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Growth, Biochemistry, Quality, and Risk Assessment of *Oreochromis nioloticus* Influenced by Crude and Processed Chicken Manure

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ABSTRACT

This study aimed to evaluate the effect of crude chicken manure and its processing on growth, biochemistry, quality, and risk assessment of *Oreochromis niloticus*, as this habit is used in several fish farms, without an adequate knowledge of its effect on fish quality. Four feeding regimes were applied, namely (1) crude chicken manure (CM), (2) processed chicken manure, (3) mixed diets [processed CM (45 days) + commercial fish diet (45 days)], and (4) commercial fish diet (control), for 90 days in glass aquaria. The fish fingerlings were acclimatized prior to the start of the experiment, followed by twice daily feeding and regular monitoring of water quality. The growth performance showed a positive trend from crude CM toward the processed CM as fish diets, leading to an increase in final weight and weight gain. The crude CM recorded the lowest final weight of 11.3g and weight gain of 10.2g, and the lowest survival percentage of 85.1%. Furthermore, mosthaematological parameters displayed the similar trend, except RBCs which showed insignificant changes. The immunological indicators such as WBCs showed anelevation starting from the dispersed diet (crude CM) toward the processed CM and mixed diets, then dropped down in the control trial, reflecting the normal trend against strange fish diets. Blood biochemistry, such as total protein and cholesterol, exhibited significant changes in the crude CM, processed CM, and the mixed trial compared to the control group. Regarding fish quality and nutrional values, fish muscle of fish fed crude CM contained the highest ash value of 7.58%, while it was 5.46% for those fed on the processed CM. However, fish muscles for mixed diets contained 5.19% ash and 62.19% protein. Human risk assessment was presented in this investigation by heavy metals determination. Pb value in fish muscles was 4.86mg/ kg for fish fed the crude CM; it was the highest one of all feeding trials. While, it decreased to 4.06mg/ kg after CM processing. The mixed feeding trial exhibited the lowest level of Arsenic (0.06mg/ kg). Processing chicken manure reduces its disadvantages by converting dispersed materials into pellets, thereby decreasing water pollution. It is not recommended to feed fish on pure crude chicken manure and pure processes of CM during the whole fish rearing, since fish need other nutrients and more energy to reduce the heavy metals formed due to chicken manure utilization. Utilizing a mixture of fish diet and processed chicken manure in fish farms, in controlled quantities, can mitigate environmental pollution. It's essential to implement processing procedures and ensure clean sourcing for optimal results.

INTRODUCTION

More than one billion people worldwide rely on fish as an important source of animal protein, providing at least 30% of the animal protein intake **(Omojowo & Omojasola, 2013)**. Fisheries and aquaculture in Egypt are important sources of animal protein **(El-Naggar *et al*., 2008)**. Aquaculture is an effective means of increasing fish production, accomplished through the augmentation of fish stocks and artificial feeding. Unfortunately, the cost of feeding is huge; therefore, the interest has been diverted to other sources of water enrichment, such as using animal manure as fertilizers. Approximately 500 to 600 birds provide sufficient waste to manage a hectare of waste in a fish pond **(Selvaraj, 1994)**. In several cases, fish pond fertilizers can improve fish production by balancing the carbon and other nutrients in ponds. Manure is directly consumed by fish, and the released nutrients support the growth of most photosynthetic organisms **(Little & Edwards, 1999)**.

Various types of natural fertilizers (manure) such as cow, poultry, and semi-liquid pig fertilizers received the highest interest **(Wohlfarth & Schroeder, 1979).** Chicken manure is preferred due to its great capacity for dissolving in addition to its high phosphorus concentrations **(Knud-Hansen *et al.*, 1991)**. However, the use of manure, which is classified as dangerous organic matter from animal excrements, poses a risk to water environment **(Mlejnkova & Sovova, 2012)**. Therefore, the physical quality of feed varies depending on the composition of the ingredients and processing conditions that can affect the intake of feed, nutrient digestibility, and growth of fish. **Srensen (2012)** has stated that the small particles are considered a problem in fish feed.

From another perspective, heavy metals encompass metal elements with relatively high density and non- biodegradability (**Mahammad & Usman, 2021**). In addition, they are toxic to all organisms including humans, even at low concentrations **(Islam *et al.*, 2018; Joda *et al.*, 2019)**, and non-degradable **)**. Bioaccumulation and biomagnification occur due to several physicochemical and biological factors. Integrated agriculture is probably one of the causes of the bioaccumulation of heavy metals and metalloids at different thermal levels **(Maurya *et al.*, 2019)**. Fish, poultry, and vegetable farming are all parts of an integrated fish farming. However, due to the complexity of biological and chemical interactions, it is difficult to accurately measure the risks.

It may undergo regular changes due to dissolving, precipitation, and absorption that affect its performance and bioavailability in the ecosystem **(Kim & Lee, 2010)**. The health risks to humans are caused by the increase in contact frequency with heavy and toxic metals, which are mainly divided into carcinogenic and non-carcinogenic consequences **(Sibal & Espino, 2018)**.

Since blood is a good indicator of the health of organisms, there is little evidence that feeding fish with chicken dung has a negative impact on the haematological parameters. It is also a pathological reflector of the whole body, therfore haematological parameters are important to diagnose the functional state of toxins exposed to animals **(Joshi *et al.*, 2002a)**. The matter of chicken manure (CM) utilization in the fish farms has extended from the fertilizers in specific time to be food or mixed food for fish in some fish farms in a random usage basis, with no care to the fish quality and environmental influences. Therefore, this study aimed to investigate the impact of chicken manure utilization in different forms on growth, biochemistry, and quality of the Nile tilapia, *Oreochromis niloticus*, as well as to determine the health risks associated with its utilization.

Furthermore, this study highlighted the effects of processing on the quality of crude CM and the probability to decrease the impact on fish and water quality.

MATERIALS AND METHODS

Experimental design

This study was performed for 90 days (from the 1st June till 1st September 2020) in the aquarium of Zoology Department, Faculty of Science, Al-Azhar University, Assiut, Egypt. Approximately, 120 fish specimens (Fingerlings) were distributed in different glass aquariums, representing four experiments of feeding the fish: crude manure CM, processed CM, processed CM + commercial feed (45+45 days), and lastly feeding on a commercial fish diet (control 30% protein). The processing of the chicken manure was applied in a simple way of food technology as follows: Initially, crude chicken manure was grinded to obtain CM powder. Then, it was mixed with water (2:1), followed by using an electric mincer to transform the CM powder into pellets instead of the dispersed crude manure powder. This may assist the water environment by reducing the pollutants and collecting all ingredients in pellets. Furthermore, it may reflect the role of processing in the quality of materials and aquatic pollution reduction.

After acclimization, fish were fed twice daily at 9:00 am and 15:00 pm for different feeding treatments. The rate of daily feeding was about 5% of total body weight of fish. During the study period, the total amount of feed consumed by the fish in each glass aquarium was determined. The main items of water quality were monitored weekly, including temperature (via a thermometer), pH (using Jenway Ltd., Model 350-pH-meter) and dissolved oxygen (using Jenway Ltd., Model 970- dissolved oxygen meter). Throught the experiment, the water quality was measured and ranged from 7.6- 8.4 for ph; 18.6- 22.6 for Tc, and 6.8- 7.9 for DO., in all feeding regimes. Due to the small size of the glass aquaria and the regular maintenance procedures such as daily care and siphoning, the effects of chicken manure utilization were not clearly recorded across different treatments.

Growth performance

The feed was halted 24 hours prior to harvest after the 45- and 90- day trials were finished. Before weighing and measuring the fish, MS 222 (100µg/ ml) was used to anaesthetize them. Body length measurements were taken using the closest 0.1mm increment. Before weighing, fish samples were blotted dry on filter paper to determine their weights. Mortality was estimated from the number of fish stocked and harvested. For growth performance, feed and chicken manure utilization were investigated via the following:

Weight gain (WG, g) = FW – IW (g / fish)

%WG = 100 × [(final fish weight (g) - initial fish weight (g)) / initial fish weight]

Specific growth rate (SGR %/ day) = 100 × [(ln final fish weight) - (ln initial fish weight)] /experimental days

Feed conversion ratio (FCR) = feed fed (g) (dry weight)/weight gain (g)

Protein efficiency ration (PER) = weight gain (g) / protein fed (g)

Blood biochemical and haematological analysis

Blood samples were drawn to conduct haematological and biochemical tests. After allowing the blood samples to coagulate at 4°C for 15- 20 minutes, the serum was separated using centrifugation for 20 minutes at 3000rpm. Before being used for biochemical and immunological tests, the serum was kept at –20°C. The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were investigated according to **Reitman and Frankel (1957)**. The levels of glucose (mg/ dl), triglycerides (mg/ dl) and cholesterol (mg/ dl), were determined according to **Trinder (1969)**, **Fridewald *et al.* (1972)** and **Thomas (1992)**, respectively. Moreover, the creatinine and urea and total protein (g/ dl) in serum were determined according to the method described by **Henry *et al*. (1964)**. Red blood cell (RBC) count, white blood cell (WBC) count, haematocrit value (Hct), and haemoglobin concentration (Hb) were measured using a Celltac MEK-6400J/K automated technical analyzer. The methods of **Dacie and Lewis (2002)** were used to estimate the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC).

Chemical composition

The main chemical composition items of fish musles were estimated according to the guidelines of **AOAC (2000)**, including the percentages of moisture and ash, in addition to the crude lipid content (Ether extract) which was analyzed using the Soxhlet system with ether as a solvent (SRPS ISO 1443:1997). Moreover, the crude protein was determined by Micro Klejdahl.

Heavy metals determination

Concentrations of Pb, Zn, and Cd in tissue were determined using a flame atomic absorption spectrophotometer (Varian Spectr AA220FS, USA). Atomic fluorescence spectrometry was used to determine the concentration of As in the manure, while a cold atomic absorption spectrophotometer was employed to measure Hg **(Liu *et al*., 2014)**.

Statistical analysis

All trials were done in a fully randomized manner with four repetitions. The treatments using PPM and biochar were assessed independently. A one-way ANOVA was used to determine the significance of the treatments by MINITAB 13 software. The means of the treatments were compared, and the significance was observed using the LSD test at $P< 0.05$ level and descriptive statistics (±SE).

RESULTS

Growth performance

Every month throughout the experiment, the growth performance metrics were assessed (Table 1). The results demonstrated that, adopting different diets (crude CM, processed CM, and processed CM+control) had a substantial impact on growth performance. The highest ultimate weight (17.7g) and weight gain were seen in fish raised on commercial diet (control) (16.6g). Fish raised on crude chicken manure had the lowest final weight (11.3g), but weight gain was still observed (10.2g). According to Table (1), fish given commercial diet had the highest FCR (2.1), followed by fish fed processed CM+ control (2.6), and fish fed on crude CM had the lowest FCR (3.1). Meanwhile, fish raised on alternative diets showed a significant increase in survival rate; fish fed on crude CM showed the lowest significant survival rate (85.1%), while the other feeding regimes showed significant increases in the survival rates of processed CM (88.3%), processed CM+ control (92.1%), and control (97.3%).

Table 1. Growth performance parameters (Mean ±SD) of *O. niloticus* fed on different formulas for 90 days (CM; Chicken manure)

Treatment / Item	90 Days			
	Crude CM	**Processed CM**	**Processed CM+ control**	**Control**
Initial body weight (IW,g/fish)	1.13±0.05	1.2±0.02	1.1±0.01	1.1±0.03
Final body weight (FW,g/fish)	11.3±0.6[a]	12.7±0.5[b]	14.4±0.7[ab]	17.7±0.2[c]
Final body length (FL,cm/fish)	6.06±0.7a	7.1±0.2b	7.2±0.05b	9.2±0.2[c]
Total weight gain (TWG,g/fish)	10.2±0.6[a]	11.6±0.5[b]	13.3±0.7[ab]	16.6±0.2[c]
Specific growth rate (SGR,%/d)	3.1±0.2[a]	2.7±0.3[a]	2.3±0.2[b]	2.4±0.1[b]
Feed conversion ratio (FCR)	3.1±0.2[a]	2.7±0.1[a]	2.6±0.1[a]	2.1±0.1[b]
Survival rate (SR, %)	85.1±0.1[a]	88.3±0.1[b]	92.1±0.3[c]	97.3±0.2[bc]

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

Blood biochemistry

Fish blood biochemistry alterations in various feeding regimens and subsequent adjustments to blood concentration were examined twice every 45 days (Table 2). Total protein, cholesterol, triglycerides, urea, ALT, and AST (in crude CM, processed CM, and processed CM + control), as well as glucose (in crude CM and processed CM) varied after 45 days of fish feeding on various regimes, showing significant changes compared to the control group. Generally, during the first 45 days, fish samples fed on the crude CM recorded the lowest values of most items, except urea, Ast, GL and TP. These parameters showed an increase and enhancement after processing via the processed CM.

On the other hand, fish fed for 90 days on various regimes, total protein, cholesterol, cholesterol and urea (in crude CM, prepared CM, and processed CM+ control), glucose (in crude CM and processed CM), ALT and AST (in crude CM and processed CM+ control) were determined and showed a significant change. Contrary to the aforementioned metrics, creatinine for the crude, processed, and processed CM + control groups were not substantially different from the control group. Generally, the processed fish diets presented results better than the crude CM.

Haematological parameters

The haematological parameters for different feeding regimes are illustrated in Table (3). After 45 days of the treatments, Hb (in processed CM), Hct, MCV, and platelets (in crude CM), MCH (in crude CM and processed CM), MCHC (in processed CM and processed CM+ control), WBCs (in crude CM, processed CM and processed CM+ Control) showed significant differences compared to the control group ($P< 0.05$), while RBCs (in crude CM, processed CM, and processed CM+ control) showed no significant differences compared to the control group ($P< 0.05$).

In comparison to the commercial diet, the haematological parameters showed a better trend for processing fish diets (control). Upon switching from the dispersed fish diet (crude CM) to the fish feeding trial (processed CM+ control), immunological indicators like WBCs showed an increase in the values after 90 days of feeding, whereas they decreased in the control feeding trial, reflecting the normal trend against unusual fish diets.

Table 2. Biochemical parameters (Mean ± SE) of *O. niloticus* fed on pure crude CM, pure processed CM, mix feedeing duration (First 45 day on processed + 45 day commercial feed) and 90 days. Feeding on commercial diet as control. (CM; Chicken manure)

Treatment	**45 Days**				**90 Days**			
Item	**Crude CM**	**Processed CM**	**Processed CM+ control**	**Control**	**Crude CM**	**Processed CM**	**Processed CM+ control**	**Control**
TP (mg/dl)	3.6±0.26^{ab}	5.5±0.20^{c}	4.9±0.06^{b}	3.1±0.12^{a}	4.47±0.33^{b}	6.00±0.22^{c}	4.73±0.24^{b}	3.1±0.0.30 ^{a}
GL (mg/dl)	111.3±3.15^{c}	74.5±3.75^{b}	68.0±2.72^{a}	55.56±2.09^{a}	147±3.22^{c}	76.26±2.95^{b}	68.1±2.22^{a}	60.23±2.04^{a}
Chol (mg/dl)	111.3±2.14^{b}	155.7±5.32^{d}	145.0±3.77^{c}	79.16±4.42^{a}	133±9.42^{b}	186.23±7.68^{c}	133.16±3.58^{b}	105.73±3.00^{a}
Tg (mg/dl)	39.9±2.86^{a}	161.4±3.52^{b}	165.3±5.00^{b}	209.33±1.57^{c}	51.33±4.17^{d}	106.63±3.28^{b}	164±5.24^{c}	197±4.22^{a}
Crt (mg/dl)	0.57±0.03^{a}	0.59±0.03^{a}	0.63±0.03^{a}	0.70±0.04^{a}	0.30±0.10^{a}	0.43±0.25^{a}	0.56±0.03^{a}	0.64±0.10^{a}
Ur (mg/dl)	29.7±2.07^{a}	34.9±4.10^{b}	30.8±0.81^{ab}	25.06±1.73^{ab}	21.33±2.08^{ab}	39.06±3.39^{c}	27±2.33^{b}	13.66±2.22^{a}
ALT(U/L)	18.73±0.61^{b}	16.3±0.57^{a}	15.6±0.38^{a}	23.56±0.60^{c}	30.46±1.88^{c}	17.6±1.26^{a}	24.86±0.59^{b}	17.3±0.50^{a}
AST(U/L)	27.73±1.12^{c}	15.6±1.17^{b}	17.3±1.38^{ab}	21.13±1.66^{b}	32.56±11.80^{c}	15.33±0.99^{a}	21.3±1.43^{ab}	26.53±2.03^{bc}

Means in the same row with different superscript letters are significantly different at $P < 0.05$.
Total protein (TP); Glucose (GL); Cholesterol (Chol); Triglyceride (Tg); Creatinine (Crt),; Urea (Ur), andAlanine aminotransferase (ALT) and Aspartate aminotransferase (AST).

Table 3. Haematological parameters (Mean ±SE) of *O. niloticus* fed pure crude CM, pure processed CM, mix feedeing duration (First 45 day on processed + 45 day commercial feed) and 90 days. Feeding on commercial diet as control. (CM; Chicken manure)

Treatment	**45 Days**				**90 Days**			
Item	**Crude CM**	**Processed CM**	**Processed CM+ control**	**Control**	**Crude CM**	**Processed CM**	**Processed CM+ control**	**Control**
RBCs (×10^{6} µl)	2.83±0.20^{a}	2.40±0.18^{a}	2.30±0.26^{a}	2.46±0.18^{a}	4.93±0.12^{c}	4.13±0.18b^{c}	2.93±0.51^{a}	3.13±0.23^{ab}
Hb (g/dl)	10.26±0.51^{a}	14.63±0.40^{c}	13.13±1.24^{ab}	14.6±1.29^{ab}	11.7±0.15^{b}	13.63±0.42^{a}	12.46±0.57^{b}	12.23±0.76^{b}
Hct (%)	23.33±0.29^{a}	39.73±1.62^{b}	37.03±3.55^{b}	35.63±1.38^{b}	33.58±0.46^{ab}	28.9±1.26^{a}	34.66±1.9^{b}	37.43±1.33^{b}
MCV (fL)	26.26±2.74^{a}	127.03±29.75^{b}	161.63±4.00^{b}	148.3±7.53^{b}	67.16±1.96^{a}	69.93±1.46^{a}	145.83±4.4^{c}	123.91±3.84^{b}
MCH (pg)	18.36±0.05^{a}	37.26±5.01^{c}	43.46±2.49^{ab}	50.16±0.82^{b}	23.36±0.67^{a}	33.23±0.47^{a}	52.4±1.93^{c}	45.86±1.93^{b}
MCHC (g/dL)	30.1±0.05^{a}	37.26±1.83^{c}	35.46±0.85^{bc}	31.33±1.19^{ab}	34.8±0.05^{b}	32.8±0.32^{b}	33±1.39^{b}	28.4±0.33^{a}
WBCs (×10^{3} µl)	112±0.90^{b}	229.23±29^{c}	246.67±15.06^{c}	34.23±2.93^{a}	129±11.2^{b}	149.66±19.46^{b}	١٥٦.2±5.76^{c}	27.96±1.07^{a}
Platelets (×10^{3} µl)	132.33±6.02^{a}	193±23.70^{b}	190.66±12.83^{b}	203.66±11.04^{b}	123±12.40^{a}	135.66±9.47^{a}	153.66±6.73^{ab}	189.33±15.50^{b}

Means in the same row with different superscript letters are significantly different at $P < 0.05$. Red blood cells (RBC), haemoglobin concentration (Hb), haematocrit value (Hct), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) and white blood cells (WBC).

Chemical composition and heavy metals of fish diets and muscles

The analysis of the chemical composition of fish muscles, representing the study's final products, and fish feeds (crude CM, processed CM, and commercial diets) in its initial phase are presented in Table (4). The crude chicken manure (CM) has the highest ash rates (٣٢.19± 0.6) and crude fat contents (ether extract), but it contains the lowest protein value (28.25%). The processed CM had protein levels of 39.625% higher than crude CM, while it decreased in its ash levels of 26.78%. The maximum concentration of protein (76.5%), and the lowest value of ash and crude fat (8.05 and 3.23%, respectively) were detected in the control groups.

Fish muscle samples previously fed on crude CM had the highest amount of ash (7.58%). The lowest value was observed in the fish muscle fed on fully processed CM, which comprised 6.92% crude fat and 5.46% ash. The fish muscle samples raised on combined diets (processed and commercial diets) exhibited 5.19% ash, 62.19% protein, and 6.45% crude fat content. The lowest levels of ash and crude fat were observed in fish muscle samples fed on pure commercial feed (control); however, the highest levels of protein were detected in these samples (75.06%).

For the human risk assessment, Table (5) illustrates the heavy metals contents in the 3 types of fish feed. It was obvious that, the fish diets analysis showed that the highest value of Pb (6.15mg/ kg), As and Cd were recorded in crude CM; meanwhile, the Hg in crude CM showed the highest value (3.17mg/ kg). Pb was still present at a high level (5.25mg/ kg) in the processed CM, which were intermediate. However, it was lower than crude CM. The control samples' levels of Cd and Hg were the lowest within the values of 0.07 and 2.58mg/ kg, respectively. The results of fish muscles in the four feeding treatments showed the heavy metal concentrations in fish muscles (Table 5). The heavy metals displayed high values, which included 0.06 for Cd, 4.86mg/ kg for Pb, and 1.98mg/ kg for Hg. However, the Pb value declined significantly with CM modification to be processed (4.06mg/ kg). The results also showed that the As in fish muscle in the mixed diet (processed CM + commercial fish diet) was 0.06mg/ kg lower than those fed on feed of fully processed CM. Although crude CM initially exhibited the highest levels of the investigated heavy metals, their concentrations declined after processing the feed material. However, the condensing of crude CM into processed CM, which contains all parts in pellets, resulted in an elevation of the percentage of some heavy metals in fish muscles, especially Hg and As. Generally, the processing of crude CM led to both negative and positive changes in both feed and fish quality.

Table 4. Chemical composition of fish diets and fish muscle samples (Mean ± SE) after 90 days of the treatment (CM; Chicken manure)

Sample	**Treatment**	**Ash (%) mean±SD**	**Protein (%)**	**Crude fat (%) mean±SD**
Fish diets	Crude CM	32.19±0.6	28.25	4.27±2.3
	Processed CM	26.78±0.4	٣9.625	3.75±1.9
	Control	8.05±0.1	76.50	3.23±1.2
Fish muscles	Crude CM	7.58±1.1	28.69	12.31±0.9
	Processed CM	5.46±0.9	55.93	6.92±1.4
	Processed CM+ Control	5.19±0.5	62.19	6.45±1.3
	Control	4.62± 2.3	75.06	5.16±0.5

Table 5. Heavy metals content in different fish formulas and fish muscles (mg/ kg) based on dry weight. (CM; Chicken manure)

Sample	Treatment	Hg	As	Pb	Cd
Fish Diets	Crude CM	3.17	0.05	6.15	0.13
	Processed CM	2.89	0.03	5.25	0.08
	Control	2.58	0.01	2.29	0.07
Fish muscles	Crude CM	1.98	0.04	4.86	0.06
	Processed CM	2.29	0.14	4.06	0.05
	Processed CM+ Control	0.47	0.06	2.91	0.04
	Control	0.44	0.04	2.06	0.02

Table 6. Maximum and standard levels in (µg/ g dry weight) of heavy metals in fish described in literature and range of concentrations found in the muscle of fish [(-) indicates not mentioned or not studied)]

Heavy metal / Organization	Pb	Cd	As	Hg	Cu	Reference
FAO	2	0.5	-	0.5	30	FAO (2000)
WHO	0.5	0.5	-	0.5	30	WHO (2000)
FDA	1.7	4	-	0.5-1		FDA (2001)
ROPME	0.01–1.28	0.01–0.75	-	1	0.05-19.5	ROPME (1999)
EC	1	0.1	-	0.5-1	10	EC Regulation
NOAA	128	4	-	0.5	149	NOAA (2009)
Present study	2.06-4.86	0.02- 0.06	0.04-0.14	0.47-3.17	-	

FAO: Food and Agriculture organization, WHO: World Health organization, FDA; Food and Drug Administration, ROPME; Regional Organisation for Protection of the Marine Enviroment, EC; Ethical consideration, NOAA; National Oceanic and Atmospheric Administration.

DISCUSSION

This study provides some scientific insights into the improper usage of conventional materials as substitutes for commercial fish diets. The utilization of manure is attributed to its inclusion in fertilizers. This finding agrees with that of **Sloan *et al.* (2003)**, who mentioned that the fertilizers are applied to produce the plant nutrients needed to be added to soil fertilizers by adding organic material. The potential risks of CM associated with human consumption must be carefully evaluated, taking into account the role of the processing for its composition. The current investigation introduced the random utilization of chicken manure in fish farms not only as fertilizers but also to be directed as fish feeds in crude and processed forms. The current results are slightly different from those reported by **Yola and Adikwu (2017)**, who found that crude protein in CM was 19.22% (lower than our obtained results), while the crude fat value was nearly identical to that obtained in this analysis (4.32%), and the ash percentage was 13.98% (lower than data in this study). On the other hand, **Yones and Metwalli (2015)** reported that the approximate protein, lipid, and ash contents of tilapia were undisturbed by the inclusion of 100% poultry manure. Moreover, **Hernandez *et al.* (2010)** reported similar outcomes for gibel carp *Carassius auratus gibelio* **(Hu *et al.*, 2008)**, tilapia, as well as sunshine bass (*Morine chrysops* × *Morone saxatilis*) **(Pine *et al.*, 2008)**, and Malabar grouper (*Epinephelus malabricus*) **(Wang *et al.*, 2008)**. The nutrient makeup of various manure types varies widely depending on the type of animal (omnivore, ruminant, etc.), sex, species, age, the meal supplied to the animal, and geographical and climatic conditions.

This disparity between our results and the literature serves as an example for such variation **(Lukehurst *et al.*, 2010)**.

The high nutrient content was found in chicken manure, which is why it created higher plankton abundances. According to **Kang'ombe *et al.* (20۰6)** and **Adewumi *et al.* (2011)** chicken manure is abundant in nitrogen, which is typically the limiting nutrient. Pigs and chickens have single-gastric animals; therefore, they only undergo one digestion of their meal. As a result, the majority of the nutrients in the feed that is given to hens are wasted in faeces **(Perkins *et al.*, 1964; FAO, 1985)**. These nutrients encourage the growth of plankton **(Piasecki *et al*., 2004; Jha *et al.*, 2008)**. To promote and stimulate the growth and thriving of the farm with plankton for life, it is important to release or assist fish farms at the beginning of their culture period. Unfortunately, farmers still use manure to feed fish farms, disregarding both their composition and the effects it has on both fish and humankind.

In this study, there was a considerable difference in how well each diet promoted growth. The data obtained in this study disagree with those of **Endebu *et al*. (2016)**, who studied the growth performance of fish under goat manure fertilized pond (T1) and poultry farms (T2). They concluded that, the daily growth rate of *O.niloticus* under poultry integration is higher than the rate in pond fertilized with goat manure under extensive pond culture, resulting in bigger- sized fish at harvest. The differences may be due the feeding style of poultry or the rearing type. Their survival rate at the end of the experiment for *O. niloticus* was 71 and 73% in T1 and T2, respectively. This is lower as compared to the 84% survival rate attained in concrete ponds (**Tugie *et al*, 2014)**. The obtained survival rate in this investigation is higher than the above mentioned, and this may be due to the small glass aquaria with the daily care. Additionally, the processing of crude CM reduced the dispearsing of particles by converting them into condensed pellets. The present growth rate showed an increase by the processing of crude CM to pure commercial fish diet, which reflected the role in processing to condensate the valuable valued rather than crude CM. This also was supported by the chemical analysis of crude and processed CM, where the protein measuring in processed CM was higher than those in crude CM. The condensing of CM particles in collective pellets may enrich the protein content which plays a role in enhancing the growth rate as presented here. In addition, **Khater *et al.* (2014)** reported that the average weight of fish increases as the size and protein ratio of the pellets increase.

The fish blood biochemistry and haematology are considered quick indicators for feeding nature. There was substantial diversity in the values of haematological variables. Nonetheless, substantial differences in the values of erythrocyte volumes across the groups were detected in this investigation. Higher metabolic demand is the cause of the elevated RBC counts and Hb concentrations, and has no effect on erythrocyte volume. The greater amount of RBCs indicates a greater need for oxygen in the tropical zone to support higher metabolic rates **(Engel & Davis, 1964)**. **Rambhaskar and Srinivasa Rao (1986)** reported similar outcomes. Moreover, **Joshi *et al.* (2002b)** reported the impact of toxicants on the blood parameters of the freshwater teleost fish *Clarias batrachus*, which coincides with the present obtained results. The findings of **Joshi *et al.* (2002b)** were supported by the rise in haematological markers detected in control fish samples on a normal diet. Fish survival has been discovered to be connected with antibodies production elevation, which is helpful for survival and recovery. When compared to fish fed with a regular diet, fish fed with poultry manure showed a decrease in haematological markers, which is an indication of blood loss. The values observed for fish fed poultry litter are lower than those reported for African catfish in studies by **Agbede *et al.* (1999)** and **Oyelese *et al.* (1999)**. The initial haematological values of the fish before the feeding trials and those fed the control diet did not differ significantly from one another although the values observed for both fell inside the boundaries for healthy juvenile catfish **(Oyelese *et al.*, 1999; Omoniyi *et al.*, 2002)**. Similar to the findings of this investigation, **Gabriel *et al*. (2004)** also observed a non-significant variation in haematological parameters for seemingly healthy *C. gariepinus* prior to and following acclimization. This may be due to the biological tolerance of catfish unlike tilapia, which is sensitive to changes in diets and its composition. B-lymphocytes are the most common cells composing leucocytes, which serve in the creation of antibodies and chemical compounds functioning as a defence against infections. The immune system is suppressed, and fish are more susceptible to illness as a result of documented changes in leucocyte count in stressed fish **(Wedemeyer & Wood, 1974)**. The reduction in RBC count, haemoglobin, and haematocrit values estimated in juvenile *C. gariepinus* fed on poultry litter is comparable to what **Gill and Pant (1981)** and **Joshi *et al.* (2002c)** reported in *C. batrachus* and *Puntius conchonius* after various toxicants exposure. The current results of haematology and biochemistry showed that the good processing of diets and manufacturing played a role in the stability of immunological status of fish rather than none or slightly processed diets.

With resoect to the fish quality and body composition, the fish muscles are affected by food materials. Fish muscle protein percentages and fish diet protein percentages followed the same patterns. This corroborates with the outcome of **Craig *et al.* (2017)** elucidating that, high fat levels can be caused by excess calories in the diet relative to protein content. Fish must consume food to meet their energy needs; therefore, high energy diets may force them to consume less food and put on less weight. Energy to protein ratios in feeds that have been properly developed and produced are balanced. The poultry litter fed to catfish had a proximate composition of less than half of 40% crude protein compared to the control diet suggested by **Faturoti *et al.* (1986)**. The current analysis of fish muscles showed the lowest protein percentages in fish fed on crude CM; at the same time, the highest ash and crude fats contents were observed. Then, an inverse trend was observed in fish fed on processed CM. This reflected the role of processing to condensate the composition in feed pellets which elevated protein content, while ash and crude fat percentages declined. In fish fed on crude CM, it needs more energy to remove/ release the extra of ash or pollutants. Hence, the current results of biochemistry, body composition, and growth are in the same trend of harmony.

In a step to the risk assessment and the danger of human consumption, heavy metals have an essential function that may impair fish quality in food manufacturing. The processing of food materials in the present study reduced the ashing and elevated the protein content. These items play the key role in fish quality and further manufacturing processing. Contrarily, the crude CM in the dispearsed form showed the highest ash content resulting in the presence of heavy metals at high levels. The maximum permissible levels for human consumption of Hg (0.5), Zn (30.0), and Cu (30.0), Pb (2.0), and (0.5) µg/g were defined by **FAO (2000)** and **WHO (2000)** based on dry samples. The gills of fish serve the dual function of filtering both water and food at the sediment interface. This can lead to the accumulation of mercury (Hg) in various tissues such as the skin & liver, which causes some metals to be retained within the fish **(Sonone *et al.*, 2020)**. Therefore, the results of heavy metals in fish muscles are lower than those in the source of feeding due to efforts of fish to filter the toxicants and release the extra substances via kidney. However, the current results gave an alarm toward Hg and Pb values particularly for fish fed on crude CM and processed CM. **Yu *et al.*** (2021) investigated the heavy metals of As, Cd, Hg, Cr, Pb, Cu, and Zn in a chicken manure-compost-soil-apple system. The concentrations of As, Cd, and Hg in chicken manure samples exceeded the National Agricultural Standard for Organic Fertilizers of China. During the composting process, heavy metal concentrations in the chicken manure were further increased. As, Cd, and Hg concentrations in chicken manure, compost, soil, and apple fruit all exceeded the standard levels (except for As in soil), while the remaining heavy metals (Cr, Pb, Cu, and Zn) were below the standard limits. These findings were higher than the present results of heavy metals, and this may be due to the concentration of chicken manure and the original food of chicken. However, the trend of increasing the metals after composting agrees with the current trend of chicken manure after processing. Given the **Yu *et al*. (2021)**, the current results are lower and below the limits of the National Agricultural Standard for Organic Fertilizers of China. While, the comparison of heavy metals between the present investigation and previous studies is shown in Table (6), as reported in **Hosseini *et al*. (2015)**. Data in Table (6) show that,, the results of Pb were higher than those reported by most of authorities except NOAA, where it showed higher values. For Cd, the current rsults are in the range of authorities, except for FDA and NOAA. Regarding Hg, the present range is higher than the ranges reported by the authorities. Finally, the chicken manure has both sides: the positive one through the organic ferilizers and protein content and the negative side due to high ash, which leads to high percentage of heavy metals. While these materials have a negative impact on fish growth, the processing of manure also offers certain benefits. For instance, it increases the protein content in pellets, which can enhance growth. However, the pelletization process may also concentrate heavy metals.

CONCLUSION

It can be concluded that the growth performance of *Oreochromis niloticus* showed the lowest growth performace rates due to the utilization of crude chicken manure (CM). This growth was enhanced after processing the CM to be formed as pellets. However, both crude CM and processed CM parameters were lower than the commercial diet (control). After CM processing, some of the features were enhanced, such as protein, ash and most haematological parameters, while some immnulogical items such as WBCs showed an elevation surpassing the normal rate. In addition to the advantages of using a mixed feeding regime, which includes processed chicken manure (CM) combined with commercial fish diet, the study demonstrated improved parameters and enhancements compared to solely using either crude CM or processed CM. Furthermore, this mixed feeding approach proved to be the most cost-effective option when compared to the expenses associated with using only a pure commercial fish diet. The processing of food source gave the positive changes in growth and some biochemistry quality. While it gave some negative changes in other biochemistry features, posing threats to fish as heavy metals are concentrated in pellets. Hence, it is not recommended to feed fish on either pure crude chicken manure or pure processed CM during the whole fish rearing period since fish need other nutrients that are not detected in CM and need more energy to remove or reduce the heavy metals occurring due to chicken manure utilization. However, the limits of risk were within the allowed permits. Hence, incorporating chicken manure into fish farms in small quantities as fertilizers is deemed acceptable. However, it is crucial to adhere to processing procedures and pollution control measures to mitigate the environmental impact of chicken waste. Additionally, combining this practice with a balanced and healthy fish diet regimen is advisable for optimal results.

REFERENCES

Abo-Elela, G. M.; Elsersy, N.A.; Elshenawy, M. and Abdel-Mawla, E. (2005). Microbial assessment of El-Max fish farm. Egypt. J. Aquat. Res., 31(1): 171-184.

Adewumi, A. A.; Adewumi, I. K. and Olaleye, V. F. (2011). Livestock waste-menace: Fish wealth-solution. African J. Environ. Sci. Technol., 5(3): 149-154.

Agbede, S. A.; Ogunsanmi,, A. O.; Taiwo, V. O.; Oso, J. A. and Ogundipe, T. I. (1999). Toxic effects of poultry faeces on *Clarias gariepinus* broodstock. Trop.Veterin., 17: 181-191.

AOAC (2000) Official methods of analysis of AOAC international, 17th edn. AOAC International, Md., USA.

Craig, S. R.; Helfrich, L. A.; Kuhn, D. and Schwarz, M. H. (2017). Understanding fish nutrition, feeds, and feeding. College of Agricult. Life Sci., Virginia Tech.

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

El-Naggar, G. O.; NasrAlla, A. M. and Kareem, R. O. (2008). Economic analysis of fish farming in Behera Governorate of Egypt. In: H. Elghobashy, K. Fitzsimmons, A.S. Diab, (Eds.), From the Pharaohs to the Future, Proceedings of 8th International symposium on Tilapia in Aquaculture. pp: 693-708.

Endebu, M.; Tugie D. and Negisho, T. (2016). Fish growth performance in ponds integrated with poultry farm and fertilized with goat manure: a case in Ethiopian Rift Valley. Inter. J. Fish. Sci. Aquacult., 3: 040-045.

Engel, D. M. and Davis, E. M. (1964). Relationship between activity and blood composition in certain marine teleosts. Copeia, 3: 586-587.

FAO (Food and Agriculture Organization) (2000). Compilation of legal limits for hazardous substances in fish and fishery products. FAO Fishery Circular No. 464 (pp. 5–10). Rome Food Agricult. Organiz. United Nations.

Faturoti, E. O.; Balogun, A. M. and Ugwu, L. L. C. (1986). Nutrient utilization and growth responses of *C. lazera* fed different dietary protein levels. Nigeria J. Appl. Fish. Hydro., 1: 41 - 45.

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

Gabriel, U. U.; Ezeri, G. N. O. and Opabunmi, O. O. (2004). Influence of sex, source, health status and acclimation on haemotology of *Clarias gariepinus* (Burch1822). African J. Biotechnol., 3(9): 463-467.

Gill, I. S. and Pant, J. C. (1981). Effect of sublethal concentrations of mercury in a teleost *Puntius conchominus* biochemical and haematological responses. Indian J. Experi. Biol., 9: 571-573.

Henry, R. J. (1964). Clinical Chemistry. New York, Harper and Row publish.

Hernandez, C.; Olvera-Novoa, A. M.; Hardy, R.W.; Hemosillo, A.; Reyes, C., et al. (2010). Complete replacement of fish meal by porcine and poultry by product meals in practical diets for fingerling Nile tilapia *Oreochromis niloticus*: digestibility and growth performance. Aquacult. Nutrit., 16(1): 44-53.

Hosseini, M.; Nabavi, S. M. B.; Nabavi, S. N. and Pour, N. A. (2015). Heavy metals (Cd, Co, Cu, Ni, Pb, Fe, and Hg) content in four fish commonly consumed in Iran: risk assessment for the consumers. Environ. Monit. Assess., 187(5): 1-7.

Hu, M.; Wang, Y.; Wang, Q.; Zhao, M.; Xiong, B.; Qian, X., ... , Luo, Z. (2008). Replacement of fish meal by rendered animal protein ingredients with lysine and methionine supplementation to practical diets for gibel carp, *Carassius auratus gibelio*. Aquaculture, 275(1-4): 260-265.

Islam, M. M.; Karim, M. R.; Zheng, X. and Li, X. (2018). Heavy metal and metalloid pollution of soil, water and foods in Bangladesh: a critical review. Inter. J. Environ. Res. Pub. Health, 15(12): 2825.

Jha, P.; Barat, S. and Nayak, C. R. (2008). Fish production, water quality and bacteriological parameters of Koi carp ponds under live-food and manure based management regimes. Zool. Res., 29 (2): 165.

Joda, B. A.; Alheloo H. S.; Al-Mankosh, H. J. A. and Maitham S. A. (2019). Determination of heavy metals arsenic, cadmium and lead in water, sediments and fish from Al Delmaj Marshes-Iraq. AIP Conference Proceedings, 2144:020012.

Joshi, P.K.; Bose, M. and Harish, D. (2002a). Changes in certain haematological parameters in a siluroid cat fish *Clarias batrachus* (Linn) exposed to *cadmium chloride.* Pollut. Res., 21(2): 129-131.

Joshi, P. K.; Harish, D. and Bose, M. (2002b). Effect of lindane and malathione exposure to certain blood parameters in a fresh water teleost fish *Clarias batrachus*. Pollut. Res., 21(1): 55-57.

Joshi, P.K.; Bose, M. and Harish, D. (2002c). Haematological changes in the blood of *Clarias batrachus* exposed to mercuric chloride. Ecotoxicol. Environ. Monit., 12(2): 119-122.

Khater, E.S. G.; Bahnasawy, A. H. and Ali, S.A. (2014). Physical and mechanical properties of fish feed pellets. J. Food Process. Technol., 5(10): 1.

Kim, N. S. and Lee, B. K. (2010). Blood total mercury and fish consumption in the Korean general population in KNHANES III, 2005. Sci. Total Environ., 408:4841-4847.

Knud-Hansen, C. F.; McNabb, C. O.; Batterson, T. R.; Harahat, S.; Sumatadinata, K. and Eidman H. M. (1991). Nitrogen input, primary productivity and fish yield in fertilized freshwater ponds in Indonesia. Aquaculture, 94:49-63.

Little, D. C. and Edwards, P. (1999). Alternative strategies for livestock-fish integration with emphasis on Asia. Ambio J. Human Environ., 28 (2): 118-124.

Lukehurst, C. T.; Frost, P. and Al Seadi, T. (2010). Utilisation of digestate from biogas plants as biofertiliser. IEA bioenergy, 2010: 1-36.

Maurya, P. K.; Malik, D. S.; Yadav, K. K.; Kumar, A.; Kumar, S. and Kamyab, H. (2019). Bioaccumulation and potential sources of heavy metal contamination in fish species in River Ganga basin: Possible human health risks evaluation. Toxicol. Reports, 6: 472-481.

Mlejnková, H. and Sovova, K. (2012). Impact of fish pond manuring on microbial water quality. Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis, 60 (3): 117-124.

Muhammad, S. and Usman, Q. A. (2021). Heavy metal contamination in water of Indus River and its tributaries, Northern Pakistan: evaluation for potential risk and source apportionment. Toxin Rev., 41:380-388.

Omojowo, F. S. and Omojasola, P. F. (2013). Microbiological quality of fresh catfish raised in ponds fertilized with raw and sterilized poultry manures. American Journal of Research Communication, 1: 1-2.

Omoniyi, I.; Agbon, A. O. and Sodunke, S. A. (2002). Effect of lethal and sub-lethal concentrations of tobacco (*Nicotiana tobaccum*) leaf dust extract on weight and haematological changes in *Clarias gariepinus* (Burchell). J. Appl. Sci. Environ. Manag., 6 (2): 37-41.

Oyelese, A. O.; Taiwo, V. O.; Ogunsanmi, A. O. and Faturoti, E. O. (1999). Toxicological effects of cassava peels on haematology, serum biochemistry and tissue pathology of *Clarias gariepinus* fingerlings. Trop. Veterin., 17:17-30.

Piasecki, W.; Goodwin, A. E.; Eiras, J. C. and Nowak, B. F. (2004). Importance of Copepoda in freshwater aquaculture. Zool. Stud., 43(2): 193-205.

Pine, H. J.; Daniels, W. H.; Davis D. A.; Jiang, M. and Webster, C. D. (2008). Replacement of fish meal with poultry by-product meal as a protein source in pond-raised sunshine bass (*Morine chrysops* × *Morone saxatilis*). Journal of the World Aquacult. Soci., 39: 586-597.

Rambhaskar, B. and Srinivasa Rao, K. (1986). Comparative haematology of ten species of marine fish from Visakhapatnam Coast. J. Fish Biol., 30:59-66.

Reitman, S. and Frankel, S. (1975). Colorimetric Determination of Glutamic Oxaloacetic and Glutamic Pyruvite Transaminase. J. Clin. Pathol., 28-56.

Selvaraj, C. (1994). Fresh water aquaculture as an important component in the integrated farming system. In Proceeding Summer institute on integrated farming system research and management for sustainable agriculture, Tamil Nadu Agricultural University Coimbatore, 31–136.

Sibal, L. N. and Espino, M. P.B. (2018). Heavy metals in lake water: a review on occurrence and analytical determination. Inter. J. Environ. Analytical Chemist., 98: 536-554.

Sloan, D. R.; Kidder, G. and Jacobs, R. D. (2003). Poultry Manure as a Fertilizer. University of Florida, Gainesville. 326 p.

Sonone, S. S.; Jadhav, S.; Sankhla, M. S. and Kumar, R. (2020). Water contamination by heavy metals and their toxic effect on aquaculture and human health through food Chain. Letters in Appl. NanoBioSci., 10(2): 2148-2166.

Sørensen, M. (2012). A review of the effects of ingredient composition and processing conditions on the physical qualities of extruded high-energy fish feed as measured by prevailing methods. Aquacult. Nutrit., 18 (3): 233-248.

Thomas, L. (1992). Enzymatic Colorimetric Method to Determine the Cholesterol. Lab and Diagnosis, 4 D.

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

Tugie, D.; Endebu, M. and Negsho, T. (2014). Comparison study of growth performance of three *O. niloticus* population under culture conditions in concrete Tanks, Ziway, Ethiopia. In: Brook Lemma and Seyoum Mengistou (Eds.) Water reservoir in Ethiopia: Ecology and their benefits. 6th Annual Conference of the Ethiopian Fisheries and Aquatic Sciences Association (EFASA), Mekelle, pp 161-178.

Wang, Y.; Li, K.; Han, H.; Zheng, Z. and Bureu, D. P. (2008). Potential using a blend of rendered animal protein ingredients to replace fish meal in practical diets for Malabar grouper (*Epinephelus malabricus*). Aquacult., 281: 133-117.

Wedemeyer, G. A. and Wood, J. (1974). Stress a predisposing factor in fish disease. U.S.Fish/Wildlife Service fish diseases leaflet. 399 Pp.

WHO (World Health Organization) (2000). Health criteria other supporting information. In Guidelines for Drinking Water Quality, vol. 2, 2nd ed., (pp. 31–388), Geneva.

Wohlfarth, G. W. and Schroeder, G. L. (1979). Use of manure in fish farming—a review. Agricult. wastes, 1(4): 279-299.

Yola, I. A. and Adikwu, A. I. (2017). Effects of organic manure on some haematological parameters of *Oreochromis niloticus*, *Clarias gariepinus* and *Cyprinus capio* in polyculture system. Bayero J. Pure and Appl. Sci., 10(2): 141-145.

Yones A.M.M. and Metwalli, A. A. (2015). Effects of fish meal substitution with poultry by-product meal on growth performance, nutrients utilization and blood contents of juvenile Nile Tilapia (*Oreochromis niloticus*). J. Aquacult. Res. Develop., 7(1): 1000389.

Yu, X.; Chen, G.; Zhang, X.; Zhang, J.; Jiang, L.; Zhu, H.; Feng, Z.; Li, Y.; Feng, T. and Zhang, X. (2021). Migration and Accumulation of Heavy Metals in a Chicken Manure-Compost-Soil-Apple System. Pol. J. Environ. Stud. Vol. 30, No. 4 (2021), 3877-3883. DOI: 10.15244/pjoes/131254