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Effect of varying replacement levels of soybean meal with watermelon (*Citrullus lanatus*) seed meal on growth and feed utilization performance of *Clarias gariepinus* (Burchell, 1822)

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Abstract
The quest to minimize cost of feeding in aquaculture operation prompted this investigation on the substitution of soybean meal (SBM) with watermelon seed (WMS) in the diets of *Clarias gariepinus* fingerlings. This study investigated impacts of the substitution on the growth performance and feed utilization of *Clarias gariepinus* fingerlings. Watermelon seeds were processed by boiling for 15-minutes, dried and milled into fine particles before incorporation into five experimental diets on graded levels of SBM-WMS replacements i.e., 0% (CTR), 25% (DT1), 50% (DT2), 75% (DT3) and 100% (DT4). 225 fish were stocked into 50-litres plastic tanks in a Complete Random Design (CRD) of 15fish per tank and each treatment in triplicates. The feeding trial was conducted under a flow-through drainage system for 56 days. Feeding was done twice daily and at 5% body weight with consequential adjustment fortnightly. Results showed significant growth differences ($P \leq 0.05$) across the treatments, with an increasing trend up to a threshold peak of 75% replacement. Thus, the DT3 group had the highest average weight gain (8.507 g), and the DT4 group with the lowest (3.245g). Similarly, feed conversion ratio, protein efficiency ratios and % survival varied significantly ($P \leq 0.05$) among the treatments with the poorest (FCR=4.89, PER=0.08 & Survival=46.35%) recorded in the DT4 group. This study establishes potential for higher replacement of SBM with WMS of 75% level but will also consider adequate treatment of the watermelon seed before incorporation in to fish feed formulations.

Keywords: Aquaculture, experiment, feed, formulation, proximate and substitution

1. Introduction

Aquaculture is a major food industry undergoing rapid growth due to the increasing global demand for fish and fisheries products [1]. In 2018, global fish production reached 178.5 million tonnes, with aquaculture contributing 45.99% (82.1 million tonnes) to this total [2]. Globally, the aquaculture sector is also struggling to meet the rapidly rising demand for fish to confront malnutrition and enhance food and nutritional security [3]. To increase fish production, aquaculture practices have transitioned from extensive to semi-intensive and intensive culture systems, where nutrition plays a pivotal role by influencing production costs, fish growth, health, and waste production [4].

The formulation and production of high-quality fish feed are crucial for the viability and profitability of aquaculture ventures, accounting for about 60% of the overall cost of fish production [5]. Nutrient requirements vary by fish species, making it imperative for fish farmers to understand the nutritional needs of their preferred aquaculture species to formulate balanced diets. In Nigeria, a major challenge faced by fish farmers is the high cost of fish feed ingredients. This necessitates the search for cheaper, locally available feed components that can serve as alternative energy sources for fish [6]. The major nutrients in fish feeds are protein and energy, but the supply of conventional feedstuffs is declining, and their prices are high due to competitive needs for human consumption, animal feed, and industrial uses [7].

Soybean meal (SBM) is widely used as a feed ingredient in aquaculture due to its high protein content, digestibility, and balanced amino acid profile [8-9]. However, the availability and wider utilization of soybean meal are limited by its increasing demand for human consumption and other animal feed industries [10]. The rapid expansion of fish culture in recent years necessitates the development of low-cost, nutritious fish feeds, as feed costs can account for 50-80% of fish production costs [10]. Consequently, there is a need to explore less expensive and readily available vegetable protein sources to replace soybean meal without compromising the nutritional quality of the feed.

Watermelon (*Citrullus lanatus*) is a drought-tolerant crop cultivated widely in tropical, semi-tropical, and arid regions. It is an economically important fruit, with global production reaching 101 million tonnes in 2020. Watermelon seeds, constituting about 2% of the fruit's weight, contain a sufficient amount of nutritional protein that could serve as an ingredient in fish feed [11]. The nutritional quality of watermelon seed meal is comparable to that of oilseed proteins, including soybean [9]. Given the inflation in prices of conventional feedstuffs like soybeans, it has become essential to explore alternative feedstuffs such as watermelon seeds. This research aims to study the replacement of soybean meal with watermelon seed meal in the diet of African catfish (*Clarias gariepinus*), investigating its potential as a suitable substitute due to its balanced nutrient profile and availability.

2. Materials and Methods

2.1. Experimental Site

The experiment took place at the fish farm complex of the Department of Fisheries and Aquaculture, Faculty of Agriculture, Bayero University, Kano. This farm is situated at latitude 11.977616°N and longitude 8.424571°E in Kano State, Nigeria [12]. The southern parts of the state receive an average annual rainfall of approximately 1,000mm, while the extreme northern regions receive just under 800mm. Kano State experiences a rainy season lasting 3-5 months, with mean ambient temperatures peaking at 41°C and dropping to 16°C during the cooler season [13-14].

2.2. Experimental Fish

Two hundred and twenty five fingerlings of *Clarias gariepinus* were procured from Salis farms located at Kwanar-dawaki, Kano State. They were transported to the study area and acclimatized for seven days (7). The fish were fed 2mm Blue Crown Catfish feed during period of the acclimatization.

2.3. Procurement of Feed Ingredients and their Processing

Feedstuffs used in compounding the experimental diets were procured from Kasuwan-rimi market in Kano city. Premix, lysine and methionine were obtained from Phed Agro-Vet shops along Zungeru road, Kano State. Soya bean was toasted to remove the trypsin inhibitor while Watermelon seeds were collected, washed with clean water and boiled for 15 minutes before sun drying for 4 days [9]. All the major feedstuffs were separately milled into fine particles.

2.4. Formulation and Preparation of Experimental Diets

Proximate analysis of each of the major ingredients was carried out (Table 1). Five experimental diets were formulated for *Clarias gariepinus* fingerlings containing 0% (CTR), 25% (DT1), 50% (DT2), 75% (DT3), and 100% (DT4) Soya bean replacements with Watermelon seed. The Five diets were formulated using Pearson square method (Table 2).

The feedstuffs were separately weighed according to the calculated amounts. They were then thoroughly mixed together to obtain a homogenous mixture. The mixture was made into dough using boiled water and starch as binder. The dough was then introduced into a palletizer to produce 2mm pellets. The pellets were dried for a day in the sun and shade dried for 3 days. The dried pellets were appropriately packaged in waterproof bags and labeled accordingly before storage at room temperature. Thereafter, proximate analysis of the different diets were also conducted using the standard procedure of A.O.A.C [15] (Table 3).

Table 1: Proximate composition of the major ingredients

Ingredients	%moisture	% ASH	% FATS	% CP	% CF	% CHO
[1]SBM	5.93	4.38	22.14	48.12	6.72	19.43
[2]YMM	7.94	1.44	7.98	13.25	3.20	66.10
[3]WSM	6.26	2.74	10.60	21.50	6.50	56.40
[4]FM	10.05	8.50	13.14	65.62	2.30	0.39

Soya bean meal
Yellow maize meal
Watermelon seed meal
Fish meal

Table 2: Percentage composition of feed stuff in each experimental diet

Ingredient	CTR	DT1	DT2	DT3	DT4
FM	24.28	24.28	24.28	24.28	24.28
YMM	34.31	34.31	34.31	34.31	34.31
SBM	36.41	27.31	18.205	9.10	0.00
WSM	0.00	9.10	18.205	27.31	36.41
Lysine	1.00	1.00	1.00	1.00	1.00
Methionine	1.00	1.00	1.00	1.00	1.00
Palm oil	0.50	0.50	0.50	0.50	0.50
Table salt	0.50	0.50	0.50	0.50	0.50
Starch	1.00	1.00	1.00	1.00	1.00
Bone meal	1.00	1.00	1.00	1.00	1.00
Total	100	100	100	100	100

Table 3: Proximate composition of the formulated feeds

Diet	% Moisture	% ASH	% FATS	% CP	% CF	% CHO
CTR	7.22	9.40	8.86	40.65	6.20	27.67
DT1	7.45	7.70	9.34	39.30	5.86	30.35
DT2	6.98	7.20	8.85	39.64	5.65	31.65
DT3	7.10	6.90	9.16	40.20	6.34	30.30
DT4	6.66	6.80	8.54	39.32	6.50	32.18

2.5 Experimental Design

Fifteen fish were randomly selected weighed and stocked into 50 liters tank in Complete Randomize Design (CRD) and were operated under flow-through drainage system. Each experimental diet was fed to fish in each unit for a period of 8- weeks. Each unit was replicated three times.

2.6. Experimental Procedure

The experiment was set according to the percentage replacement of Soya bean meal (SBM) with Water melon seed meal (WSM) as 0% WSM (CTR), 25% WSM (DT1), 50% WSM (DT2), 75% WSM (DT3) and 100% WSM (DT4). Fish were starved for 2 days before commencing the experiment. Feeding was done twice daily (8:00am -10:00 am and 2:30pm to 4:00pm) and fed 5% body weight which was consequentially adjusted fortnightly in accordance with changes in fish weight. Water quality monitoring was done through siphoning of uneaten feed and fecal materials on daily basis and ensuring the flow- through system throughout period of the experiment.

2.7. Data Collection
Estimation of the feed consumed by fish was done by subtracting quantity of uneaten feed within 15 minutes of feeding from the quantity administered. The initial and bi-weekly weights of the fish were done using digital weighing scale and recorded grams. The data were recorded in 2016 Microsoft Excel sheet.

2.8. Calculated Growth and Feed Utilization Parameters
The calculated growth parameters are Mean Weight Gain (MWG) and Specific Growth Rate (SGR). Feed Conversion Ratio (FCR) and Protein Efficiency Ratio (PER) were calculated to infer the feed utilization of the diets by the experimental fish. The formulae used for the calculations are;

Weight Gain (WG)
This is the difference between the final fish weight and fish initial weight and is expressed as;

$$WG\ (\%) = Final\ weight\ gain - Initial\ weight\ gain$$

Specific Growth Rate (SGR)
This is the mean percentage increase in body weight per day over a given time interval [16].

$$SGR\ (\%) = \frac{LnW2 - LnW1}{T} \text{ x } 100$$

Where: W_1 = weight of the fish at the initial stage; W_2 = weight of the fish at the final stage, T = experimental period, Ln = Natural Logarithm.

Feed Conversion Ratio (FCR)
This is defined as weight of feed fed in dry weight per fish live weight gain and was evaluated as:

$$FCR\ (\%) = \frac{\text{Feed fed (gram/dry weight)}}{\text{live weight gain (g)}}$$

Protein Efficiency Ratio (PER)
Protein Efficiency Ratio (PER) This was calculated by using the relationship between the increases in the body weight of fish (i.e. the weight gain of fish) and protein consumed. It is an expression which relates the gram of weight gained to the gram of crude protein fed.

$$PER = \frac{Weight\ gain\ of\ fish}{Crude\ protein\ in\ diet}$$

Survival Rate (%)

$$SR = \frac{Initial\ number\ of\ fish\ stock - Mortality}{Initial\ number\ of\ fish} \text{ x } 100$$

2.9. Statistical Analysis
Data obtained from the experiment were expressed in mean ± SEM and subjected to one-way analysis of variance (ANOVA) using JMP statistical software (ver.17). LSD tests were used to compare differences among individual treatment means to reveal significant differences ($P < 0.05$).

3. Results and Discussion
3.1. Results

3.2. Growth Response of the Fish
Analysis of variance of the growth and feed utilization parameters between the treatments are presented (Table 4). The initial weight of *Clarias gariepinus* fingerlings employed for the feeding trial shows no significant difference ($P \geq 0.05$) between the treatments. There mean initial weight ranged from 7.000 to 7.0679 g. The final weight ranged from 10.293 to 15.651 g with significant differences ($P \leq 0.05$) between the treatments' means. The mean weight gain indicated DT3 (75% WSM) made highest mean weight gain (8.507g) followed by the CTR and DT2 (50% WSM) at 6.959 g and 5.895g respectively. The test of significant among the treatments' means of weight gain indicated a significant difference ($P \leq 0.05$) with DT4 (100%) having the lowest value. The Specific growth rate (SGR) among the treatments also indicated similar pattern of significant difference ($P \leq 0.05$) among the treatments. The DT4 treatment recorded lowest SGR of 0.677 while the highest SGR of 1.406 comes from the DT3. Treatments DT1 and DT2 respectively produced the second and third highest SGR at 1.232 and1.083.

3.3. Feed Utilization Parameters
The feed utilization parameters were also contained in Table 4.1. There are significant differences ($P \leq 0.05$) among the treatments' feed conversion ratio (FCR) and Protein efficiency ratio (PER). The best FCR was with fish in CTR group (2.151) while DT4 gave the poorest (4.891). On the other hand, DT4 gave the lowest PER of 0.081 while the highest was recorded with DT3 at 0.213.

3.4. Survival Percentage of the Fish Fed the Different Diets
The survival percentages of the fish throughout the 8-weeks feeding indicated significant difference ($P \leq 0.05$) between all the treatments and treatment DT4. The later recorded the lowest survival rate of 46.347% while treatment DT3 had the highest survival of 84.387%.

3.5. Biweekly Weight of the Experimental Fish
The fish fed different inclusion levels of water melon seed (WMS) had their weight at the same pace at the beginning (week 0) of the experiment. Variation in growth occurred right from week 2 on ward. Treatment DT3 recorded the highest weight while DT4 had lowest weight (figure 4.).

Table 4: Growth and feed utilization parameters of the experimental fish

Parameter	CTR	DT1	DT2	DT3	DT4
	Mean (±SEM)	Mean (±SEM)	Mean (±SEM)	Mean (±SEM)	Mean (±SEM)
Initial weight (g)	7.000±0.004	7.033±0.004	7.067±0.004	7.067±0.004	7.033±0.004
Final weight(g)	13.963±0.055[ab]	11.826±0.055[bc]	12.964±0.055[abc]	15.651±0.055[a]	10.293±0.055[c]
Weight Gain (g)	6.959±0.134[a]	4.611±0.134[ab]	5.895±0.134[ab]	8.507±0.134[a]	3.245±0.134[b]
SGR	1.232±0.106[a]	0.892±0.106[ab]	1.083±0.106[ab]	1.406±0.106[a]	0.677±0.106[b]
FCR	2.151±0.142[b]	3.793±0.142[ab]	2.569±0.142[ab]	2.320±0.142[b]	4.891±0.142[a]
PER	0.174±0.135[a]	0.115±0.135[ab]	0.147±0.135[ab]	0.213±0.135[a]	0.081±0.135[b]
Survival (%)	82.164±0.057[a]	68.586±0.057[a]	84.387±0.057[a]	66.444±0.057[a]	46.347±0.057[b]

Treatments' means with different superscripts along the same row are significantly different from each other ($p<0.05$)

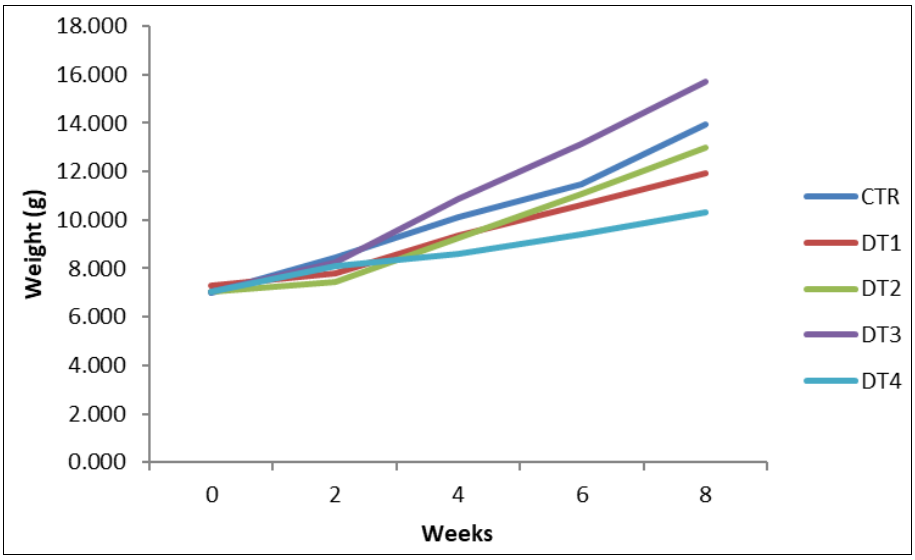

Fig 1: Bi-weekly growth curve of *C. gariepinus* fed the different diets

Discussion

The findings of this study on the aspects of growth and feed utilization responses of *Clarias gariepinus* fingerlings to increasing replacement levels of soybean meal (SBM) with watermelon seed meal (WSM) align with an earlier study [9]. Comparison between the two studies revealed a peak replacement threshold of 60% in the earlier study while the present study established a higher peak replacement level at 75%. The observed low growth and feed efficiency at 100% replacement diet may be attributed to the high presence of antimetabolites in the diet, which could reduce its palatability and the bioavailability of essential amino acids that are typically lacking in plant-based proteins [17].

However, the rate of growth response to the experimental diets differed significantly between the studies. In the present study, the mean weight gain ranged from 3.245 g to 8.507 g, whereas findings from a similar study reported a higher range of 10.49 g to 21.65 g [9]. This discrepancy may be attributed to differences in the pre-formulation treatments of watermelon seed meal, specifically the oil extraction process, which was not performed in the current study. Additionally, the higher fish meal (27.7%) and soybean meal (44.4%) content incorporated in the diets of the reference study [9] likely contributed to the observed higher fish growth rates [1].

Conclusion

The current study demonstrates a potential for higher replacement levels of SBM with WSM. The differences in growth rates highlight the importance of considering pre-formulation treatments and the overall diet composition. Further research is necessary to optimize the use of WSM in aquafeeds, taking into account the balance of essential nutrients and the reduction of anti-nutritional factors.

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