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Amino Acids Content Comparison with Different Processing Methods (Cook, Raw and Fermented) and Inclusion Levels of *Delonix regia* in Formulated Fish Diets

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Authors' contributions

This work was carried out in collaboration among all authors. Author BO designed the study. Author ORO performed the statistical analysis. Author YA managed the literature searches. Author IA proofread the manuscript. Author SDO managed the data cleaning. All authors read and approved the final manuscript.

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ABSTRACT

This study investigated the effects of different processing methods of *Delonix regia* seeds on amino acids composition of experimental diets. Ten isonitrogenous diets (40% crude protein) were formulated with cooked, raw and fermented *Delonix regia* seeds at 0% (Control), 10%, 20% and

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30% inclusion levels respectively. Data were analysed using Analysis of Variance, significant differences in means were separated using Duncan Multiple Range Test. All the essential amino acids (lysine, arginine, threonine, valine, methionine, isoleucine, leucine and phenylalanine) differs significantly among the treatments except histidine which was statistically similar (P > 0.05) across the dietary treatments. The activity of essential and non- essential amino acid concentration was higher in cooked than the fermented and raw *Delonix regia* seeds. It was concluded that cooked *Delonix regia* seeds at 10% inclusion levels had the highest activities of essential and non-essential amino acids and could be used to supplement conventional feedstuff for livestock especially in fish nutrition and bioenergetics.

Keywords: Amino acids; Delonix regia; fermentation; cooking; bioenergetics.

1. INTRODUCTION

Fish feeds constitute 60-75% of the total cost of aquaculture production which is expensive and has led to studies on how to reduce the high cost of fish feed production using alternative feed ingredients [1]. The competition between humans and livestock for the conventional crop feed ingredient which has made it scarce and expensive. The use of unconventional plant sources of protein such as seeds, leaves and other agricultural by-products in the formulation of fish feed to attain a more economically sustainable, environmentally friendly and cheap fish feed have become desirable among the fish farmers. Generally, the ingredients that are essential for fish feed formulation are proteins and amino acids, lipids, carbohydrates, vitamins and minerals. Protein is the major concern during the formulation of fish feed. It is the most expensive components in fish feed and the important factors contributing to the growth performance of cultured species [2]. Protein requirement in fish diet can be related to the general energy requirement of the fish at certain water temperature and the ability to gain weight at present capacity [3]. Diet formulation based on digestible amino acids will allow the use of alternative protein sources with low digestibility coefficients because such formulation will improve the precision of least-cost diets and reduce nitrogen excretion from livestock operations [3]. Although the advantages of the digestible amino acid are recognized, diet formulation based on the total amino acid content is still widely used in many parts of the world. In the future, however, economic reasons will compel the fish industry to increase the use of an array of cheaper, alternative protein supplements with low digestibility coefficients such as *Delonix regia* in feed formulation. Amino acids are the substance derived from the ultimate product of digestion. There are about twenty naturally available amino acids which includes arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, protein, threonine, alanine, aspartic acid, asparagine, cystine, glutamic acids, glutamine, proline, tryptophan, valine, serine, tyrosine but only the first ten are essential for fish growth because they are not synthesized in fish and must be provided in the feed, because of this they are called “essential or indispensable" amino acids [4]. A particularly important amino acid for growth is lysine. Protein-rich in lysine can be obtained from a legume, fish meal, blood meal, or meat and bone meal. Tryptophan is very important for the growth of all cells and normal development, it is used in cell profile ration. Arginine and histidine play an important role in maintaining a normal and healthy bloodstream and has other complex functions [5]. Fish and other animals can synthesize their non-essential amino acids from carbohydrates and lipids and other nitrogen compounds but mainly from other non-essential amino acids. Any diet deficient in any of the essential amino acid will cause depressed appetite and growth rate of fish. Therefore, this study is designed to evaluate the effect of different processing methods of *Delonix regia* seeds on the amino acid composition of diets.

2. MATERIALS AND METHODS

The experiment was conducted at the aquaculture production technology unit of the Skill Acquisition and Development Centre, NAERLS, Ahmadu Bello University, Zaria, located at latitude 11º09^{1}45.2^1 N and longitude 7º38^{1}17.9^1 E. The experiment was conducted from September 2015 to March 2016.

2.1 Collection of *Delonix regia* (Flamboyant) Seeds

Matured and dry pods of *Delonix regia* (flamboyant) containing the seeds were collected from the annex campus of Nuhu Bamalli

Polytechnic Zaria. The seeds were collected by opening the pods manually. The average seeds per pod were between 30-37, the weight of 100 seeds was 42.5 g. The collected seeds were handpicked for the selection of healthy seeds.

2.2 Processing of Seeds

Seeds with identification number 1971 were weighed separately for processing, one part for cooking, the second for fermentation and the third left as raw.

2.2.1 Fermentation of *Delonix regia* (Flamboyant) seeds

The seeds were soaked in water for 12 hours. The drained soaked seeds were allowed to ferment naturally by tying in a polythene bag and kept in a dark cupboard for 72 hours without the addition of yeast [6]. The fermented seeds were allowed to dry for two days before grinding into homogenous powder using a hammer mill.

2.2.2 Cooking of *Delonix regia* (Flamboyant) seeds

The seeds were boiled to 100ºC for 80 minutes and were allowed to cool and dried for two days and later ground to homogenous powder using a hammer mill [7].

2.2.3 Raw *Delonix regia* (Flamboyant) seeds

The raw seeds were dried for two days and milled into a homogenous powder using a hammer mill.

2.3 Analysis of Differently Processed *Delonix regia* (Flamboyant) Seeds

The differently processed seeds, cooked flamboyant seeds (CFS), fermented flamboyant seeds (FFS) and raw flamboyant seed (RFS) were taken for analysis of amino acid profile. All analysis was carried out in triplicates.

2.4 Determination of Amino Acid Profile of Raw and Processed *Delonix regia* (Flamboyant) Seeds

The amino acid profile in the known sample was determined using the methods described by NRC [8]. The known sample was dried to constant weight, defatted, hydrolysed, evaporated in a rotary evaporator and loaded into the Technicon Sequential Multi-sample Amino Acid Analyser (TSM).

2.5 Defatting Sample

The sample was defatted using a chloroform/methanol mixture of ratio 2:1. 4 g of the sample was put in extraction thimble and extracted for 15 hours in the soxhlet extraction apparatus [8].

2.6 Nitrogen Determination

Two hundred milligramme (200 mg) of ground sample was weighed, wrapped in Whatman filter paper (No. 1) and put in the Kjeldahl digestion flask. 10ml of concentrated sulphuric acid was added. Catalyst mixture (0.5 g) containing sodium sulphate (Na_2SO_4), copper sulphate ($CuSO_4$) and selenium oxide (SeO_2) in the ratio of 10:5:1 was added into the flask to facilitate digestion and four pieces of anti-bumping granules were added.

The flask was then put in Kjeldahl digestion apparatus for 3hours until the liquid turned light green. The digested sample was cooled and diluted with distilled water to 100 ml in a standard volumetric flask. An aliquot (10 ml) of the diluted solution with 10 ml of 45 % sodium hydroxide was put into the Markham distillation apparatus and distilled into 10 ml of 2% boric acid containing 4 drops of bromocresol green/methyl red indicator until about 70 ml of distillate was collected.

The distillate was then titrated with standardized 0.01N hydrochloric acid to a grey colour.

$$\text{Percentage of Nitrogen} = \frac{(a-b)\times 0.01 \times 14 \times V \times 100}{W \times C}$$

Where;

a = Titre value of the digested sample
b = Titre value of the blank sample
v = Volume after dilution (100 ml)
W = Weight of the dried sample (mg)
C = Aliquot of the sample used (10 ml)
14= Nitrogen constant in mg

2.7 Hydrolysis of the Sample

A known weight of the defatted sample was weighed into glass ampoule. Seven (7) ml of 6NHCl was added and oxygen was expelled by passing nitrogen into the ampoule. This is to avoid possible oxidation of some amino acids during hydrolysis (e.g. methionine and cysteine). The glass ampoule was then sealed with Bunsen burner flame and put in an oven preset at 105ºC ± 5ºC for 22 hours. The ampoule was allowed to cool before broken open at the tip and the content was filtered to remove the humins. It should be noted that the tryptophan is destroyed by 6NHCl during hydrolysis. The filtrate was then evaporated to dryness in a hot air oven. The residue was dissolved with 5ml to acetate buffer (pH 2.0) and stored in plastic specimen bottles which were kept in the freezer.

2.8 Loading of the Hydrolysate into TSM Analyzer

The amount loaded was between 5 to 10 microliter. This was dispended into the cartridge of the analyzer. The TSM analyzer is designed to separate and analyze free acidic, neutral and basic amino acids of the hydrolysate. The period of an analysis lasted for 76 minutes.

2.9 Method of Calculating Amino Acid Concentration

An integrator attached to the analyzer calculates the peak area proportional to the concentration of each of the amino acids which include lysine, histidine, arginine, proline, glycine, alanine, cystine, valine, methionine, isoleucine, leucine, tyrosine and phenylalanine.

2.10 Statistical Analysis

Data obtained were subjected to one-way analysis of variance (ANOVA) using a general linear model (GLM of SAS 9.2). Duncan Multiple Range Test (DMRT) was used to test the difference between levels of means and mean separation was considered significant at $P < 0.05$.

3. RESULTS AND DISCUSSION

Table 1 shows the amino acid profile of diets fed *H. bidorsalis* fish at different inclusion levels of raw *Delonix regia* seeds. All the essential amino acids (lysine, arginine, threonine, valine, methionine, isoleucine, leucine and phenylalanine) differs significantly at different inclusion levels among the treatments except for histidine which was statistically similar ($P > 0.05$) across the dietary treatments. Lysine had the highest concentration (4.53g/100g protein) in $RFSM_1$, while the least concentration (4.19g/100g protein) was observed in $RFSM_3$. $RFSM_1$ and $RFSM_2$ recorded the highest arginine content (6.53g/100g Protein and 6.45g/100g Protein) and were statistically similar ($P > 0.05$) but differs significantly ($P < 0.05$) from the control (6.10g/100g Protein) and $RFSM_3$ (5.16g/100g Protein). Threonine and valine recorded similar statistical trend with arginine. Methionine and leucine had the highest concentration in the control group (2.29g/100g Protein and 9.60g/100g Protein) which was statistically different from other treatments. $RFSM_1$ had the highest concentration of isoleucine (3.14g/100g Protein) and phenylalanine (4.17g/100g Protein) which differs statistically ($P < 0.05$) from other treatments.

Non-essential amino acid (Glutamic acid, serine, aspartic acid, proline, glycine, alanine, cystine and tyrosine) were statistically different ($P < 0.05$) among the treatments. $RFSM_1$, had the highest concentration of glutamic acid (12.87g/100g Protein) while the least concentration was observed in $RFSM_3$ (11.50g/100g Protein). $RFSM_1$ had the highest value for serine (3.59g/100g Protein), $RFSM_2$ was intermediate (3.43g/100g Protein) while the control (3.08g/100g Protein) and $RFSM_3$ recorded the lowest concentration (3.00g/100g Protein) and were statistically similar ($P > 0.05$). Aspartic acid was statistically different ($P < 0.05$) among the treatments except for $RFSM_3$ (7.69g/100g Protein). $RFSM_1$ had the highest levels of proline (3.25g/100g Protein) and glycine content (3.32g/100g Protein) which differs significantly ($P < 0.05$) from $RFSM_2$ (3.14g/100g Protein and 3.25g/100g Protein), control (3.04g/100g Protein and 3.16g/100g Protein) respectively. $RFSM_1$ had the highest concentration for alanine (3.98g/100g Protein) which differs significantly ($P < 0.05$) from $RFSM_2$ (3.90g/100g Protein), control and $RFSM_3$ respectively, though the control and $RFSM_3$ were similar ($P > 0.05$). $RFSM_1$ had the highest level of cysteine (1.15g/100g Protein) which differs significantly from the control, $RFSM_2$ and $RFSM_3$ respectively. Tyrosine concentration was highest in $RFSM_1$ (2.75g/100g Protein) and $RFSM_2$ (2.75g/100g Protein) which differs statistically from control (2.41g/100g Protein) and $RFSM_3$ (2.41g/100g Protein) which were both statistically similar ($P > 0.05$).

Table 2 shows the amino acid profile of diets fed *H. bidorsalis* fish at different inclusion levels of fermented *Delonix regia* seeds. All the essential amino acids were statistically different ($P < 0.05$) among the treatment for lysine, arginine, threonine, valine, isoleucine, leucine and phenylalanine except for histidine and methionine. $FFSM_1$ had the highest lysine content (4.85g/100g Protein), followed by $FFSM_2$ (4.77g/100g Protein), $FFSM_3$ (4.67g/100g Protein) and the control group (4.37g/100g Protein). $FFSM_1$ and $FFSM_2$ had the highest levels of arginine (6.88 and 6.7g/100g Protein) while the control group recorded the least level (6.10g/100g Protein).

Table 1. Mean amino acid profile of raw *Delonix regia* seeds fed *H. bidorsalis* at different inclusion levels of diet

Amino acid (g/100 g Protein)	0% (Control)	$RFSM_1$	$RFSM_2$	$RFSM_3$	SEM
EAA					
Lysine	4.34^c	4.53^a	4.45^b	4.19^d	0.014
Histidine	2.23^a	2.27^a	2.23^a	2.23^a	0.017
Arginine	6.10^b	6.53^a	6.45^a	5.16^c	0.031
Threonine	2.99^b	3.11^a	3.08^a	2.61^c	0.008
Valine	3.80^b	3.97^b	3.94^b	3.74^c	0.016
Methionine	2.29^a	2.19^a	2.19^b	2.19^b	0.018
Isoleucine	2.94^c	3.14^a	3.07^b	2.68^d	0.021
Leucine	9.60^a	6.94^b	6.59^c	5.25^d	0.034
Phenylalanine	3.72^d	4.17^a	4.08^b	3.81^c	0.018
NEAA					
Glutamic acid	11.96^c	12.87^a	12.72^b	11.50^d	0.032
Serine	3.08^c	3.59^a	3.43^b	3.00^c	0.034
Aspartic acid	7.78^{ab}	7.90^a	7.87^a	7.69^b	0.046
Proline	3.04^c	3.25^a	3.14^b	2.84^d	0.020
Glycine	3.16^c	3.32^a	3.25^b	3.08^d	0.021
Alanine	3.79^c	3.98^a	3.90^b	3.79^c	0.011
Cysteine	1.09^b	1.15^a	1.09^b	1.09^b	0.007
Tyrosine	2.41^b	2.75^a	2.75	2.41^b	0.020

[abcd] Means with different superscripts cross the groups differed significantly (P<0.05)
EAA - Essential amino acid, NEAA - Non- essential amino acid

Table 2. Mean amino acid profile of fermented *Delonix regia* seeds fed *H. bidorsalis* at different inclusion levels of diet

Amino acid (g/100 g Protein)	0% (Control)	$FFSM_1$	$FFSM_2$	$FFSM_3$	SEM
EAA					
Lysine	4.34^d	4.85^a	4.77^b	4.67^c	0.014
Histidine	2.23^a	2.30^a	2.30^a	2.30^a	0.032
Arginine	6.10^c	6.88^a	6.79^a	6.62^b	0.038
Threonine	2.99^b	3.19^a	3.16^a	3.19^a	0.016
Valine	3.80^c	4.24^a	4.18^a	4.09^b	0.019
Methionine	2.29^a	2.21^b	2.21^b	2.21^b	0.017
Isoleucine	2.94^c	3.30^a	3.21^b	3.21^b	0.023
Leucine	9.60^a	7.79^b	7.50^c	7.18^d	0.043
Phenylalanine	3.72^c	4.25^a	4.25^a	3.99^b	0.017
NEAA					
Glutamic acid	11.96^d	13.47^a	13.32^b	13.17^c	0.015
Serine	3.08^b	3.81^a	3.81^a	3.78^a	0.023
Aspartic acid	7.78^c	8.18^a	8.09^b	8.06^b	0.016
Proline	3.04^b	3.25^a	3.25^a	3.25^a	0.018
Glycine	3.16^b	3.37^a	3.35^a	3.42^a	0.020
Alanine	3.79^c	4.21^a	4.09^b	4.17^a	0.019
Cysteine	1.09^c	1.21^b	1.27^a	0.97^d	0.016
Tyrosine	2.41^c	3.09^a	2.92^b	2.93^b	0.017

[abcd] Means with different superscripts across the groups differed significantly (P<0.05)
EAA - Essential amino acid; NEAA - Non- essential amino acid

$FFSM_1$, $FFSM_2$ and $FFSM_3$ recorded higher concentration of threonine which differs significantly from the control group (2.99g/100g Protein). $FFSM_1$ and $FFSM_2$ were statistically higher (4.24 and 4.1g/100g Protein) than $FFSM_3$ (4.09g/100g Protein) and the control (3.80g/100g protein) for valine concentration. $FFSM_1$ had the highest concentration (3.30g/100g Protein) of isoleucine which was statistically different ($P < 0.05$) from $FFSM_2$ (3.21g/100g Protein), $FFSM_3$ (3.21g/100g Protein) and the control treatment (2.94g/100g Protein). Leucine was highest (9.60g/100g Protein) in the control which differs statistically ($P < 0.05$) from other dietary treatments. $FFSM_1$ and $FFSM_2$ recorded the highest concentration (4.25g/100g Protein) of phenylalanine which was statistically different ($P < 0.05$) from $FFSM_3$ (3.99g/100g Protein) and the control diets (3.72g/100g Protein). All the non-essential amino acids (glutamic acid; serine, aspartic acid, proline, glycine, alanine cysteine and tyrosine) were statistically different ($P < 0.05$) across the dietary treatments. $FFSM_1$ had the highest levels (13.47g/100g Protein) of glutamic acid which was statistically different ($P < 0.05$) from $FFSM_2$ (13.32g/100g Protein), $FFSM_3$ (13.17g/100g Protein) and the control (11.96g/100g Protein). $FFSM_1$, $FFSM_2$ and $FFSM_3$ were statistically higher ($P < 0.05$) than the control group (3.08g/100g Protein) in serine. $FFSM_1$ had the highest level of aspartic acid (8.18g/100g Protein) which was statistically different ($P < 0.05$) from $FFSM_2$ (8.09g/100g Protein) and $FFSM_3$ (8.06g/100g Protein) which were similar though higher than the control group (7.78g/100g Protein). Proline and glycine recorded a similar trend for $FFSM_1$, $FFSM_2$ and $FFSM_3$ respectively and were statistically different ($P < 0.05$) from the control group. $FFSM_1$ and $FFSM_3$ had the highest levels of alamine (4.2g and 4.17g/100g Protein) and were statistically different ($P < 0.05$) from $FFSM_2$ (4.0g/100g Protein) and the control group (3.79g/100g Protein). $FFSM_2$ had the highest cysteine concentration (1.27g/100g Protein) which differs statistically from control (1.09g/100g Protein), $FFSM_1$ (1.21g/100g Protein) and $FFSM_3$ (0.97g/100g Protein). Tyrosine level was higher in $FFSM_1$ (3.09g/100g Protein) which differs significantly ($P < 0.05$) across the dietary treatments. Amino acid profile of diets fed *H. bidorsalis* at different inclusion levels of cooked *Delonix regia* seeds are shown in Table 3. Essential amino acids concentration were statistically different ($P < 0.05$) across the dietary treatments. $CFSM_1$ had the highest lysine content while the least was recorded in the control diet (4.34g/100g Protein). $CFSM_1$ and $CFSM_2$ were statistically similar ($P > 0.05$) in the concentration of histidine but were significantly different ($P < 0.05$) from $CFSM_3$ and the control. $CFSM_1$ had the highest arginine content

Table 3. Mean amino acid profile of cooked *Delonix regia* seeds fed *H. bidorsalis* at different inclusion levels of diet

Amino acid (g/100 g Protein)	0% (Control)	$CFSM_1$	$CFSM_2$	$CFSM_3$	SEM
EAA					
Lysine	4.34^{c}	5.17^{a}	5.04^{b}	5.09^{b}	0.016
Histidine	2.23^{b}	2.36^{a}	2.23^{a}	2.27^{b}	0.017
Arginine	6.10^{d}	7.31^{a}	7.14^{b}	6.54^{c}	0.036
Threonine	2.99^{c}	3.24^{b}	3.22^{b}	3.36^{a}	0.017
Valine	3.80^{c}	4.44^{a}	4.30^{b}	4.30^{b}	0.035
Methionine	2.29^{a}	2.21^{b}	2.19^{b}	2.24^{ab}	0.020
Isoleucine	2.94^{b}	3.40^{a}	3.34^{a}	3.30^{a}	0.032
Leucine	9.60^{a}	8.29^{b}	8.08^{c}	7.21^{d}	0.033
Phenylalanine	3.72^{d}	4.43^{a}	4.25^{b}	4.08^{c}	0.017
NEAA					
Glutamic acid	11.96^{d}	14.31^{a}	14.23^{b}	13.32^{c}	0.020
Serine	3.08^{c}	4.10^{a}	3.89^{b}	3.08^{c}	0.023
Aspartic acid	7.78^{c}	8.50^{a}	8.40^{a}	8.00^{b}	0.032
Proline	3.04^{c}	3.35^{a}	3.35^{a}	3.25^{b}	0.022
Glycine	3.16^{c}	3.56^{a}	3.39^{b}	3.34^{b}	0.021
Alanine	3.79^{c}	4.47^{a}	4.28^{b}	4.28^{b}	0.038
Cysteine	1.09^{b}	1.21^{ab}	1.27^{a}	0.90^{c}	0.040
Tyrosine	2.41^{c}	3.09^{a}	3.09^{a}	2.75^{b}	0.023

^{abcd} Means with different superscripts across the groups differed significantly (P<0.05)
EAA- Essential amino acid, NEAA- Non- essential amino acid

(7.31g/100g Protein) which was significantly different ($P < 0.05$) from $CFSM_1$ (3.24g/100g Protein), $CFSM_2$ (3.22g/100g Protein). Control had the highest leucine concentration (9.60g/100g Protein) which differs significantly ($P < 0.05$) among the treatments. $CFSM_1$ had a higher level of phenylalanine content (4.43g/100g Protein) than the control (3.72g/100g Protein), $CFSM_2$ (4.25g/100g Protein) and $CFSM_3$ (4.08g/100g Protein) respectively. Nonessential amino acids (glutamic acid, serine, aspartic acid, proline, glycine, alanine, cysteine and tyrosine) differs significantly ($P < 0.05$) among the treatments $CFSM_1$ recorded the highest concentration (14.31g/100g Protein) of glutamic acid which differs significantly ($P < 0.05$) from $CFSM_2$ (14.23g/100g Protein), $CFSM_3$ (13.32g/100g Protein). Serine had the highest concentration (4.10g/100g Protein) in $CFSM_1$ which was statistically different ($P < 0.05$) from other dietary treatments. $CFSM_1$ and $CFSM_2$ recorded the highest concentration of aspartic acid, proline and tyrosine which were statistically different ($P < 0.05$) from $CFSM_3$ and the control group. Glycine (3.5g/100g Protein) and alanine (4.47g/100g Protein) levels were highest in $CFSM_1$ which was statistically different from $CFSM_2$, $CFSM_3$ and the control.

$CFSM_2$ and $CFSM_3$ were statistically similar ($P > 0.05$) for glycine and alanine, though significantly different from the control. $CFSM_1$ and $CFSM_2$ recorded significantly ($P < 0.05$) the highest concentration of cysteine (1.21g/100g Protein and 1.27g/100g Protein) which were statistically different ($P < 0.05$) from control (1.09g/100g Protein) and $CFSM_3$ (0.90g/100g Protein).

4. DISCUSSION

The raw, cooked and fermented *Delonix regia* seeds were rich sources of essential amino acids which make it a useful supplement for cereal grains which are generally low in these amino acids [9]. The lower level of lysine (4.19- 5.17 g/100 g cp) as compared to the reports of several researchers [9] may be due to reaction with oxidized lipids. Highest digestibility of amino acids observed in cooking as a processing method in this study could be linked to breaking down of the proteinaceous toxins such as typsin inhibitors and haemagglutinin and downregulation of sulphur-containing compounds which enhance the high digestibility of amino acids. All the range observed in this study for both essential and non- essential amino acids in *Delonix regia* seeds were higher than the minimum recommended levels of [10] for amino acids in diets. The range of proline contents (2.84 – 3.35g/100g cp) in the analyzed samples of *Delonix regia* seed meal is notably lower than the values reported in the literature (4.02 g/g cp) [11]. The increase in amino acid content in the fermented *Delonix regia* seed meal as compared to the raw *Delonix regia* seed meal could be linked to the higher ability to hydrolyze the antinutritional components during fermentation which will then allow more release of amino acids and could be used to supplement conventional feedstuff for livestock [12].

5. CONCLUSION

Essential and non-essential amino acid concentrations were higher in the cooked *Delonix regia* seeds than the fermented *Delonix regia* seeds. Cooking of *Delonix regia* seeds at 10% inclusion levels had the highest activities of essential and non-essential amino acids.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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