



Effect of different processing methods on nutritional composition of *Leucaena leucocephala* (Lam De Wit) leaves as inclusion in Fish Feed

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ABSTRACT: The potentials of *Leucaena leucocephala* leaf-meal as a supplementary protein in fish feed was evaluated. *Leucaena* leaves were subjected to two processing methods (sun-drying and boiling) to determine the effect of these treatments on proximate composition and anti-nutritional factors. Five samples of *Leucaena* leaves were analyzed, sundried for 24 hours (SD₁), sundried for 48 hours (SD₂), sundried for 72 hours (SD₃), soaked in boiled water for five minutes at 100°C (SB₅) and unprocessed (SU). The tests were carried out in triplicate for each treatment. Data obtained were subjected to one way analysis of variance. Sun-drying (SD₁, SD₂, SD₃) led to a significant (P<0.05) increase in crude protein, ash, phosphorus, potassium, magnesium and sodium when compared with the soaked in boiled water (100°C) for 5 minutes (SB₅) treatment. The sample soaked in boiled water for 5 minutes (SB₅) gave 73, 90, 81, 97 and 36 % reduction in the levels of phytate, cyanide, tannin, saponin and mimosine respectively, which was better than the sundried treatments. © JASEM

<https://dx.doi.org/10.4314/jasem.v21i4.12>

Key Words: *Leucaena leucocephala*, anti-nutritional factors, nutritional composition, supplementary protein, mimosine, fish feed.

The use of plant protein sources such as soyabeans and groundnut in fish diet may not be sustainable because they are good sources of protein for humans. There is therefore an urgent need to identify other protein rich plant resources that could substitute or supplement conventional protein feed ingredients. The nutritional interest in some of these vegetable species stems from their rich contents of essential amino acids, vitamins and minerals (Aye, 2012). However, the presence of inherent toxic factors or anti-nutritional components in plants has been one major obstacle in harnessing the full benefits of the nutritional value of plant proteins.

Leucaena leucocephala is a fast growing multipurpose legume that has been introduced to many tropical and subtropical countries (FACT, 1997). *L. leucocephala* belongs to the family *Fabaceae*, sub-family *Mimosoidae*. It has been documented that *L. leucocephala* was used in dry season feeding of ruminants, agro-forestation, wind breakers, live fence, alley farming, roadside landscaping, fuel production, paper production, timber production and nitrogen fixation (Hetrampf and Piedad-pascual, 2003). *Leucaena* leaf has crude protein values ranging from 15.2-34.3% dry matter (FACT, 1997; Monoj and Bandyopadhyay, 2007; Ayssiwede *et al.*, 2011). The nutritional value of *Leucaena* in fish feed formulation has been limited due to the presence of a toxic non-protein amino acid called mimosine (Hetrampf and Piedad-pascual, 2003). The level of mimosine can be

reduced through processing (Monoj and Bandyopadhyay, 2007). Thus simple and cost effective processing methods which the small-scale fishfarmers can adopt should be employed to reduce the levels of anti-nutritional factors.

The research evaluated the effects of processing on the proximate composition and anti-nutrients of *L. leucocephala* leaf-meal thereby assessing its potentials in supplementing conventional protein feed ingredients.

MATERIALS AND METHODS

Sample Collection: *Leucaena* leaves were collected from trees planted at the College of Agriculture and Animal Sciences, Mando, Kaduna and identified at the forage section of the National Animal Production Research Institute (NAPRI), Zaria. The leaves were handpicked and leaflets separated from the long leaf stalk.

Sample Treatment: The leaves were divided into five portions of 500g each and subjected to two processing methods, namely sun-drying and boiling. The first 500g of leaves were spread on a concrete slab outside the hatchery and sundried for 24 hours, another 500g was sundried for 48 hours while the last 500g was sundried for 72 hours (Ayssiwede *et al.*, 2011). These were coded SD₁, SD₂ and SD₃, respectively. Another leaf sample of 500g was immersed in 1000 litres of

boiled water (100° C) for 5 minutes. The boiled *Leucaena* leaf sample was then sun-dried for 24 hours until it became crispy and coded SB₅. The last sample (unprocessed) consists of 500g *Leucaena* leaves and was coded SU which served as control. All the five leaf samples were ground into fine powder using a hammer mill, labeled and separately packed into polythene bags. These were stored for proximate, mineral and anti-nutrients analysis.

Proximate Analysis: Determination of ash content: Crucibles were cleaned and dried in the oven. After drying they were corked in the desiccator and weighed (W₁). After which 2g of the grounded sample was placed in the crucible and weighed (W₂). These were transferred into furnace and set to 550°C. The Sample was incinerated in the furnace for 8 hours. The crucible containing the ash was removed and cooled in the desiccator and weighed (W₃). The weight of the residue in the crucible corresponds to the organic matter content (AOAC, 1990).

Determination of moisture content (AOAC, 1990): Crucibles were washed and dried to a constant weight in an air oven at 100°C, these were later removed and cooled in a desiccator and weighed (W₁). Two grams of the grounded sample was placed in the weighed moisture dish (W₂) the crucible containing the sample was kept in an oven at 100° C for 24 hours and weighed. It was kept back in the oven and reweighed after about 3 hours to ensure a constant weight (W₃). The moisture content was calculated as:

$$\% \text{Moisture} = \frac{W_2 - W_3}{W_2 - W_1} \times \frac{100}{1}$$

Determination of lipid content (AOAC, 1990): Two round bottom flasks were cleaned and few anti bump granules were added to prevent bumping. 300mls of petroleum ether (40-60° C) boiling point were poured into the flask. These were fitted into the soxhlet extraction units. Extraction thimbles were weighed and approximately, 2.0 g of samples were weighed into it (W₁), the thimble was fixed into the soxhlet extraction unit with forceps and cold water circulation put on the heating mantle was switched on and solvent refluxing was adjusted at a steady rate. Extraction was carried out for eight hours. The thimble was removed and dried to constant weight in an oven at 70° C and was weighed (W₂). The extractible lipid was calculated as:

$$\% \text{Lipid} = \frac{\text{Wt of lipid extracted}}{W_2 - W_1} \times \frac{100}{1}$$

Wt of dried sample

Where the weight of lipid extracted is given by the loss in weight

W₁-W₂= weight of thimble content after extraction.

Determination of nitrogen and crude protein: The crude protein content of the samples was determined using the Microkjeldahl method of (AOAC, 1990) which involved protein digestion and distillation. **a. Protein Digestion:** About 2g of the sample was weighed using Mettler 50 weighing balance into 100ml kjeldahl flask and few antibump granules, one gram of catalyst (K₂SO₄+anh CuSO₄) was added to speed up the reaction. The flask was fixed on the Kjeldahl digestion rack and heated slowly at first until fretting subsides and then more rigorously with occasional rotation of the flask to ensure even digestion and avoid over heating of content. **b. Protein Distillation:** After a clear solution was obtained, the sample was transferred to 100ml volumetric flask and diluted to mark with distilled water after cooling. 10mls of the diluted sample or digest was pipetted into Markham semi macro nitrogen still and 10mls of 40% NaOH solution added. The sample was distilled liberating NH₃ into 100ml conical flask containing 10ml of 40% Boric acid and 2 drops of methyl red indicator. Distillation was continued until the pink colour of the indicator turned greenish. The control was titrated with 4% Boric acid with end point indicated by a change from greenish to pink colour. The volume of acid used for each sample distillate was noted as well as that of blank.

% total N per sample was calculated as:

$$\% \text{N} = \frac{V_1 - V_0 \times M \times 14 \times 100 \times 100}{0.2 \times 1000 \times 10 \times 1}$$

V₀ = vol. of HCl required for blank

V₁ - vol. of the HCl required for 10ml sample solution.

M = Molarity of acid (0.1m)

The crude protein was calculated as % crude protein (cp) = 6.25xN

NOTE: Protein contains 16% N₂. This makes the general conversion factor to be 6.25

Determination of crude fibre (AOAC, 1990): Two grams of the grounded sample was placed in a round bottom flask. 100ml of 0.25M H₂SO₄ was added and the mixture boiled under reflux for 30minutes. The

insoluble matter was washed several times with hot water until it was acid free. Thereafter, it was transferred into a flask containing 100ml of hot 0.312M NaOH solution. The mixture was boiled again under reflux for 30 minutes and filtered under suction the insoluble residue was washed with hot water until it was base free. It was dried to constant weight in an oven at 100°C, cooled in a desiccator and weighed (C₂). The weighed sample was incinerated in a furnace at 550°C for 2 hours. It was put off and allowed to cool down. It was removed cooled in a desiccator and weighed (C₃). The crude fibre was calculated as the loss in weight on ashing:

Weight of the original sample = W

$$\% \text{ crude fibre} = \frac{C_2 - C_3}{W} \times \frac{100}{1}$$

Nitrogen free extract: The nitrogen-free extract (NFE) which is the digestible portion of the carbohydrate contained in the sample and was determined by subtracting the percentage sums of moisture, crude protein, crude fat, crude fibre and ash from 100.

Energy values: The energy value of the leaf samples was calculated as described by Ponzenga (1985). Crude protein x 37 + ether extract x 81.8 + nitrogen free extract x 35.

Determination of Anti-nutritional Compounds
Cyanogenic glycoside: the procedure of AOAC (1990) was used in the determination of cyanogenic glycosides. Five grams of sample was placed in a 200ml conical flask, 50ml of distilled water was added. The content of the flask was stirred and allowed to stand for 4 hours. The filtrate was collected into a beaker using glass wool which was placed in a funnel. The filtrate was steam distilled into 20ml of 2.5% Sodium hydroxide. About 75ml of the distillate was collected. This was titrated with 0.02N Silver nitrate after the addition of 8ml of 6N Ammonium hydroxide and 2ml of 2% Potassium iodide. Permanent turbidity indicated end point.

1ml 0.02N AgNO₃ = 1.08 mg HCN

Phytic acid: Phytate phosphorus was determined by the method of Wheeler and Ferrel (1971). Approximately 2.0g of the dried sample was soaked into 100ml of 2% HCl for 5 hours and filtered 25cm³ of the filtrate was taken into a conical flask. 5cm³ of 0.3% potassium thiocyanate solution was added. The mixture was titrated with standard solution of FeCl₃. A standard

curve was prepared expressing the results as Fe (NO₃)₃ equivalent.

Tannin: Tannin was determined using the standard method described by AOAC (1990). Two grams of the dried sample was boiled with 300ml of distilled water. This was diluted in a standard volumetric flask and filtered through a non-absorbent cotton wool. A volume of 25ml of the infusion was measured into a 2 litre porcelain dish and titrated with 0.1N potassium permanganate (0.1N potassium permanganate was standardized against 0.1N oxalic acid) until the blue solution changed green, then few drops of 0.1N-9.005 potassium permanganate was added. The titre was multiplied by 0.0066235 to obtain the amount of tannin in the sample as shown in the equation is below:

0.1N oxalic acid = 0.0066235g tannin

Saponin: The standard method of AOAC (1990) was used to determine saponin in the samples. A gravimetric method employing the use of a Soxhlet extractor and two different organic solvents was used. The first solvent extracts lipids and interfering pigments while the second solvent extracts the saponin proper. Five grams of the dried ground sample was weighed into a thimble and transferred into the Soxhlet extraction chamber fitted with a condenser and round bottom flask. Some quantity of acetone enough to cause a reflux was poured into the flask. The sample was exhaustively extracted of its lipids and interfering pigments for 3 hours by heating the flask on a hotplate and the solvent was distilled off. For the second extraction, a pre-weighed round-bottom flask was fitted into the Soxhlet apparatus which bore the thimble containing the sample. Then methanol was poured into the flask. The methanol was enough to cause reflux. The saponin was then exhaustively extracted for 3 hours by heating the flask on a hotplate after which the solvent was distilled off. The flask was reweighed. The difference between the final and initial weights of the flask represented the weight of the saponins extracted.

$$\% \text{ Saponins} = \frac{\text{Weight of saponins}}{\text{Weight of sample}} \times 100$$

Determination of mimosine: The mimosine content of the leaf meal was determined by the rapid colorimetric method of Megarity (1978). To extract the mimosine, about 2g of sample was digested with 0.1N HCl, filtered and clarified by removing plant pigments with activated carbon. Ferric chloride (0.5%) was then added to the mimosine extracts to develop a coloured compound, the intensity of which was estimated by measuring the absorbance at 535nm in a Pye Unicam

PU 8650 visible spectrophotometer. The concentration of mimosine was then calculated from a standard curve using pure mimosine.

Determination of the Mineral Content: Mineral Content samples were determined by the methods of (AOAC, 1990). Digestion: One gram of each sample was weighted into a clean dried crucible and placed in a furnace. The temperature was raised to 550°C and allowed to ash for 3 hours. The contents were cooled, and 10ml of Aqua regia was added to the sample in crucible and then filtered into a 25ml volumetric flask to make up to volume with de-ionised water. Calcium, magnesium and sodium were read with PG 990 Atomic Absorption Spectrophotometer and potassium was read using Jenway flame photometer. Phosphorus was analysed when a sample of 1ml aliquot of the plant extract was pipette into a 25ml volumetric flask and 10ml distilled water was added, 4ml of Molybdate reagent was then added to make up to 25ml with distilled water and allowed to stand for 15 minutes for the colour to develop. The phosphorus content in

solution was then determined on a Jenway 6705 uv/vis spectrophotometer.

Statistical analysis: One way analysis of variance (ANOVA) was used to analyze data collected. The means were separated using Duncan's multiple range test at 95% confidence limit.

RESULT AND DISCUSSION

The result from Table 1 shows that the crude protein in the sun-dried treatments were higher than the sample soaked in boiled water (100°C) for 5 minutes (SB₅). Treatment SD₃ (sundried for 72 hours) had the highest crude protein of 33.22% followed by SD₂ (sundried for 48 hours) with 31.83% and then SD₁ (sundried for 24 hours) 30.66% while (SB₅) soaked in boiled water (100°C) for 5 minutes had 28.33%. The crude protein value in the sundried treatments (SD₁, SD₂ and SD₃) were significantly different (P ≤ 0.05) from the (SB₅) treatment. The crude fibre content of the sundried treatments was significantly different (P ≤ 0.05) from the soaked in boiled water 100°C for 5 minutes (SB₅) treatment.

Table 1: Effects of processing on the Proximate Composition of *Leucaena leucocephala* Leaves

Treatment (g/100g)	SU	SD ₁	SD ₂	SD ₃	SB ₅
Crude protein	11.82±2.68 ^c	30.67±0.58 ^{ab}	31.67±0.58 ^a	33.00±0.00 ^a	28.33±1.15 ^b
Ash	4.6±1.98 ^b	7.00±1.00 ^a	7.00±0.00 ^a	7.00±0.00 ^a	6.33±0.58 ^{ab}
Ether extract	6.03±1.97 ^a	5.00±0.00 ^a	5.67±0.58 ^a	5.67±0.58 ^a	5.33±0.58 ^a
Crude fiber	4.71±3.29 ^d	12.67±0.58 ^{ab}	10.67±0.58 ^{bc}	9.33±0.58 ^c	14.33±0.58 ^a
Moisture	66.10±11.60 ^a	10.00±0.00 ^b	10.00±1.00 ^b	10.67±0.58 ^b	7.67±0.58 ^b
Nitrogen free extract	6.75±5.59 ^b	34.33±1.53 ^a	34.67±1.15 ^a	33.67±0.58 ^a	37.67±2.08 ^a
Kcal	1166.84±455.29 ^b	2747.00±27.71 ^a	2843.00±29.87 ^a	2881.33±26.03 ^a	2807.10±40.27 ^a
<i>Minerals(mg/kg)</i>					
Phosphorus	10.02±0.01 ^d	10.46±0.01 ^b	10.06±0.04 ^c	12.63±0.02 ^a	9.85±0.01 ^c
Potassium	30.50±0.14 ^a	30.38±0.13 ^a	29.38±0.38 ^b	30.63±0.13 ^a	16.25±0.00 ^c
Magnesium	11.59±0.56 ^a	11.58±0.48 ^a	11.61±0.49 ^a	11.51±0.54 ^a	11.03±0.55 ^b
Calcium	61.96±1.77 ^a	64.47±3.20 ^a	61.52±3.13 ^a	53.10±2.49 ^b	51.25±1.81 ^b
Sodium	6.88±0.36 ^d	7.04±0.03 ^{cd}	7.29±0.02 ^b	7.73±0.04 ^a	5.25±0.07 ^c

Note: Means with the same superscript within the same row are not significantly different (P>0.05)

SU – raw

SD₁ –Sundried for 24 hours

SD₂ –Sundried for 48 hours

SD₃ –Sundried for 72 hours

SB₅ – Soaking in boiled water (100°C) for 5 minutes

The sample soaked in boiled water at 100°C for 5 minutes (SB₅) had the highest crude fibre value of 14.33% while the sundried treatments were between 9.33-12.67%. SB₅ had the highest nitrogen free extract (37.67%) and SD₃ (sundried for 72 hours) had the least (33.61%). However, the nitrogen free extract was not significantly different (P>0.05) for sundried and soaked in boiled water samples.

The mineral composition of differently processed *Leucaena* leaves as presented in Table 1 shows that

processing led to a reduction in the minerals. The sundried treatments had higher values for all the minerals evaluated when compared with the soaked in boiled water treatment.

The anti-nutrients composition of differently processed *Leucaena* leaves as presented in Table 2 shows that there was a reduction in anti-nutrients of processed leaves when compared with the raw sample (SU). Furthermore the anti-nutrients were higher in the sundried treatments (SD₁, SD₂ and SD₃) than the

treatment soaked in boiled water 100 °C for 5 minutes (SB₅). The anti-nutrients values in the sundried treatments were significantly different (P≤0.05) from the treatment soaked in boiled water 100 °C for 5 minutes (SB₅) with the exception of cyanide. The phytate content was highest (0.71mg/100g) in SD₃ followed by SD₂ 0.66mg/100g, then SD₁ with 0.61mg/100g, while SB₅ was least with 0.25mg/100g. Treatment SB₅ gave 73% reduction of phytate, while SD₁, SD₂ and SD₃ gave 23-33% reduction respectively. The tannin content was highest in SD₃ with a value of 0.62mg/100g SD₁ had 0.61mg/100g and SD₂ with 0.51mg/100g, while SB₅ had the least 0.2mg/100g. The sundried treatments gave a tannin reduction of 41-50% while SB₅ gave 81%. The highest value of 0.64mg/100g for saponin was recorded in SD₁ while SB₅ had the least (0.05mg/100g). The percentage reduction of saponin for the treatment soaked in boiled water, SB₅ was 97%, while that of the sundried was 55-57% as shown in Table 2. The mimosine content of the sundried treatments was highest (1.28%) in SD₃ and least (1.07%) in SB₅. The percentage reduction of mimosine was 23-27% for SD₁, SD₂ and SD₃ while the treatment soaked in boiled water 100 °C for 5 minutes SB₅ had (36%) reduction of mimosine.

The result shows that processing methods (sun-drying and soaking in boiled water) affected the proximate composition of *Leucaena* leaves. The high crude protein in the sundried treatments may be as a result of

concentration of soluble solids thereby increasing the drymatter. Mbah *et al.* (2012) reported a similar increase in protein when *Moringa oleifera* leaves were sundried. The reduction of crude protein in the soaked treatment (SB₅) may be due to leaching of soluble nutrients (Nuttaporn and Naiyatat, 2009). The low crude protein value observed in the SB₅ treatment could also be associated with loss of amino acids especially sulphur amino acids, methionine + cysteine. Madalla *et al.* (2013) reported a reduction in values of methionine and cysteine by 67% when *Moringa* leaves were soaked in water overnight. The variation in the crude protein values of the different processing methods in this study and that of previous studies could be attributed to differences in duration of sun-drying, hours of soaking, temperature of water and method of soaking (Masafu, 2006). The high value of crude fibre in SB₅ treatment may be due to loss of nutrients that are soluble in water, leaving insoluble fibre. The nitrogen free extract values were higher in the *Leucaena* leaves soaked in boiled water when compared with the sundried *Leucaena* leaves. This observation may be due to the lower levels of other proximate components (crude protein, crude fibre, ash and ether extract) since nitrogen free extract values are obtained by subtracting other components. Previous studies also reported high nitrogen free extract values for soaked and cooked *Leucaena* leaves when compared with sundried *Leucaena* leaves (Osman *et al.*, 1996; Adekojo *et al.*, 2014).

Table 2: Effects of processing on the Anti-nutrients Composition of *Leucaena leucocephala* Leaves

Anti-nutrients (mg/100g)	Treatments				
	SU	SD ₁	SD ₂	SD ₃	SB ₅
Phytate	0.91±0.2 ^a	0.61±0.05 ^b	0.66±0.02 ^b	0.71±0.09 ^b	0.25±0.03 ^c
% Reduction	-	33	28	22	73
Cyanide	0.50±0.1 ^a	0.07±0.01 ^b	0.05±0.01 ^b	0.07±0.01 ^b	0.05±0.01 ^b
% Reduction	-	86	90	86	90
Tannin	1.03±0.33 ^a	0.61±0.01 ^b	0.51±0.23 ^b	0.62±0.01 ^b	0.2±0.02 ^c
% Reduction	-	41	50	40	81
Saponin	1.43±0.48 ^a	0.64±0.01 ^b	0.46±0.04 ^b	0.62±0.04 ^b	0.05±0.01 ^c
% Reduction	-	55	68	57	97
Mimosine (%)	1.66±0.02 ^a	1.25±0.01 ^b	1.22±0.01 ^b	1.28±0.03 ^b	1.07±0.04 ^c
% Reduction	-	25	27	23	36

Note: Means with the same superscript within the same row are not significantly different (P>0.05)

SU – raw

SD₁ – Sundried for 24 hours

SD₂ – Sundried for 48 hours

SD₃ – Sundried for 72 hour

SB₅ – Soaking in boiled water (100 °C) for 5 minutes

The positive effects of the processing methods in the line with the reports of Fayemi *et al.* (2011) and reduction of most anti-nutritional compounds are in Adekojo *et al.* (2014). Similar reductions of various

anti-nutritional compounds were reported when *Leucaena* leaves were subjected to sun-drying, soaking and fermentation (Fayemi *et al.*, 2011; Adekojo *et al.*, 2014). Soaking in boiled water (100°C) for 5 minutes was able to reduce the levels of various anti-nutritional compounds better than sun-drying. The lower value of mimosine and other anti-nutritional compounds recorded for soaking in boiled water (100°C) for 5 minutes processing method has been reported by previous authors when *Leucaena* leaves were soaked in hot or cold water (Osman *et al.*, 1996; Baraigi *et al.*, 2004; Fayemi *et al.*, 2011). The high value of minerals such as phosphorus, potassium, magnesium and calcium in the sundried treatments when compared with SB₅ treatment could be as a result of reduction of moisture while the low minerals value observed in SB₅ treatment could be due to leaching. Adekojo *et al.* (2014) gave a similar report when *Leucaena* leaves were soaked in 60°C water for 24 hours.

Conclusion: Soaking in boiled water (100°C) for 5 minutes increased palatability and digestibility of the *Leucaena* leaf meal due to the low levels of anti-nutrients therefore is recommended for inclusion in fish diet. The crude protein in the processed *Leucaena* leaves indicates it could serve as a supplement to other conventional proteins in fish feed.

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