0.01
Ash	4.44±0.02	4.77±0.02	4.81±0.02	4.94±0.09	4.88±0.26
Fiber	13.62±0.68	11.23±0.09	8.93±0.20	8.71±0. 20	8.71±0.03
NEF	35.14±0.94	37.08±0.57	37.91±0.50	38.14±0.61	38.85±0.32
Total energy(kJ g_1)	18.94±0.89	18.66±0.13	19.17±0.19	19.67±0.49	19.26±0.22

MATERIALS AND METHODS

Experimental Diets: Five iso-nitrogenous and iso-caloric feeds incorporated with different levels of maltose (0%, 20%, 25%, 30% and 35%) which extracted from barley by germination process [5]. All feeds were formulated with ingredients commonly used, including fish meal, soya bean, wheat flour, cellulose which extracted from barley husk, sunflower oil, mineral premix, vitamin premix, ascorbic acid, binder and chromic oxide, were presented in Table 1. Table 2 is the proximate analysis of the experiment diets in percentages.

Fish and Experimental Conditions: The study was conducted at the Fresh Water Hatchery, Faculty of Fisheries and Aqua-Industry.University Malaysia Terengganu, Malaysia. *Oreochromis niloticus* were obtained from Pusat Pengembangan Akuakultur Jitra, Kedah, Malaysia and acclimated to laboratory conditions for 2 weeks. During that period, the fish were fed with commercial feed. A total 450 *O. niloticus* fingerlings, with a mean initial weight of 2.1±0.2 g, were randomly distributed into 15 tanks with 150-L capacity. The fish were fed on the five test diets for 12 weeks during experiment.

Haematological Techniques: At harvest, blood samples were taken using one ml syringes from the caudal vein of a set of three *O. niloticus* fingerlings from each treatment tank (randomly chosen) and put separately in heparinized tubes and taken to the laboratory for determination of haematology parameters. During the blood collection, no anaesthetized was used to avoid the increases in PCV and potassium concentrations and urinary electrolyte loss in teleost fish [13]. Haematocrit (Hct) was determined by the microhematocrit centrifugation technique. The haemoglobin concentration was determined with Drabkin's reagent as absorbance at 540 nm. The red blood cell count (RBCs) and white blood cells (WBCs) were determined optically with a Neubauer chamber [14]. The Giemsa staining method was used for the differential count of WBCs and calculated as a percentage [15]. The haematological indices of mean cell haemoglobin concentration (MCHC), mean cell haemoglobin (MCH) and mean cell volume (MCV) were calculated using the total red blood cell count (RBCs), haemoglobin concentration (Hb) and hematocrit (Hct) according to the following formulae [14].

$$MCHC\ (g/dl) = [Hb\ (g\ dl^{-1}) \times 10] / Hct \times 100$$
$$MCH\ (pg) = [Hb\ (g\ dl^{-1}) \times 10] / RBC\ (10^6\ \mu l^{-1})$$
$$MCV\ (fl) = Hct / RBC\ (10^6\ \mu l^{-1})$$

Statistical Analyses: All the results were subjected to analysis of variance (ANOVA). Duncan multiple range test [16] was further used to evaluate the mean differences at 0.05 significant levels.

Table 3: Haematological parameters of fish after 12 weeks of feeding the experimental diets

Diets	PCV(HCT) (%)	RBCs (10^6/mm^3)	Hb (g/dl)	MCH (pg)	MCHC (g/dl)	MCV (µ3)
Feed A	13.74±0.82	4.33±0.01a	4.63 ± 0.25	10.70±0.57	33.75±0.18	31.73±1.83
Feed B	13.16±1.12	4.10±0.01b	4.41± 0.41	10.73±0.96	33.50±0.90	32.03±2.62
Feed C	13.33±0.10	3.95±0.02c	4.36± 0.02	11.03±0.07	32.71±0.21	33.72±0.43
Feed D	12.95±0.13	4.26±0.03a	4.28± 0.08	10.05±0.12	33.09±0.65	30.38±0.26
Feed E	12.18±0.57	3.64±0.02d	4.25± 0.11	10.97±0.23	32.88±0.89	33.44±1.40

Means with the same letter in the same column is not significantly different at P<0.05

Table 4: Differential counting of leucocytes in the blood of Nile tilapia feed different levels of maltose

Diets	WBCs (x10^3/mm^3)	Lymphocyte (%)	Monocytes (%)	Neutrophils (%)	Eosinophils (%)	Basophils (%)
Feed A	28.65±0.81a	56.66±0.88a	1.50±0.29ab	29.50±2.22abc	2.75±0.25a	0.75±0.25a
Feed B	17.65±0.51c	55.33±0.66ab	2.25±0.48a	35.75±1.88a	2.25±0.63ab	0.75±0.25a
Feed C	20.95±0.71b	54.67±0.33ab	1.50±0.28ab	27.25±2.02bc	1.50±0.50bc	1.00±0.00a
Feed D	29.75±0.49a	53.33±1.20bc	1.50±0.28ab	31.50±2.10ab	1.50±0.29bc	0.75±0.25a
Feed E	1.95±0.45b	51.66±0.88c	1.25±0.48a	24.25±1.25c	0.75±0.25c	0.50±0.28a

Letters in the column indicate significant difference among treatments (P < 0.05)

RESULTS

The haematological parameters indices of Nile tilapia fed on different levels of maltose were presented in Table 3 and Table 4 .The results showed no significant (P < 0.05) differences between all groups in packed cell volume (PCV), hemoglobin (Hb), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), monocytes, neutrophils and basophiles, but the red blood cells count (RBCs) and white blood cells (WBCs),was lower significant (P < 0.05) between groups B,C and E respectively for fish feed on 20, 25 and 35% of maltose in diets compared with control and addition, the percentage of lymphocytes and eosinophils in fish group E which fed on 35% maltose in diet was significantly (P < 0.05) lower than that of the other treatments.

DISCUSSION

The haematological parameters of fish are reported to be affected by a range of factors, which include species, size, age, physiological status, environmental conditions and dietary regime, e.g. quality and quantity of food, dietary ingredients, protein sources, vitamins, probiotics [17-19]. Results show that there a slight decrease in the values of haematological parameters of the *O. niloticus* fed with maltose compared to that fed with control feed. However, the haematological values obtained were within acceptable limits for Nile tilapia, according to the reported of Badawi and Said [20] on blood parameters of Nile tilapia, *tilapia zilli* and *tilapia aureus*. These result were also in agreement with the reported of blood parameters in freshwater teleost fish by Joshi, [21].

Erythrocytes (RBCs) count at range (3.64 – 4.63 10^6/$mm^{3)}$ in the studied *O. niloticus* for all groups were within the range described by Homytowska *et al*. [22] (3.14-5.71 x10^6 /mm^3) and a similar observation was noted with another species such as Jundia, *Rhamdia quelen* as reported by Borges *et al*. [23] and Sunbleak, *Leucaspius delineates* by Homytowska *et al*. [22] and *Clarias gariepinus* by Sotolu and Faturoti, [24].

The hemoglobin concentration (4.25 - 4.63 g/dl) for all groups in studied fish was similar to those reported by Gbore *et al*. [25]; Ispir *et al*., [26] and Ada *et al*. [27], but was lower than those of *Clarias garipeinus* (6.29g/dl) and *Curimbata* (9.7 g/dl) as reported by Adam and Agab [28] and Teixeira [29]. Haemoglobin concentration a slight decreased with increasing dietary maltose, such that fish fed the control diet had the highest values that were different from the values obtained from fish fed other diets.

The mean values of packed cell volume (PCV) in *O. niloticus* obtained from the present study were within the range of the corresponding values by Sotolu and Faturoti; [24] and Ada *et al*. [27]. Several of these studies attempted to determine whether significant variations from normal values of these parameters do exist and could be attributable to some internal or external factors.

The range of corpuscular volume (MCV) 30.36 -33.72 μ^3 and corpuscular haemoglobin (MCH) 10.05- 10.97pg, obtained from this study were similar to previous study by Zaki, *et al*. [30] and Gaafar *et al*. [31].

The numbers of leukocytes (WBCs) in fish blood are extremely variable even among conspecific individuals, even in similar conditions and depend on many factors. in the present study the number of Leukocytes is a higher and it was at range (17.65– 29.75 x10^3/mmG^3) for all groups

were within the range described by Osuigwe, *et al*. [32] (19.23-24.88 x103mmG^3) which fed Juvenile *Heterobranchus longifilis* on raw and boiled jackbean seed meal. In addition, there was found a significantly reduced number ($P < 0.05$) of leukocytes in fish feed on 20% maltose in diet compare with control, it was (17.65 x10^3/mmG^3) a similar observation was noted with another species, such as *Clarias gariepinus* (18.33 x10^3/ mm^3) as reported by Ayoola [33] and juvenile beluga by Hoseinifar *et al*. [34], it was (19.88 x10^3/mm^3) in fish fed on 3% oligofructose.

The higher WBC levels in the present study hint at a possible immunomodulatory effect or due to the stressor effect [35]. However, additional studies are required to assess the effects of maltose on the immune response and stressor of tilapia. The specific reason for this is unclear, but it has been suggested previously that might be a result of inability of the intestinal microbiota to ferment and possible accumulation within intestinal enterocytes by Olsen *et al*. [36] and Olsen *et al*. [36], they hypothesized this after observing that high-level dietary inulin (15% dietary inclusion) had negative effects on the gastrointestinal tract.

On the other hand, differential leukocytes counts were characterized by predominance of lymphocytes. Five types of leucocytes, namely lymphocytes, neutrophils, monocytes basophiles and eosinophils were identified in the circulating blood of Nile tilapia. The percentage of lymphocytes, Neutrophils, basophiles and eosinophils in fish feed on 35% maltose in diet was significantly ($P < 0.05$) lower than that of the other treatments. This could be due to optimal health conditions in fish feed on the maltose diet. On the other hand, the monocytes were a similar in all groups and within the normal range. Kelly [11] reported that monocytes comprise less than 10% of the total WBCs production in animals of all species. Lymphocytes were the highest proportion of the WBCs in the blood of fish in this study. This is in contrast with the WBCs composition of most livestock with neutrophil being the highest in dog, horse, pig and cat while the highest in ruminants was eosinophils.

CONCLUSION

It can be concluded that high dietary levels of maltose as feed to Nile tilapia (*O. niloticus*) *a* slight decrease in the haematological parameters of Nile tilapia but not lower the normal levels. Maltose has no negative impact on the health status of Nile tilapia (*O. niloticus)*. Therefore direct of maltose as feed should be encouraged.

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