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Growth performance, nutrient utilization and survival rate of *Clarias gariepinus* fed varied inclusion of processed *Moringa oleifera* diets

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Abstract
This study was conducted to test the effect of *Moringa oleifera* processed diet processed with Aqueous, Ethanol and Hexane solvents on the growth performance, nutrient utilization and survival rate of *Clarias gariepinus*. Fish having average initial weight of 9.8±0.1 g were stocked in triplicate at the stocking density of 20 fish. 7 diets were formulated with a Commercial Reference Diet (CRD) in which CRD and ZSD serving as control. Others such as MAQ, MET and MHX were formulated with 3% and 5% Moringa inclusion rate. Fish were fed twice daily at 5% of their body weight, water quality parameters were monitored and weight of the fish were taken biweekly. At the end of the feeding trial, the growth performance and nutrient utilization of fish fed 3% Moringa based diets were significantly (p>0.05) higher than 5% Moringa based diets. All experimental samples grew uniformly at the beginning of the experiment and at 2. Diet 3% MAQ, 3% MHX, ZSD, 3% MET and CRD gave the best weight beginning from week 2. All the 5% diets administered to the fish did not produce increase in weight at the same pace as 3% diets. Fish fed 3%MAQ consistently maintained superior performance followed by 3% MHX, ZSD and 3% MET. There was a significant (p>0.05) effect of diet on the survival of the experimental fish. All samples fed 5% inclusion and those fed 3% MAQ had maximum survival while CRD had the least survival rate. Findings in the study shows that inclusion of processed Moringa have positive impact on the diet of *Clarias gariepinus* as it promotes fish growth performance and nutrient utilization at optimum inclusion level.

Keywords: *Moringa oleifera*, aqueous**,** ethanol**,** hexane and *Clarias gariepinus*

Introduction
In fin fish aquaculture production, aqua feed production accounts for 60-70% operating cost depending on the level of aquaculture intensification. Protein is the most critical and most expensive ingredient in aqua feed production in the world and usually, protein sources represents about 60% or more in the cost of feed production (Bake *et al.*, 2014) [3]. The aim of fish farmers are to produce table-sized fish within the shortest possible time. In Nigeria, the aquaculture industries has focused attention on the Clarias species because they occupies a unique position in the commercial aquaculture due to their nature of being hardy, tasty, tolerance to poor water quality conditions, highly fecund, early growth performance, efficient feed conversion ratio especially in the male and thereby attract high market value (Afia and Ofor, 2016) [1]. The essential nutrients for fish are amino acids, fatty acids, vitamins, minerals and energy-yielding macronutrients (protein, lipid and carbohydrate). Diets for fish must supply all essential nutrients and energy required to meet the physiological needs of growing animals. Guidelines for nutrient adequacy for some farmed fish species suggest the minimum nutrient requirement to promote growth and prevent signs of nutrient deficiency (Hixson, 2014) [6]. *Moringa* oleifera belongs to the family of *Moringaceae* and it can survive in harsh climatic condition including impoverished soil without being affected by drought (Zinorova *et al.*, 2015). It has beneficial properties from the root to the leaves and the leaf contain various phytochemicals constituents such as crude protein, amino acids, vitamins, minerals, fatty acids and other nutrient (Ajantha *et al.*, 2020) [2]. This research seek to investigate the growth performance of *Clarias gariepinus* fingerlings fed Processed *Moringa oleifera* diets (Aqueous, Ethanol and Hexane) and their impact upon aquaculture production.

Materials and Methods

This research feeding trial was conducted at the farm complex, Department of Fisheries and Aquaculture, New Site, Gwarzo Road Bayero University Kano, Nigeria. The experimental units were located at the indoor hatchery unit under latitude 11.978422°N, longitude 8.424395°E and altitude 528.48 m (figure 3.1).

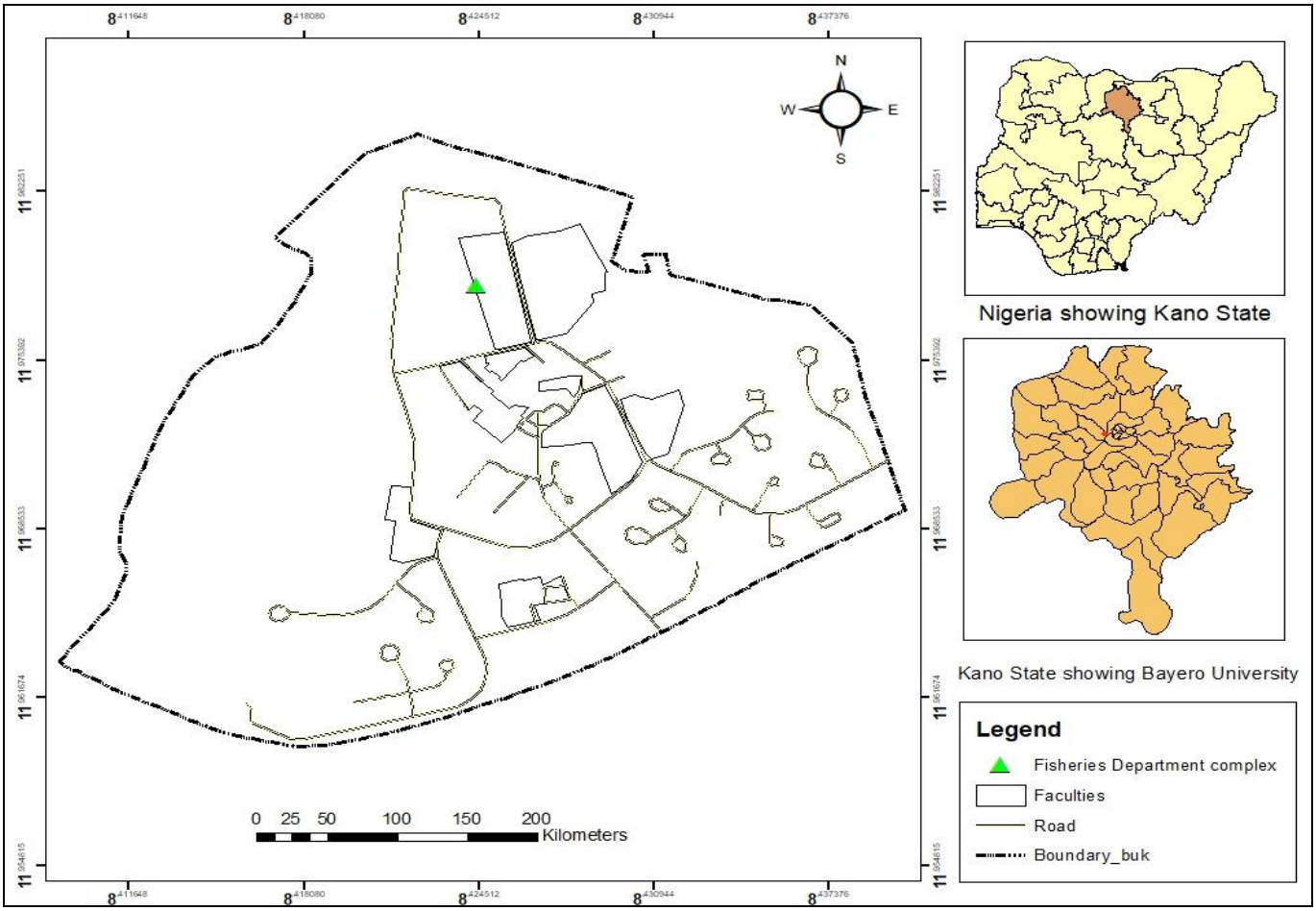

Fig 1: Showing Research Location

Processing of *Moringa oleifera*

M. oleifera fresh leaves were procured from Kure Market, Along Dutsen Kura Road, Minna, Nigeria. Sellers were targeted early in the morning prior to their arrival at the market to ensure fresh leaves were obtained. They were taken to the Laboratory of the Department of Water Resources, Aquaculture and Fisheries Technology (WAFT), Federal University of Technology, Minna, Niger State, Nigeria. The leaves were then detached manually from the branches, thoroughly washed to remove the dirt and prevent deterioration of phytochemicals as observed by Ochang *et al.*, 2015; Sahira Banu & Cathrine, 2015 [9, 10]. They were drained properly and air dried at room temperature to prevent nutrient loss for seven (7) days (Suleiman *et al.*, 2018) [11, 12]. The leaves were reduced to smaller quantities using pistle and mortar and further fed into electrical motorised hammer mill, milled into fine powder, package in polythene and stored at -4 °C prior to usage (Suleman *et al.*, 2018) [15].

Processing of *M. oleifera* Powder

The milled moringa was processed using aqueous, ethanol and hexane solvent. 250 g was measured using Ohaus sensitive weighing balance (PA313) and mixed with 500 ml volume of each solvents in a 1000 ml bottle. The bottles were tightly closed to prevent evaporation of the solvents and were placed on the laboratory table. They were agitated within an interval of 8 hours to enable effective dissolution of soluble matters into the solvents (Ezearigo *et al.*, 2014) [5]. After 72 hours, muslin cloths were laid in triple layers, labelled according to each treatment in a different containers while the contents were poured, filtered and macerated. Solvent of each treatment was added to rinse the concentrated liquid from the residues. The filtrates were evaporated to dryness under pressure at 45 ºC using a rotary evaporator (RE300). The extracts were labelled as Moringa Aqueous (MA), Moringa Ethanol (ME) and Moringa Hexane (MH). The extracts were preserved at -4 °C until further usage (Chakraborty *et al.*, 2018) [4].

Diet Formulation

Seven (7) iso-nitrogenous diets were formulated for the experiment to contain 40% Crude protein each with a Commercial Reference Diet (CRD) for the experiment. The CRD and Zero Supplement Diet (ZSD) serve as the control diet while Diets such as Moringa Aqueous Diet (MAD), Moringa Ethanol Diet (MED) and Moringa Hexane (MHD) were the tested diets formulated at 3% and 5% inclusion levels respectively (Table 1). Prior to formulation and compounding of the experimental diets, major ingredient were milled to a fine particles and sample were taken for proximate analysis before inclusion level of each ingredient was determined. Each ingredient was weighed in accordance with

the calculation (Table1) and were thoroughly mixed together to ensure homogeneity (Suleiman *et al.*, 2018) [11, 12]. The extract were dissolved in 350 ml of warm water which was used to form a kilogram of dough. The feeds were pelleted to 2 mm size and dried at 26 °C for 72 hours. They were packaged in a labelled airtight containers, stored at -4 °C prior to feeding trial (Ochang *et al.*, 2015) [9].

Table 1: Feed Formulation Showing Inclusion Levels and Proximate Compositions of Moringa Diets

Ingredients	CRD	ZSD	3%MAD	3%MED	3%MHD	5%MAD	5%MED	5%MHD
Fishmeal	-	25.71	24.90	24.90	24.90	24.36	24.36	24.36
Soybean Meal	-	51.42	49.80	49.80	49.80	48.71	48.71	48.71
Maize Meal	-	17.87	17.30	17.30	17.30	16.93	16.93	16.93
Moringa	-	0.00	3.00	3.00	3.00	5.00	5.00	5.00
Oil	-	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Bone Meal	-	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Lysing	-	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Methionine	-	0.50	0.50	0.50	0.50	0.50	0.50	0.50
VMP	-	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Total	-	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Proximate Analysis of the Test Diets at 3 and 5% Inclusion Levels of Moringa								
Ash (%)	10.35	11.63	12.65	9.45	11.92	12.75	10.31	13.92
CF (%)	5.55	1.07	2.28	5.83	6.05	5.35	4.88	4.45
Ether (%)	15.25	19.52	8.95	10.84	10.54	14.24	12.65	13.09
CP (%)	40.71	54.22	42.34	42.00	41.50	39.2	38.82	40.36
MC (%)	5.30	7.05	5.93	5.03	6.10	5.40	5.48	6.09
NFE (%)	22.84	6.51	24.85	26.85	23.89	23.06	27.86	22.09
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Where, VMP = Vitamin and Mineral Premix, CF = Crude Fibre, CP = Crude Protein, MC = moisture Content and NFE = Nitrogen Free Extract

Experimental Design

Experimental fish (fingerlings) were sourced and purchased from Gerrit Fleuren Hatchery, Kuje, Abuja. The fish were stocked in 2 x 3 x 1.5 tank at the indoor hatchery of Fish Farm Complex, Department of Fisheries and Aquaculture, BUK for two weeks to undergo acclimatization. Flow through System (FTS) was the set up used for this experiment. Each experimental unit consist of 50 litres capacity round plastic tank with centrally drained pipe measured to 30 litres calibration to control all the water volume and all the tanks depends on the same water source with independent inlets and outlets. The tanks were covered with 4mm mesh nets to prevent fish from escaping and possible invasion of predators. Water flow rate were equally maintained at 1.5 L per minute. Fish were starved 24 hours prior to the commencement of feeding trials in order to increase their appetite and eliminate variation in weight due to residual feed content that may be left in the gut and also to prepare the gastrointestinal tract for the experimental diets (Ochang *et al.*, 2015) [9]. Fish were weighed equally and distributed into various experimental units using an electronic digital sensitive weighing balance (MP300) at a stocking density of 20 fish per experimental unit. Water quality parameters were monitored weekly, fish growth data were taken fortnightly and they were fed 5% of their body weight daily with equal meals being fed between 10am and 16 pm. Feed quantity were adjusted according to their biweekly weight for a period 56 days.

Growth Parameters

At the end of the experiment, data obtained were used to determine Mean Weight Gain (MWG), Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER), Apparent Net Protein Utilization (ANPU) and Survival Rate using the following formulae;

MWG = Final Weight – Initial Weight

$$SGR\ (\%) = \frac{(\ln W2 - \ln W1)}{T} \times 100$$

Where, W1 = Fish Initial Weight
W2 = Fish Final Weight
Ln = Natural Logarithm
T = Number of days in the experiment

$$FCR\ (\%) = \frac{\text{Feed fed (g)}}{\text{Weight gain (g)}} \times 100$$

$$PER = \frac{\text{Live weight gain (g)}}{\text{Crude protein fed (g)}}$$

$$ANPU = \frac{(P2 - P1)}{\text{Total protein consumed (g)}} \times 100$$

Where;
P2 = Protein in fish carcass (g) at initial level of experiment
P2 = Protein in fish carcass (g) at the end of the experiment

$$\text{Survival Rate} = \frac{\text{Initial Number of Fish Stocked} - \text{Mortality}}{\text{Number of Fish stocked}} \times 100$$

Statistical Analysis

Mean value and standard deviation (S.D.) were calculated from the results. All growth data obtained were subjected to one way analysis of variance (ANOVA) using Minitab version 19, 2020 and Originlab Professional, 2022 software. The effect of inclusion levels of Moringa at 3% and 5% was determined using student's t-test.

Results

The growth parameters and nutrients utilizations of different processed Moringa for Aqueous, Ethanol and Hexane based diets are shown in Table 2. There were significant difference (p>0.05) among the mean initial weights of the fish in controls (CRD and ZSD) and processed Moringa based diets between 3% and 5%. Also, in the Mean Final Weight, fish of

3% MAD based diet were significantly (p>0.05) higher than the fish of all the experimental based diets while the fish fed 3% MET (74.48) and 3% MHX (75.83) based diets were not significantly different (p>0.05) in terms MFW. Least significant values were obtained across all the fish fed 5% Moringa processed based diets. The Mean Weight Gain (MWG) of the fishes of 3% Moringa processed based diet were significantly higher than that of 5% Moringa processed based diets and were significantly lower (p>0.05) than control based diets (CRD and ZSD) respectively. The Specific Growth Rate (SGR), Food Conversion Ratio (FCR) and Protein Efficiency Ratio (PER) showed significant difference among all the tested diets. The percentage Net Protein Utilization of 3%MAD (93.30%) and 3%MHD (94.94%) were not significantly different (p>0.05) from each other and were next to ZSD (96.25%) which was highest significantly (p>0.05).

Comparison of survival rate of the experimental fish fed between 3% levels of Moringa inclusion was not possible because all survival rates under 5% inclusion were the same i.e. no variation exists. There was a significant (p>0.05) effect of diet on the survival of the experimental fish. All samples fed 5% inclusion and those fed 3% MAQ had maximum survival while CRD had the least survival rate.

All experimental samples grew uniformly between the start of the experiment and week 2. Diet 3% MAQ, 3% MHX, ZSD, 3% MET and CRD gave the best weight beginning from week 2. All other diets administered to the fish did not produce increase in weight at the same pace as the diets mentioned above. Fish fed 3%MAD consistently maintained superior performance followed by 3%MHX, ZSD and 3%MET as shown in Figure 2.

Table 2: Growth parameters and Nutrient Utilization for *Moringa oleifera* Diets at 3 and 5% Inclusion levels

Treatment	MIW (g)	MFW (g)	MWG (g)	SGR (%)	FCR	PER	ANPU (%)	SR (%)
CRD	9.86±0.00^{a}	52.30±0.17^{c}	42.44±0.17^{c}	2.98±0.01^{c}	1.36±0.01^{d}	3.48±0.01^{a}	84.20±0.37^{d}	85.00±0.00^{d}
ZSD	9.83±0.01^{b}	76.40±0.10^{b}	66.57±0.10^{b}	3.66±0.00^{b}	1.08±0.01^{f}	1.66±0.00^{c}	96.25±0.40^{a}	88.33±0.31^{c}
3%MAQ	9.83±0.00^{b}	95.34±0.95^{a}	85.50±0.95^{a}	4.05±0.02^{a}	0.97±0.01^{g}	2.14±0.02^{b}	93.30±0.70^{b}	100.00±0.00^{a}
3%MET	9.83±0.00^{b}	74.48±0.61^{b}	64.64±0.61^{b}	3.61±0.01^{b}	1.07±0.00^{f}	1.62±0.02^{c}	86.80±1.17^{c}	96.67±0.61^{b}
3%MHX	9.83±0.00^{b}	75.83±0.78^{b}	66.01±0.78^{b}	3.64±0.02^{b}	1.14±0.01^{e}	1.65±0.02^{c}	94.94±0.24^{ab}	96.90±0.61^{b}
5%MAQ	9.82±0.01^{b}	24.03±0.15^{de}	14.21±0.15^{de}	1.60±0.01^{e}	2.91±0.02^{b}	0.36±0.00^{de}	50.22±0.29^{e}	100.00±0.00^{a}
5%MET	9.85±0.01^{a}	25.23±0.17^{d}	15.37±0.17^{d}	1.68±0.01^{d}	2.76±0.02^{c}	0.38±0.00^{d}	40.95±0.29^{f}	100.00±0.00^{a}
5%MHX	9.86±0.01^{a}	22.25±0.14^{e}	12.40±0.14^{e}	1.45±0.01^{f}	3.07±0.03^{a}	0.31±0.00^{e}	49.88±0.41^{e}	100.00±0.00^{a}
p-value	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Where, CRD = Commercial Reference Diet, ZSD = Zero Supplemented Diet, MAQ = Moringa Aqueous, MET = Moringa Ethanol, MHX = Moringa Hexane, MIW = Mean Initial Weight, MFW = Mean Final Weight, MWG = Mean Weight Gain, FCR = Food Conversion Ratio, PER = Protein Efficiency Ratio, ANPU = Apparent Net Protein Utilization and SR = Survival Rate.

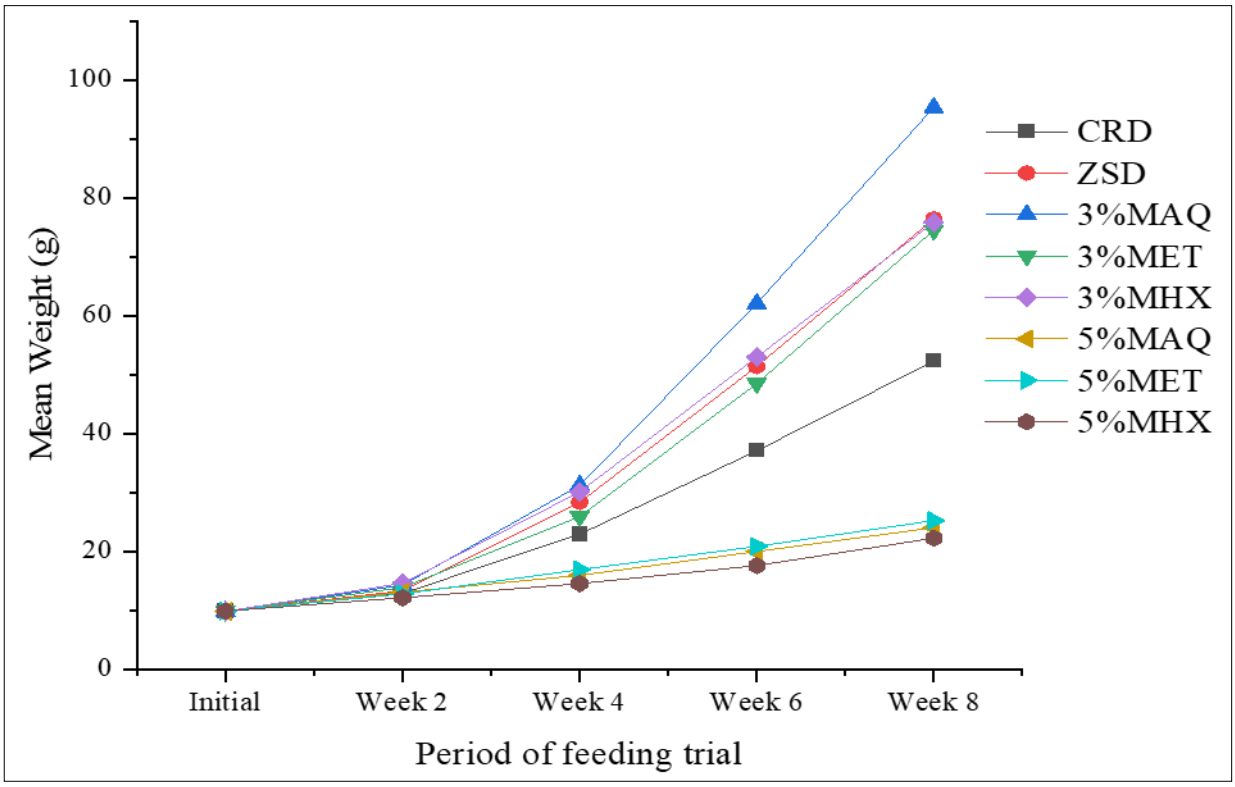

Fig 2: Biweekly growth performance of *Clarias gariepinus* fed 3% and 5% inclusion of Moringa Processed diets

Discussion

The ranges of water qualities such as dissolved oxygen (3.9-6.9 mg/l), temperature (29.9-30.8 °C), conductivity (224-250 µs/cm), total alkalinity (93.3-98.5) and pH (7.4-7.51) were within the requirement for fish culture and were monitored thorough out the experimental period. In the present study,

from Table 2, it was obvious that fish fed 3% Moringa Processed based diets exhibited the best performance as compared to that of 5% Moringa Processed based diets. Findings also shows that, inclusion of processed Moringa at 5% levels of Aqueous, Ethanol and Hexane retarded the growth performance and utilization of nutrient of *Clarias gariepinus*. These findings are in agreement with Nsofor *et al.* (2012) [14] who says fish fed with Moringa Leaf Meal (MLM) in replacement of fishmeal at 60% inclusion rate resulted in the lowest growth response due to the low palatability of the diets which may lead to poor feed intake.

However, Tabassum *et al.* (2021) [13] reported that partial replacement of fishmeal with *Moringa oleifera* Leaf Meal (MOLM) was not effective beyond 10% inclusion levels in terms of growth performance and nutrient utilization. The highest weight gain was achieved in 3% MAD based diet, followed by 3% MHD and 3% MED based diets respectively. The variation in their performance may be attributed to different solvent used for processing MLM which shows that aqueous processing technique improves palatability and acceptability of the diet at 3% inclusion rate. The significant (p>0.05) growth performance observed in 3% MAD based diet may also be attributed to the retention of nutritional constituent of Moringa leaf by using Aqueous processing method which seems to be natural while some traces of the solvent of Ethanol and Hexane processing method may denature some of the nutritional constituent of Moringa leaf even though finding shows that, the performance of Ethanol and Hexane processing method were better than the previous research of whole leaf meal inclusion.

The 3% levels of processed Moringa were observed to be the best in terms of Protein Efficiency Ratio (PER), Apparent Net Protein Utilization and Mean Weight Gain (MWG). The least performance were observed in 5% MHD based diet. These were also in agreement with the observation made earlier by aforementioned authors affirming that higher inclusion level of Moringa lead to reduce growth performance. The Significant successes achieved in survival rate for all the fish of experimental based diets were confirmation of Suleiman *et al.* (2018) [11, 12] who reported that, proper monitoring of water quality and Phytonutrient contents of medicinal plant additive diets improves survival rate of fish fed.

Conclusion

Findings in this study shows that inclusion of processed Moringa have positive impact on the diet of *Clarias gariepinus* as it promotes fish growth performance and nutrient utilization at 3% inclusion rate than the use of whole Moringa leaf meal used by previous aquaculture researchers.

Recommendation

It will be recommended that inclusion of 3% inclusion level of Moringa processed with Aqueous solvent is optimum and appropriate for *Clarias gariepinus* production and cost of processing it will be cheaper and affordable to peasant aquaculturist than using Ethanol and Hexane solvent for processing as all the processes aimed at affordable and sustainable aquaculture.

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