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Effect of dietary inclusion levels of watermelon (*Citrullus lanatus*) peel meal on growth and haematological parameters of *Clarias gariepinus* Juveniles

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Abstract
The nutritional value of feeding *Clarias gariepinus* with various levels of *Citrullus lanatus* peel meal was evaluated in this study for 70 days. Five diets of 40% crude protein were formulated with different levels of *C. lanatus* peel meal at 0.0, 1.0, 1.5, 2.0 and 2.5g/kg inclusion denoted as (DI-D5). Two hundred and twenty five (225) *C. gariepinus* juveniles with mean weight of 8.15±0.05g were randomly selected and distributed into 15 glass Tanks (70litre) each measuring (70cm x45cm x45cm) at the rate of 15 fish per tank representing five treatments and three replicates. Fish were weighed at the end of the experiment. Blood samples from each treatment were collected for haematological examination. The results revealed that significant differences (P<0.05) were observed in all the growth performances. Fish on Diet 4 had the best growth performance compared to other treatments and control. A reduction in growth as the *C. lanatus* peel meal increased beyond 2.0g/kg was observed. Varying inclusion levels of *C. lanatus* in the diets improved the blood profile as there were significant differences (P<0.05) in the parameters tested. The inclusion level of 2.0g/kg of *C. lanatus* peel meal in the diet of *C. gariepinus* is found to be ideal for enhancing growth and better nutrient utilization.

Keywords: *Citrullus lanatus* peel, growth, haematology, *Clarias gariepinus*

1. Introduction
The African catfish *(Clarias gariepinus)* is appreciated by customers for the quality of its meal [1]. The fish is an excellent species for aquaculture as it is omnivorous, grows fast, and tolerates relatively poor water quality [2]. Production of quality fish feed is considered a critical factor in aquaculture as it ensures growth efficiency, quality flesh and feed utilization [3]. The higher cost and competition imposed on some feed ingredients, such as soybean, groundnut cake, maize and sorghum used by human population as food, have necessitated the use of unconventional material for fish feed formulation. Gabriel *et al.* (2007) [4] reported that fish feed account for 50-60% of aquaculture production, hence has necessitated the search for cheap and locally available feed stuffs that can serve as alternative feed for fish. Fish haematology is gaining increasing importance in fish culture because of its importance in monitoring the health status of fish [5]. Watermelon (*Citrullus lanatus*) contains proteins, carbohydrates, vitamins, minerals and fats [6]. The peel contains impressive concentrations of most nutrients like phenolic antioxidants, flavonoids and lycopene [7]. The peel has being reported to be a good dietary energy source in the diet of Nile tilapia [8]. Usually, *C. lanatus* peel is discarded as agricultural food waste. Shazali *et al.* (2013) [9] reported that the inclusion of *C. lanatus* seed meal in broiler diets induced better growth and feed utilization efficiency. *C. lanatus* seed peel meal has also been reported to increase growth and nutrient utilization in *Cyprinus carpio* and *Oreochromis niloticus* as reported by Lateef *et al.* (2014) [10] and Iheanacho *et al.* (2018) [11]. Therefore, the present study is geared towards the prospects of *C. lanatus* peel on growth and haematological parameters of *C. gariepinus* juveniles.

2. Materials and Methods
2.1 Collection and Preparation of *Citrullus lanatus* Peels
C. lanatus peels were collected from the watermelon fruit seller at the School of Agriculture and Agricultural Technology, the Federal University of Technology Akure, Ondo State where

it was heaped. Identification and authentication was carried out at the Department of Crops, Soil and Pest Management, Federal University of Technology, Akure, Ondo State, Nigeria. *C. lanatus* pulp was carefully scraped off to obtain the peel. The peels were thoroughly washed in sterile distilled water to removed sand particles after which it was sliced into smaller bits using a home European knife, spread on a foil and sundried for 5 days. The dry weight sample was ground using electric grinder (Model 242) into powder and stored in an airtight container at 4ºC prior to use.

2.2 Experimental Fish
The *Clarias gariepinus* juveniles were obtained from the Federal university of Technology fish farm Akure, Ondo State. Three hundred (300) juveniles of mean 7.85±0.06g weight were acclimatized for two weeks in the laboratory, during this period fish were fed commercial diet twice daily.

2.3 Preparation of Experimental Diets.
Five iso-nitrogenous diets (40%CP) were formulated for *C. gariepinus* to contain *C. lanatus* peel at different inclusion levels (0.0, 1.0, 1.5, 2.0 and 2.5 g/kg) and labelled as D1-D5. Diets were fan dried under room temperature for 48 hours packed in polyethylene bags, sealed, and marked according to treatments and stored at 4ºC before use.

2.4 Experimental Design and Management
The experimental design was a complete randomized design. After the acclimation period fish were weighed, Two hundred and twenty five (225) *C. gariepinus* juveniles with mean weight of 8.15 ± 0.05g were randomly selected and distributed into 15 glass Tanks (70litre) each measuring (70cmx45cmx45cm) at the rate of 15 fish per tank representing five treatments and three replicates.
The diets were fed to the fish twice daily between 08:00 and 09:00 and 16:00 and 17:00 hours to apparent satiation for 70days. The fish were weighed biweekly until the experiment was terminated. Water in the tank was removed partly by siphoning and replaced with fresh water every three days to avoid fouling resulting from faeces and uneaten food. Water quality parameters (temperature, dissolved oxygen and pH) were determined twice a week. Calculation of the growth performance was according to Iheanacho *et al*. (2017) [12]. Haematological values were measured according to adapted method of Dacie and Lewis (2011) [113]. Proximate analysis of *C. lanatus* and feed were carried out according to AOAC (1990) [14] methods.

Table 1: Gross composition of the experimental diets (g/kg) for culturing *C. gariepinus* juveniles

Ingredients	D1	D2	D3	D4	D5
Fish meal	25.4	25.4	25.4	25.4	25.4
Soybean meal	35.4	35.4	35.4	35.4	35.4
Groundnut cake	12.1	12.1	12.1	12.1	12.1
Yellow maize	16.1	16.1	16.1	16.1	16.1
Vitamin premix**	2.00	2.00	2.00	2.00	2.00
Fish oil	5.00	5.00	5.00	5.00	5.00
Methionine	1.00	1.00	1.00	1.00	1.00
Lysine	1.00	1.00	1.00	1.00	1.00
Starch	2.00	2.00	2.00	2.00	2.00
C. lanatus peel	0.00	1.00	1.50	2.00	2.50

Vitamins/Minerals premix supplies 100g Diet. Palmat A: 1000IU; Cholecalceferol (D):1000IU; G-Tocopherolacetate (E): 1.1mg; Menadilione (K): 0.02mg; Thiamine B1: 0.63mg; Riboflavin (B12): 0.001mg; Nicotinic Acid: 3.0mg; Folic Acid: 0.1mg; Choline: 31.3mg; Ascorbic Acid ©: 0.1mg; Iron (Fe): 0.05mg; Cu: 0.25mg; Mn:6.00mg; Co: 0.5mg; Zn: 5.0mg; Sn:0.02mg.

2.5 Data Analysis
All data were expressed in terms of mean±standared error. Treatment effects on different parameters were analysed by one-way analysis of variance (ANOVA) as described by Steel and Torrie [15]. Duncan's New Multiple Range Test Duncan (1955) [16] was used to separate differences among the means using the computer software SPSS (Statistical Package for Social Science Version 20) and (Microsoft office Excel programme 2010).

3. Results and Discussion
The result of the proximate composition of the *C. lanatus* peel meal is shown in Table 2. *C. lanatus* peel meal had a moisture level of 10.63%, crude lipid 1.97%, crude protein 11.28%, crude fibre 20.54%, total ash was 7.91%. These values were comparable with those reported in other plants such as Jack beans, pear and cowpea by Olalekan and Bosede [17]. This suggests the potential of *C. lanatus* peel meal as animal feed agree with other plants from nutritional point of view.

Table 2: Proximate Composition of dry *Citrullus lanatus* peel meal

Parameters (%)	Values
Moisture	10.63
Lipid	1. 97
Protein	11.28
Fibre	23.54
Ash	7.91
NFE	44.67

NFE, Nitrogen free extract.

Proximate composition of experimental diets is presented in table 3. Moisture content was highest in diet 3 (7.22%) while diet 1 had the lowest moisture content (7.08%). The lipid, ash and fibre contents increased with increasing levels of *C. lanatus* in the diets which was highest in D5 while the lowest value was seen in D1. Protein content decreased with increasing levels of *C. lanatus* in the diets which was highest in D1 while the lowest value was seen in D5. The observed protein content in the fish diet ranged from 39.57 to 40.15%. Akiyama (1999) [18] reported that the dietary protein requirements of warm water fish have been estimated to vary from 20 to 40%. Therefore, protein content of the test diets used in this study were within the suitable range for *C. gariepinus* culture.

Table 3: Proximate Composition of Diets containing different inclusion levels of *C. lanatus* peel meal

Parameters	D1 (0.00)	D2 (1.00)	D3 (1.50)	D4 (2.00)	D5 (2.50)
Moisture	7.08	7.13	7.19	7.22	6.85
Lipid	10.91	11.47	11.51	11.59	11.73
Protein	40.15	40.03	39.76	39.62	39.57
Ash	9.26	9.39	9.42	9.54	9.83
Fibre	4.01	4.12	4.16	4.21	4.36
NFE	28.59	27.86	27.96	27.82	27.66

Means in the same row followed by different superscripts letters differed significantly (P<0.05)
NFE, Nitrogen free extract.

The results of Physico-chemical parameters are presented in Table 4. This showed significant difference ($p<0.05$) in Dissolved oxygen and pH while there was no significant difference between temperature among the treatments. The physico chemical parameters of water were within the acceptable range recommended for rearing and culture of most tropical fishes, including the *C. gariepinus* for optimum growth, survival and reproduction [19].

Table 4: Physico-chemical parameters for culture of *Clarias gariepinus*

Parameters	D1 (0.00)	D2 (1.00)	D3 (1.50)	D4 (2.00)	D5 (2.50)
Temperature (°C)	26.95±0.35^{a}	26.90±0.71^{a}	26.90±0.28^{a}	26.05±0.21^{a}	26.40±0.71^{a}
Dissolved oxygen (mg/l)	7.21±0.42^{b}	7.15±0.64^{b}	7.60±0.28^{b}	7.45±0.35^{b}	6.80±0.14^{a}
pH	6.90±0.28^{ab}	6.85±0.71^{ab}	6.50±0.14^{a}	7.20±0.14^{b}	6.85±0.71^{ab}

Means in the same row followed by different superscripts letters differed significantly ($P<0.05$)

Result on growth performances of *C. gariepinus* fed with different dietary inclusion levels of *C. lanatus* peel meal diets are presented in Table 5. The high percentage survival recorded in the study is an indication that the peel meal was palatable and well digested and absorbed by the fish. This result compared favourably with the reports of Thanikachalam *et al.* (2010) [20] who reported significantly higher survival (%) in *C. gariepinus* fed garlic peels basal diet. The results revealed that significant differences (P<0.05) were observed in all the growth performances. Fish on D4 had the best growth performance compared to other treatments and control. A reduction in growth as the *C. lanatus* peel meal increased beyond 2.0g/kg was observed. This result agrees with the reports of Ochang *et al.* (2017) [21] who reported decreased in growth of *C. gariepinus* with increasing levels of *Afzelia africana* in the diet. This growth reduction observed at higher inclusion levels of *C. lanatus* peel meal could be related to the presence of anti-nutritional factors or high fibre levels. This observation agrees with the findings of Jimoh and Aroyehun (2011) [22] for *Clarias gariepinus* fed sesame seed meal-based diet. Jimoh *et al.* (2013) [23] reported that higher inclusion levels of *Luffa cylindrical* meals resulted in poor growth and nutrient utilization by *Clarias gariepinus*. The growth performance recorded in this study was higher than (13.47%) reported by Lateef *et al.* (2014) [10] on *Cyprinus carpio* fed different inclusion levels of *C. lanatus* seed meal for 56 days. The high SGR and low FCR values of fish fed D4 confers it with better advantages for growth and efficiency of feed utilization over the rest of the experimental formulated diets. This result agrees with the report of Olaniyi *et al.* (2009) [24] who indicated that the higher the SGR the smaller the FCR values, the better the feed quality on *Clarias gariepinus* fed different inclusion levels of mucuna seed meal.

Table 5: Growth Parameters of the *C. gariepinus* juveniles Fed different dietary inclusion levels of *C. lanatus* peel meal diets

Parameters	D1 (0.00)	D2 (1.00)	D3 (1.50)	D4 (2.00)	D5 (2.50)
MIW(g)	8.15±0.03^{a}	8.16±0.02^{a}	8.15±0.01^{a}	8.15±0.02^{a}	8.15±0.03^{a}
MFW(g)	29.79±1.66^{a}	31.94±0.93^{b}	33.98±1.29^{c}	34.23±0.35^{c}	28.44±1.18^{a}
MWG (g)	21.64±1.70^{a}	23.78±0.85^{b}	24.83±1.27^{c}	26.08±0.09^{c}	20.29±1.55^{a}
SGR (%)	1.85±0.11^{a}	1.95±0.03b^{ab}	2.04±0.07^{b}	2.05±0.01^{b}	1.79±0.08^{a}
FI	36.57±3.82^{ab}	38.48±4.53^{b}	39.56±3.25^{b}	41.13±2.69^{c}	34.63±1.70^{a}
FCR	1.69±0.31^{a}	1.62±0.13^{a}	1.59±0.20^{a}	1.58±0.09^{a}	1.71±0.23^{a}
Survival (%)	100.00±0.00^{a}	96.65±4.74^{a}	96.65±4.74^{a}	100.00±0.00^{a}	90.00±14.14^{a}

Means in the same row followed by different superscripts letters differed significantly ($P<0.05$)
MIW: Mean Initial Weight; MFW: Mean Final Weight; MWG: Mean Weight Gain; FI: Feed Intake, SGR: Specific Growth Rate, FCR: Feed Conversion Ratio.

The haematological response of blood of *C. gariepinus* at the end of the experimental period is presented in table 6. Varying inclusion levels of *C. lanatus* in the diets improved the blood profile as there were significant differences (P<0.05) in the parameters tested. This agrees with the reports of Dada (2017) [25] where *C. gariepinus* fingerlings fed *Telfaria occidentalis* leaf powder supplemented diets showed significantly improved haematological parameters when compared with the control group. Values for PCV and Hb was highest in fish fed D4 followed by D3 while fish fed D1 had the lowest values for the same parameters. Significant increase (P<0.05) in RBC was observed in fish fed D3 compared other treatments and the control. Fish fed D2 had the highest value for WBC while fish fed D3 had the lowest WBC value. This implies that *C. lanatus* added to the diet of *C. gariepinus* had no negative effect on blood parameters. The results obtained in the present study is in agreement with the reports of Sogbesan and Ahmed (2018) [26] who recorded highest red blood cell (RBC), white blood cell (WBC), haemoglobin (Hb) and packed cell volume (PCV) in fish fed diet containing 4% *O. gratissimum* and lowest values in the fish on control group. The range of RBC observed in the present study were higher than that reported by Ibidun *et al.* (2017) [27] on *C. gariepinus* fed diets containing *Zingiber officinale* root at different inclusion levels. The mean corpuscular volume (MCV) range (62.90 to 74.10 fl) recorded in this experiment was lower than 87.50 to 210.00fl reported by Dienye and Olumuji (2014) [19] on *Clarias gariepinus* fed dietary levels of *Moringa oleifera* leaf meal), meanwhile the mean corpuscular haemoglobin concentration (MCHC) range (42.26 to 45.44%) recorded in this study was higher than (30.70%) reported for *C. gariepinus* fed *Telfaria occidentalis* leaf powder [25]. The MCH range (28.21 to 32.62 pg) obtained in this study was higher than the range (20.82 to 26.60 pg) reported for *Heteroclarias* fed *Carica papaya* leaf meal incorporated feed [28].

Table 6: Haematological parameters of *Clarias gariepinus* juveniles fed different levels of *C. lanatus* meal diet.

Parameters	D1 (0.00)	D2 (1.00)	D3 (1.50)	D4 (2.00)	D5 (2.50)
Hb(g/dl)	10.70±1.41^{a}	12.50±0.28^{ab}	12.75±0.49^{ab}	13.70±1.41^{b}	11.60±0.71^{ab}
PCV (%)	25.32±4.24^{a}	27.51±0.7 ^{ab}	28.43±1.41^{ab}	31.12±4.24^{b}	26.50±2.1^{ab}
RBC(10^6/mm^3)	3.55±0.50 ^{a}	4.15±0.07^{b}	4.52±0.14^{b}	4.20±0.46^{b}	3.83±0.25^{a}
WBC(10^2/mm^3)	5.27±1.27^{a}	5.45±0.50^{a}	4.70±0.28^{a}	5.09±0.50^{a}	5.27±1.13^{a}
MCV(fl)	71.32±0.62^{b}	66.29±0.16^{ab}	62.90±0.32^{a}	74.10±0.17^{c}	69.19±0.29^{c}
MCH(Pg)	30.14±0.22^{b}	30.12±0.17^{b}	28.21±0.16^{a}	32.62±0.50^{c}	30.29±0.11^{b}
MCHC (%)	42.26±0.01^{a}	45.44±0.13^{b}	44.85±0.54^{b}	44.02±0.01^{b}	43.77±0.18^{a}

Means in the same row followed by different superscripts letters differed significantly (P<0.05).
Hb= Haemoglobin, PCV = Pack Cell Volume, RBC =Red Blood Count, WBC= White Blood Cell, MCV= mean corpuscular volume, MCHC= mean cell haemoglobin concentration, MCH= mean cell haemoglobin.

Conclusion

The feeding trial on varying inclusion levels of *C. lanatus* peel meal revealed that D4 which had 2.0g/kg inclusion level emerged as the best in both growth performance and nutrient utilization. As evidenced by the findings of this study, *C. lanatus* peel can be successfully used in *C. gariepinus* feed at 2.0g/kg without adverse effects on the growth responses and nutrient utilization of the fish. The present study has clearly shown that *C. lanatus* peel increased growth and also improved haematological indices of the fish. Further studies should be performed on processing *C. lanatus* peel so as to improve growth potential in fish.

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