Full Length Research Paper

Effect of extruded soy-cocoa and corn starch based complementary food on some haematological, biochemical and histopathological parameters of rats

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Extruded soy-cocoa and corn-starch based complementary diet with a protein-energy ratio of 21% holds great promise in alleviating malnutrition so prevalent among Nigerian children under five years. There is however, paucity of scientific information on its safety- a necessary prelude to trials on human subjects. This study reports a controlled feeding trial involving 29 adult wistar rats housed in standard cages and acclimatization for 10 days under tropical room temperature conditions. Following a subsequent daily intubate feeding, 50 - 5000 mg/kg body weight for 21 days, sub-acute toxicity studies (anthropometric, biochemical, haematological and histopathological) were done. Results show that the diet had a statistically significant salutary effect on growth (weight gain 56.9%) of test rats when compared with control (37.85) at an optimum daily intake of 100 mg/kg body weight. Haematological characteristics such as mean corpuscular hemoglobin concentration (MCHC) range from 276 - 294 g/l for treatment groups as against 282 g/l for control with no significant difference (P ≤ 0.05). The values (0.56 - 7.74 µ/kat/l) obtained for alkaline phosphate (ALP) - a key biochemical marker in liver function tests were within permissible limits. Moreover, rat biopsy (histopathology) revealed no necrosis. Evidently extruded soy-cocoa corn starch-based complementary food has no established deleterious effect and may therefore be safe for humans.

Key words: Complementary food, extruded soy-cocoa, sub-acute toxicity, food safety, rat biopsy.

INTRODUCTION

The end of the 20th century was marked by drastic increase in the incidence of chemical hazards as well as other safety issues (Motarjemi and Lelieveld, 2013). In a bid to establish the safety or otherwise of raw or processed foods for human consumption, it is customary to test same on animal models which could provide relevant indirect information. New complementary foods meant to provide nutritional support for improving growth and for continued reduction in child morality/morbidity are by no means an exception (SACN/COT, 2012). Against backdrop of research findings such as enlargement of pancreas and inhibited growth of rats fed sub-optimally

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processed soybean foods (Lusas, 2004), theobromine (as in cocoa) induced fetal malformation (Eteng et al., 1997), preclinical experimentation using animals are therefore in order.

Ogazi et al. (1990) in reporting the effects of 'soya musa' (an extruded soybean-plantain baby food) on sixteen wistar rats observed that they had normal growth, packed cell volume and white blood cell counts. No immunological reaction occurred following haemaglutination tests. In evaluating the biological effects of a cassava-soy weaning food on rat organs (small intestines, pancreas, liver and heart), Babajide et al. (2001) saw no significant difference in the organ weight of the rats fed the test diet as compared to those fed cerelact - a commercial weaning food. Rats fed on extruded weaning foods based on peanut, maize and soybean were observed by Plahar et al. (2003) to have between 60-100 fold increases in mean weight gain over the control. It was reported that haematological data of test animals showed normal values for white blood cells (WBC) count, red blood cell (RBC) count, haemoglobin (Hb) levels and packed cell volume (PCV) for all the weaning foods studied except the control. In an experiment involving the nutrient status of the protein of corn-soy based extruded products evaluated by rats bioassay, Baskaran and Battacharaya (2004) reported higher body weight gain by rats fed the test diet as compared to the control (fed skimmed milk powder). Information on the possible safety or otherwise of a complementary food based on extruded soy-cocoa is virtually non-existent. There is need to ascertain this in view of the likely potential benefit of such a product harnessing properties of these two agricultural produce (Dillinger et al., 2000; Lusas, 2004).

**Table 1.** The complementary food stored under similar condition as the basal diet had the following composition.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Composition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>0.1±0.09</td>
</tr>
<tr>
<td>Protein</td>
<td>21.7±0.4</td>
</tr>
<tr>
<td>Fat</td>
<td>3.7±0.2</td>
</tr>
<tr>
<td>Ash</td>
<td>3.0±0.1</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>0.5±0.1</td>
</tr>
<tr>
<td>Carbohydrate **</td>
<td>71.6±0.8</td>
</tr>
<tr>
<td>Potassium</td>
<td>1.4±0.2</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0.39±0.1</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.21±0.1</td>
</tr>
<tr>
<td>Magnesium</td>
<td>0.15±0.1</td>
</tr>
<tr>
<td>Sodium (mg/10 g)</td>
<td>8.3±0.4</td>
</tr>
<tr>
<td>Manganese</td>
<td>2.5±0.2</td>
</tr>
<tr>
<td>Iron (mg/100 g)</td>
<td>13±1.0</td>
</tr>
<tr>
<td>Copper (mg/100 g)</td>
<td>3±0.1</td>
</tr>
<tr>
<td>Zinc (mg/100 g)</td>
<td>2.1±0.1</td>
</tr>
<tr>
<td>Polyphenol (mg/100 g)</td>
<td>50.41±0.1</td>
</tr>
<tr>
<td>Energy Kcal/100 g</td>
<td>406.5±5.6</td>
</tr>
</tbody>
</table>

**Carbohydrate was by difference. Source: Arueya (2013).**

**MATERIALS AND METHODS**

**Experimental animals**

The animals used in this study were adult male and female albino wistar rats (100-200g) obtained from the animal house, department of Clinical Pharmacy, University of Ibadan. The animals were used after an acclimatization period (10 days) to well ventilated room with temperature 30 ± 4°C and relative humidity of 60%. They were housed in standard cages.

**Basal diet**

The animals were maintained on standard animal pellets (obtained from Ladokun Feeds Ltd., Ibadan, Nigeria) and potable water ad libitum. The proximate composition of the pellet as stored under room temperature (28±2°C): protein 21%, fat 3.8%, fibre 6.0%, calcium 0.8%, phosphorus 0.8%.

**Complementary food**

The complementary food stored under similar condition as the basal diet had the following composition (Table 1).

**Acute toxicity studies**

Following clearance by the UI/UCH ethical Review Committee, the fixed dose procedure of Boyd (1976) and organization for European cooperation and development (O.E.C.D) as described by (Botham, 2004) was followed. Gruels (20% w/v) were prepared from the complementary food by making a smooth cold paste and gradually pouring same into boiling water. This was stirred vigorously until the entire mass became viscous. The resulting mass was distilled daily for twenty one (21) days immediately after which the blood samples were taken through induced bleeding (from the orbital sinus) into heparinised and ethylene-diaminetetraacetic (EDTA) coated bottles.

**Sub-acute toxicity**

The intubate feeding reported continued daily for twenty-one (21) days immediately after which the blood samples were taken through induced bleeding (from the orbital sinus) into heparinised and ethylene-diaminetetraacetic (EDTA) coated bottles.

**Biochemical analysis**

Some biochemical parameters (total protein, albumin, aspartate (AST), alanine amino transferase (ALT) alkaline phosphatase (ALP) urea and creatinine) were determined using Roche diagnostic test kits. (Indiana, USA).

**Haematological analysis**

Haematological (mean corpuscular volume (MCV), mean corpuscular
hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), neutrophils, lymphocytes, packed cell volume (PCV), hemoglobin (Hb), (RBC), (WBC), platelets) were evaluated by Sysmex automated hematology analyzer KX - 21 (Sysmex corporation, kobe, Japan).

Histopathological analysis

After the induced bleeding, rats from each group (1 - 6) were sacrificed through vascular dislocation. Three organs namely liver, kidney and spleen were removed from the animals. They were weighed and subjected to histopathological analysis (toxicity signs) after fixation in slides using the methods of Adesiji (1999). The prepared slides were viewed under the light microscope (model:420- 420AHF-10) and examined by a pathologist.

Stool consistency

Samples of stool from the various groups were also compared for consistency.

Mortality rate

This was done by visual observation and counting of any death among the rats.

Statistical analysis

All the data obtained were statistically analyzed using the analysis of variance (ANOVA) SPSS software package (No. 13). Means were separated using Duncan's multiple range test (P<0.05).

RESULTS AND DISCUSSION

Acute toxicity studies

There were no visible signs of toxicity such as gasping or writhing within the first 24 h of diet administration. There was however decreased motor activity (reduced movement) evident through direct observation within the first 2 min of administration of the diet at 5000 and 2000 mg/kg levels. These animals became more active, ostensibly having overcome the initial shock of sudden expansion of the gastric (stomach) chamber of the rat. There was no death of animal recorded.

Sub acute toxicity

Feed treatment effects on mean weights of rats

Changes ranging from 144 - 232.5 g were observed in weights of rats fed the test diet and the control (Table 2). While there was significant gain across all the test groups and control, rats fed 100 mg/kg body weight were exceptionally high, attaining a 56.9% weight gain. This is the only grouping where the figure obtained was greater than the control. The progressive increase in percentage weight gain from 50 mg/kg body weight diet fed rats to 100 mg/kg body weight and thereafter a decrease as compared to the control may indicate that the diet had an optimum salutary effect on rat growth at the 100 mg/kg body weight feeding level. Increase in weight gain is an indication that the diet supported growth and this is in agreement with the findings of Plahar et al. (2003). In the work, they observed between 60-100 fold increases in mean weight gain over control when rats were fed extruded weaning foods based on peanut, maize and soybean. Apparently beyond this level (100 mg/100 g body weight), rats can no longer optimize the benefits of the diet. This may be due to increasing density of antinutritional factors such as polyphenols per unit weight of diet intake. A similar conclusion has been reached in some studies where anti-nutritional factors affected the activity of digestive enzymes in vivo (Anantharaman and Finot, 1993). It has also been noted that above serum levels of 25 µg/ml, cocoa polyphenols becomes inhibitory (Mao et al., 2003). The study showed that this level is a critical threshold for some stimulatory/inhibitory effect on growth related secretions from peripheral blood mononuclear cells.

Organ weights relative to body weights of rats after feeding duration

There was no significant difference (P ≤ 0.05) in the weights of liver and spleen across the test groups and control after a feeding duration of 21 days (Table 3). This also holds true for kidney weights of the animals fed at 50, 2,000 and 5,000 mg/kg body weight. The implication is that the test diet intake may not have deleterious

### Table 2. Feed treatment effects on mean weights.

<table>
<thead>
<tr>
<th>Mean weight (g)</th>
<th>Soyco meal (mg/kg body wt)</th>
<th>50</th>
<th>100</th>
<th>300</th>
<th>2,000</th>
<th>5,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>164±2.6</td>
<td>138±1.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Final</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>226±2.3</td>
<td>167±2.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight gain (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>37.8</td>
<td>21.0</td>
<td>56.9</td>
<td>34.8</td>
<td>13.3</td>
<td>16.1</td>
<td></td>
</tr>
</tbody>
</table>

Means not having the same superscript (a, b or c) within a column are significantly different at P≤ 0.05.
soybean (control) and the treatment groups (50, 100, 200, 300, and 500 mg/kg). A significant decrease in creatinine and urea levels was observed in the treatment groups compared to the control. However, the effects of varying levels of feed intake on some hematological parameters (Table 4) were observed. The mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) showed a trend towards decrease in the treatment groups compared to the control. The packed cell volume (PCV) was not significantly different between the groups. The hematocrit (Ht) showed a significant decrease in the treatment groups compared to the control. The mean corpuscular hemoglobin concentration (MCHC) was not significantly different between the groups. The white blood cell (WBC) count was significantly higher in the treatment group compared to the control. The red blood cell (RBC) count was not significantly different between the groups. The platelet count was not significantly different between the groups. The mean values not accompanied by the same superscript (a, b, or c) across the rows are significantly different at P < 0.05.

Table 4. Feed treatment effects on mean values of some hematological parameters between groups of rats.

<table>
<thead>
<tr>
<th>Haematological parameters</th>
<th>Control</th>
<th>50</th>
<th>100</th>
<th>300</th>
<th>2,000</th>
<th>5,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean corpuscular volume (MCV) (fl)</td>
<td>61.4±1.2abc</td>
<td>59.2±1.9a</td>
<td>65.0±1.6ab</td>
<td>63.3±1.5b</td>
<td>65.0±2.9b</td>
<td>69.4±1.6c</td>
</tr>
<tr>
<td>Mean corpuscular hemoglobin (MCH) (pg)</td>
<td>17.4±1.9a</td>
<td>17.4±2.1a</td>
<td>18.4±1.7abc</td>
<td>17.8±1.6ab</td>
<td>18.8±1.7bc</td>
<td>19.2±1.7c</td>
</tr>
<tr>
<td>Mean corpuscular haemoglobin concentration (MCHC) (g/l)</td>
<td>282±2.6a</td>
<td>294±1.8a</td>
<td>280±1.3a</td>
<td>280±1.7a</td>
<td>276±1.5a</td>
<td>276±1.5a</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>18.0±2.1a</td>
<td>21.8±1.8a</td>
<td>13.2±2.1b</td>
<td>21.3±1.6a</td>
<td>26.6±2.3a</td>
<td>20.6±0.9a</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>80.8±3.4a</td>
<td>77.4±3.1a</td>
<td>86.0±2.8a</td>
<td>77.5±3.0a</td>
<td>71.8±3.2a</td>
<td>79.0±3.0a</td>
</tr>
<tr>
<td>Packed cell volume (PCV) (%)</td>
<td>54.4±2.2abc</td>
<td>52.2±2.1a</td>
<td>56.6±2.8a</td>
<td>61.0±2.0c</td>
<td>57.6±1.8bc</td>
<td>50.4±2.1a</td>
</tr>
<tr>
<td>Haemoglobin (g/l)</td>
<td>154.8±2.8abc</td>
<td>152.6±2.7b</td>
<td>157.8±3.0c</td>
<td>170.5±1.5c</td>
<td>158.8±2.5c</td>
<td>140.6±1.8a</td>
</tr>
<tr>
<td>Red blood cells (x10^12/µl)</td>
<td>8.8±0.1b</td>
<td>8.70±0.1b</td>
<td>8.70±0.2b</td>
<td>9.60±0.1c</td>
<td>8.48±0.2b</td>
<td>7.34±0.1c</td>
</tr>
<tr>
<td>White blood cells (x10^9/µl)</td>
<td>12.2±2.0ab</td>
<td>10.3±1.6ab</td>
<td>11.44±1.5c</td>
<td>11.48±1.8a</td>
<td>13.32±2.1a</td>
<td>15.3±1.7a</td>
</tr>
<tr>
<td>Platelets (x10^12/µl)</td>
<td>771.6±28.0abc</td>
<td>916.8±26.1c</td>
<td>673±23.0a</td>
<td>741±30.1abc</td>
<td>659±27.3a</td>
<td>861.6±28.4abc</td>
</tr>
</tbody>
</table>

Mean values not accompanied by the same superscript (a, b, or c) across rows are significantly different at P ≤ 0.05.

Effect of varying levels of feed intake on some hematological parameters in rats

The mean values obtained for 50, 100, 300, 2,000 mg/kg body weight treatment groups were not significantly different (p ≤ 0.05) from the control (Table 4). The parameters reported for the group fed with 5,000 mg/kg body weight of the rat appears unique. However, the mean corpuscular haemoglobin (MCH) for this treatment group compared favourably with those of 100 and 2,000 mg/kg body weight group but were significantly different (P ≤ 0.05) from those of the control group. The 294 g/L mean corpuscular hemoglobin concentration (MCHC) obtained for 50 mg/kg body weight treatment group was the highest. This is also true for the neutrophils excluding the 100 mg/kg body weight group where this is lower than others. The reason for this is not obvious especially with the significant weight gained but may be traceable to non-dietary factors (Michael et al., 2008). The highest value of 61.0% obtained as packed cell volume (PCV) for the 300 mg/kg body weight test group compared favourably with the treatment group of 2,000 mg/kg body weight. The haemoglobin concentrations followed the same pattern with the PCV. The red blood cell levels for 50, 100 and 2,000 mg/kg body weight treatment groups of rats were not significantly different from the control. This was also true for white blood cells and platelets.

The MCV, MCH, MCHC values including RBC, PCV and Hb- major indicators of assessment of tendency towards anaemia shows that the diet was neither toxic toward red blood cells nor an impediment towards erythropoiesis (Bain et al., 2011). The difference in WBC values of test groups and the control may be attributable to a suppression of leucocytosis in the bone marrow (Antia et al., 2006). The lower platelets levels as compared to control may be due in part to bioavailability of cocoa polyphenols. This conclusion is borne out of a study reported by Rein et al. (2000), where after a cocoa
drink high in polyphenols was taken, a reduction in platelet surface marker (PAC-1) was found in human subjects, an indication of decreased platelet activation. Platelets carry blood-clotting factors and are important for wound healing.

Effect of feed treatment on some mean values of biochemical parameters in rats

The alkaline phosphatase (ALP) values for treatment groups (0.56-7.74 µkat/l) were generally not significantly different from the control (6.67 µkat/l) (Table 5). The group of 50 and 100 mg/kg body weight feed treatment were though lower in real values. It is however instructive that the highest weights gained among the rat groupings are associated with this low values which may just be the optimum for fastest growth rate. Experimental results established for alkaline aminotransferase (ALT) across all the groupings were not significantly different at (P ≤ 0.05). A similar pattern was evident for alanine amino transferase. The diet appears to be having a slight depressing activity on these enzymes linked principally with liver function, in view of their comparatively reduced values (Antia et al., 2006). Increased values are associated with higher activity and may likely indicate damage or hyperplasia of liver cells (Benjamin, 1978).

The total protein for groups on 100 and 5,000 mg/kg body weight feed intake compared favourably with that of the control, but differ significantly from the 50, 300 and 2000 mg/kg body weight groups. The albumin levels of 100, 300, and 2,000 mg/kg treatment groups (45.8-49 g/l) were essentially similar to control (45 g/l). Fluctuations in the levels of total protein and albumin in the rats’ blood serum are reflections of likely deviation from the normal liver function (Ahmed et al., 1992).

The urea profile for treatment groups 300, 2000 and 5,000mg/kg body weight were slightly higher but significantly different from that of the control group. The 50 and 100 mg/kg test groups were similar in this respect (21.4 and 19mmol/l respectively). With the exception of the 5,000 mg/kg body weight treatment groups (84.9 µmol/l) all others had creatinine level which were not significantly different (P ≤ 0.05).

Histopathological findings

The absence of necrosis in the tissues examined as shown in photomicrographs (Figures 1 to 3) clearly established the safety of the diet at the levels administered following feeding duration.

Mortality rates

There was no recorded death among the rats. This reinforces to some extent the safety of the diet.

Stool consistency

The treatment groups had better stool consistency (Figure 4). There were no sign of diarrhoea or watery stool. This is significant as a number of food induced diarrhoea have been reported (Motarjemi and Lelieveld, 2011). Additionally, excessive gas production by hetero-fermentative degradation of carbohydrate are known to cause loose stool and speed up the intestinal passage. In both cases, the absorption of nutrient become less efficient.

Absence of this occurrence in this study might be linked to the polyphenols/tannins content of the test diet. These have a tendency to interfere with microbial activities by immobilizing their extracellular enzymes and proteins on cell membranes (Gupta and Haslam, 1993; Shahidi and Naczek, 2002). Cocoa procyanidins (condensed tannins) are high in molecular weight and form strong complex with proteins. They are resistant to digestive enzymes and get transported down the intestinal colon (Jimenez-Ramayse et al., 1994). The foregoing may explain the better stool consistency from the test group as compared to the control as the administered diet is the only identified variable factor.
Figure 1. Photomicrographs indicating the null effect of the diet on the histopathology of the liver cells (liver architecture intact).

Figure 2. Photomicrographs indicating the null effect of the diet on the histopathology of the spleen cells (spleen architecture intact).

Figure 3. Photomicrographs indicating the null effect of the diet on the histopathology of the kidney cells (kidney architecture intact).
Conclusion
Evidently, the cocoa based complementary diet is safe and beneficial to growth especially at lower feeding levels of administration.

Conflict of Interests
The authors did not declare any conflict of interests.

ACKNOWLEDGEMENT
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REFERENCES


