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In-vivo and in-vitro nutrients digestibility of raw and processed *Hibiscus sabdariffa* (Roselle) seed meal fed to the Nile Tilapia *(Oreochromis niloticus)*.

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Target Audience: *Fish farmers; Fish feed millers; Aquaculturist*

Abstract

This study was conducted to determine the in-vivo and in-vitro apparent digestibility coefficient of nutrients in raw and processed Hibiscus sabdariffa (Roselle) seed meals. In the in-vivo digestibility, Acid Insoluble Ash (AIA) was used as an indicator. Five experimental diets were formulated using raw roselle, (RR) soaked roselle, (SR) boiled roselle,(BR) sprouted roselle (SR) and fermented roselle (FR) to feed Oreochromis niloticus fingerlings inside fifteen plastic aquaria of 60cm x 50cm x 30cm dimension with 120L water holding capacity to determine the effect of raw and processed roselle on the digestibility of the oil seed. Ten fingerlings of O. niloticus (initial total weight 0.60kg) were stocked in each aquarium with three replicates. The results obtained revealed that the apparent digestibility coefficient (ADC) for protein were highest in fermented roselle (88.20%) followed by boiled roselle (86.40%), sprouted roselle (85.74%), soaked roselle was 81.43% and the raw roselle was the least with 79.57%. The ADC for Crude Lipid was 57.90%, 50.17%, 57.80%, 50.54% and 49.75% for fermented roselle, sprouted roselle, boiled roselle, soaked roselle and raw roselle respectively. The results for the digestibility coefficient of roselle seed meals analyzed in-vitro using casein as the control showed that the ADC for protein were also highest in fermented, roselle 90.50%, followed by boiled roselle (85.00%), soaked roselle (75.00) and lastly raw roselle (70.00%). There were significant differences ($p < 0.05$) in all the processing methods. In both the in-vivo and in-vitro digestibility, fermented roselle presented the highest digestibility of 88.20% and 90.50% respectively. These values compares favorably with other oil seed meals and legumes used in aqua-feeds.

Key Words: Digestibility, Nile Tilapia, Roselle

Description of Problem

Compounded feeds for The Nile Tilapia (*Oreochromis niloticus*) can be balanced with all the dietary essential nutrients, but still not producing the desired growth because the nutrients may not be readily available (1). Digestibility of the feed is directly related to the ability of the fish to digest the feed. It is a relative measure of the extent to which ingested feed and its nutrients component have been digested and metabolized by the fish (2). According to (3), diet design, feeding strategy, faecal collection and method of calculation all have important implications for determination

of the digestible value of nutrient from any ingredients.

Digestion of feed in fish depends on a number of factors; the ingested feed, susceptibility to the effects of the digestive enzymes and time of exposure to the action of the digestive enzymes (4). These factors are affected by many secondary factors, such as fish species, age of the fish, fish size, physiological condition, and environmental condition such as water temperature, pH, and feed composition such as ingredients used, particle size and the quantity eaten (5).

Digestibility of nutrients in fish diets need to be studied because it is the digested feed, which is absorbed, that is made available to cellular metabolism. The resultant of which will be tissue synthesis, repair of worn-out tissues and various energy utilization channels (6). The most important characteristics of feedstuffs are the bioavailability of nutrients, hence reliable data on different ingredients for each species need to be well considered as a necessary prerequisite (7).

Various direct and indirect methods have been used to study the digestibility of *Hibiscus sabdariffa*, in this study the direct method used involves the use of a non-digestible exogenous marker/indicator (AIA) it is assumed that the amount of marker in the feed and faeces remains constant throughout the experimental period and that all of the ingested markers appear in the faeces. The digestibility of the nutrient in question can then be determined by assessing the difference between the feed and faecal concentrations of the marker and the nutrient (8).

The present study aimed to evaluate the apparent digestibility coefficient of nutrients present in *Hibiscus sabdariffa* in formulated feeds for *Oreochomis niloticus* and also the *in-vitro* assays of the raw and processed seed meals.

Materials and Methods

Experimental Site, Procurement and Processing of Feedstuffs

The study was conducted at the Aquaculture Unit, of the Skills Acquisition and Entrepreneurial Development Centre, National Agricultural Extension and Research Liaison Services, Ahmadu Bello University, Zaria. The area is located within the Northern Guinea Savannah Zone of Nigeria at latitude 11^{o} 09' 06'' N and longitude 7^{o} 38' 55'' E, at an altitude of 706m above sea level.

Groundnut cake, maize, soybean cake, bone meal were purchased from Labar feed mills Samaru, Palm oil, salt, maize bran, wheat offal were purchased from Samaru market in Zaria. Imported fish meal, vitamin and mineral premix were purchased from an agro- based shop (PIMO VENTURES) in Zaria. Roselle seeds were purchased from Sabon-Gari Market, Zaria. The ingredient composition of the experimental diet is shown in Table 1. Two and half kilogrammes (2.5kg) of raw roselle (RR) seeds were thoroughly cleansed, milled, and incorporated in the diets of *O. niloticus*. Another 2.5kg of the seeds were boiled for 30 minutes at 100^{o}C, the boiled roselle (BR) seeds were later sundried for 3 days and milled into powder using hammer mill. A third 2.5kg roselle seeds were boiled for 30 minutes at 100^{o}C washed and kept in an air tight container to ferment for three days. The fermented seeds were sundried for 3 days, then milled and incorporated into the experimental diet (FR). Lastly, the fourth 2.5kg of seeds were cleansed, washed and soaked in water for 24 hours and kept in kaka ban sack for three days to sprout. The sprouted roselle (SR) were dried for 72 hours grinded into powder and incorporated into the diets of *Oreochromis niloticus*.

A small portion of differently processed Roselle seed meals were taken to the laboratory and analysed for *In-vitro* digestibility. The remaining part were mixed

with other feed ingredients to formulate feed the feed were pelleted using a locally fabricated industrial pellet. The proximate composition of the ingredients used in experimental diets are shown in Table 2.

Determination of *In-vivo* Digestibility Coefficient

Digestibility of diets was determined using the indirect method which relies on use of an inert marker (Lovell, 1989). The marker concentrates in faeces relative to the digestible material. Digestibility was determined by the relative quantities of the marker in feed and faeces. In this study chromium (III) oxide (BDH 10234) was used as a marker at an inclusion level of 0.5%.

Acid insoluble ash (AIA) analysis

AIA analyses were carried out on the diets and faeces. AIA was obtained by adding 25 ml of 10% HCl to the weighed ash content of sample. This was covered with a water-glass and boiled gently over a low flame for five minutes. This was then filtered using ash less filters and washed with hot distilled water. The residue from the filter was returned to the crucible and ignited until it was carbon free after which it was weighed. Percentage AIA was calculated as;

$$\% AIA = \frac{weight\ of\ AIA}{weight\ of\ ash} \times 100$$

Determination of digestibility coefficient

This was calculated on the percentage of AIA in feed and in faeces and the percentage of nutrient on diets and feaces (5).

$$Apparent\ organic\ matter\ digestibility\ (\%) = 100 - 100\left(\frac{AIA\ in\ Diets}{AIA\ in\ faeces}\right)$$

$$Apparent\ digestibility\ (\%) = 100 - 100\left(\frac{AIA\ in\ Diets}{AIA\ in\ faeces}\right) \times \left(\frac{Nutrients\ in\ faeces}{Nutrient\ in\ diets}\right)$$

Determination of *In-vitro* Digestibility Coefficient

To determine *in-vitro* digestibility the modified procedure by (10) was used. The drop of pH of casein (control) and the samples after 20minutes hydrolysis by proteolytic enzymes was measured using an Orion research digital ionalyser/ 501 (USA). The enzymes used were trypsin type IX from porcine pancreas, chymotrypsin type II from bovine pancrease, peptidase type III from porcine intestine and protease type VI from Streptomyces griseus. All enzymes were purchased from (Sigma Chemical Company ST. Louis, MO USA) Percent *in-vitro* digestibility was calculated from the pH drop using the following equation:

% *in-vitro* digestibility= 234.84-22.56 (X)

Where X = the pH after 20 minutes hydrolysis

Experimental Design

This experiment was conducted to determine the apparent digestibility coefficient (ADC) of nutrients in *Hibiscus sabdariffa* seed meal. The feeding trial was conducted under laboratory condition in the aquaculture production unit of Skills Acquisition and Entrepreneurial Development Centre, National Agricultural Extension and Research Liaison Services (NAERLS). Fifteen plastic aquaria of 60cmx50cmx30cm dimension with 120L water holding capacity was used. Continuous aeration using whirl charging electro-magnetic aerator (model: ACO 3) were used. Ten fingerlings (Initial weight 0.60g) were stocked in each aquarium with three replicates. The fish were fed twice in a day with the compounded experimental diet (2mm) by 9:00am and 6:00pm.at a feeding rate of 3% of

the total body weight. The daily ration were adjusted every forth night after weighing the fish. The uneaten feed was siphoned off 6hrs after each feeding and oven dried at 100^0C for 24hrs to calculate the feed conversion ratio. The uneaten feeds remained almost intact due to the binder (CMC) the faecal samples released by the fish were siphoned from each aquarium by pipetting (11). The faecal samples were oven dried at 60^0C and analysed for digestibility estimation.

Growth and Nutrient Utilization Parameters Analysis

Weight gain (WG) = $w_2 - w_1$ (g)

Where w_2 = final mean weight (g)

w_1 = initial mean weight (g)

Percentage weight gain (PWG (%)) = $\frac{\text{MWG}_{(g)}}{\text{1WG}_{(g)}} \times 100$

Specific Growth Rate (SGR)

Specific growth rate will be calculated according to the method of Brown (1957) as:

$$\mathbf{SGR} = \frac{\log_e W_2 - \log_e w_1}{T_2 - T_1} \times 100$$

Where w_2 = weight of fish at time T_2 in days

w_1 = weight of fish at Time T_1 in days

Loge = Natural Log to base e

Daily Rate of Growth (DRG)

This will be calculated according to formula: -

$$\mathbf{DRG} = \frac{\text{Mean increase in weight per day}}{\text{Body weight of Fish}}$$

Average Daily Gain (ADG)

$$\mathbf{ADG} = \frac{\text{WTG (g)}}{\text{Experimental Period (d)}}$$

Food Conversion Ratio (FCR)

The food conversion ratio **(FCR)** is expressed as the proportion of dry food fed per unit live weight gain of fish (Reich, 1975)

$$\mathbf{FCR} = \frac{\text{Weight of dry feed fed(g)}}{\text{Live weight gain}}$$

Gross Food Conversion Efficiency (GFCE)

The gross food conversion efficiency will be calculated according to Stickney, 1979 as a percentage of the reciprocal of conversion ratio

$$\mathbf{GFCE} = \frac{1}{\text{FCR}} \times 100$$

Protein Intake

The protein intake will be calculated according to Harling and Wilson 1976 in the formula

PI = Feed intake (g) x % protein in the diet

Protein Efficiency Ratio (PER)

Protein efficiency ratio will be calculated using the method of Osborne and Mendel (1919) as:

$$\mathbf{PER} = \frac{\text{Gain in weight of test fish(g)}}{\text{Protein consumed(g)}}$$

The PER. expresses the measure of dietary protein utilization in by the fish.

Nitrogen Metabolism (NM)

This will be calculated using the method of Dabrowski (1977) as: -

$$\mathbf{NM} = \frac{(0.54)(b - a)h}{2}$$

Where a = initial weight of fish

b = final weight of fish

0.54 = Experimental constant

h = Experimental periods in days

Apparent Net Protein Utilization (ANPU) or Productive Protein Utilization (PPV)

The **ANPU** or **PPV** is expressed as a percentage of protein retained in the fish body to the total protein ingested and does not take into consideration the endogenous protein losses.

$$\mathbf{ANPU} \text{ or } \mathbf{PPV} = \frac{B - BO}{PI} \times 100$$

Where B = Final protein content of fish body

BO = Initial Protein content of Fish body.

Statistical Analysis

All determinations were conducted in triplicate and the means were subjected to

analysis of variance, where the ANOVA revealed a significant difference, Duncan multiple range test was used to compare differences among individual treatment means using SASS version 13.

Table 1: Nutrients Composition of the Formulated Diets

Ingredients (g/100g)	RRSM	FRSM	BRSM	SP. RSM	SRSM
Fishmeal	26.0	26.0	26.0	26.0	26.0
H. sabdariffa	26.0	26.0	26.0	26.0	26.0
Wheat offal	20.0	20.0	20.0	20.0	20.0
Maize	20.0	20.0	20.0	20.0	20.0
Vit./min.Premix	1.0	1.0	1.0	1.0	1.0
Lysine	0.2	0.2	0.2	0.2	0.2
Methionine	0.2	0.2	0.2	0.2	0.2
Oil	0.8	0.8	0.8	0.8	0.8
DCP	0.2	0.2	0.2	0.2	0.2
Salt	0.5	0.5	0.5	0.5	0.5
GNC	5.0	5.0	5.0	5.0	5.0
AIA	0.1	0.1	0.1	0.1	0.1

RRSM – Raw roselle seed meal
FRSM – Fermented roselle seed meal
BRSM – Boiled roselle seed meal
SP.RSM – Sprouted roselle seed meal
SRSM – Soaked roselle seed meal
DCP – Di calcium phosphate
GNC – Groundnut cake
AIA-Acid Insoluble Ash

Table 2: Proximate Composition of the Feedstuff used in formulating Experimental Diets (%)

INGREDIENTS	CP	CF	CL	Ash	NFE
Fishmeal	72.23	0.10	8.50	10.00	3.96
Roselle seed meal	39.95	6.44	20.65	6.20	29.20
Wheat offal	16.34	12.34	1.69	6.58	22.50
Maize	10.80	3.50	3.60	8.40	64.10
GNC	43.50	6.15	5.36	6.10	31.00

CP—Crude Protein
CF—Crude Fibre
CL—Crude Lipid
NFE—Nitrogen Free Extract
GNC—Groundnut cake

Results and Discussion

The apparent protein digestibility coefficient in this study for all the experimental diets were 79.57, 81.43, 86.40, 85.74 and 88.20 for raw, soaked, boiled, sprouted and fermented Roselle respectively as showed in Table 3: The protein digestibility values for protein rich feed ingredients are

usually in the range of 75-95%. For example, the protein digestibility value of sunflower cakes was in the range of 86-89% and wheat bran 75% (12). The digestibility values recorded in this study (ranged from 79.57 to 88.20) which agrees with the findings of (13) who worked on defatted soybean meal. Similarly, (14) reported values that ranged from 70-89% in *O. niloticus* fed cottonseed meal. (27), observed the apparent protein digestibility of Jack bean meal (*Cannavalia ensiformis)* for *O. niloticus.* The results of the apparent protein digestibility for the seed meal was similar to that reported in (7), for the same seed meal fed to Nile Tilapia but lower than the values reported by (15), who fed the same seed meal to *O. mosambicus.* The little variation could be attributed to variability of nutrients as well as differences in nutrients processing, experimental methodology or differences in species (2).

Table 3: *In- vivo* apparent digestibility of *O.niloticus* fed processed Roselle seed meals

Parameters (%)	RR	SR	BR	SP.R	FR
Crude Protein	79.57^{e}	81.43^{d}	86.40^{b}	85.74^{c}	88.20^{a}
Crude Lipid	49.75^{e}	50.84^{d}	57.80^{b}	56.17^{c}	57.90^{a}
Crude Fibre	12.84^{a}	8.73^{d}	12.43^{b}	9.16^{c}	7.80^{e}
Ash	21.13^{a}	18.00^{b}	16.65^{c}	10.14^{d}	6.80^{e}
Dry Matter	85.50^{e}	86.00^{d}	86.19^{c}	87.39^{b}	91.91^{a}

Means on the same row with the same superscripts were not significantly different (p>0.05)
RR---Raw Roselle
SR---Soaked Roselle
BR---Boiled Roselle
SP.R-Sprouted Roselle
FR---Fermented Roselle

The results of dry matter digestibility for *H. sabdariffa* seed meals in this study was generally high (85.5-91.91). The higher digestibility values obtained in this study agreed with those reported by (16) who also obtained a higher value for digestibility coefficient of the same oil seed. Other workers however recorded a lower dry mater apparent digestibility coefficient of soybean for red drum (*Sciaenops ocellatus)* (18). Higher apparent digestibility co-efficient for dry matter (ADC $_{DM}$) were reported for Nile Tilapia (*O. niloticus)* fed pelleted diets (19). Similar higher values were reported for fishmeal in rainbow trout (20) and Piavucu (*Leporinus macrocephalus*) (21). The dry matter apparent digestibility coefficient estimates the amounts of solids waste released to the environment and could be used to rate the gross environmental impact in aquaculture production. Fish use around 80 percent of dietary dry matter (ADC $_{DM}$) which describes how efficiently the feeds or feed ingredients are digested, and how much of their nutrient contents can be made available to fish for maintenance and growth. In addition, (ADC $_{DM}$) generally provides a better estimate of the quantity of indigestible material in the feeds or feed ingredients, rather than that of the individual nutrient. Several studies have reported the correlation between dry matter and gross energy (GE) digestibility (20). This effect could already be related to the lipid content of feedstuff since in this study the

ingredients, which presented the highest protein digestibility values had the highest lipid contents. The high lipid digestibility by *O. niloticus* was found to be in line with what was reported by (22) for rainbow trout. A range of 76 to 97% fat digestibility of various sources of fat has been reported for channel catfish (9). Andrew *et al.* (23) reported that the ability to digest fat is apparently influenced by temperature and the level of fat in the diet. The result for the crude indicates no significant difference (P>0.05) occurred for crude fibre digestibility of *O. niloticus* fed *H. sabdariffa* seed meal at different processing techniques except for the fish fed with diets prepared with soaked (7.73) and sprouted Roselle (9.16). The crude fibre digestibility coefficient in this study was higher than that of *O. niloticus* in another study by (7). This might be attributed to the natural feeding habit of tilapia that consists mainly of plant material (24). *O. aureus* was found to digest highly fibrous feedstuffs such as Alfalfa meal (25). Fermented (13.80) and boiling (12.43) processing methods improved the digestibility of crude fibre in this study. This finding conforms to the report of (26) that fibre digestibility of *O. niloticus* fed Lima bean diet was improved with toasting and autoclaving.

Table 4: *In-vitro* Apparent Digestibility of Raw and Processed Roselle Seed Meals

Parameters	Casein	Raw Roselle	Soaked Roselle	Boiled Roselle	Sprouted Roselle	Fermented Roselle
CP	95.00^{a}	70.00^{f}	75.00^{e}	85.00^{c}	80.50^{d}	90.50^{b}
CL	97.00^{a}	80.00^{f}	90.00^{d}	90.00^{c}	80.50^{e}	95.90^{b}
GE	92.00^{a}	70.50^{f}	75.00^{e}	85.50^{c}	75.50^{d}	85.90^{b}
P	95.00^{a}	64.00^{c}	60.50^{d}	60.50^{d}	60.00^{e}	64.30^{b}
DE	97.00^{a}	14.50^{f}	14.90^{e}	16.00^{b}	15.00^{d}	15.50^{c}
DM	95.00^{a}	60.00^{e}	65.50^{d}	70.00^{c}	70.50^{b}	70.50^{b}

Means on the same row with the same superscripts were not significantly different (p>0.05)
CP-Crude protein
CL-Crude lipid
GE-Gross energy
P-Phosphorus
DE-Digestible energy
DM-Dry matter

The *in-vitro* protein digestibility values for *H. sabdariffa* seed meals were given at 70.00%, 75.00%, 85.00%, 80.00% and 90.50) for raw, soaked, boiled, sprouted, and fermented roselle respectively. Although these values were lower than that of Casein which was the controlled, it is however equal to or higher than most legumes and other oil seeds. This agrees with the work of (27), and (28), who reported *in-vitro* digestibility of *H. sabdariffa* defatted flour as 76.0%. Maina *et al.* (12) reported *in-vitro* apparent digestibility of 76.32, to 88.45 for soy defatted flour soy-protein concentrate and (29) also reported the *in-vitro* soybean meal was 85.8% for cotton seed meal was 77.6% and for protein in sesame seed meal.

Table 5: Growth performance and Nutrient Utilization of *O. niloticus* fed raw and processed roselle seed meals.

Parameters	TREATMENTS				
	RR	SR	BR	S.PR	FR
Initial weight of fish (g)	0.60	0.60	0.60	0.60	0.60
Final weight of fish(g)	349.78^{e}	350.28^{d}	360.24^{b}	359.34^{c}	369.71^{a}
Mean weight gain (g)	349.18^{e}	349.68^{d}	359.64^{b}	358.74^{c}	369.11^{a}
DRG(g)	12.82^{c}	12.00^{e}	13.00^{b}	12.40^{d}	13.50^{a}
SGR(g)	0.26^{d}	0.28^{c}	0.29^{b}	0.28^{c}	0.30^{a}
FCR(g)	2.00^{a}	1.98^{b}	1.70^{d}	1.90^{c}	1.50^{e}
GFCE (%)	50.00^{d}	50.51^{d}	58.82^{b}	52.63^{c}	66.67^{a}
PPV	75.55^{b}	79.01^{c}	84.35^{b}	80.00^{c}	89.02^{a}
SR. (%)	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}

DRG—Daily Rate of Growth
SGR---Specific Growth Rate
FCR---Feed Conversion Ratio
GFCE—Growth Feed Conversion Efficiency
PPV---Productive Protein Value

The result for the growth performance and nutrient utilization is showed in Table 5. The results revealed that *O. niloticus* fed fermented Roselle seed meal were superior to the fish fed raw and other methods of processing. The fermented roselle had a mean weight gain of 369.11g, followed by boiled Roselle (360.24) then sprouted Roselle (359.34), soaked Roselle (350.28) and lastly the raw Roselle (349.18). A qualitative comparison of the processing techniques showed nutritional superiority of fermented Roselle seeds on the other methods. This was due to the fermentation process which is recognized as converter of food compound into structurally related but financially more viable food through the activities of microbial cells (30). This finding is supported by various reports on the effect of different fermentation methods on the health and growth responses of broiler chickens (31). The synergistic effect of beneficial fermentation microbes and host micro-organisms must have led to reduction in the content of pathogenic bacteria and increased the population of useful micro flora in the gut, resulting to improvement in the gastro-intestinal health and performance of broilers as reported by (32 and 30). Other factors that may be responsible could be the positive effects of fermentation originating from enzymes of the seed itself (30).

Conclusion and Applications

It can be concluded that

1. The different processing methods had significant variation (P<0.05%) in the apparent nutrients digestibility of *Hibiscus sabdariffa* seed meal fed to *Oreochromis niloticus* fingerlings.
2. In both the *in-vivo* and the *in-vitro* apparent digestibility coefficient the fermentation process gave the best digestibility coefficient.
3. Fermentation gave the best growth performance of *O. niloticus*

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