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Bio-Composition of Time-Based Effects of Fish Feed Bioformulated with Unfermented Agricultural Wastes

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ABSTRACT

Bioformulated feeds from agricultural wastes were studied to determine the percentage bio-composition and nutritional value of crude protein, maize and wheat shaft, crude fibre, moisture, and carbohydrate levels in formulated feed. Time-based effects were conducted to determine the material content of these feed materials. Results showed a significant difference ($P \geq 0.05$) in protein, moisture, and fibre, all in line with global nutritional requirements. The value highlights the potential inherent in biotechnology usage in feed formulation. Carbohydrate content ranged from 14.58 to 40.58%; crude protein ranged from 40.70% to 47.03%; moisture ranged from 10.20% to 12.74%; ash content ranged from 9.56& to 9.07%; crude fibre ranged from 10.75% to 12.75%; fat ranged from 11.50% to 11.50%. These values show a balanced nutritional profile to ensure stability and potential for nurturing fermented agro-waste not only as essential components in animal feed, but as an alternative to known imported feeds which are scarcely affordable.

Key words: Fermented wastes, Agricultural waste, Time-based effect, Bioformulated feeds, Bio-composition.

INTRODUCTION

Efficient management of agricultural wastes from soybean, maize, and bambara nut will lead to sustainable agricultural systems whereby waste is transferred into valuable resources (Chivenge *et al.* 2017). This assertion was explained by Ogundipe *et al.* (2018), who showed that unfermented wastes from agricultural products could serve as organic fertilizer to improve soil health and crop yield. Closely related, Nzayisenga *et al.* (2020) and Tiamiyu *et al.* (2018) explained that animal feed, when incorporated with products from unfermented agricultural products, can help mitigate feed shortage and reduce feed costs in livestock production. Also, Akinfemi *et al.* (2017) explained that Agricultural waste from maize, bambara-nut, and soya beans is rich in nutritional value and is common in all our local communities. They are also of high quality and nutritional value, noting the high protein nature of the soya bean and carbohydrate value of maize. In all, Kumar *et al.* (2016) showed that simplified composition will ensure balanced ratios promote growth and productivity. This research proposes a systematic of feed formulation using agricultural by-products for increased production, as a means of inward invention as an alternative to imported feeds, which are beyond the purchase of the average farmer.

MATERIALS AND METHODS

Collection, processing, and formulation of feed

The basic agricultural materials used in this research are: maize, soya bean, wheat, and bone meal. These were collected from Ose Market in Onitsha. Feed Formulation was conducted locally at Dennis Osadebay University Feed Mill in Asaba. Various feed materials, including white maize, soya bean, bambara nut meal, groundnut cake, palm oil, starch, salt, and

vitamin premix, at five different rations – 0%,25%, 50%, 75%, and 100% – were used for the experimental diet before taking them to the feed mill for processing via grinding, mixing, pelleting, and drying

Table 1: percentage composition of experimental diet.

S/N		0% Control	25%	50%	75%	100%
1.	Groundnut cake	31.00	30.50	20.00	13.00	10.50
2.	Fish Meal	30.00	30.50	20.50	11.50	6.50
3.	Bambara millet waste	12.00	5.70	6.50	11.75	10.50
4.	Soybean waste	8.00	6.50	12.50	20.70	18.00
5.	Maize waste	6.00	6.30	10.50	19.00	20.00
6.	Vitamin C / Primix	20.0	1.50	2.50	0.50	2.50
7.	Blood meat	20.0	1.50	1.50	1.00	1.00
8.	Lysine	1.00	2.50	1.00	2.00	1.50
9.	Vegetable oil	1.50	1.50	1.50	1.55	4.00
10.	Starch	1.00	5.50	3.00	2.50	5.00
11.	Salt	0.50	0.50	2.50	0.50	3.00
12.	Wheat/Oak	4.00	7.50	18.00	16.00	18.50
13.	Total	100.00	100.00	100.00	100.00	100.00

Analysis
The material contents of crude protein, fibre content, carbohydrate, lipids, and moisture were determined using the Association of Official Analytical Chemists (AOAC) (2015).

Moisture Content
A petri dish was washed and dried in the oven. Exactly 2g of each sample was weighed into the petri dish. The weight of the petri dish and sample was noted before drying. The petri-dish and sample were put in the oven and heated at 100 °C for 1 hour. The result was noted, and then heated for another 1 hour until a steady result was obtained, and the weight was noted. The drying procedure was continued until a constant weight was obtained.

Calculation
% Moisture content = $W_1 - W_2 \times 100$
Where:
W_1 = weight of petri dish and sample before drying
W_2 = weight of petri dish

Ash Content
An empty platinum crucible was washed, dried, and the weight was noted. Exactly 2 g of wet sample was weighed into the platinum crucible and placed in a muffle furnace at 500 °C for 3 hours. The sample was cooled in desiccators after burning and weighed.

Calculation
% Ash content = $\frac{W3-W1}{W2-W1} \; X \; \frac{100}{1}$
Where:
W_1 = weight of empty platinum crucible
W_2 = weight of platinum crucible and sample before burning
W_3 = weight of platinum crucible and ash

Fibre Content
De-fat about 2 g of material with petroleum ether (if the fat content is more than 10%), boil under reflux for 30 minutes with 100 ml of a solution containing 1.25% of H_2SO_4 per 100 ml of solution. Filter the solution through linen or several layers of cheesecloth on a fluted funnel, wash with boiling water until the washings are no longer acidic. Transfer the residue to a

beaker and boil for 30 minutes with 100 ml of a solution containing 1.25 NaOH per 100 ml. Filter the final residue through a thin but close pad of washed and ignited asbestos in a Gooch crucible. Then, dried in electric oven and weighed. It was later, after cooling, then weighed again.

Calculation

The loss in weight after incineration × 100 is the percentage of crude fiber.

%Crude fiber = $\frac{weight\ of\ Fibre}{Weight\ of\ sample}\ x\ 100$

Protein Content

Exactly 0.5 g of sample was weighed into a 30 mL Kjeldahl flask (gently to prevent the sample from touching the walls of the flask, and then the flask was stoppered and shaken. Then 0.5 g of the Kjeldahl catalyst mixture was added. The mixture was heated cautiously in a digestion rack under fire until a clear solution appeared. The clear solution was then allowed to stand for 30 minutes and allowed to cool. After cooling, about 100 mL of distilled water was added to avoid caking, and then 50 mL was transferred to the Kjeldahl distillation apparatus. A 100 mL receiver flask containing 5 mL of 2% boric acid and an indicator mixture containing 5 drops of bromocresol blue and 1 drop of methylene blue is placed under a condenser of the distillation apparatus so that the tap was about 20 cm inside the solution. The 5 mL of 40% sodium hydroxide was added to the digested sample in the apparatus, and distillation commenced immediately until 50 drops entered the receiver flask, after which it was titrated to pink colour using 0.01 N hydrochloric acid.

Calculations

% Nitrogen = $\frac{Titre\ Value\ x\ 0.01\ x\ 14\ x\ 100}{Weight\ of\ Sample}$

% Protein = %Nitrogen x 6.25

Fat Content

Dry 250 mL clean flasks in an oven at 105 to 110 °C for about 30 minutes. Transfer into a desiccator and allow to cool. Fill the flasks with about 300 mL of petroleum ether (boiling point 60 °C), plug the extraction thimble lightly with cotton wool, assemble the Soxhlet apparatus, and allow it to reflux for about 6 hours. Remove the thimble with care and collect petroleum ether in the top container of the set-up and drain into a container for re-use. When the flask was almost free of petroleum ether, remove and dry at 105 to 110 °C for 1 hour. Transfer from the oven into a desiccator and allow to cool and then weigh.

Calculations

% Fat = $\frac{Weight\ of\ fat}{Weight\ of\ Sample}\ X\ 100$

Carbohydrate Content

Carbohydrate content was determined according to the standard method of AOAC (2015).

%Carbohydrate = 100 - (%Protein + %Moisture + %Ash + %Fat + %Fibre)

Statistical Analysis

Data obtained were subjected to analysis of variance (ANOVA), and the treatment means were separated from significance differences following the procedure of Duncan's Multiple Range Test (Steel and Torrie, 1980). All the analyses were carried out using the computer software Statistical Package for the Social Sciences Version 20 for Windows (SPSS, 2013)

RESULTS

Table 2 results show a significant difference in moisture content. The highest value was recorded in 100% (11.47), followed by 75% (10.85), 50% (10.83), 25% (10.77), and the control (8.47). For ash content, there was a significant difference ($P < 0.05$) among 25%, 50%, and 100%, but no significant difference between the control, 75%, and 100%. For crude

fiber, no significant difference was observed among 50%, 75%, and 100%, but a significant difference was noted between the control and 25%. For fat content, a significant difference was observed among the control, 25%, and 50%, while no significant difference was found between 75% and 100%. Lastly, for carbohydrates, there was no significant difference between the control and 75%, but a significant difference was observed among 25%, 50%, and 100%.

Table 2. Food Composition of Formulated Feeds

Parameters	0%	25%(UF)	50%(UF)	75%(UF)	100%(UF)	P-value
Moisture	8.47±0.3^{b}	10.77±0.28^{b}	10.83±0.04^{b}	10.85±0.03^{b}	11.47±0.03^{a}	0.05
Ash	9.86 ± 0.06^{a}	8.81 ± 0.03^{c}	9.04± 0.04^{bc}	9.41 ± 0.11^{b}	9.02±0.05b^{c}	0.05
Crude Protein	47.10±0.15^{a}	33.49±0.04^{d}	34.59±0.02^{c}	35.41±0.12^{b}	40.71±0.07^{b}	0.05
Crude Fibre	10.75±0.12^{c}	7.26 ± 0.22^{d}	11.67± 0.17^{b}	11.83± 0.05^{b}	12.35± 0.06^{a}	0.05
Fat	11.35±0.36^{c}	9.39 ± 0.08^{d}	13.74± 0.21^{b}	14.45± 0.07^{a}	11.50± 0.02^{c}	0.05
Carbohydrate	14.50±0.13^{d}	30.25±0.39^{c}	40.21±0.16^{a}	18.15±0.15^{b}	40.58±0.06^{a}	0.05

DISCUSSION

Food material composition

Moisture content: Table 2 shows that the composition of fish fed with formulated unfermented soybean, maize, and bambara nut waste changes as the level of unfermented waste increases. The moisture content progressively rises from 8.47% in the control to 11.74% in the 100UF(unfermented) treatment. Also, ash content increases slightly as a result of mineral composition; more unfermented waste is being added, with values higher than those reported by Fasaki *et al*. (2015) for bambara nut. However, the crude protein content decreases significantly, with the control group having the highest protein level. This contradicts the report of Anhwange *et al*. (2006), in line with Olanipekun et.al. (2012), who stated that legumes typically have protein contents between 17% and 30%. There is also a noticeable increase in crude fiber content as the unfermented waste levels rise, with the (unfermented) treatment having the highest fiber content. Fat content shows a significant rise, with the highest value in the (unfermented) treatment. On the other hand, carbohydrate content declines with increasing levels of unfermented waste. The control group has the highest carbohydrate content, while the (unfermented) treatment shows a marked reduction.

Moisture content of the samples ranges from 8.47 to 11.47%. This is in line with Jipara *et al.* 2021, who reported moisture content in feed ranging between 8.20 and 10.00%. The moisture content observed in this study falls within the desirable range, as Joshi *et al.* (2021) reported that higher moisture levels promote mold growth and feed decomposition, while lower moisture levels enhance the shelf life of fish feed. Among the samples, the (unfermented) treatment had the highest moisture content at 11.74%, whereas the control exhibited the lowest moisture content at 7.40%. The (unfermented) treatment saw a significant increase in moisture (7.40%) compared to the control, while 50% and 75% showed minimal differences (10.77% and 10.83%). A significant difference was observed between the control and 100UF% treatments, while no significant differences were found between the 25UF%, 50UF%, and 75UF% treatments.

Ash Content: Ash content ranges from 9.86% to 9.02%, indicating the mineral composition in the fish feed, as t reported by Solomon and Oyategbe (2016), between 6% and 9%. It is known that these minerals contribute essential nutrients and assist in the breakdown of fats, proteins, and carbohydrates. There was a significant difference in ash content between the 25UF, 50%UF, and 100%UF treatments, indicating substantial ash contents in these treatments. However, no significant difference was found between the control and 75UF treatments.

Crude Protein: Protein is an essential primary growth-promoting factor in fish feed. The protein requirement of fish is influenced by various factors: fish size, water temperature, feeding rate, the availability and quality of natural foods, and the overall digestibility of the diet. As shown in Table II, the crude protein content in the feed ranges from 40.71 to 47.10%. The control treatment exhibits the highest crude protein content at 47.10%, while the 25UF% (unfermented) treatment has the lowest at 33.49%. This is probably due to the use of lower-quality inputs for protein sources. For instance, bambara nut and soybean are legumes, whereas maize is a cereal. According to Anhwange and Atoo (2016), Bambara nut has a protein content of 18%, while soybean contains 40%. When combined with maize, which has a crude protein content of 8-11%, the overall protein content of the formulated feed can range from 30% to 48%. The results from this study indicate that the formulated feed using unfermented agricultural waste has a relatively low protein content when included in fish meal. This is consistent with the findings of Fasakin *et al.* (2015), who reported that *Clarias gariepinus* requires 40-45% crude protein in its diet for optimal growth.

Crude fibre: The crude fiber content, as shown in Table 1, ranged from 10.75% to 12.55%. The treatment with the highest crude fiber is 75UF (unfermented) at 11.83%, while the lowest crude fiber content was recorded in 25UF (unfermented) at 7.26%. However, no significant difference was observed between the 50%UF, 75%UF, and 100%UF.In fish feed, the recommended crude fiber content is between 3-6% (Food and Agriculture Organization (FAO 2018, 2019). The values obtained in this study are relatively high; increased fiber content could aid digestion, but it should be moderated, as fish have a limited capacity to digest fibrous material (Olaniyi and Onyenuga, 2013; Oyekale *et al.*, 2012).

Fats: The results presented in Table 1 show that the fat content ranges from 11.53% to 11.50%, with the highest at 0%UF (unfermented) at 14.56% and the lowest value observed at 25%UF (unfermented) at 9.36%. Joshi *et al.* (2021) reported that lipids/fats generally make up 10-15% of the feed, which aligns with the findings of this study.

Carbohydrates: The carbohydrate content in the table ranges from 11.50% to 40.58%, with the highest level observed in the 100%UF (unfermented) treatment (40.58%) and the lowest in the 75% UF (unfermented) treatment (18.15%). These results indicate that the carbohydrate levels in the feed are within the desirable range, consistent with the findings of Joshi *et al.* (2021), who reported that carbohydrates typically make up 20-30% of commercial fish feed. There was a significant difference in carbohydrate content between the control and 75%UF treatments, while the 25%UF, 50%UF, and 100% UF treatments did not show significant differences (Table II).

CONCLUSION AND RECOMMENDATIONS

The analysis of fish feed formulated using unfermented waste from soybean, maize, and bambara nut showed that agricultural by-products can be effectively utilized as sustainable and cost-efficient feed sources for aquaculture, thereby reducing feed cost. The waste materials provided a considerable amount of crude protein, fats, carbohydrates, and minerals, which are essential for the growth and health of fish. The feed also showed a low

moisture content, enhancing its shelf stability and reducing the risk of spoilage.
It is recommended that fish feed production in Nigeria should be encouraged to adopt this fish formulation method, given its cost-effectiveness, accessibility, and nutritional balance. This will contribute to enhancing fish growth and production while reducing feed cost, and it will also help to ensure a balanced diet for different fish species and improve overall fish health and performance.

Government agencies, agricultural organizations, and aquaculture stakeholders should offer both financial and technical support through these simple methods to promote the adoption of time-based feed production to contribute to sustainable aquaculture practices and effective waste management in our local communities.

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