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Growth performance and nutrient utilisation of *Clarias gariepinus* fed on sweet orange peels (*Citrus sinensis*) as carbohydrate source

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ABSTRACT: The effect of substitution of maize meal with orange peel in the diet of *Clarias gariepinus* was investigated. Proximate composition, anti-nutritional content of differently processed orange peels and their effects on the growth of *Clarias gariepinus* were investigated. Soaked sweet orange peel meal was used in the formulation of the experimental diet and replaced maize at 25, 50, 75, and 100% inclusions respectively while the control diet was at 0% SOPMs replacement. The experiment consists of five treatments (1, 2, 3, 4 and 5) with two replicates in a completely randomized design (CRD). Two feeding trials lasted for a period of 8 weeks (56 days) and was carried out in a concrete experimental pond measuring 2 m x 1 m x 0.5 m. Two hundred *Clarias gariepinus* fingerlings, 10 g average weight were randomly distributed at 40 fish per replicate. Growth responses were checked and recorded weekly with a sensitive weighing balance (Mettler 5000) to the nearest grams after which feed quantity was adjusted. Fish were fed experimental diet twice daily (08:00 am and 05:00 pm) at 5% body weight, pond water was changed weekly during weight recording and the water quality parameters were monitored. The results of the proximate analysis of orange peel meals under different processing methods indicated that moisture content was highest in sundried peels (6.74) and lowest in cold-soaked peels (5.8%). Lipid content was highest in boiled peels (8.47) and lowest in fermented peels (7.71%). Protein content was highest in boiled peels (7.43) and lowest in sundried peels (6.40%). The highest NFE was recorded in the sundried peels (60.90) while lowest NFE value was recorded in the fermented (54.38). The anti-nutritional factors determined from the various processing method showed that boiling was more effective in reducing tannin (46.4% reduction), phytic acid (46.5% reduction), oxalate (54% reduction) and was least effective in reducing saponin (20% reduction). Fermentation was least effective in ANF reduction, tannin (21%) and phytic acid (15%); cold-soaking was more effective in saponin reduction (46.5%). Result obtained revealed that there was no adverse effects on the growth performance of the fish fed orange peel meals at 50% inclusion, thus sweet orange peel meals can replace maize up to 50% level of inclusion in the diet of *Clarias gariepinus* fingerlings without adverse effect with a corresponding SGR of (2.64±0.00). Apparent net protein utilization differed among the treatments ($p<0.05$).

Keywords: Anti-nutritional factors, carbohydrate, growth performance, proximate composition.

INTRODUCTION

Fish feeds constitute about 40 to 60% of the recurrent cost of most intensive fish farming ventures and can sometimes negate the economic viability of a farm if suitable feeds are not used (NRC, 1993). Carbohydrates are used in fish diets primarily as energy source and for their binding properties; it can be added in excess of the amount required by fish (Krogdahl *et al*., 2005) but rising costs and its scarcity is making it uneconomical to feed animals

including fish (Falaye *et al*., 1998). Producers have intensified the search for less costly and readily available alternative feed materials (Hon *et al*., 2009). The use of alternative feed sources especially plant feed stuffs will help solve the challenges on annual increase in fish production, rising cost, competition and scarcity of carbohydrates and protein feed ingredients. The need to solve the problems of feeding in aquaculture has been demonstrated through various researches in the utilization of vegetables and agricultural feedstuff such as toad meal (Annune, 1990), maggot meal (Faturoti *et al*., 1995; Fasakin *et al*., 2004), fermented shrimp head waste meal (Nwanna, 2003), poultry offal (Fasakin, 2008), water hyacinth meal (Sotolu, 2008), fermented locust bean (*Parkia biglobosa*) seed meal (Obum, 2008).

Anti-nutritional factors have significant negative effects on livestock production; these effects include reduction in palatability, digestibility and utilization of ration, intoxication of different classes of livestock resulting in mortality or decreased production of animal and reduction in the quality of meat, egg, and milk due to the presence of hazardous residues (Amuchie, 2001). Orange peels possess some anti-nutritional factor(s) which do not promote the deposition of fat in the body (Oluremi *et al*., 2008). The presence of endogenous anti-nutritional factors in plant feedstuff is believed to be the largest single factor limiting their uses within animal and fish feeds. Although these factors vary in their individual toxicity to fish, a large proportion can be destroyed or inactivated by heat treatment (Tacon and Jackson, 1985). Maize has been a traditional energy source in formulated feeds, but rising costs and its scarcity is making it uneconomical to feed animals, including fish (Falaye *et al*., 1998).

Many factors limit the utilisation of non-conventional feedstuffs in livestock feeds (Oluremi and Andrew, 2007), these include low protein (Gohl, 1981), high fibre content (McDonald *et al*., 1988; amino acid imbalance and presence of anti-nutritional factors (Tacon and Jackson, 1985). Anti-nutrients are natural or synthetic compounds that interfere with the absorption of nutrients; one common example is phytate, which forms insoluble complexes with Calcium, Zinc, Iron, and Copper. Protein inhibitors trypsin and lectins found in legumes (Gilani *et al.*, 2005), flavonoids and tannins inhibit and reduces the absorption of metals such as iron and zinc; they also inhibit digestive enzymes and may precipitate protein (Beecher, 2003).

High cost and shortage of high quality feeds needed to sustain fish growth is especially a major challenge to the industry in developing countries, however, crop residues, agro-industrial by-products and non-conventional feed resources are being evaluated to access their nutritive potentials to support fish production at low production cost.

In view of the increasing demand for fish, high cost of conventional feed ingredients and competition for maize by humans and animals it is necessary to investigate the possibility of using unconventional feeds such as orange peels that are arbitrarily discarded where they are a pollution risk to the environment. This study will investigate the use of orange waste in fish feed as a replacement for maize with the purpose of cutting down the cost of fish production while increasing total yield.

Objectives

The objective of this study are to determine:

1. the proximate composition of various processed sweet orange peels (*Citrus sinensis*).
2. the anti-nutritional factors in processed orange peel.
3. the effect of processed orange peel on the growth and nutrient utilisation of *Clarias gariepinus* fingerlings.
4. the effect of inclusion of various levels of orange peel in the diet of *Clarias gariepinus*.

MATERIALS AND METHODS

Source of test ingredient

Sweet orange peels were obtained through manual peeling from the oranges (*Citrus sinensis*) bought from the orange orchard of the College of Agriculture, Lafia and moved to the Fisheries Laboratory of the same Institution where it was processed and used as replacement for maize in the diet of *Clarias gariepinus* fingerlings.

Preparation of test ingredient

Collected orange peels were processed by four methods: (1) Sun-dried until they became crispy hard and dry and labelled SA. (2) Boiled for 10-15 minutes and cooled at room temperature, thereafter peel was drained out of the water using a colander, sundried and labelled SB. (3) The third portion of sweet orange peel was soaked in cold water for 24 hours, drained, sundried and labelled SC. (4) The fourth and last portion was covered in a basket for 72 hours to ferment under room temperature and thereafter sundried and labelled SD.

Each processed dried peel sample was ground into powder using Thomas Willy Miller with a 2 mm sieve to obtain fine particles of sweet orange peel meal (SOPMs) sample and labelled as SOPMA, SOPMB, SOPMC, and SOPMD respectively and taken for proximate analysis.

Proximate analysis of sweet orange peels

Each sweet orange peel meal sample; A, B, C, and D was assayed for the proximate composition according to AOAC (2000).

Determination of moisture content

Moisture or water content of the samples was determined using the hot oven method. Two (2) grams of samples were transferred into labelled crucibles of known weight and the crucibles with the two (2) grams samples were covered with a lead. On placing the crucible in the oven, the lead was removed and the temperature of the oven was set at 100°C to effect proper drying of the samples. The samples were allowed to remain in the oven until when dried to a constant weight. Then the samples were removed and cooled in a desiccator prior to weighing. The percentage moisture content was calculated at this point.

$$\%\, Moisture\ Content = \frac{\mathrm{W1} - \mathrm{W2}}{Weight\ of\ sample} \times \frac{100}{1}$$

Where: W1-W2 = Difference between initial and final weight

Crude protein

The protein content was obtained through the determination of total nitrogen by micro kjeldahl's method. The value of nitrogen obtained was multiplied by 6.25 to get the crude protein value.

Determination of ash content

Procedure: The required number of silica dishes was ignited in the furnaces at a temperature of 600^0C for 15 minutes. These were removed using tongs, put in a dedicator and weighed to the nearest milligram, 2 g was placed on a hot plate under a fume cupboard and the temperature increased gradually until smoking cases and the sample becomes thoroughly charred. Then the dishes were removed using tongs and placed in a muffle furnace. The temperature was increased for 6 to 24 hours until a whitish grey ash remains. The dishes were removed and placed in desiccators to cool to room temperature. Each dish and the remains were weighed to the nearest milligram. A calculation of the weight of ash was sustained.

$$Calculation\colon \%\, ash = \frac{\mathrm{Weight\ of\ ash}}{Weight\ of\ sample} \times \frac{100}{1}$$

Determination of ether extract

Procedure: Washed flask was put over a temperature of 100°C for 1 hour. The flask was put in the desiccators and cooled then weighed. 2 g of dried samples were wrapped with filter paper, put in a thimble and plug with cotton wool. The thimble was placed in the extractor, and then a weighed flask containing about 100 ml of petroleum spirit was connected and a reflux condenser.

Extraction was done for six hours. A thimble was removed and most of the solvent was distilled from the flask into the desiccators, recovering each fraction. The flask was disconnected and then placed in the oven at 100°C for 2 hours. This was cooked in the desiccators and then weighed.

$$Calculation\colon \%\, fat = \frac{\mathrm{Weight\ of\ fat}}{Weight\ of\ sample} \times \frac{100}{1}$$

Determination of crude fibre

Procedure: 2 g of sample were washed to the nearest mg. Extraction of oil was done using a soxhlet unit. The extracted sample was dried by air and transferred into 1000 ml conical flask.

Acid digestion: 20 ml of sodium hydroxide solution was added and it was boiled for 30 minutes. Then left standing for 1 minute, then it was filtered and washed with boiling water, 1% of hydraulic acid and then boiling the water until the washing are neutral to litmus. The insoluble matter was transferred into a crucible dried at 100°C to constant weight; this was cooled in the dedicator and weighed again.

Determination of nitrogen free extract (NFE)

This was done by subtracting the percentages of crude protein, crude fat, ash, moisture and crude fibre from a hundred per cent of the fish flesh i.e.;100 – percentage crude protein + percentage crude fibre + percentage ash + percentage moisture + percentage fat.

Determination of anti-nutritional factors

Anti-nutritional factors such as; saponin, phytates, tannins and oxalates inhibitors were determined according to the method outlined by Kakade *et al*. (1972). 0.2 g of sample ingredient was weighed into screw cap centrifuge tube each 10ml of 0.1m phosphate buffer was added and the content was shaken at room temperature for 1 hour on a shaker. Each suspension obtained was centrifuged at 5000 rpm for 5 minutes and filtered through Whatman No.42 filter paper. The volume of each filtrate was adjusted to 2 ml with phosphate buffer in test tubes. The test tubes were transferred to water bath maintained at 37°C. 6 ml of 5% TCA solution was poured into a test-tube to serve as a blank. 2ml of casein solution was added to each of the test-tube which was previously kept at 37°C, then incubated for 20 minutes by adding 6ml of TCA solution to the experimental tubes and shaken.

The reaction was allowed to proceed for 1 hour at room temperature. Each mixture was filtered through Whatman No 42 filter paper. Absorbance of filtrates from each sample and trypsin standard solution was read in a spectro-photometer at 280 nm.

Formulation of experimental diets

Soaked sweet orange peel meal was used in the formulation of the experimental diet and replaced maize at 25, 50, 75, and 100% inclusions respectively while the control diet was at 0% SOPMs replacement. Diet was prepared using feed ingredients such as soybean meal 39.24% (Bayero *et al.,* 2019), groundnut cake, fish meal 68.4% (Olayemi *et al.,* 2011), maize 8.75% (Ape *et al.,* 2016), vitamins and minerals premix.

The various feedstuffs were mixed together in a bowl and thereafter made into a dough and pelletized using pelleting machine and then sun-dried. Each diet sample was taken for proximate analysis before feeding trial.

Experimental design

The experiment consists of five treatments (1, 2, 3, 4 and 5) with two replicates in a completely randomized design (CRD). Two feeding trials lasted for a period of 8 weeks (56days) and was carried out in a concrete experimental pond measuring 2 m x 1 m x 0.5 m. Two hundred *Clarias gariepinus* fingerlings, 10 g average weight were randomly distributed at 40 fish per replicate. Fish were acclimatized for 10 days; the fish were starved for two days before the start of the experiment. Growth responses were checked and recorded weekly with a sensitive weighing balance (Mettler 5000) to the nearest grams after which feed quantity was adjusted. Fish were fed experimental diet twice daily (08:00 am and 05:00 pm) at 5% body weight, pond water was changed weekly during weight recording and the following water quality parameters; water temperature was monitored using a simple thermometer while pH was monitored using the digital pH meter.

Growth parameters evaluation

The following parameters were determined from the feeding trials from each of the treatment groups and the following formulae were used for the computation of the parameters.

$\text{Mean Weight Gain} = W1 - Wo$ (Saviour and Gift, 2021).

$\text{Specific Growth Rate} = \frac{Logew2 - Logew\ 1}{t2 - t1}$ (Brown, 1957).

Where; e = natural logarithm, w2 = final weight, w1 = initial weight, t2 & t1 = time duration (days).

$$\text{Percentage weight gain} = \frac{\text{Weight gain}}{Initial\ weight} \times 100$$

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{Weight gain}}{Weight\ of\ feed\ consumed} \times 100$$

$$\text{Efficiency Ratio (PER)} = \frac{\text{Weight gain by fish}}{Weight\ of\ protein\ intake}$$

$$\text{Protein intake} = \frac{\%\ \text{of protein in diet} \times \text{total feed intake}}{100}$$

$$\text{ANPU} = \frac{\text{Protein gain}}{Protein\ intake} \times 100$$

Where ANPU = Apparent Net Protein Utilisation

$$\text{Protein gain} = \text{Final carcass protein} - \text{Initial carcass protein}$$

(Brown, 1957; Osborne *et al*., 1919; Miller and Bender, 1955; NRC, 1993).

Data analysis

Data generated from the study were subjected to one-way analysis of variance (ANOVA), using the SPSS (statistical package computer software 2000 version). Using the methods of Steel *et al*. (1997), data generated was presented as mean ± standard error and significance was declared ($p<0.05$).

RESULTS

The results of the proximate composition of processed orange peels are presented in Table 2. Boiled orange peels had the highest crude protein (7.43±0.38) while it was least with Sundried samples (6.40±0.50). For Lipids content, boiled processed orange peels had the highest (8.47±1.41), it was least with fermented orange peels (7.71±0.86). For moisture content, sundried orange peels were highest (6.74±0.88) while it was least with Cold Soak orange peels (5.82±0.41). NFE was higher with sundried orange peels (60.90±2.45) while it was least with boiled orange peels (57.57±4.23).

The result on the proximate composition of the processed orange peels as shown in Table 2 shows no significant difference ($p<0.05$) in nutrient composition. The fibre content was highest (21.43) in fermented processed orange peels but lowest (15) in sundried orange peels. NFE was highest (60.90) in sundried orange peels but lowest (54.38) in fermented orange peels. However, cold soaked orange peel was selected for inclusion in the

Table 1. Gross composition of diet for *Clarias gariepinus* of various levels of percentage inclusion of orange peel meal.

Feed ingredients	Control (0% SOPM)	Diet 1 (25% SOPM)	Diet 2 (50% SOPM)	Diet 3 (75% SOPM)	Diet 4 (100% SOPM)
Dried orange peel meal	0	7.5	15	22.5	30
Rice bran	10	10	10	10	10
Fish meal	54	54	54	54	54
Maize meal	30	22.5	15	7.5	0
Vegetable oil	2.0	2.0	2.0	2.0	2.0
Bone meal	1.0	1.0	1.0	1.0	1.0
Vitamin premix	1.0	1.0	1.0	1.0	1.0
Lysine	2.0	2.0	2.0	2.0	2.0
Total	100	100	100	100	100

SOPM: Sweet orange peel meal.

Table 2. Proximate composition of processed sweet orange peels (%).

Processing method	Protein	Lipid	Ash	Fibre	Moisture	NFE
Boiled	7.43±0.38a	8.47±1.41a	9.11±1.60d	17.42±0.8b	6.18±0.66c	57.57±4.2b
Cold soak	7.32±0.21b	7.97±0.09c	9.62±1.30c	15.45±0.35c	5.82±0.41d	59.64±1.33c
Fermented	6.80±0.09c	7.71±0.86d	9.68±1.56a	21.43±2.33a	6.52±0.79b	54.38±4.68d
Sundried	6.40±0.50d	8.07±0.94b	9.63±0.60b	15.00±1.87d	6.74±0.88a	60.90±2.45a

Table 3. Proximate composition of soaked sweet orange peel based diets (%).

Diet	Control	Diet 1	Diet 2	Diet 3	Diet 4
Parameters	0 %	25%	50 %	75%	100
Protein	43.39±0.00b	43.57±0.00a	43.42±0.02b	43.17±0.01c	%43.21±0.01c
Lipid	4.41±0.01d	5.71±0.01c	5.89±0.02b	6.49±0.00a	6.47±0.00a
Ash	5.46±0.01e	7.28±0.00d	7.74±0.01c	8.67±0.00b	8.77±0.02a
Fibre	5.04±0.00e	5.54±0.00d	6.53±0.01c	7.46±±0.01b	7.82±0.01a
Moisture	4.52±0.00d	4.88±0.01c	5.19±0.01b	5.33±0.00a	5.32±0.01a
NFE	41.70±0.01a	41.70±0.01a	36.43±0.05c	34.21±0.01d	33.73±0.01e

Means different column followed by different superscripts differ significantly ($p<0.05$).

experimental diet due to its combination of protein and fibre content as well as the reduced saponin content.

Feeding of fish was carried out using experimental diets formulated with soaked orange peels at various levels of inclusion ranging from 0 to 100%. Gross composition of diet for *Clarias gariepinus* at various levels of percentage inclusion of soaked orange peel meal is presented in Table 1. The proximate composition of the experimental diets (Table 3) shows that significant differences exist in terms of all the nutrients. Protein content was highest (43.57) in the diet with 25% inclusion level and lowest (43.17) in the diet with 75% inclusion while lipid was highest (6.49) in the diet with 75% and 100% levels of inclusion of the orange peel but lowest (4.41) at 0% level of inclusion. Carbohydrate content was found to be highest (41.7) in the diet with no orange peel inclusion and lowest (33.73) in the diet that was totally replaced with orange peel meal. Fibre was highest (7.82) in the diet with full replacement with orange peels and lowest (5.04) at 0% inclusion. Full replacement of orange peel also produced a diet with the highest (8.77) level of ash while the diet without orange peels gave the lowest (5.46) level of ash.

Generally, the lipid content of the diet increased with level of inclusion but declined with full inclusion/ replacement. However, protein content of the diets reduced with increasing inclusion of orange peel meals, it is best at 25% inclusion. Fibre content of the feed increased with increase in inclusion of orange peel in the formulated diets with carbohydrate content of the diet showed a converse pattern.

Table 4. Anti-nutritional factors in processed sweet orange peels *Citrus sinensis* obtained from Lafia.

Treatment	Tannin	%Red in ANF	Phytic Acid	%Red in ANF	Oxalate	%Red in ANF	Saponin	% Red in ANF
Raw Fresh	0.660±0.020a	-	0.114±0.002a	-	0.097±0.003a	-	0.080±0.002a	-
Sundried	0.367±0.009cd	44.4	0.076±0.001c	33.3	0.052±0.001c	46.4	0.048±0.000c	40.0
Boiled	0.354±0.040d	46.4	0.061±0.002d	46.5	0.045±0.005c	53.6	0.064±0.002b	20.0
Cold soaked	0.437±0.009c	33.8	0.082±0.001c	28.1	0.075±0.001b	22.7	0.045±0.001c	46.5
Fermented	0.520±0.005b	21.2	0.096±0.004b	15.8	0.077±0.001b	20.6	0.062±0.001b	22.5

Means values in the same column with different superscript differs significantly ($p<0.05$). **Key:** ANF = Anti nutritional factors, Red = Reduction, Treat = Treatment.

Table 5. Growth performance Indices of fish fed various levels of sweet orange peel (*Citrus sinensis*) in the diet.

Growth parameters	Control 0%	Diet 1 25%	Diet 2 50%	Diet 3 75%	Diet 4 100%
MIW (g)	10.02±0.00	10.02±0.00	10.02±0.00	10.02±0.00	10.02±0.00
MFW (g)	41.54±0.88c	43.51±0.76ab	44.03±0.03a	41.97±0.21bc	41.52±0.33c
MWG (g)	31.52±0.8c	33.49±0.76ab	34.01±0.04a	31.95±0.21bc	31.50±0.33c
SGR (%/day)	2.54±0.04c	2.62±0.03ab	2.64±0.00a	2.56±0.01bc	2.54±0.01c
FCR	1.90±0.06b	1.98±0.01ab	2.02±0.01a	1.88±0.03b	1.76±0.04c
PER	1.21±0.04bc	1.30±0.03a	1.23±0.02ab	1.16±0.01bc	1.15±0.00c
ANPU	0.78±0.02b	0.83±0.02a	0.73±0.00bc	0.71±0.00c	0.74±0.02bc
Feed Intake (g)	62.65±0.15c	62.07±0.27c	71.01±0.34a	68.30±0.33b	62.44±0.45c
Survival (%)	100	100	100	100	100

Means in the same row followed by different superscripts differ significantly ($p<0.05$). **Key:** Mean initial weight (MIW), Mean final weight (MFW), Mean weight gain (MWG), Specific growth rate (SGR), Food conversion ratio (FCR), protein efficiency ratio (PER), Apparent net protein utilization (ANPU).

Anti-nutritional factors

The result of the anti-nutritional factors present in different processed orange peels as presented in (Table 4) showed a significant difference in the anti-nutritional factors determined for each of the treatments ($p>0.05$). Heat treatment by boiling was more effective in reducing tannin, phytic acid, and oxalate. While cold-soaking was more effective in reducing saponin content, fermentation on the other hand was least effective in reducing anti-nutritional factors.

Reduction in percentage content of the anti-nutritional factors depended on the treatments as compared to the raw fresh peel given in Table 4 shows that boiling was more effective against tannin (46.4%), phytic acid (46.5%), oxalates (54%) but was least effective in reducing saponin 20%. Fermentation was least effective in reducing the anti-nutritional factors (20.6%). Cold soaking on the other hand was more effective against saponin (44% reduction).

Growth performance

Result from Table 5 revealed that with increasing levels of sweet orange peel meal inclusion Diet 2 (50%) sweet orange peel inclusion gave the best SGR (2.64±0.00), While Diet 4 (100%) sweet orange peel meal inclusion produced the least SGR (2.54±0.01).

Protein utilisation indices such as PER AND ANPU were highest in diet1 (25%), (1.30±0.03) and (0.83±0.02) respectively, protein utilization value decreased with increase in sweet orange peel from 75 to 100% inclusion respectively.

Experimental fish grew at a rate between 2.54 to 2.64% per week with the best specific growth rate being recorded for fish fed with diet containing 50% orange peels while total inclusion and zero inclusion produced the same specific growth rate. Significant differences exist in terms of the specific growth rate among fish fed the various diets ($p<0.05$). The specific growth rate increased with increasing inclusion and then declined after 50% inclusion. This trend led to a better weight gain among fish fed 25 and 50% orange peel in the diet while the 100% inclusion level as well as the zero inclusion levels produced similar weight gains with significant differences observed among the treatments ($p<0.05$). Furthermore, the final weight of fish across all diets followed a similar pattern with increasing trend as inclusion increased but declined after the level of inclusion exceeded 50%.

Utilization indices for the feeds exhibited a different

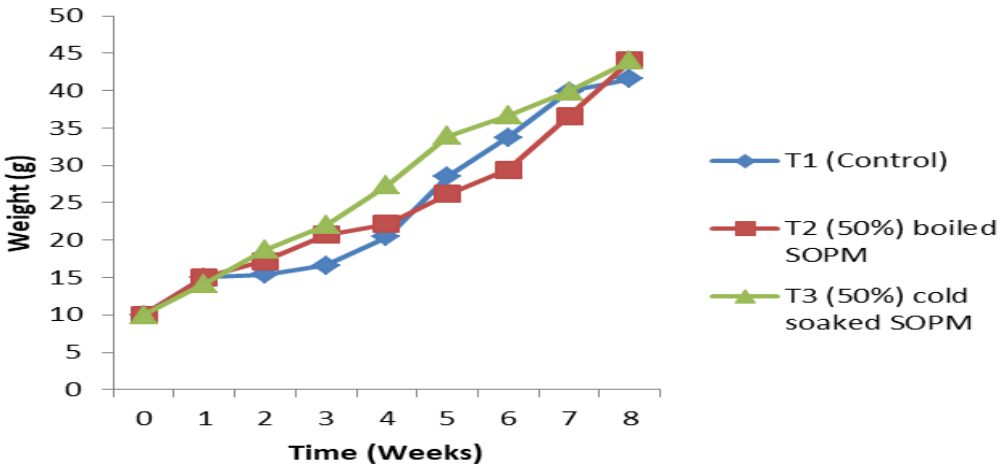

Figure 1. J-curved graph showing weekly weight increase of fish fed various sweet orange peel levels in the diet.

trend. Significant differences were observed among fish fed the different diets in terms of FCR, PER and ANPU ($p<0.05$). The 25% level of orange peel in the diet gave better feed conversion efficiency than either higher or lower levels and protein efficiency is better at the 25% level of inclusion. The apparent net utilization of proteins is also better at 25% level of orange peel in the diet and least at 75% level of inclusion. However, feed intake was highest in the group of fish fed 50% orange peel but there was a high feed conversion ratio in this group.

The trend of weight gained across the eight weeks of study (Figure 1) showed that growth performance of fish fed 0, 25 and 50% inclusions of orange peel in their diet were higher than fish fed both the 50 and 75% orange peel inclusion in their diets. Fish fed 100% of orange peels inclusion maintained a fairly stable weight gain between the first week and the fifth week, those fed 0 and 25% inclusion of orange peel in their diet exhibited reduction in weekly weight gain between weeks three and six. However, the fish fed with 25% orange peel in the diet increased weight gain between week 6 and week 8 hence coming close to the fish fed with orange peel at 50% inclusion in the diet.

Water quality

Water quality parameters (Table 6) were monitored throughout the eight weeks of study and the values were at recommended levels for fish. Dissolved oxygen ranged from 7.02 mg/l at 75% level of inclusion to 7.34 mg/l at 25% level of inclusion. The temperature varied between 26.24°C at 25% level of inclusion to 26.76°C at 100% level of inclusion. pH ranged from 6.27 to 6.40 for 100% and 25% inclusion levels respectively. Weekly variation in water quality shows that pH was highest in week 7 (6.55) and lowest in week 2 (6.21). The temperature was highest in week 4 (226.68°C) and lowest in weeks 6 and 7 (26.44°C). Dissolved oxygen was highest at the start of the experiment (7.32 mg/l) and lowest in weeks 4 and 5 (7.06 mg/l).

DISCUSSION

Proximate composition

The proximate composition of the orange peel reveals a high concentration of carbohydrates with lower protein and this result disagree with the findings of Hutton (1987), that orange peels has virtually no starch. Reason for this difference may be because the amount of soluble carbohydrate of peel and pulp of citrus fruits are correlated with stages of fruit maturity (Mohamed, 1999).

The result of proximate composition of sweet orange peel meal (*Citrus sinensis*) across the various processing methods showed that the moisture content of the sample was between (5.82±0.41and 6.74± 0.88), the lowest moisture content was recorded in the soaked peel; this is expected since the various samples were subjected to drying to reduce the moisture contents, high moisture content is an index of spoilage. The protein content was between (6.40 ± 0.50 and 7.43± 0.38) with the highest value recorded in the boiled peel the value obtained is lower than the value (10.73%) obtained by Agu *et al*. (2010), the crude fiber was (15.00±1.87 and 21.43±2.33) with lowest value obtained in the cold soaked sweet orange peel and the highest value obtained from the fermented peels, fat content was between (7.71±0.86 and 8.47±1.41) an indication that the orange peel contained much oil, carbohydrate content by difference was between (54.38±4.68 and 60.90±2.45).

Table 6. Mean weekly water quality parameters.

Variable	Initial	Wk1	Wk2	Wk3	Wk4	Wk5	Wk6	Wk7	Wk8
DO (mg/L)	7.32±0.08	7.12±0.11	7.22±0.14	7.26±0.07	7.06±0.11	7.06±0.10	7.22±0.11	7.30±0.09	7.24±0.12
Temp (°C)	26.50±0.08	26.68±0.08	26.48±0.20	26.36±0.18	26.68±0.14	26.54±0.15	26.44±0.22	26.44±0.07	26.62±0.23
PH	6.39	6.41	6.21	6.38	6.44	6.28	6.28	6.55	6.24

The proximate value as obtained in this study is slightly above the value recorded by Osarumwense *et al*. (2013), who reported a proximate composition of sundried sweet orange peels to be protein content (4.05± 0.25), ash content (14.35±0.35), fibre content (26.50 ± 0.20), moisture content (2.20±0.20), carbohydrate (42.90±1.00), and fat content (10.00±0.00). The present result however agrees with the findings of Braddock and Crandall (1981) who reported a 6% protein value of dry citrus peel.

The result on the proximate composition of fermented orange peel as obtained in this study are lower than the values obtained by Ani *et al*. (2015) who reported a composition of fermented sweet orange peel meal to be protein content (10.00), fibre content (14.60), ash content (4.47), NFE (67.90) respectively, however in the present study boiling happen to be the best form for processing orange peels for feeding trials.

The differences in the value of proximate composition in the present study revealed that various processing methods impacted on the value of the proximate composition obtained, processing by boiling gave the best protein value (7.43±0.38), while sun drying gave the least protein value (6.40±0.50).

Anti-nutritional factors

The results revealed that boiling was 54% effective against oxalates while fermentation was least effective (20.6%). Cold soaking was 44% effective against saponin but was not as effective as boiling and sun drying in terms of reduction of tannins, phytic acid and oxalate. This observation is in line with the findings of Smith (2010) that anti-nutritional factors can either be heat labile (phytate , trypsin inhibitors, heamoglotinin, lectins and anti-vitamin) or heat stable (saponin, taninns inhibitors allegiens, lysine and alanin Fermentation was least effective in dealing with the anti-nutritional factors.

Tannin, saponin, phytate, oxalate, as contained in this study agrees with the findings of (Oluremi *et al*., 2007) that orange peels contains tannin, saponin, phytate, oxalate, flavonids and limonene. Following the classification by Francis *et al*. (2001), tannins as found in this study is a factor affecting protein utilization and digestion, phytate and oxalate affects mineral utilization while saponin is a miscellaneous substance. Anti-nutritional factors are substances that either on their own or in combination with other biochemical products of metabolism hamper feed digestion and uptake and adversely affect animal health (Kumar, 1992, Makkar, 1993, Aganga and Tshwenyane, 2003). The toxicity of anti-nutritional factors also depends on the digestive anatomy and physiology of the animal (Kumar, 2009).

Fermentation as used in this study showed the least percentage of removals for ANF's; this suggests that fermentation is not a very good method of removing ANF's.

Effect of processing on the feed utilization as reported by Oluremi *et al*. (2008) shows that fermented sweet orange peel when used to replace maize in the diet of broilers had a negative effect on feed intake, weight gain and live weight of the broilers. The palatability of orange peel is reduced due to saponin content and this can be removed via soaking as seen in this study. According to Callaway *et al*. (2011), palatability affected the intake of orange peel based diets by sheep at 20% inclusion level but reported the efficacy of orange peel against salmonella in the gut of sheep. Similarly, dried sweet orange peels was reported to promote feed intake in broilers within the first 21 days of age at 1.5% inclusion but reduced feed intake at 3% level of inclusion (Abbas *et al*., 2013). The various processing methods as used in this study revealed that heat treatment by boiling did not reduce the saponin content and phytic acid was highest in the fermented orange peel followed by the cold soaked peel. Although fermentation has been reported to reduce the phytate content in grains (Mukhopadhyay and Ray, 1999), fermentation seems to be ineffective for the orange peel. The reason for this is unclear, but it is known that phytates are found in high concentrations on the seed coats of grains and can be reduced significantly by milling to remove the coat (Francis *et al*., 2001). The result in this study correspond with the findings that anti-nutritional factors can be denatured or deactivated using different processing methods. Heat is the most commonly used method (Francis *et al*., 2001). The bitter taste of saponin has been identified as a factor that reduces palatability of feeds made with feedstuff

that contain saponin (Oluremi *et al*. (2007). The heat treatment in this study was effective in reducing phytic acid content while saponin which is heat stable (Rumsey *et al*., 1993) was reduced with the aid of cold soaking. Adegunwa *et al*. (2012) reported cooking as a better method for dealing with phytates in Beniseed (*Sesamu mindicum*) Flour, with a 39% reduction from the raw state while roasting and autoclaving gave similar results (28% reduction). This is similar to the trend observed here with the orange peels. Boiling reduced phytic acid content by 47% while fermentation reduced this content by merely 16%. However, these results are lower than 72 to 74% reduction in phytic acid content of sesame seed meals as reported by Hossain and Jauncey (1990) with the use of autoclaving. Virginia *et al*. (2012) also reported the efficacy of cooking in the reduction of phytate and oxalates in green vegetables and pulses. With a 54% reduction in oxalate content, boiling as observed from this study is quite effective in reducing oxalates in orange peels. However, roasting was found to be more effective in reducing oxalate content of beniseed with a 42% reduction compared to 39.1% and 32% for autoclaving and cooking respectively (Adegunwa *et al*., 2012).

Saponin content was used as a yardstick for selecting the treatment method for orange peel that made it suitable for incorporation in the diet of fish. Saponins have amphiphilic properties that confer on them the ability to alter the surface tension of cells by reduction and hence cause haemolysis of erythrocytes (Tacon, 1997; Soetan and Oyewole, 2009). The action of saponin as a surfactant affects the gills of fish and cause severe damage to the respiratory surface (Francis *et al*., 2001).

Tannins give an astringent sensation in the mouth hence a drying puckering sensation (Lee and Lawless, 1991). These compounds can either be in hydrolysable or condensed forms (Francis *et al*., 2001). The hydrolysable form is biodegradable and can be assimilated into the circulatory system where bioaccumulation predisposes the ingesting animal to toxicity (Kumar, 1992) especially in the kidney and liver (Francis *et al*., 2001). However, condensed tannins reduce digestibility via binding to enzymes or formation of protein complexes that are indigestible. Various methods have been suggested for the removal of tannins from feed ingredients, including heat treatment (autoclaving), alkali treatment (Pettersson and Åman, 2012), fermentation with lactic acid bacteria (Mukhopadhyay and Ray, 1999) and aqueous extraction (Dominguez *et al*., 1993). The reduction in tannin content in this study was highest (46%) when heat was applied to the orange peels in the form of boiling and least with fermentation. Although fermentation has been recommended for use in the removal of tannins (Mukhopadhyay and Ray, 1999), this study did not isolate lactic bacteria specifically to carry out the fermentation process hence the disparity in results coupled with the fact that the authors carried out their test on sesame seed meal.

Orange peel utilization (for growth)

The utilisation of orange peel meal in the diets at various levels of inclusion impacted on various growth parameters. This study reveals a wobbling FCR with increasing levels of inclusion of cold soaked orange peel meal in the diet as 25% level of inclusion produced the best FCR while the worst was recorded at 75% level of inclusion. Akinkumi (2011) reported similar feed conversion ratios for *Oreochromis niloticus* fed various levels of banana peels in the diet with PER ranging from 1.15 to 1.27 at 50% and 75% levels of inclusion respectively. Literature on the use of orange peel meal in feeding fish species is scarce. However, numerous researches on its use in animal feeds abound. Oyewole *et al*. (2013) reported the worsening of FCR values with increasing levels of substitution of sweet orange peel meal for maize meal in the diet of growing pullets. In other studies, it was observed that treatments did not affect growth parameters. Ojabo *et al*. (2012), reported that dried sweet orange peel meal did not affect growth parameters of rabbits except for water intake. Also, Agu *et al*. (2010), reported that sweet orange peels in the diet of broilers did not affect feed intake, body weight gain, FCR as well as water intake. However, inclusions of sweet orange peel meal in diets for cockerel chicks significantly reduced growth and feed intake as levels of the peel increased but FCR and PER were not affected (Ojabo and Adenkola, 2013). Close (1993) reported a reduction in energy intake with increased fibre intake which reduces both growth and energy indices in broiler diet.

Conclusion

The evaluation of the proximate composition of sweet orange (*Citrus sinensis*) peels showed that this peel is high in valuable nutrients (moisture, ash, fibre, fat, crude and protein) however high fibre content was recorded in the fermented peels. The results obtained have shown that the various processing methods as used in this study affected the proximate composition and also impacted in the reduction of anti-nutritional factors (tannin, pytic acid, saponin, and oxalate) present in sweet orange (*Citrus sinensis*) peels. The performance of *Clarias gariepinus* fed diets in which maize was substituted at 0, 25, 50, 70, and 100 % with sweet orange (*Citrus sinensis*) peel meal and result revealed that up to 50% dietary maize in Clarias diet can be replaced with sweet orange peel meal without adverse effects on the performance of the fish. As a high energy and protein source, its inclusion in fish feed formulation would help to reduce the cost of fish production.

Recommendations

1. The effect of orange peel on physiology and digestive

tract anatomy should be subject to further investigation.
2. The practical abundance should be exploited as a significant leap to reduce the high demand on maize, its accompanying economic feasibility of substituting orange peels with maize and direct effect on the cost of finished table sized fish products.
3. Further studies are recommended to elucidate appropriate processing treatments to apply to sweet orange peels to improve its nutritive value in order to enhance its suitability as a feed resource in fish production.
4. The use of cold soaking and boiling is recommended to reduce the bitterness brought about by the presence of saponin to make the feed more acceptable and palatable thereby resulting in their enhanced performance.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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