

4 **EFFECTS OF DIFFERENT PROCESSING METHODS OF *D.regia* SEEDS**
5 **ON AMINO ACID COMPOSITION OF EXPERIMENTAL DIETS FED**
6 ***Heterobranchus bidorsalis* FISH**

7
8 **Abstract**

9 This study investigated the effects of different processing methods of *Delonix regia* seeds on amino acids
10 composition of experimental diets fed *H.bidorsalis* . Ten isonitrogenous diets (40% crude protein) were
11 formulated with cooked, raw, and fermented *Delonix regia* seeds at 0% (Control), 10%, 20% and 30%
12 inclusion levels respectively. Data was analysed using Analysis of Variance, significant differences in
13 means were separated using Duncan Multiple Range Test. All the essential amino acids (lysine,
14 arginine, threonine, valine, methionine, isoleucine, leucine and phenylalanine) differs
15 significantly at different inclusion levels among the treatments with the exception of histidine
16 which was statistically similar ($P > 0.05$) across the dietary treatments. The activity of essential and
17 non- essential amino acid concentration was higher in cooked than the fermented and raw *Delonix regia*
18 seeds. It was concluded that cooked *Delonix regia* seeds at 10% inclusion levels had the highest
19 activities of essential and non-essential amino acids.

20 **Key words: Amino acids, *Delonix regia*, fermentation, cooking**
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24 INTRODUCTION

25 Fish feeds constitutes 60-75% of total cost of aquaculture production (Oyegbile, 2018; Yola and
26 Onuora, 2012) which is expensive and has led to studies on how to reduce the high cost of fish
27 feed manufacturing using alternative feed ingredients (Benitez, 1989). Generally, the ingredients
28 that are essential for fish feed formulation are proteins and amino acids, lipids, carbohydrates,
29 vitamins and minerals. In addition, preservatives and attractants are also incorporated into fish
30 feeds to extend shelf life and enhance feed palatability respectively (Robinson et al., 2006).
31 Protein is the major concern during formulation of fish feed. It is the most expensive components
32 in fish feed and the important factors contributing to the growth performance of cultured species
33 (Deng et al., 2011). It is the basic nutrient needed in fish feed ingredients (Ayinla, 1991). Protein
34 requirement in fish diet can be related with the general energy requirement of the fish at certain
35 water temperature and the ability to gain weight at present capacity (Findley and Ludin, 1980).
36 The quality of a feed protein depends not only on nitrogen content, but also on constituent amino
37 acids and their digestibility (Benitez, 1989). Diet formulation based on digestible amino acids
38 will allow the use of alternative protein sources with low digestibility coefficients, because such
39 formulation will improve the precision of least-cost diets and reduce nitrogen excretion from
40 livestock operations (Fagbenro, 1998). Although the advantages of the digestible amino acid are
41 recognized, diet formulation based on the total amino acid content is still widely used in many
42 parts of the world. In the future, however, economic reasons will compel the fish industry to
43 increase the use of an array of cheaper, alternative protein supplements with low digestibility
44 coefficients in feed formulation. Amino acids are the substance derived from the ultimate
45 product of digestion. There are about twenty naturally available amino acids which includes
46 arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, protein, threonine,

47 alanine, aspartic acid, asparagine, cystine, glutamic acids, glutamine, proline, tryptophan, valine,
48 serine, tyrosine (Hardy, 1990) but only the first ten are essential for fish growth because they are
49 not synthesized in fish and must be provided in the feed, because of this they are called “essential
50 or indispensable” amino acids (Hardy, 1990). A particularly important amino acid for growth is
51 lysine. Protein rich in lysine can be obtained from legume, fish meal, blood meal, or meat and
52 bone meal. Tryptophan is very important for growth of all cells and normal development, it is
53 used in cell profile ration. Arginine and histidine play an important role in maintaining a normal
54 and healthy bloodstream and has other complex functions (NRC, 1993). Fish and other animals
55 are able to synthesize their non-essential amino acids from carbohydrates and lipids and other
56 nitrogen compounds but mainly from other non-essential amino acids. Any diet deficient in any
57 of the essential amino acid will cause depressed appetite and growth rate of fish (Hardy, 1990).
58 Therefore, this study is designed to evaluate the effect of different processing methods of
59 *Delonix regia* seeds on the amino acid composition of diets fed *H.bidorsalis* fish.

60 **MATERIALS AND METHODS**

61 The experiment was conducted at the aquaculture production technology unit of the Skill
62 Acquisition and Development Centre, NAERLS, Ahmadu Bello University, Zaria, located at
63 latitude $11^{\circ} 09' 45.2''$ N and longitude $7^{\circ} 38' 17.9''$ E. The experiment was conducted from
64 September 2015 to March 2016.

65 **Collection of *Delonix regia* (Flamboyant) Seeds**

66 Matured and dry pods of *Delonix regia* (flamboyant) containing the seeds were collected from
67 the annex campus of Nuhu Bamalli Polytechnic Zaria. The seeds were collected by opening the
68 pods manually. The average seeds per pod was between 30-37, weight of 100 seeds was 42.5g.
69 The collected seeds were hand picked for selection of healthy seeds.

70 **Processing of Seeds**

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

73 **Fermentation of *Delonix regia* (flamboyant) Seeds**

74 The seeds were soaked in water for 12 hours. The drained soaked seeds were allowed to ferment
75 naturally by tying in polythene bag and kept in a dark cupboard for 72 hours without the addition
76 of yeast (Udensi and Okonkwo, 2006). The fermented seeds were allowed to dry for two days
77 before grinding into homogenous powder using a hammer mill.

78 **Cooking of *Delonix regia* (flamboyant) Seeds.**

79 The seeds were boiled to 100 °C for 80 minutes and were allowed to cool and dried for two days
80 and later ground to homogenous powder using a hammer mill (Bake et al., 2013).

81 **Raw *Delonix regia* (flamboyant) Seeds**

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

84 **Analysis of Differently Processed *Delonix Regia* (Flamboyant) Seeds**

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

88 **Determination of Amino Acid Profile of Raw and Processed *Delonix regia* (flamboyant)**
89 **Seeds**

90 The amino acid profile in the known sample was determined using the methods described by
91 Benitez (1989). The known sample was dried to constant weight, defatted, hydrolysed,

92 evaporated in a rotary evaporator and loaded into the Technicon Sequential Multi-sample Amino
93 Acid Analyser (TSM).

94 **Defatting sample**

95 The sample was defatted using chloroform/methanol mixture of ratio 2:1. 4g of the sample was
96 put in extraction thimble and extracted for 15 hours in soxhlet extraction apparatus (Balogun et
97 al., 2001).

98 **Nitrogen determination**

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

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

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

$$111 \text{ Percentage Nitrogen} = \frac{(a-b) \times 0.01 \times 14 \times V \times 100}{W \times C}$$

112 Where;

113 a= Titre value of the digested sample

114 b= Titre value of blank sample

115 v= Volume after dilution (100ml)

116 W= Weight of dried sample (mg)

117 C= Aliquot of the sample used (10ml)

118 14= Nitrogen constant in mg

119 **Hydrolysis of the Sample**

120 A known weight of the defatted sample was weighed into glass ampoule. Seven (7) ml of 6NHCl
121 was added and oxygen was expelled by passing nitrogen into the ampoule . This is to avoid
122 possible oxidation of some amino acids during hydrolysis (e.g. methionine and cysteine). The
123 glass ampoule was then sealed with Bunsen burner flame and put in an oven preset at $105^{\circ}\text{C} \pm 5$
124 $^{\circ}\text{C}$ for 22 hours. The ampoule was allowed to cool before broken open at the tip and the content
125 was filtered to remove the humins. It should be noted that the tryptophan is destroyed by 6NHCl
126 during hydrolysis. The filtrate was then evaporated to dryness in hot air oven. The residue was
127 dissolved with 5ml to acetate buffer (pH 2.0) and stored in plastic specimen bottles which were
128 kept in the freezer.

129 **Loading of the hydrolysate into TSM analyzer**

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

133

134

135 **Method of calculating Amino Acid Concentration**

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

139 **Statistical Analysis**

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

143 **1. RESULTS AND DISCUSSION**

144 Table 1 shows the amino acid profile of diets fed *H. bidorsalis* at different inclusion levels of
145 raw *Delonix regia* seeds. All the essential amino acids (lysine, arginine, threonine, valine,
146 methionine, isoleucine, leucine and phenylalanine) differs significantly at different inclusion
147 levels among the treatments with the exception of histidine which was statistically similar ($P >$
148 0.05) across the dietary treatments. Lysine had the highest concentration (4.53 g/100g protein) in
149 RFSM₁, while the least concentration (4.19g/100g protein) was observed in RFSM₃. RFSM₁ and
150 RFSM₂ recorded the highest arginine content (6.53g/100g Protein and 6.45 g/100 g Protein) and
151 were statistically similar ($P > 0.05$) but differs significantly ($P < 0.05$) from the control
152 (6.10g/100g Protein) and RFSM₃ (5.16g/100g Protein). Threonine and valine recorded similar
153 statistical trend with arginine. Methionine and leucine had the highest concentration in the
154 control group (2.29g/100g Protein and 9.60g/100g Protein) which was statistically different from
155 other treatments. RFSM₁ had the highest concentration of isoleucine (3.14g/100g Protein) and
156 phenylalanine (4.17g/100g Protein) which differs statistically ($P < 0.05$) from other treatments.

157 Non-essential amino acid (Glutamic acid, serine, aspartic acid, proline, glycine, alanine, cystine
158 and tyrosine) were statistically different ($P < 0.05$) among the treatments. RFSM₁, had the
159 highest concentration of glutamic acid (12.87g/100g Protein) while the least concentration was
160 observed in RFSM₃ (11.50g/100g Protein). RFSM₁ had the highest value for serine (3.59g/100g
161 Protein), RFSM₂ was intermediate (3.43g/100g Protein) while the control (3.08g/100g Protein)
162 and RFSM₃ recorded the lowest concentration (3.00g/100g Protein) and were statistically similar
163 ($P > 0.05$). Aspartic acid was statistically different ($P < 0.05$) among the treatments with the
164 exception of RFSM₃ (7.69g/100g Protein). RFSM₁ had the highest levels of proline (3.25g/100g
165 Protein) and glycine content (3.32g/100g Protein) which differs significantly ($P < 0.05$) from
166 RFSM₂ (3.14g/100g Protein and 3.25g/100g Protein), control (3.04g/100g Protein and 3.16 g/100
167 g Protein) respectively. RFSM₁ had the highest concentration for alanine (3.98g/100g Protein)
168 which differs significantly ($P < 0.05$) from RFSM₂ (3.90g/100g Protein), control and RFSM₃
169 respectively, though the control and RFSM₃ were similar ($P > 0.05$). RFSM₁ had the highest
170 level of cysteine (1.15g/100g Protein) which differs significantly from the control, RFSM₂ and
171 RFSM₃ respectively. Tyrosine concentration was highest in RFSM₁ (2.75g/100g Protein) and
172 RFSM₂ (2.75g/100g Protein) which differs statistically from control (2.41g/100g Protein) and
173 RFSM₃ (2.41g/100g Protein) which were both statistically similar ($P > 0.05$).

174 Table 2 shows the amino acid profile of diets fed *H. bidorsalis* at different inclusion levels of
175 fermented *Delonix regia* seeds. All the essential amino acids were statistically different ($P <$
176 0.05) among the treatment for lysine, arginine, threonine, valine, isoleucine, leucine and
177 phenylalanine with the exception of histidine and methionine. FFSM₁ had the highest lysine
178 content (4.85g/100g Protein), followed by FFSM₂ (4.77g/100g Protein), FFSM₃ (4.67g/100g
179 Protein) and the control group (4.37g/100g Protein). FFSM₁ and FFSM₂ had the highest levels of

180 arginine (6.88 and 6.7g/100g Protein) while the control group recorded the least level (6.10
181 g/100g Protein).

182 FFMS₁, FFMS₂ and FFMS₃ recorded higher concentration of threonine which differs
183 significantly from the control group (2.99g/100g Protein). FFMS₁ and FFMS₂ were statistically
184 higher (4.24 and 4.1g/100g Protein) than FFMS₃ (4.09g/100g Protein) and the control
185 (3.80g/100g protein) for valine concentration. FFMS₁ had the highest concentration (3.30 g/100g
186 Protein) of isoleucine which was statistically different ($P < 0.05$) from FFMS₂ (3.21 g/100g
187 Protein), FFMS₃ (3.21g/100g Protein) and the control treatment (2.94g/100g Protein). Leucine
188 was highest (9.60g/100g Protein) in the control which differs statistically ($P < 0.05$) from other
189 dietary treatments. FFMS₁ and FFMS₂ recorded the highest concentration (4.25g/100 g Protein)
190 of phenylalanine which was statistically different ($P < 0.05$) from FFMS₃ (3.99g/100 g Protein)
191 and the control diets (3.72g/100 g Protein). All the non-essential amino acids (glutamic acid;
192 serine, aspartic acid, proline, glycine, alanine cysteine and tyrosine) were statistically different (P
193 < 0.05) across the dietary treatments. FFMS₁ had the highest levels (13.47g/100g Protein) of
194 glutamic acid which was statistically different ($P < 0.05$) from FFMS₂ (13.32g/100g Protein),
195 FFMS₃ (13.17g/100g Protein) and the control (11.96g/100g Protein). FFMS₁, FFMS₂ and FFMS₃
196 were statistically higher ($P < 0.05$) than the control group (3.08g/100g Protein) in serine. FFMS₁
197 had the highest level of aspartic acid (8.18g/100g Protein) which was statistically different ($P <$
198 0.05) from FFMS₂ (8.09g/100g Protein) and FFMS₃ (8.06g/100g Protein) which were similar
199 though higher than the control group (7.78g/100g Protein). Proline and glycine recorded similar
200 trend for FFMS₁, FFMS₂ and FFMS₃ respectively and were statistically different ($P < 0.05$) from
201 the control group. FFMS₁ and FFMS₃ had the highest levels of alamine (4.2g and 4.17g/100g
202 Protein) and were statistically different ($P < 0.05$) from FFMS₂ (4.0g/100g Protein) and the

203 control group (3.79g/100g Protein). FFMS₂ had the highest cysteine concentration (1.27g/100g
204 Protein) which differs statistically from control (1.09g/100g Protein), FFMS₁ (1.21g/100g
205 Protein) and FFMS₃ (0.97g/100g Protein). Tyrosine level was higher in FFMS₁ (3.09g/100g
206 Protein) which differs significantly ($P < 0.05$) across the dietary treatments. Amino acid profile
207 of diets fed *H. bidorsalis* at different inclusion levels of cooked *Delonix regia* seeds are shown in
208 Table 3. Essential amino acids concentration were statistically different ($P < 0.05$) across the
209 dietary treatments. CFMS₁ had the highest lysine content while the least was recorded in the
210 control diet (4.34g/100g Protein). CFMS₁ and CFMS₂ were statistically similar ($P > 0.05$) in the
211 concentration of histidine but were significantly different ($P < 0.05$) from CFMS₃ and the
212 control. CFMS₁ had the highest arginine content (7.31g/100g Protein) which was significantly
213 different ($P < 0.05$) from CFMS₁ (3.24g/100g Protein), CFMS₂ (3.22g/100g Protein). Control
214 had the highest leucine concentration (9.60g/100g Protein) which differs significantly ($P < 0.05$)
215 among the treatments. CFMS₁ had higher level of phenylalanine content (4.43g/100g Protein)
216 than the control (3.72g/100g Protein), CFMS₂ (4.25 g/100g Protein) and CFMS₃ (4.08g/100g
217 Protein) respectively. Nonessential amino acids (glutamic acid, serine, aspartic acid, proline,
218 glycine, alanine, cysteine and tyrosine) differs significantly ($P < 0.05$) among the treatments
219 CFMS₁ recorded the highest concentration (14.31 g/100g Protein) of glutamic acid which differs
220 significantly ($P < 0.05$) from CFMS₂ (14.23g/100 g Protein), CFMS₃ (13.32g/100g Protein).
221 Serine had the highest concentration (4.10g/100g Protein) in CFMS₁ which was statistically
222 different ($P < 0.05$) from other dietary treatments. CFMS₁ and CFMS₂ recorded the highest
223 concentration of aspartic acid, proline and tyrosine which were statistically different ($P < 0.05$)
224 from CFMS₃ and the control group. Glycine (3.5 g/100g Protein) and alanine (4.47g/100g

225 Protein) levels were highest in CFMS₁ which was statistically different from CFMS₂, CFMS₃
 226 and the control.

227 **Table 1: Mean Amino Acid Profile of Raw *Delonix regia* Seeds Fed *H. bidorsalis* at**
 228 **Different Inclusion Levels of Diet**

Amino acid (g/100gProtein)	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

229 ^{abcd} Means with different superscripts cross the groups differed significantly (P<0.05).

230 EAA - Essential amino acid
 231 NEAA - Non- essential amino acid
 232

233 **Table 2: Mean Amino Acid Profile of Fermented *Delonix regia* Seeds Fed *H. bidorsalis* at**
 234 **Different Inclusion Levels of Diet**

Amino acid (g/100gProtein)	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

235 ^{abcd} Means with different superscripts across the groups differed significantly ($P < 0.05$).

236 EAA - Essential amino acid

237 NEAA - Non- essential amino acid

238

UNDER PEER REVIEW

239 **Table 3: Mean Amino Acid Profile of Cooked *Delonix regia* Seeds Fed *H. bidorsalis* at**
 240 **Different Inclusion Levels of Diet**

Amino acid (g/100gProtein)	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

241 ^{abcd} Means with different superscripts across the groups differed significantly (P<0.05).

242 EAA - Essential amino acid

243 NEAA - Non- essential amino acid

244

245 CFMS₂ and CFMS₃ were statistically similar ($P > 0.05$) for glycine and alanine, though
246 significantly different from the control. CFMS₁ and CFMS₂ recorded significantly ($P < 0.05$) the
247 highest concentration of cysteine (1.21g/100g Protein and 1.27g/100g Protein) which were
248 statistically different ($P < 0.05$) from control (1.09 g/100g Protein) and CFMS₃ (0.90g/100g
249 Protein). The raw, cooked and fermented *Delonix regia* seeds were rich sources of essential
250 amino acids which make it a useful supplement for cereal grains which are generally low in these
251 amino acids (Iyayi and Taiwo, 2003). The lower level of lysine (4.19- 5.17 g/100g cp) as
252 compared to report of several researchers (Balogun et al., 2001) maybe due to reaction with
253 oxidized lipids (Findley and Ludin, 1980). Highest digestibility of amino acids observed in
254 cooking as a processing method in this study could be linked to break down of the proteinaceous
255 toxins such as tpsin inhibitors and haemagglutinins (Liener, 1980) and down regulation of
256 sulphur containing compounds which enhance the high digestibility of amino acids. All the range
257 observed in this study for both essential and non- essential amino acids in *Delonix regia* seeds
258 were higher than the minimum recommended levels of (NRC, 1993) for amino acids in diets.
259 The range of proline contents (2.84 – 3.35g/100g cp) in the analyzed samples of *Delonix regia*
260 seed meal are notably lower than the values reported in literature (4.02 g/g cp). The increase in
261 amino acid content in the fermented *Delonix regia* seed meal as compared to the raw *Delonix*
262 *regia* seed meal could be linked to the higher ability of hydrolyzing the antinutritional
263 components during fermentation which will then allow more release of amino acids.

264 CONCLUSION

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

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