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NUTRITIONAL COMPOSITION OF TOASTED SOYBEAN, BLANCHED AND SUN-DRIED DUCKWEED MEALS USED AS PROTEIN SOURCES

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ABSTRACT

This study was conducted to compare the nutritive values and antinutrient contents of plant protein sources such as toasted soybean meal (TSBM), blanched duckweed meal (BDM) and sun-dried duckweed meal (SDM) with the requirement for Nile tilapia feeding. Determination of all samples was done in triplicate. TSBM had significantly higher ($P \leq 0.05$) protein content (38.00%) while raw duckweed meal (RDM) had the least (35.08%). The total essential amino acid ranged from 32.77 to 41.11 g/16gN of crude protein and the total essential amino acid to crude protein content ratio ranged from 102.00 to 128.00 g/16gN. There was a significant difference ($P \leq 0.05$) in the macro mineral concentrations of all the samples except in magnesium which was not significantly different ($P > 0.05$) in all the treatments. BDM gave the best reduction of antinutrient content by 95.26%, 92.18%, 61.47%, 33.33% and 12.50% for tannin, saponin, alkaloid, oxalate and phytate, respectively. All the nutrients in TSBM, BDM and SDM are sufficient as regards the nutrient requirement for species like Nile tilapia Oreochromis niloticus except for methionine which was the only limiting amino acid. Therefore, should these feedstuffs be considered for inclusion in the diet of such species, DL-methionine supplementation would be required to maximise production characteristics.

Keywords: Nutrients requirement, Nile tilapia feed, total essential amino acid

INTRODUCTION

The optimal growth of fish requires different feed composition in its formulations; this includes protein (amino acids) sources, energy sources, lipids, minerals and vitamins. To achieve fast growth in fish, the requirement for the development of new body tissues must be satisfied (Abushweka, 2018). Thus, feed must supply not only protein and lipids to build new tissue, but also energy which is important for protein synthesis. The total requirement of protein and energy for growing fish differ for different species and are the sum of demands for growth and maintenance (Abushweka, 2018). Many conventional and unconventional feedstuffs have been used in animal nutrition following different processing method to improve their utilization in the diet of the fish.

An example of such unconventional feedstuff is duckweeds, a monocotyledonous, free-floating aquatic plants that can be easily cultured. They are one of the world's simplest and smallest flowering plants that grow well in static and nutrient-rich freshwater or a brackish water environment (Abdullahi *et al*., 2023). The biomass of duckweed doubles in 2 to 3 days under ideal conditions of nutrient availability, sunlight, pH (6.5-7.5), and temperature (20 °C to 30 °C) (Christine *et al*., 2018). There are about 40 species of duckweed plant species worldwide and the major ones are of four (4) main genera namely, *Lemna*, *Spirodela*, *Wolfilla* and *Wolffiella* (Dorothy *et al*., 2018). The plant consists of a combination of leaves and stems, a little more than two, poorly differentiated fronds (Abdullahi *et al*., 2022). The tissue is composed principally of chlorenchymatous cells, separated by large intercellular spaces that provide buoyancy (Abdullahi *et al*., 2022). The upper epidermis is cutinized and sheds water. An important feature of their structure is the almost total lack of woody tissue.

Plant proteins are considered to be economically vital to fish feed production because of their relative abundance and low cost (Maundu, 2020). However, they must possess high protein levels, good palatability, low fibre and reduced antinutritional factors (Maundu, 2020). This means plant protein sources must provide feeds that are capable of producing high-quality fish flesh with minimum deterioration of the environment. According to Tantikitti (2014), plant protein feedstuffs possess several challenges which range from antinutrients to inadequate amino acid contents and poor palatability. Thus, the objective of this research was to compare the nutritive values and antinutrient contents of conventional plant protein i.e., toasted soybean meal to processed unconventional protein sources i.e., blanched duckweed meal and sun-dried duckweed meal with specific reference to the nutritional requirement for Nile tilapia *Oreochromis niloticus*.

MATERIALS AND METHODS

Sample Collection and Treatment

Fresh duckweed (*Leman paucicostata*) was collected from a burrow pit at Hanwa Low-cost, Kwangila, Zaria, Kaduna State, with a hand net and conveyed in nylon bags. The collected duckweed was cultured in a fertilized concrete pond for two months before it was processed. While soybean was obtained from Sabon - Gari market, Zaria and packaged in a polythene bag.

Blanching and sun-drying methods were used to process the cultured duckweed samples while toasting was used to process the soybean. Blanching was done by boiling duckweed in water for 5 minutes at 100°C as described by Akpodiete and Okagbare (1999). The blanched duckweed was oven dried and milled into a fine powder and sieved through a 0.5 mm mesh screen. The second treatment involved sun drying duckweed for three (3) days. After which the sun-dried sample was then milled into a fine powder and sieved through a 0.5 mm mesh screen. All the samples were analyzed for proximate composition, amino acid profile, mineral and antinutrient contents in triplicate.

Determination of Proximate Composition

The proximate composition of the raw, blanched duckweed meal, sun-dried duckweed meal and toasted soybean meal was determined using the methods of the Association of Official Analytical Chemists (A.O.A.C., 2019). Parameters determined includes moisture, crude protein, fat, fibre and ash.

Gross Energy Values

The gross energy values in kilo-calories of the leaf samples and feed were calculated as described by Pauzenga (1985).

GE = (Crude protein x 37) + (ether extract x 81.8) + (nitrogen-free extract x 35) Kcal.

Determination of Amino Acids Contents

The amino acid contents of the raw, blanched duckweed meal, sun-dried duckweed meal and toasted soybean meal were determined using methods described by Benitez (1989). The known sample was dried to weight, defatted, hydrolysed, evaporated in a rotary evaporator and loaded into the Technicon Sequential Multi-Sample Amino Acid Analyzer (TSM). The TSM Analyzer is designed to separate and analyze free acidic, neutral and basic amino acids of the hydrolysate. The amino acid values was then calculated from the Chromatogram Peaks gotten.

Total Essential Amino Acid to Crude Protein Content Ratio (TEAA: CP) %

$$\text{TEAA: CP} = \frac{\text{Total essential amino acid of the sample}}{\text{Crude protein of the diet}} \times 100$$ (Sogbesan, 2014)

Mineral Determination

Mineral contents of the raw, blanched duckweed meal, sun-dried duckweed meal and toasted soybean meal were determined by atomic absorption spectrometry, flame photometry and spectrophotometry according to the methods of AOAC (2019).

Determination of Antinutrients

Antinutritional contents of the raw, blanched duckweed meal, sun-dried duckweed meal and toasted soybean meal were determined by standard methods as described by AOAC (2019).

RESULTS

The result of the proximate analysis of raw, blanched, sun-dried duckweed meal and toasted soybean meal is presented in Table 1. Toasted soybean meal had significantly highest ($P \leq 0.05$) protein content of 38.00% while raw duckweed meal recorded significantly least crude protein content of 35.08%. There was no significant difference ($P > 0.05$) in the ether extract the content of the raw duckweed, blanched duckweed meal and the toasted soybean meal but differed significantly ($P > 0.05$) with sun-dried duckweed meal.

Table 1: Proximate composition of raw, blanched, sun-dried duckweed meals and toasted soybean meal

Parameters (g/100g)	RDM	BDM	SDM	TSBM
Moisture	10.23 ± 0.63^{a}	8.52 ± 0.63^{b}	9.10 ± 0.63^{ab}	10.00 ± 0.63^{a}
Crude protein	35.08 ± 0.62^{b}	37.13 ± 0.62^{ab}	36.75 ± 0.62^{b}	38.00 ± 0.62^{a}

Crude fibre	4.72±0.62[ab]	3.34±0.62[b]	3.62±0.62[b]	5.00±0.62[a]
Ether extract	6.20±0.59[a]	6.07±0.59[a]	5.90±0.59[ab]	6.60±0.59[a]
Ash	18.18±0.60[b]	21.90±0.60[a]	20.48±0.60[a]	18.00±0.60[b]
Nitrogen free extract	25.60±0.63[a]	23.04±0.62[b]	24.15±0.62[ab]	22.4±0.62[c]
Gross energy (Kcal)	2701.12±33.57[a]	2678.22±33.57[a]	2687.62±33.57[a]	2433.88±33.57[b]

Means with the same superscript across the same row were not significantly different (P>0.05)
Keys: RDM = Raw duckweed meal; BDM = Blanched duckweed meal; SDM = Sun-dried duckweed meal; TSBM = Toasted soybean meal

The amino acid composition of raw, blanched, sun-dried duckweed meal, toasted soybean meal and amino acid requirement of Nile tilapia as presented in Table 2 showed that there was a significant reduction (P≤0.05) in all the essential amino acid values for the processed duckweed meal and toasted soybean meal when compared to the raw duckweed meal. The total essential amino acid ranged from 32.77 to 41.11 g/16gN of crude protein and the total essential amino acid to crude protein content ratio ranged from 102.00 to 128.00 g/16gN.

Table 2: Essential amino acid composition of raw, blanched, sun-dried duckweed meal, toasted soybean meal and EAA requirement of Nile tilapia

Amino acid (g/16gN)	RDM	BDM	SDM	TSBM	EAA requirement of Nile tilapia[1]
Lysine	5.51±0.54[a]	5.46±0.54[a]	5.71±0.54[a]	4.66±0.54[b]	5.12
Histidine	2.27±0.54[a]	2.16±±0.54[b]	2.04±0.54[b]	1.94±±0.54[c]	1.72
Arginine	4.51±0.45[a]	4.22±0.45[c]	4.39±0.45[b]	4.64±0.45[a]	4.20
Valine	5.14±0.61[a]	4.97±0.61[b]	5.09±0.61[b]	3.58±0.61[c]	2.80
Methionine	1.39±0.42[a]	1.23±0.42[b]	1.38±0.42[a]	1.03±0.42[c]	2.68
Isoleucine	4.32±0.43[a]	3.91±0.43[c]	4.23±0.43[b]	3.49±0.43[c]	3.11
Leucine	7.68±0.45[a]	6.14±0.45[b]	6.71±0.45[b]	5.65±±0.45[c]	3.39
Threonine	4.22±0.76[a]	3.84±0.76[b]	4.17±0.76[a]	2.93±0.76[c]	3.75
Tryptophan	1.24±0.49[a]	1.16±0.49[b]	1.19±0.49[b]	1.01±0.49[c]	1.00
Phenylalanine	4.83±0.74[a]	4.53±0.74[b]	4.48±0.74[b]	3.84±0.74[c]	3.76
TEAA	41.11±0.14[a]	37.62±0.14[c]	39.39±0.14[b]	32.77±0.14[d]	-
TEAA:CP (%)	128.00±0.11[a]	117.00±0.11[c]	123.00±0.11[b]	102.00±0.11[d]	-

1 – Source: FAO (2022). Note: Means with the same superscript across the same row were not significantly different (P>0.05)
Keys: RDM = Raw duckweed meal; BDM = Blanched duckweed meal; SDM = Sun-dried duckweed meal; TSBM = Toasted soybean meal; EAA = Essential amino acid; TEAA = Total essential amino acid**;** TEAA: CP = Total essential amino acid to crude protein content ratio

The macro and micro mineral compositions of raw, blanched, sun-dried duckweed meal and toasted soybean meal are presented in Table 3. Among all the macro minerals determined potassium (K) had the highest concentrations in all the samples. There was a significant difference (P≤0.05) in the macro mineral concentrations of all the samples except in magnesium which was not significantly different (P>0.05) in all the treatments.

Table 3: Macro and micro mineral composition of raw, blanched, sun-dried duckweed meal, toasted soybean meal and mineral requirement of Nile tilapia

Minerals (g/100g)	RDM	BDM	SDM	TSBM	Mineral requirement of Nile tilapia[1]
Calcium	2.24±0.45[a]	1.35±0.45[ab]	2.10±0.45[a]	0.28±0.45[b]	0.7

Magnesium	0.31±0.05[a]	0.25±0.05[a]	0.28±0.05[a]	0.22±0.05[a]	0.06-0.08
Sodium	0.60±0.06[a]	0.51±0.06[a]	0.57±0.06[a]	0.05±0.06[b]	NA
Potassium	6.78±0.77[a]	6.11±0.77[a]	6.69±0.77[a]	1.62±0.77[b]	0.21-0.33
Phosphorus	1.81±0.04[a]	1.67±0.04[b]	1.51±0.04[c]	0.61±0.04[d]	0.8-1.0
Iron	0.09±0.11[b]	0.08±0.11[b]	0.09±0.11[b]	7.40±0.11[a]	6
Zinc	5.70±0.0.29[a]	5.41±0.29[a]	5.52±0.29[a]	3.54±0.29[b]	3-7.9
Copper	0.30±0.35[a]	0.21±0.35[a]	0.27±0.35[a]	1.42±0.35[a]	0.2-0.3
Manganese	197±29.33[a]	182±29.33[a]	184±29.33[a]	8.91±29.33[b]	1.2

1 – Source: FAO (2022). Note: Means with the same superscript within the same row were not significantly different (P>0.05)
Keys: RDM = Raw duckweed meal; BDM = Blanched duckweed meal; SDM = Sun-dried duckweed meal; TSBM = Toasted soybean meal; NA = Not available

The antinutrient composition of raw, blanched, sun-dried duckweed meal and toasted soybean meal is represented in Table 4. Processing significantly reduced alkaloid, saponin, tannin and trypsin inhibitor levels. Blanched duckweed meal gave the best reduction of antinutrients content by 95.26%, 92.18%, 61.47%, 33.33% and 12.50% for tannin, saponin, alkaloid, oxalate and phytate, respectively.

Table 4: Antinutrients composition of raw, blanched, sun-dried duckweed meal and toasted soybean meal

Antinutrients (g/100g)	RDM	BDM	SDM	TSBM	Recommended Limit[1]
Alkaloid	1.22±0.07[a]	0.47±0.07[b]	0.51±0.07[b]	0.48±0.07[b]	0.5
% Reduction	-	61.47	58.19	-	-
Oxalate	0.18±0.04[a]	0.12±0.04[a]	0.14±0.04[a]	0.07±0.04[a]	2
% Reduction	-	33.33	22.22	-	-
Phytate	0.16±0.07[a]	0.14±0.07[a]	0.15±0.07[a]	0.32±0.07[a]	0.5
% Reduction	-	12.50	6.25	-	-
Saponin	11.00±0.91[a]	0.86±0.91[b]	1.50±0.91[b]	0.60±0.91[b]	10.00
% Reduction	-	92.18	86.36	-	-
Tannin	1.90±0.28[a]	0.09±0.28[b]	0.14±0.28[b]	0.03±0.28[b]	0.15
% Reduction	-	95.26	92.63	-	-
Trypsin inhibitor	1.30±0.10[a]	0.43±0.10[b]	0.42±0.10[b]	0.50±0.10[b]	0.5
% Reduction	-	66.92	67.69	-	-

1 – Source: Francis (2001) Note: Means with the same superscript across the same row were not significantly different (P>0.05)
Keys: RDM = Raw duckweed meal; BDM = Blanched duckweed meal; SDM = Sun-dried duckweed meal; TSBM = Toasted soybean meal

DISCUSSION

Toasted soybean meal had significantly highest (P≤0.05) protein content while raw duckweed meal (RDM) recorded significantly low crude protein content. The essential amino acid composition showed that blanching and sun-drying may not have affect the amino acid composition of duckweed differently, however, their values were higher compared to the toasted soybean meal. The low amino acid content in the blanched duckweed meal might be attributed to the loss of free water-soluble amino acids as a result of blanching. Hasan (2001) reported that when there is a loss of amino acids in water, the sulphur-containing amino acids (methionine + cysteine) are the most affected. These sulphur-containing amino acids are important in forming di-sulphide bonds and initiating the synthesis of other proteins (Brosnan and Brosnan, 2006). Arginine, histidine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan and valine contents obtained in this study were sufficient in the blanched duckweed meal, sun-dried duckweed meal and the toasted soybean meal to meet *Oreochromis niloticus* needs based on essential amino acids requirement of Nile tilapia recommended by FAO (2022). Methionine was the only limiting amino acid among the essential amino acids observed in this study.

The macro and micro mineral compositions of the raw, blanched, sun-dried duckweed meal and toasted soybean meal showed a significant difference in their concentrations except in magnesium and copper which had no significant difference (P>0.05). This study revealed that blanching and sun-drying methods had a significant effect on the macro and micro mineral concentrations of duckweed meal, and the concentrations of macro minerals obtained were also significantly higher (P≤0.05) when compared to those in the toasted soybean meal. This might be a result of the nutrient-rich medium where duckweed was cultured. Potassium (K) had the highest concentration among the macro minerals followed by Calcium (Ca) in the raw, blanched and sun-dried duckweed meal. Among the micro minerals, Manganese (Mn) had the highest concentration in the raw, blanched and sun-dried duckweed meal. The concentrations of the macro and micro minerals of the blanched and sun-dried duckweed meal obtained in this study were higher than the mineral requirement of Nile tilapia recommended by the FAO (2022). Only the iron (Fe) concentration of the blanched and sun-dried duckweed meal had not met the requirement for Nile tilapia as recommended but it could be obtained in the other ingredients of the feed and the water medium.

Antinutrients are chemical substances present in ingredients that by themselves or their metabolic products arising in the system interfere with the feed utilization, and growth performance or affect the health of the fish (Francis *et al.,* 2001). They are also called toxic factors or incriminating factors (Ramchandra *et al*., 2019). The results of the antinutritional factors of the blanched and sun-dried duckweed meal showed a significant reduction of these factors following the processing methods. Alkaloid, oxalate, phytate, saponin, tannin, and trypsin inhibitor were reduced significantly in the blanched and sun-dried duckweed meal compared to the control. The positive effects of the processing methods in the reduction of the antinutritional compounds are in line with the reports of Kandonga (2019). A similar reduction of various antinutritional compounds was reported when vegetable leaves were subjected to blanching and sun-drying by Ilelaboye *et al.* (2013). The antinutrients of the blanched, sun-dried duckweed meal and the toasted soybean meal in this study were within tolerable limits of Nile tilapia. The recommended limit of alkaloid, oxalate, phytate, saponin, tannin and trypsin inhibitors in the diet of *Oreochromis niloticus* according to Francis *et al.* (2001) are less than 0.5g/100g, 2g/100g, 0.5g/100g, 10.00g/100g, 0.15g/100g and 0.5g/100g, respectively.

CONCLUSION

This research revealed that the nutrient compositions of toasted soybean meal, blanched duckweed meal and sun-dried duckweed meal can meet the nutritional requirement for Nile tilapia feed except for the limitation of methionine observed in this study. Therefore, if the feed ingredient is ever considered for use for this species, DL-methionine would have to be supplemented to improve the performance characteristics of the reared species.

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