Nutritional Evaluation and Effect of varying fortified feeds of *Ziziphus mauritina* fruit (mesocarp) on Growth performance of albino rats.

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**ABSTRACT:** This study evaluated the nutritional value of *ziziphus mauritina* fruit (mesocarp) and its effect on the growth of experimental animals. Proximate analysis of the mesocarp was carried out using standard methods. The fat content were analysed using GCMS, carbohydrate content were analysed using carbohydrate Digestibility assay and the protein content were determined using amino acids profile analysis. The mesocarp of *Ziziphus mauritiana* was dried and added to standard commercial feed (mesocarp: commercial feed) at 0% (0:100), 20% (20:80), 30% (30:70), 40% (40:60) and 50% (50:50) respectively and were fed to albino rats to evaluate the effects on growth performance of the animal. The results revealed the presence of carbohydrate (55.00 ± 0.24%), crude fat (5.13 ± 0.02%), crude protein (17.50 ± 0.32%), crude firbre (11.40 ± 0.04%), ash content (6.27 ± 0.31%) and moisture (4.70 ± 0.25%). The amino acids profile reveals the presence of essential amino acids with leucine (5.60 g per 100 g sample) as the most abundant and tryptophan (0.74g/100g sample) as the least concentrated while the non essential amino acids present in the sample reveals cystine as the lowest (1.09 g per 100 g sample) and glutamic acid is the most concentrated and abundant non essential amino acid with 10.00 g per 100 g sample. GCMS analysis of the crude fat reveals the presence of hexanoic acid, hepta-2,4-dienioic acid, hexadecanioic acid(palmitic acid), 9,12-Octadecadienioic acid,(Linolelaaidic acid), 11,14,17-Eicosatrienoic acid, octadecanoic acid (Stearic acid), Di-n-octyl phthalate (1, 2-Benzenedicarboxylic acid, dioctyl ester) while the carbohydrate digestibility assay reveals resistant starch (1.64 mmol/L per 100mg) and digestible starch (18.81 mmol/L/ 100mg) of the sample. The sum of the RS content and DS content gives the total starch of 20.44 mmol/L per 100 mg of the sample. Protein efficiency ratio (PER), feed efficiency ratio and consumption index analyses showed that there is no significant difference in all the blends of the feed samples used. The effect of mesocarp of *ziziphus mauritiana* fruit on growth exhibit low growth performance and this is corroborated by the profiles of the major component of the fruit which consist mainly of non-essential biomolecules which can improve growth.

**KEYWORDS:** *Ziziphus mauritiana*, fruit mesocarp, proximate analysis, growth performance, GCMS.
INTRODUCTION

Plant parts have always been used for food and remedies for disease conditions. Medicinal plants play a significant role in traditional herbal treatments. Despite these wide uses, only a few African medicinal plants have been studied (Lee et al., 2003; Ogle et al., 2003; Adebooye & Opabode, 2004; Ayodele 2005). It is important to find alternative food sources as a result of food shortage and drought especially in rural communities. All organisms must obtain nutrients from their environments to live and these nutrients include organic chemicals, such as carbohydrates, proteins, lipids, and vitamins, and inorganic substances, such as minerals and water. The nutrients are essential for the maintenance of biological functions, including metabolism, growth, and repair (Dongyeop et al., 2015).

*Ziziphus mauritiana* Lam. belongs to the family *Rhamnaceae* (Wunderlin & Hansen 2008). It is called jujube tree or Indian jujube (Morton,1987; Michel, 2002). The plant is commonly known as Magarya in Hausa and Whuya in Kilba (Nigeria) (Dahiru et al., 2005). It is called Chinese apple or Indian Jujube in English (Heuze et al., 2017). The leaves of the plant are used in the treatment of diarrhoea, wounds, abscesses, swelling and gonorrhoea (Michel, 2002). It is also used in the treatment of liver diseases, asthma and fever (Morton, 1987) while the fruit mesocarp are eaten as wild fruit.

The plant is an evergreen shrub or small thorny tree that can grow to 3-15 meters (Heuze et al., 2017), making it a good source of food in arid regions and during drought. The fruit is an ovoid drupe, about 6 cm x 4 cm in size (Heuze et al., 2017). The fruit skin may be smooth or rough, glossy, yellowish to reddish or blackish with white, juicy, slightly acid to sweet, turning mealy when fully ripe (Latiff, 1991). The stone is single central hard, oval with rough surface containing 2 elliptic brown seeds 6mm long (Morton, 1987).

The aim of this study is to evaluate the nutritional value of *Ziziphus mauritiana* fruit mesocarp and to identify possible influence of growth performance due to its intake.

MATERIALS AND METHODS

Plant material and authentication

The fruit of *Ziziphus mauritiana* used in this study were obtained from Wudil community and Rimi market in Kano, Nigeria and authenticated at the Department of Plant Biology, Bayero University Kano, where a voucher (BUKHAN 0233) was allocated and the specimen deposited.

Experimental animals

Healthy male and female albino rats (*Rattus norvergicus*) weighing 50–80 with age ranging from 4–6 weeks obtained from the Small Animal Holding Units of the Department of Physiology, Bayero University Kano were used for the studies. Prior and during the experiments, the animals were kept in well-ventilated house conditions (temperature: 28–31 °C; Photoperiod: 12 hour natural light and 12 hour darkness cycle; Humidity: 50–55%) and the animals were supplied with feed pellets (vita feeds) and tap water was provided *ad libitum*.

Proximate analysis of the fruit (Mesocarp)

Proximate analyses of the fruit samples were evaluated using the standard methods in AOAC, (2003) and Asean manual for food analysis, (2011).
Determination of amino acid profile
The amino acid profile in Z. mauritiana mesocarp was determined using methods described by Benitez (1989). The sample was dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Applied Biosystems 120A PTH Amino Acid Analyzer (model: 120A PTH California, USA).

Determination of fatty acid profile
The GC-MS analysis of oil extract from the fruit mesocarp was performed using a GC-MS QP2010 PLUS Shimadzu, Japan. Interpretation of GC-MS data was done using the database of National Institute of Standard and Technology (NIST). The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST (NARICT) library. The name, molecular weight and structure of the components of the test oil sample were ascertained.

Determination of carbohydrate digestibility
The resistant starch (RS) contents of the samples were determined by the direct method described by Goni et al. (1996).

Experimental diets formulation
The mesocarp of Ziziphus mauritiana fruit sample were dried, pulverized and packed in glass jars and labeled. The experimental diets were formulated using the method described by Alagbaoso et al., (2015) with modification. Standard commercial feed was fortified with the mesocarp of Ziziphus mauritiana fruit sample.

Statistical analysis
The data were analysed using Graphpad Instat version 3.05. The mean and standard error of means (SEM) of the triplicate analyses of the samples were calculated. The analysis of variance (ANOVA) was performed to determine significant differences between the means of proximate composition, carbohydrate profile, amino acid compositions, and growth performance parameters; while the means were separated using the Turkey-kraemar range test at p<0.05.

RESULTS AND DISCUSSION
Proximate Analysis of the Fruit (Mesocarp)
Food analysis is the determination of the component of food into its parts most especially the major components of food (Aja et al., 2015; Onwuka, 2005). These components are moisture, ash, crude fat, crude protein, crude fiber and carbohydrate. Moisture content of food sample is an index of its water activity and it can be used to measure its stability and susceptibility to microbial contamination (Uyoh et al., 2013). The moisture content of Z. mauritiana fruit (mesocarp) determined in this study (Table 1) is lower than those of other species of the same genus (Owolarafe et al., 2016, Heba et al., 2011). Hence it may have high stability and low susceptible to microbial contamination that can prolong their shelf life (Aruah et al., 2012).

In living system proteins are necessary for growth and replacement of lost tissues, and their efficiency in providing these basic functions largely dependent on the quality of the protein (Monteiro et al., 2014). The quality of a dietary protein can be described in terms of its percentage content of certain essential amino acids relative to some standard dietary protein in appropriate amount (Monteiro et al., 2014). The
protein content of *Ziziphus mauritiana* fruit mesocarp (Table 1) is significantly higher than some wild fruits reported by Locket *et al.*, (2000). A diet is nutritionally satisfactory if it contains high caloric value and a sufficient amount of protein (Nwofia *et al.*, 2012) and the protein content of *Z. mauritiana* is higher than protein content in pulp of monkey orange (*S. innocula*) reported by Hassan *et al.*, (2014), *ziziphus spina-christi* fruit reported by Heba *et al.*, (2011).

### Table 1: Proximate composition of *Ziziphus mauritiana* (Magariya) fruit mesocarp

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>% Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>4.70 ± 0.25</td>
</tr>
<tr>
<td>Ash content</td>
<td>6.27 ± 0.31</td>
</tr>
<tr>
<td>Crude Protein</td>
<td>17.50 ± 0.32</td>
</tr>
<tr>
<td>Crude Fat</td>
<td>5.13 ± 0.02</td>
</tr>
<tr>
<td>Crude Fibre</td>
<td>11.40 ± 0.04</td>
</tr>
<tr>
<td>Carbohydrate content</td>
<td>55.00 ± 0.24</td>
</tr>
</tbody>
</table>

*Mean and standard deviation. Readings were taken in triplicates.

### Table 2: Carbohydrate profile of mesocarp of *ziziphus Mauritiana* fruit

<table>
<thead>
<tr>
<th>Carbohydrate digestibility Parameters</th>
<th>Concentration (per 100mg sample (mmol/L))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digestible Starch</td>
<td>18.81 ± 2.46</td>
</tr>
<tr>
<td>Resistant Starch</td>
<td>1.64 ± 0.29</td>
</tr>
<tr>
<td>Total Starch</td>
<td>20.44 ± 2.29</td>
</tr>
</tbody>
</table>

Readings are in triplicates. Values are shown as mean and standard error of mean.

One of the functions of dietary fat is to improve palatability of food by absorbing and retaining flavours (Anita *et al.*, 2006) and a diet that supplies 1–2% of calorie value food intake is reported to be sufficient for humans (Aruah *et al.*, 2011). Excess consumption of fat has been reported to cause certain cardiovascular disorders such as atherosclerosis and aging. The crude fat in the *Z. mauritiana* (Table 1) is lower than the amount reported by Locket *et al.*, (2000) but higher than the value reported for *Ziziphus spina-christi* fruit by Heba *et al.*, (2011). This may be due to differences in species and geographical location and may be an indication that *Z. mauritiana* contain low fat content and its consumption may have less or no effect on the health risk mentioned above.
Crude fibre promotes the growth of beneficial intestinal micro-flora and accelerates ulcer-healing process by increasing the secretion of gastric mucus which protect the gastric mucosa of the lining of the intestine (Achoba et al., 1992, Monterio et al., 2014). In addition, it is known that high intake of fibre reduce the risk of colon cancer (Dawczynski et al., 2007). The crude fibre content of the fruit mesocarp of Z. mauritiana (Table 1) is relatively lower than Z. spina-christi fruit (Hebbas et al., 2011; Owolarafe et al., 2016). Hence, Z. mauritiana may not improve the repression of colon cancer, absorption processes in the large intestine, and stimulation of peristalsis. Hence, its consumption in large amounts may lead to constipation (Ogungbenle & Omosola, 2015). There is also the possibility that it may bind some essential trace elements leading to deficiency of some minerals such as iron and zinc (Ekwumemgba et al., 2014).

The ash content of food sample gives an insight into the mineral composition and is an index to measure nutritionally important mineral content of the food sample (Achoba et al., 1992) Nnamani et al., 2009). The ash content of about 6% of the sample of Z. mauritiana mesocarp investigated (Table 1) shows that it compares favourably with S. Innocua (Monkey Seed) (Bello et al., 2008, Hassan et al., 2014). It follows that the mesocarp maybe a good source of minerals which are important in enzymatic reaction and optimum enzyme activity in the body system (Nelson & Cox, 2005).

The carbohydrate content of Z. mauritiana (55%) (Table 1) is comparable to that of Z. spina-christi (77%) (Owolarafe et al., 2016) and 68.75% for Parkia biglobosa pulp (Bello et al., 2008). Hence, Z. mauritiana can be considered a good source of carbohydrate when compared with the carbohydrate content of some conventional sources like cereal which has 72–90% carbohydrate (Elinge, et al., 2012). However the value obtained is lower when compared with what was reported for Z. spina-christi fruit by Heba et al., (2011), this may be due to difference in species and geographical location.

Evidence from various research suggests that dietary fiber consumption is essential for optimal health and well-being (Jenkins et al., 2012; Murphy et al. 2012; Sun et al., 2010; Yao et al., 2014) and concentration of resistant starch in food maybe use as a parameter for the beneficial effect of the starch or carbohydrate present in food sample (Haub et al., 2012). The carbohydrate digestibility assay of Z. mauritiana (Table 2) shows a predominantly digestible starch. Therefore the mesocarp of Z. mauritiana may not be a good source of resistant carbohydrate and large intake may lead to constipation which is one of the disadvantages of food sample with low resistant starch (Office of Disease Prevention and Health Promotion, 2011).

Essential amino acids are very important to biological systems since they are the major contributor to growth and replacement of tissue. Also the quality of protein in a food sample is determined by the presence of essential amino acids (EAs) in the right proportion (Monterio et al., 2014). Leucine functions to counterbalance isoleucine thereby assisting in the regulation of the thymus, spleen, pituitary gland, metabolism, and formation of haemoglobin. Lysine aids the functions of the liver, gallbladder and pineal and mammary glands. Tryptophan, threonine, and valine help in the generation of cells, red and white blood corpuscles, proper functioning of the mammary glands and ovaries (Shaba et al. 2015). Table 3 shows the amino acid composition of the plant extract. The amino acid profile of the protein content in Z. mauritiana mesocarp reveal the presence of isoleucine, threonine, valine, methionine, tryptophan and phenylalanine respectively while major non-essential amino acids are glutamic acid, alanine, aspartic acid, proline and serine, glycine, cystine, tyrosine. Arginine and Histidine are also present and essential for growing children. The concentration of these amino acids do not meet the recommended dietary allowance for children and adolescent however their concentration compared favourably with other fruits reported by (Bello et al., 2008, Hassan et al., 2014).
The presence of essential fatty acids (EFAs) in fat content of a food sample may be used as one of the parameters for evaluating the quality of the food source. Essential fatty acids serves in various processes such as inhibition of the synthesis of pro-inflammatory cytokines, HMG-CoA reductase activities (Kumar and Das, 1994), improve cell membrane fluidity upon its incorporation into the cell membrane and also possess antibacterial, antiviral, and antifungal properties (Das, 2006). The essential fatty acid presence in the oil of *Ziziphus mauritiana* (Table 4) mesocarp are relatively low and may not be a good reservoir of EFAs.
Table 4: Fatty Acid composition of extracted oil of *Ziziphus mauritiana (Magariya)* fruit mesocarp

<table>
<thead>
<tr>
<th>Retention Time</th>
<th>Name of Compounds</th>
<th>Molecular Formula</th>
<th>Molecular weight</th>
<th>Peak Area (%)</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 3.192</td>
<td>Hexanoic acid, Methyl Ester</td>
<td>C(<em>7)H(</em>{14})O(_2)</td>
<td>130</td>
<td>0.94</td>
<td><img src="image1" alt="Structure" /></td>
</tr>
<tr>
<td>2 6.025</td>
<td>Hepta-2,4-dienoic acid, methyl ester</td>
<td>C(<em>8)H(</em>{16})O(_2)</td>
<td>140</td>
<td>3.96</td>
<td><img src="image2" alt="Structure" /></td>
</tr>
<tr>
<td>3 14.585</td>
<td>Hexadecanoic acid, methyl ester (Palmitic acid, methyl ester)</td>
<td>C(<em>{17})H(</em>{34})O(_2)</td>
<td>270</td>
<td>12.17</td>
<td><img src="image3" alt="Structure" /></td>
</tr>
<tr>
<td>4 16.526</td>
<td>9,12-Octadecadienoic acid, methyl ester, (Linolelaic acid, methyl ester)</td>
<td>C(<em>{19})H(</em>{36})O(_2)</td>
<td>294</td>
<td>35.71</td>
<td><img src="image4" alt="Structure" /></td>
</tr>
<tr>
<td>5 16.755</td>
<td>Octadecanoic acid, methyl ester (Stearic acid, methyl ester)</td>
<td>C(<em>{18})H(</em>{36})O(_2)</td>
<td>298</td>
<td>3.24</td>
<td><img src="image5" alt="Structure" /></td>
</tr>
<tr>
<td>6 17.051</td>
<td>11,14,17-Eicosatrienoic acid, methyl ester</td>
<td>C(<em>{21})H(</em>{38})O(_2)</td>
<td>320</td>
<td>4.86</td>
<td><img src="image6" alt="Structure" /></td>
</tr>
<tr>
<td>7 20.401</td>
<td>Di-n-octyl phthalate (1,2-Benzenedicarboxylic acid, diocyl ester) or Phthalic acid, diocyl ester</td>
<td>C(<em>{24})H(</em>{38})O(_4)</td>
<td>390</td>
<td>1.80</td>
<td><img src="image7" alt="Structure" /></td>
</tr>
</tbody>
</table>
The growth performance of the experimental animal model fed with fortified feed of the mesocarp of *Ziziphus mauritiana* fruit (Table 5) shows that the feed efficiency ratio reveals no significant difference among the groups despite an increased feed intake and the protein efficiency ratio which is a factor that determines the digestibility of the protein content of the feed shows no significant difference with the control group. This may be an indication of presence of anti-nutrients (Pearson, 2007) and increase in weight gained by the experimental animals which is of no statistical difference when compared with the control group maybe an indication of inability of the experimental animal to utilize the protein consumed. This also corroborate with low essential amino acid observed in the amino acid profile of the fruit mesocarp.

**Table 5: Growth performance ratio of albino rats fed with Ziziphus mauritiana fruit (mesocarp) and commercial feed.**

<table>
<thead>
<tr>
<th>Feed Intake (g)</th>
<th>Average Feed Intake (g)</th>
<th>Average Weight Gained/week (g)</th>
<th>Growth Rate</th>
<th>Consumption Index</th>
<th>Feed Efficiency Ratio</th>
<th>Protein Efficiency Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.111</td>
<td>277.75 ± 7.49</td>
<td>10.28 ± 1.66</td>
<td>7.63 ± 0.96</td>
<td>0.19 ± 0.02</td>
<td>1.42 ± 0.20</td>
</tr>
<tr>
<td>20% fortified</td>
<td>1.608</td>
<td>402.0 ± 26.52</td>
<td>12.36 ± 4.71</td>
<td>11.46 ± 3.09</td>
<td>0.17 ± 0.06</td>
<td>1.26 ± 0.47</td>
</tr>
<tr>
<td>30% fortified</td>
<td>1.398</td>
<td>349.50 ± 25.38</td>
<td>12.65 ± 5.67</td>
<td>8.29 ± 2.99</td>
<td>0.16 ± 0.08</td>
<td>1.08 ± 0.54</td>
</tr>
<tr>
<td>40% fortified</td>
<td>1.518</td>
<td>379.50 ± 34.52</td>
<td>11.70 ± 4.02</td>
<td>7.99 ± 1.91</td>
<td>0.13 ± 0.04</td>
<td>0.85 ± 0.32</td>
</tr>
<tr>
<td>50% fortified</td>
<td>1.597</td>
<td>399.25 ± 32.43</td>
<td>7.8 ± 3.31</td>
<td>6.26 ± 1.75</td>
<td>0.08 ± 0.03</td>
<td>0.49 ± 0.21</td>
</tr>
</tbody>
</table>

\( n = 5, \overline{X} \pm \text{SEM.} \) \(^{abc}\text{ test values carrying superscripts different from the control across the column for each parameter are significantly different at } p< 0.05\)

The growth pattern reflects the quality of the protein contained in the diet in which the animals are fed (Osagie, 1998). The amino acid composition of the food sample used to fortified feeds show presence of non essential amino acid which is higher than essential amino acid present give insight into the non statistical difference shown in weight gained as the week increases and might be an indication of toxicity due to presence of anti-nutrients that are bound in plant food (Lewu et al, 2007). Therefore the growth recorded in the experiment can be attributed to poor quality of protein in the mesocarp of the fruit studied or maybe due to reduction in protein synthesis because of low amount of essential amino acids in the protein content of the feed which are needed in the physiological process that is entailed in the synthesis of cellular protein (Lewu et al., 2011).

The consumption of the fruit of *Ziziphus mauritiana* mesocarp maybe rampant in the northern Nigeria, its nutritional value may not meet the nutrition need of the populace and care need to be exercise in its consumption to avert it adverse effects which may come along with its intake over a long period.
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