1

Nutritional Evaluation of Extruded Complementary Diet from Quality Protein Maize and Soybean Protein Concentrate Using In-Vivo Bioassays

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Abstract

Complementary meals were formulated from quality protein maize, soybean protein concentrate and cassava starch in the ratio 72:18.94:9.06 respectively. The flour blend was extruded to give three meals by varying three extrusion conditions (Temperature, moisture content and screw speed). The formulated diet had protein content values ranging from 20.09% in one of the extruded samples to 22.09% (dry weight basis) in the unextruded sample. The formulated meal (extruded and unextruded) and control diets were fed to rats for 28 days and the effects on body weight, organ weight, clinical signs, histopathology, haematology, and serum biochemical parameters were evaluated. There was no significant difference in the weight of the liver, kidney, pancreas, spleen, stomach and heart of the rats fed the three extruded samples and the casein diet at $p \leq 0.05$. High protein Efficiency Ratio was recorded for the extruded meals (ranging from 2.24 to 2.45). There was significant difference at $p \leq 0.05$ between the organ weights of the extruded meal and the unextruded meals. The rats fed the basal diet had the lowest (24%) Packed Cell Volume value while the values recorded for the extruded formulated meals were comparable to that recorded for the casein diet. Portal congestion and shrunken hepatocytes were however noticed in the liver of the rats fed the unextruded and basal diets. Extrusion cooking had significant effects on the biological response of the rats to the formulated diet.

Keywords: Extrusion cooking, protein quality, nutritional assessment, in vivo digestibility, haematology

Introduction

Cereals and cereal products are one of the major sources of energy in human nutrition worldwide (Katina *et al.,* 2005; Arendt and Nunes, 2010). Several million people, particularly in the developing countries, derive their protein and calorie requirements from maize (Mbuya *et al.,* 2011). Quality Protein Maize (QPM) is high yielding high lysine maize released for alleviating hunger and malnutrition of the poorer sections of the maize dependent populations. QPM is a superior grain and can find specific end uses in food product development and nutritional interventions. Supplementation of cereals with legumes improve the protein quality of formulated meals (Forbes *et al.,* 2014)

Soybeans have an excellent reputation for their elevated protein contents, amino acids quality, high nutritional value, and low production costs. Soy products consumption has increased because of their large beneficial properties such as being free of cholesterol, gluten and lactose, making them suitable for lactose-

intolerant consumers and vegetarians (Liu and Lin, 2001). Despite these merits, the use of soybeans has been limited by the presence of several biologically active anti nutritional factors. Soybeans reportedly contain phytates, which are considered antinutrients because they diminish the bioavailability of certain minerals including magnesium, calcium, iron and zinc. Soy foods are normally regarded as nutritious, cholesterol free health foods and play an important role in combating the protein calorie malnutrition. (Tripathi *et al.,* 2004). Soy foods are not widely accepted because of the beany flavour, difficulty in cooking, flatulence factors, and anti-nutritional factors such as trypsin inhibitors (TI), hemagluttinins and phytic acid. The presence of these factors is causing a major concern. These anti-nutritional factors must be inactivated to make them fit for consumption and to ensure maximum utilization of this nutrient rich food crop. This is achieved through the processing of soybeans into protein concentrate. Heat treatment is an effective method used to reduce antinutritional factors present in raw soybeans and other pulses, in this case, extrusion cooking (Charunuch *et al*., 2003).

Food with balanced amino acid profile can be obtained by mixing legumes and cereal grains. The retention of these essential nutrients would be enhanced through the application of extrusion cooking processes (Iwe, 2003; Omosebi *et at*., 2018). The anti-nutritional factors present in legumes are reduced to the barest minimum in the protein concentrates (Endres, 2001). Cassava starch may be included in composite food products as it serves as a binder whilst improving its consumption level in developing countries (Laswai *et al.,* 2017). Utilization of the protein concentrate of soybeans in food formulation and the extrusion cooking process is expected to improve the digestibility of the food and bioavailability of the essential nutrients present in the food.

This study aimed at assessing the nutritional quality and bioavailability of protein of the formulated complementary diet from quality protein maize, soybean protein concentrates and cassava starch using *in-vivo* assays.

Materials and Methods

Consumer friendly variety of Cassava roots (TMS 4(2) 1425) was purchased from International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria. Soybean (TGx1987-10F) was purchased from International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria. Quality protein maize (ART/98/SUWN/SR) was purchased from Institute of Agricultural Research and Technology (IAR&T), Ibadan, Nigeria.

The cassava starch was processed using the method described by Osundahunsi *et al.,* (2011). The cassava roots were peeled, washed and grated. The grated cassava was mixed with water, filtered and allowed to settle. Starch washing was carried out. It was allowed to settle again and dewatered. The resultant starch was dried at 35°C and fine milled. The soybean protein concentrate was processed using the method described by Adebowale and Lawal (2003). Defatted soybean flour was mixed with water in ratio 1:10. The pH was regulated to 8.0-8.5 with 0.1M NaOH. The mixture was stirred for 4-5 hours continuously to allow for protein solubulization. The mixture was centrifuged and the sediment was discarded. The pH of the supernatant was adjusted to pH 4 with 0.1m HCl and centrifuged. The supernatant was discarded while the sediment was freeze-dried. The quality protein maize was cleaned and milled to give a fine meal. The maize meal, soybean protein concentrates and cassava starch (72%, 18.94% and 9.06% respectively) were mixed in proportions to reach the target protein content of at least 18% (using regression analysis). The mixture was extruded by varying extrusion conditions (Temperature (T); Moisture Content (MC) and Screw Speed (SS). The extruded meals were produced by varying these three extrusion conditions (T_{180oC}, $MC_{20\%}$, SS_{230rpm}). The feed conditions (temperature (T), moisture content (MC) and Screw Speed (SS) for the three samples (A,B,C) were $T_{180\ ^{o}C}$, $MC_{20\%}$, SS_{230rpm}; $T_{170\ ^{o}C}$, $MC_{20\%}$, SS_{200rpm} and $T_{180\ ^{o}C}$, $MC_{18\%}$, SS_{200rpm} respectively.

Basal diet

A basal diet was prepared according to the method described by Ingale and Shrivastava, (2011). The composition (g/kg) of the experimental and control diets are presented in Table 1. Experimental and control diets were prepared by incorporating the weaning foods and casein (control) into the basal diet to achieve an isonitrogenous diet at 10% protein level.

Table 1. Composition of experimental diet (g/100 g)

Ingredients	Basal Diet	Casein Diet	Sample A	Sample B	Sample C	Sample D
Corn starch	59.75	48.27	7.59	5.20	5.43	7.66
Casein	-	11.48				
Sample A	-		52.16			
Sample B	-			54.55		
Sample C	-				54.32	
Sample D	-					52.09
Glucose	5.00	5.00	5.00	5.00	5.00	5.00
Sucrose	15.00	15.00	15.00	15.00	15.00	15.00
Non-nutritive Cellulose	5.00	5.00	5.00	5.00	5.00	5.00
Vegetable oil	10.00	10.00	10.00	10.00	10.00	10.00
Oyster shell	1.00	1.00	1.00	1.00	1.00	1.00
Bone meal	2.00	2.00	2.00	2.00	2.00	2.00
NaCl	2.50	2.50	2.50	2.50	2.50	2.50
Vitamin premix	2.00	2.00	2.00	2.00	2.00	2.00
Total	100	100	100	100	100	100

Experimental animals

Thirty weaning albino rats (male) weighing between 40 g and 60 g and about 25 – 26 days old were obtained from the Animal breeding Centre, Department of Veterinary Physiology and Pharmacology, Faculty of Veterinary Medicine, University of Ibadan. They were randomly distributed into six groups each consisting of five replicates, placed in metabolic cages and fed a stabilizing diet containing 4% casein (Sigma Chemical Company Vitamin Free) for a period of 5 days. After the 5 – day period, the animals were reweighed and regrouped for control, basal and experimental diets so that the average weight difference did not exceed ±2g. Water and food were given *ad libitum*. The diets were fed to the animals for a period of 28 days. This period is nutritionally accepted to be long enough to observe biological and chemical changes in animal tissue. Weighed diet was given and unconsumed diet was collected and weighed daily, while live weight of the animals was determined and recorded twice a week throughout the experimental period. At the end of the

test period, the rats were reweighed. After a starving period of 18 hours, the rats were sacrificed.

The feed efficiency ratio (FER), protein efficiency ratio (PER), the net protein retention (NPR) and protein retention efficiency (PRE) were calculated using the formulae given below (Osundahunsi *et al.,* 2003; Ingale and Shrivastava, 2011; Ikya *et al.,* 2013).

$$Feed\ Efficiency\ Ratio\ (FER) = \frac{Gain\ in\ body\ weight\ (g)}{Feed\ Intake\ (g)} \quad (1)$$

$$Protein\ Efficiency\ Ratio\ (PER) = \frac{Weight\ gain\ of\ test\ animal\ (g)}{Protein\ consumed\ by\ test\ animal\ (g)} \quad (2)$$

$$Net\ Protein\ Ratio\ (NPR) = \frac{weight\ gain\ of\ test\ animal + avg\ weight\ loss\ of\ animal\ fed\ basal\ (non-protein)}{Protein\ consumed\ by\ test\ animal} \quad (3)$$

$$Protein\ Retention\ Efficiency\ (PRE) = NPR \times 16 \quad (4)$$

$$Feed\ Conversion\ Ratio = \frac{Feed\ Consumed\ (g)}{Gain\ in\ body\ weight\ (g)} \quad (5)$$

Haematological Analysis

The haematological analysis was carried out using the method described by Harishankar *et al.,* (2011). The haematocrit method was used in determining the packed cell volume. Capillary tubes were filled with blood by capillary action up to 2/3 the length of the tube and the vacant end sealed in the flame of a burner. The sealed tubes centrifuged for 10 minutes at 3000 rpm in Hawskley microhaematocrit centrifuge. Each tube was then read using the special graphic reader. The value obtained was expressed as a percentage of the total blood volume and recorded (Aniagu *et al.,* 2005). The method of counting suggested by Savala *et al.,* (2012) was used to evaluate the differential leucocyte count. The counting was started along the outer margin of the smear for about 3 fields moving inward a short distance, then paralleling the margin. This procedure was repeated as many times as necessary until a hundred cells were examined and counted. The number of each type of leucocyte was recorded for each 100 cells counted. The grass total was also recorded. The differential count was derived by finding the respective percentages of each set of the cells in the total count.

To determine the haemoglobin concentration, 0.2ml of blood sample was pipetted into a test tube containing 5 ml of Drabkin's solution. The content was then mixed thoroughly by rotation. The optical density at 540 nm of the resulting solution was read using the spectrophotometer. The corresponding

haemoglobin concentration was read on a standardized calibration curve and recorded.

The improved Neubauer Haemocytometer was cleaned properly before determination of the red blood cell (erythrocyte) count. Blood was drawn into the pipette as far as 0.5 marks. The Hayems diluting fluid was sucked up to the 101 mark. The blood was mixed thoroughly with the diluting fluid using the mechanical shaker. The fluid in the stem of the pipette was expelled. Placing a finger over the upper end of the pipette, the lip of the pipette was placed between a slide and the counting chamber. Fluid was then allowed to run by capillary action into the haemocytometer. The cells were allowed to settle for a few minutes before examining and counting under the high power of a light microscope. The total number of red blood cells in eighty small squares was counted. Usually, cells in the four corner squares (made of 16 smaller squares each) and the central square were also counted. Cells on the upper line and left side of each square were included in the count for the square (Akpanabiatu *et al.*, 2013).

For the white blood cell count determination, blood was drawn up to 0.5 mark on the stem of the white blood cell pipette and followed up with diluting fluid sucked up to the 11 mark. The pipette content was shaken for 3 minutes in a mechanical shaker. Some unmixed fluid in the stem of the pipette was expelled and the Neubauer counting chamber was filled as done for red blood cell count. The cells were allowed to settle for 1 minute. The cells in the ruled site center square of the chamber were counted under high power magnification. The rule for excluding and including cells touching lines as done for erythrocytes was employed (Akpanabiatu *et al.*, 2013).

From the values of total red blood cell count (RBC), packed cell volume (PCV) and haemoglobin concentration (Hb). The following indices were determined by formulas reported by Yakubu et al., (2007).

$$Mean\ Corpuscular\ Volume\ (MCV)(\mu 3) = \frac{PCV\ (\%) \times 10}{RBC\ (per\ cumm\ blood)} \quad (6)$$

$$Mean\ Corpuscular\ Haemoglobin\ (MCH)(\mu mg) = \frac{Hb\left(\frac{g}{100ml}\right) \times 10}{RBC\ (per\ cu.mm\ Blood} \quad (7)$$

$$MCH\ Concentration\ (MCHC)(\%) = \frac{Hb\left(\frac{g}{100ml}\right) \times 100}{RBC\ (per\ cu.mm\ Blood} \quad (8)$$

Biochemical Tests

Each of the blood samples collected was centrifuged and the plasma separated into Bijon bottle. The plasma was kept at 20 °C until analyzed. The total protein was determined by the Biuret method using Jenway 6100 spectrophotometer. Exactly 0.1 ml of serum was mixed with 2.9 ml of distilled water and 3 ml of Biuret reagent was added. Another 3 ml of Biuret reagent mixed with 3 ml of distilled water was used as blank. The sample was read against the blank for determination of

optical densities at wavelength 540 nm. The instrument was standardised using sample of known optical density and zeroed with the blank. The corresponding total protein concentration in g/100 ml was read off a calibration curve (Ogundeji *et al.*, 2013).

Clear serum (0.2 ml) was pipetted into a centrifuge tube and 5.8 ml of sodium sulphite solution was added. The solution was mixed thoroughly. 2.0 ml of the mixture was pipetted into a tube and another 2.0 ml of sodium sulphite was used as blank. The suspension in the centrifuge tube was added to 1.0 ml span ether reagent. The tube was finally capped and shaken vigorously for 10 s and allowed to stand for 10 min. The tube was then centrifuged at 2800 G for 10 min. A volumetric pipette was inserted down to the albumin layer of the mixture was sucked up and ran into the albumin tube. To each of the tube was added 3.0 ml of Biuret reagent. The mixture was placed on 37 °C water bath for 10 min. The optical density of the total albumin at 540 nm was read using a spectrophotometer. The instrument was zeroed using the blank (Ogundeji *et al.*, 2013).

Globulin fraction was determined by subtracting the albumin concentration from the total protein content (Ogundeji *et al.*, 2013)

For the determination of serum transaminases, test sera were ensured to be free of haemolysis or bacterial contamination. These were kept in the fridge 0 °C (Not Frozen) to retain their activity for several days. If haemolysis occurred, enzyme activity would be inhibited. Enzyme substrate was prepared. Exactly 1.0ml of appropriate substrate was pipetted into each tube of 'test' and 'blank' and placed in water bath at 37 °C for about 5 min. About 2 drops (0.06 – 0.08 ml) of aniline citrate reagent was added to the blank followed by mixing. Exactly 0.2 ml of serum was added to each of the 'test' materials. The mixture was allowed to stand for 60 min for Aspartate Transaminase (AST) formally known as serum glutamic oxaloacetate transaminase (SGOT) and for 30 min for Alanine Transaminase (ALT) formerly referred to as serum glutamic – pyruvate transaminase (SGPT). Following the addition of 2 drops of aniline citrate reagent and mixing, the 'test' was removed from the water bath. 1.0 ml of dinitrophenyl – hydrazine reagent was added immediately in case of ALT, while it was added after 20 min in the case of AST and was made to stand for another 20 min. About 10.0 ml of 0.4N sodium hydroxide was finally added and the mixture allowed to stand for another 10 min. The optical densities of the samples were determined at 540 nm. The 'blank' was used to zero the instrument and values were read off calibration (standard) curves (Devaki et al., 2012).

Histopathological examination

At the end of the feeding test period, the liver, kidney, spleen, pancreas and adrenal gland of the experimental rats were quickly excised, weighed fresh and used for histopathological study. The slides were prepared according to the methods described by Eweka (2007) and Akinnawo *et al.,* (2005).

Results and Discussions

The result of the biological response of rats fed the extruded and control diets are presented in Tables 2 and 3. The mean final body weight of the rats ranged from 41.35 g for the rats placed on the basal diet to 124.28g for the rats fed diet A. The rats fed the basal diet lost an average of 11.68 g over the test period while the rats fed with diet A gained 71.38 g, the rats fed with diet B gained 62.08 g, the rats fed with diet C gained 59.83 g, the rats fed with diet D gained 26.04 g and the rats fed with casein diet gained 70.02 g. The casein diet had the highest Feed Efficiency Ratio (FER) (0.274) followed by sample A (0.268), then sample C (0.251), followed by Sample B (0.245) and then sample D (0.132). The corrected Protein Efficiency Ratio (PER) ranged from 1.08 for sample D to 2.44 for sample A. The Net Protein Retention (NPR) ranged from 1.92 for sample D to 3.21 for the casein diet.

Organ weights (Table 4) measured in rats fed basal diet were significantly ($P \leq 0.05$) lower than those on either the casein diet or the extruded meals. There was no significant difference in the weight of the liver, kidney, pancreas, spleen, stomach and heart of the rats fed the three extruded samples and the casein diet. The weight of the excised liver ranged from 2.29 g for the rats fed the basal diet to 4.80 g for the rats fed sample A. The rats fed the casein diet had the highest value for kidney weight (1.15 g). However, the rats fed sample D recorded the lowest kidney weight (0.49 g). The weight of pancreas of rats fed the basal diet and sample D was the same (0.10 g). The weight of the spleen ranged from 0.16 g in the rats fed the basal diet to 0.60 g for the rats fed sample A. There was no significant difference among the stomach weights recorded for the formulated diets and the casein diet. The rats fed the basal diet recorded a stomach weight of 0.47 g. The weight of the intestine ranged from 3.68 g for the rats fed the basal diet to 8.50 g for the rats fed sample C. The weight of the heart also ranged from 0.26 g for the rats fed the unextruded formulated meal (sample D) to 0.43 g for the rats fed sample A.

The results of the haematological parameters of the rats fed the various diets and the serum constituents and serum transaminase activities are presented on Table 5 and Table 6

respectively. The haemoglobin concentration (Hb) ranged from 8.3 to 11.82 g/100 ml in the rats fed basal diet to diet B. The rats fed the basal diet had the lowest (24%) Packed Cell Volume (PCV) while the rats fed sample B had the highest (35.6%) PCV value. The red blood cell (RBC) count ranged from 4.03 μL in the rats fed the basal diet to 5.82 μL in the rats fed sample C. The Mean corpuscular haemoglobin concentration ranged from 32.65% for the rats fed the casein diet to 34.58% for the rats fed the basal diet.

Histopathological changes observed on the animal organs are shown on Plates 1 to 8. There was no significant lesion in the pancreas of all the rats. No significant lesion was observed in the spleen of all the rats. There was no significant lesion in the kidneys of all the rats except one of the rats fed the casein diet that shows protein casts in several tubular lamina and two of the rats fed the basal diet that exhibited moderate renal cortical congestion. One of the kidneys showed multiple foci of interstitial cellular infiltration. There were also protein casts in the lumen of several adjoining renal tubules while there was no lesion in the liver of the rats fed the casein and the extruded meal.

The micrograph of the liver of animals fed the extruded diets showed presented no lesions (Plate 1). Portal congestion and shrunken hepatocytes were however noticed in the liver of the rats fed the unextruded diets as presented on Plate 2. There was moderate diffuse hepatic degeneration and necrosis. There was severe periportal cellular infiltration by mononuclear cells of animals fed the basal diet (Plate 3 and 4). Very mild hydropic degeneration of hepatocytes was noticed in one of the livers. The liver of one of the rats fed the casein diet showed a diffuse hepatic vacuolar degeneration and necrosis, with mild periportal fatty infiltration. Plate 6 and 7 represent the micrograph of kidney of animals fed the extruded diet.

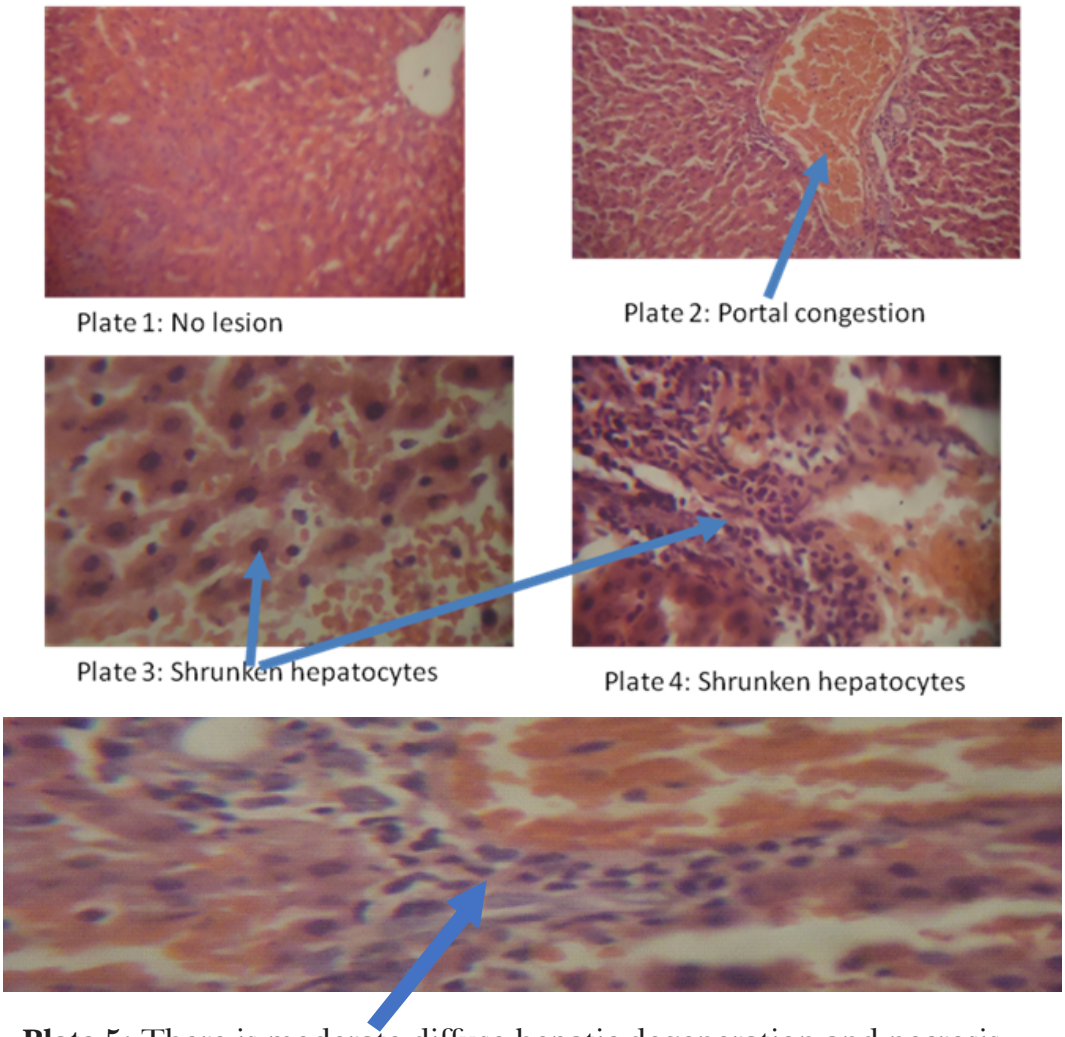

Plate 5: There is moderate diffuse hepatic degeneration and necrosis. There is severe periportal cellular infiltration by mononuclear cells.

The results recorded in this research showed that the extruded meals produced from whole maize and soybean protein concentrate had higher PER (ranging from 2.45 to 2.68) than ordinarily adding soybean flour to the maize (2.10) as reported for meal produced from 70% maize +30% soy flour by Ikya *et al*., (2013). The higher PER for groups fed the casein diet and the extruded diet showed that the diet contained desirable pattern of EAA, which the animals used to synthesize new protein (Alabi *et al.,* 2001; Osungbaro *et al.,* 2009; Chai *et al.,* 2011). The PER of rats fed the extruded diet was comparable to that of rats fed the

casein diet which tends to suggest that its protein quality was comparable to that of the control (casein) (Osundahunsi and Aworh, 2003).

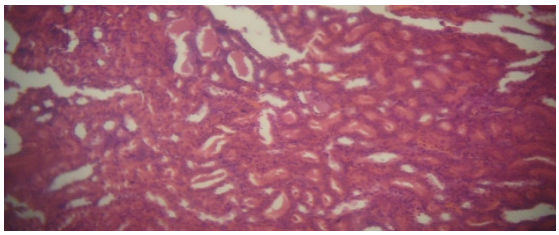

Plate 6: Protein casts in several tubular lamina

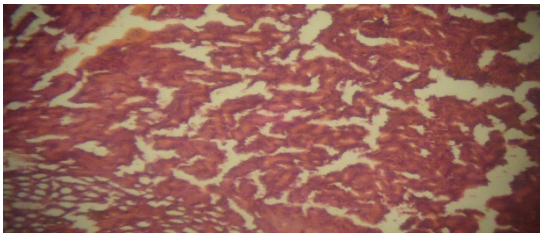

Plate 7: No visible lesion

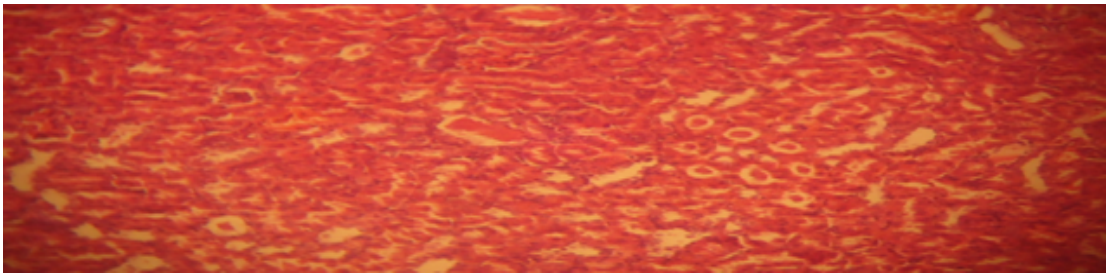

Plate 8: There is severe diffuse tubular degeneration, with protein casts in several tubular lumina of kidney of rats fed the basal diet.

Table 2. Weight gain, feed and protein intake of rats fed the formulated meals of quality protein maize, soybean concentrate and cassava starch and control diets

Diets	Mean initial weight (g)	Mean final weight (g)	Mean body weight gain (g)	Feed intake (g)	Protein intake (g)
A	52.90±0.5a	124.28±0.3a	71.38±0.3a	266.0±2.5a	26.60 ±0.2a
B	53.14± 0.4a	115.22 ±0.7a	62.08 ±0.5a	252.4 ±1.6a	25.24 ±0.8a
C	53.00 ±0.3a	112.83 ±0.5a	59.83 ±0.4a	238.1 ±1.5a	23.81 ±1.5b
D	53.06 ±0.5a	79.10 ±1.0b	26.04 ±0.6b	196.8 ±1.2b	19.68 ±1.3c
Casein	53.88 ±0.2a	123.90± 0.4a	70.02 ±0.3a	254.8 ±2.0a	25.48 ±1.0a
Basal	53.03 ±0.3a	41.35 ±0.3b	-11.68 ±0.2c	193.2 ±0.7b	-

A: (T180oC, Mc20%, SS230rpm); B: (T170oC, MC20%, SS200rpm); C: (T180oC, MC18%, SS200rpm); D: (unextruded meal)
*Values with different superscript on the same column are significantly different ($P<0.05$)

Table 3. Biological response of rats fed the formulated meals of quality protein maize, soybean concentrate and cassava starch and the control diets

Diet	Feed Efficiency ratio (FER)	Protein Efficiency ratio (PER)	Protein PER** Efficiency	Corrected Protein Retention Ratio	Net Protein Retention (g)	Protein gain / day	Weight Feed Conversion
A	0.268±0.01a	2.68±0.02a	2.45a	3.12±0.01a	49.92±0.02a	2.55a	3.72
B	0.245±0.02a	2.45 ±0.02a	2.24a	2.92± 0.01a	46.72 ±0.01a	2.22a	4.10
C	0.251±0.02a	2.51± 0.01a	2.29 a	3.00 ±0.02a	48.00 ±0.03a	2.14a	3.98
D	0.132±0.01b	1.32± 0.02b	1.20b	1.92 ±0.01b	30.72 ±0.02b	0.93b	7.56
Casein	0.274±0.03a	2.74± 0.02a	2.5a	3.21 ±0.01a	51.36 ±0.01a	2.50a	3.64
Basal	-0.060±0.02c	-0.60±0.01c	-0.65c	-	-	-0.42c	16.54

A: (T180oC, Mc20%, SS230rpm); B: (T170oC, MC20%, SS200rpm); C: (T180oC, MC18%, SS200rpm); D: (unextruded meal)
*Values with different superscript on the same column are significantly different (P ? 0.05)
**Based on value 2.5 as standard for casein

Table 4: Organ weight of animals fed extruded and unextruded meals of quality protein maize, soybean concentrate and cassava starch.

Sample ID	Liver (g)	Kidney (g)	Pancrease (g)	Spleen (g)	Stomach (g)	Intestine (g)	Heart (g)
A	4.80±0.02a	0.98± 0.03a	0.30±0a	0.60±0a	1.28±0.02a	8.44± 0.08a	0.43±0.01a
B	4.56 ±0.03a	0.87 ±0.05a	0.29 ±0.01a	0.42 ±0a	0.82 ±0a	6.33 ±0.10b	0.39 ±0a
C	4.07 ±0.04a	0.93± 0.04a	0.28 ±0.02a	0.40 ±0a	0.92 ±0a	8.50 ±0.14a	0.36 ±0a
D	2.62 ±0.04b	0.49± 0.03b	0.10 ±0.01b	0.24 ±0.01b	1.00 ±0.01a	5.27± 0.06b	0.26 ±0.01b
Casein	4.70 ±0.04a	1.15± 0.03a	0.31 ±0.01a	0.52 ±0.02a	1.22 ±0a	7.63 ±0.05a	0.38 ±0.01a
Basal	2.29 ±0.01b	0.54± 0.04ab	0.10 ±0.01b	0.16 ±0b	0.47 ±0.01b	3.68 ±0.06c	0.28 ±0.02b

A: (T180oC, Mc20%, SS230rpm); B: (T170oC, MC20%, SS200rpm); C: (T180oC, MC18%, SS200rpm); D: (unextruded meal)
*Values with different superscript on the same column are significantly different (P ? 0.05)

Table 5. Haematological parameters of rats fed control diets, extruded and unextruded meals of quality protein maize, soybean concentrate and cassava starch.

Sample ID	(Hb) g/100ml	RBC (μL)	PCV (%)	MCV (μL)	MCH (μmg)	(MCHC) (%)	WBC (μL)	Neutrophils(%)	Lymphocytes(%)	Monocytes(%)	Eosinophils (%)
A	11.68 ±0.40a	5.78 ±0.02a	35.2±0.03a	60.90±1.50ab	20.21±0.50ab	33.18±1.70b	5.20±0.15a	40.00±0.40b	63.00±0.5b	2.40±0.10a	1.40±0.02b
B	11.82 ±0.22a	4.84 ±0.1a	35.6±0.01a	73.55±2.50a	20.21±0.78ab	33.20±1.00b	5.28±0.20a	34.40±1.20c	60.40±1.0b	1.80±0.20b	0.80±0.01c
C	11.00 ±0.15a	5.82 ±0.15a	32.5±0.02a	55.84±1.80b	18.90±0.40b	33.85±0.90b	5.35±0.10a	37.00±0.80b	69.00±0.9a	2.00±0.05a	1.00±0.01c
D	9.80 ±0.09b	5.16 ±0.50a	29±0.10b	56.20±1.55b	18.99±0.35b	33.79±1.05b	5.20±0.08a	45.00±0.50a	52.75±1.2c	1.50±0.02b	1.00±0c
Casein	11.10 ±0.20a	5.49 ±0.02a	34±0.05a	61.93±1.50ab	20.22±0.40ab	32.65±0.75b	5.45±0.70a	43.00±1.00a	64.00±0.5b	1.50±0.05b	1.75±0a
Basal	8.30 ±0.02c	4.03 ±0.03b	24±0.02b	59.55±1.80ab	20.60±0.50a	34.58±0.95a	4.68±20b	46.00±0.50a	61.00±0.8b	1.00±0.05c	1.25±0.01b

A: (T180oC, Mc20%, SS230rpm); B: (T170oC, MC20%, SS200rpm); C: (T180oC, MC18%, SS200rpm); D: (unextruded meal). Haemoglobin (Hb); Red Blood Cell (RBC); Packed Cell Volume(PCV); Mean Corpuscular Volume (MCV); Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Conc (MCHC), White Blood Cells (WBC)
*Values with different superscript on the same column are significantly different (P ? 0.05)

Table 6. Serum constituents and serum transaminase activities of blood samples of rats fed the control diets, extruded and unextruded meals of quality protein maize, soybean concentrate and cassava starch.

Diets	Total protein (g/100ml)	Albumin (g/100ml)	Globulin (g/100ml)	Alanine transaminase (IU/L)	Aspartate transaminase (IU/L)
A	7.78 ±0.02a	5.54±0.04a	2.24±0.01b	32.00±1.50a	44.8±1.80b
B	7.0 ±0.04a	4.68± 0.05a	2.32±0.03b	27.60±1.95b	38.8±1.20b
C	7.63 ±0.05a	5.80± 0.45a	1.83±0.02c	28.50±1.25b	38.5±2.50b
D	7.26 ±0.01a	5.46±0.01a	1.80±0c	27.5±1.00b	39.8±1.70b
Casein	7.99 ±0.07a	6.18±0.03a	1.81±0.01c	29.75±0.90a	40.0±3.20b
Basal	5.18 ±0.05b	1.73±0.02b	3.45±0.01a	28.00±0.70b	69.0±2.55a

A: (T180oC, Mc20%, SS230rpm); B: (T170oC, MC20%, SS200rpm); C: (T180oC, MC18%, SS200rpm); D: (unextruded meal)
*Values with different superscript on the same column are significantly different (P ? 0.05)

Generally, a PER below 1.5 suggests that the protein is of low or poor quality (Womeni *et al,* 2012; Ogundeji *et al.,* 2013). According to Abdul-Hamid *et al*., (2002) though, the PER of protein have no proportional relationship to one another or to the suitability of the protein source analysed for maintenance of protein nutrition. It also lacks precision, has poor reproducibility and it is expensive.

The rats fed the basal diet had the lowest biological response. The FER, PER and NPR of the extruded meal was comparable to the casein diet (Table 3). The extrusion process improved the digestibility of the meal which is indicated by high PER values. NPR is a more accurate measure of protein quality than PER as it allows the evaluation of maintenance of requirement and results are independent of food intake. The NPR values ranged from 1.92 in the rats fed the unextruded sample to 3.21 in the rats fed the casein diet. There was no significant difference between the PER of the rats fed the extruded meals and those fed the casein diet. This is similar to the findings of Osundahunsi and Aworh, (2003). The high quality of the protein of the extruded meal makes the product particularly attractive for Nigeria and other developing countries where the prevalence of protein-energy malnutrition is due largely to the poor nutritional quality of traditional weaning foods made from locally available crops such as maize (Osundahunsi and Aworh, 2003).

The rats fed the basal diet lost an average of 11.68 g in body weight over the study period. This implies that consumption of nutritionally deficient diet leads to stunting. Hair loss was also observed in the rats that were fed the basal diet. The rats fed the raw formulated meal gained 26.04 g as against the rats fed Sample A, which gained 71.38 g. the rats fed the casein diet (control) gained 70.02 g during the study period. The results of the in vitro digestibility agree with the findings of the rat study that uncooked meals are less digestible than the cooked foods. The inherent nutrients were not available for utilization by the rats fed the unextruded samples.

Lower liver weights recorded for the rats fed the uncooked meal may be presumably due to necrosis arising from the potent antinutritional factors still present in the meal (Omaye, 2004). Reduced liver weight was reported in rats fed raw lima beans presumably because of the presence of anti-nutritional factors in the raw beans (Aletor and Fetuga, 1986). Food processing reduces these anti-nutritional factors. The extrusion cooking of the formulated meal reduced the effect of these factors as evidenced in the weight gains obtained in rats given the extruded meals. This result is supported by the findings of Akinnawo *et al.,* (2005).

Quite apart from growth inhibition, poor protein utilisation and alteration in organ development, the result of this study revealed that the ingestion of inadequate protein may be accompanied by derangement in haematological traits. The overall general appearance of the malnourished animals placed on basal diet showed extensive muscle wasting and serious atrophy, skin and hair changes. The animals showed signs of apathy but tended to be alert to any intruder. The liver weight of the rats fed the extruded meal and the casein diet were higher than those of the rats fed the basal diet and uncooked meal. The liver weight of the rats fed the extruded meal and the casein diet were within the range of 4.07 g to 4.80 g. The liver weights of the animals fed the basal diet and uncooked meal were 2.29 g and 2.62 g respectively. The same trend was observed for the other organs checked. The organ weights of the rats fed the extruded meals were similar to those of the rats fed the casein diet. The presence of a growth inhibitory factor in the raw food may be responsible for the growth depression observed (Akinnawo *et al.,* 2005) in the test rats which inadvertently transmitted to the lower organ weights recorded.

The white blood cells count of the rats fed the basal diet was significantly lower than that of the rats placed on the other diets. This could point to the fact that good nutrition is necessary for good health as the white blood cells are responsible for fighting germs in the body. Good nutrition is necessary to boost the immune system because it is responsible for increasing the population of defensive white blood cells (Aniagu, *et al.,* 2005). Low PCV, Hb and serum protein have been associated with protein deficiency. The PCV and Hb levels were reduced in rats fed the basal diet and unextruded meal indicating that malnutrition and feeding of unprocessed food may have anaemic effects. However, it is recognised that these parameters, although useful in routine assessment of iron and protein status, are not sensitive enough to detect changes over a short period of time (Osundahunsi and Aworh, 2003). The reduction in lymphocyte count was compensated for by an increased neutrophil count suggesting that lack of proper nutrition leads to anti-lymphocytic activity

Liver cell damage is sparingly characterized by a rise in plasma enzymes Aspartate Aminotransferase, (AST) and Alanine Aminotransferase (ALT). The results of this research showed that AST concentrations were consistently higher than ALT levels which are expected since body cells contain more AST than ALT (Aniagu *et al.,* 2005) Usually, about 80% of AST is found in the mitochondria whereas ALT is a purely cytosolic enzyme. Therefore, AST appears in higher concentrations in a number of tissues (liver, kidneys, heart and pancreas)

and is released slowly in comparison to ALT. But since ALT is localized primarily in the cytosol of hepatocytes, this enzyme is considered a more sensitive marker of hepatocellular damage than AST and within limits can provide a quantitative assessment of the degree of damage sustained by the liver (Al-Mamary *et al.,* 2002). Both the enzymes are present in the cytosol of the hepatocytes. AST is also found in the mitochondria. ALT is present primarily in the liver and to a lesser extent in kidney and skeletal muscle. AST is found in all body tissues especially heart, liver and skeletal muscle. Neither enzyme is specific for the liver but as ALT is found in much higher concentration in liver than in other organs. Increase in its activity indicates that the liver is damaged. Therefore, in the absence of acute necrosis or ischaemia of other organs such as myocardium, high serum ALT and AST activity suggest liver cell damage. (Srivastava and Chosdol, 2007). In cases of severe liver damage, ALT is found to be higher than AST. This is however not the case with samples assessed in this study.

The rats that were fed the extruded formulated meal and those fed the casein diet experienced close to no histopathological changes when compared to the rats that were fed the unextruded sample. The histopathological changes observed in the liver and kidney of rats given the unextruded meal reveals that antinutritional factors in the feed may have had adverse effects on the organs of the rats. The shrunken hepatocytes could be linked to the weight loss caused by undernutrition of the rats fed the basal diet. The liver, being the first organ that encounters all absorbed materials from the gastrointestinal tract, has been shown to respond to toxicological insults in a number of ways including cellular degeneration and necrosis, bile duct hyperplasia and fibrosis (Jubb *et al*., 1995). The kidney is an excretory organ that removes metabolised and nonmetabolised toxic materials from the body (Robbins *et al*., 1985; Akinnawo *et al.,* 2005). Hence, this organ would be exposed to high concentrations of the noxious materials that could have caused the lesions (Plate 8).

Conclusion

The Protein Efficiency Ratio (PER), the Net Protein Ratio (NPR), the Protein Retention Efficiency (PRE) and the Feed Conversion Ratio of the extruded formulated meals were comparable to those of casein diet (control). The protein rich feed enhanced the growth of the animals and bioavailability of the protein was enhanced by the extrusion cooking employed in the processing of the feed. Organ weights of the rats fed the

basal diet were significantly lower than rats on the other experimental diets indicating the inadequacy of the non-protein diet to maintain normal growth and body functions. Histopathological analysis of the organs of the test animals showed that there was no significant lesions in all the organs of rats fed the experimental diets. There is potential for using the formulated extruded complementary as an effective diet in the treatment of Protein Energy Malnutrition.

References

Abdul-Hamid E.I., Gengaihi S.E., Salem A, Bashandi S.A., Ibrahim N.A. and Abdel- Hamid S.R. (2002). Hypolipidemic effect of some vegetable oils in rats. *Journal of Food, Agriculture and Environment* 2: 88-93.

Adebowale, K.O. and Lawal, O.S. (2003). Foaming, gelation and electrophoretic characteristics of mucuna bean (Mucuna pruriens) protein concentrates. *Journal of Food Chemistry.* 83:237-246

Akinnawo, O.O., Taiwo, V.O., Ketiku, A.O. and Ogunbiyi, J.O. (2005). Weight Changes and Organ Pathology in Rats Given Edible Larvae of *Cirina Forda* (Westwood). *Afri J. Biomedical Res. 8:35 – 39*Aletor, V.A. and Fetuga, B.L. (1986). Some pathological aspects of raw lima beans (Phaseolus lunatus Linn) feeding to growing rats. *Nigerian Journal of Animal Production* 13: 48-57

Akpanabiatu, M.I., Ekpo, N.D., Ufot, U.F., Udoh, N.M., Akpan, E.J. and Etuk, E.U.I. (2013). Acute toxicity, biochemical and haematological study of *Aframomum melegueta* seed oil in male wistar albino rats. *Journal of Ethnopharmacology* 150(2):590-4.

Alabi M.O., Anuonye J.C., Ndaeji C.F. and Idowu A.A. (2001). Comparison of the growth and development of selected children in soybean and non soybean producing and utilization of villages in Niger State, Nigeria. *Polymath.* J. 2:8-12.

Al-Mamary M, Al-Habori M, Al-Aghbari A.M. and Baker M.M. (2002). Investigation into the toxicological effects of *Catha edulis* leaves: a short - term study in animals. *Phytotheraphy Research.* 16:127 – 132.

Aniagu, S.O., Nwinyi, F.C., Akumka, D.D., Ajoku, G.A., Dzarma, S., Izebe, K.S., Ditse, M., Nwaneri, P.E.C., Wambebe, C. and Gamaniel, K. (2005). Toxicity studies in rats fed nature cure bitters. *African Journal of Biotechnology* 4 (1):72-78.

Arendt, E. K. and Nunes, M. H. B. (2010). Processing gluten free foods in Allergen Management in the Food Industry, J. I. Boye, S. B. Godefroy, Ed. New Jersey: John Wiley & Sons, pp. 333-354.

Chai C., Ju H.K., and Kim S.C. (2011). Determination of bioactive compounds in fermented soybean products using GC/MS and further investigation of correlation of their bioactivities. *Journal of Chromatography B, Analytical Technologies in the Biomedical and Life Sciences*. 880(1):42-49.

Charunuch, C. Boonyasirikool, P. and Tiengpook, C. (2003). Using of Extrusion Process for Preparation of Instant Cereal Beverage Powders based on Corn and Soybean. Kasetsart *Journal of Natural Science*. 37: 72-83.

Devaki, K., Beulah, U., Akila, G. and Gopalakrishnan (2012). Effect of aqueous extract of *Passiflora edulis* on biochemical and haematological parameters of wistar albino rats. *Toxicology International*. 19(1): 63-67

Endres, J.G. (2001). Soy Protein Products: Characteristics, Nutritional Aspects, and Utilization. AOCS Press, Champaign, IL, USA.

Eweka, A.O. (2007). Histological studies of the effects of monosodium glutamate on the kidney of adult wiatar rats. *The International Journal of Health*. 6:2

Forbes, S.C., McCargar, L., Jelen, P., and Bell, G.J. (2014). Dose response of whey protein isolate in addition to a typical mixed meal on blood amino acids and hormonal concentrations. *International Journal of Sports Nutrition and Exercise Metabolism* 24(2): 188-195.

Harishankar, N., Kumar, P.U., Sesikeran, B. and Guridharan, N. (2011). Obesity associated pathophysiologyical and histological changes in WNIM obese mutant rats. *Indian Journal of Medical Research*. 134(3): 330-340.

Ikya, J. K., Gernah, D. I. and Sengev, I. A. (2013). Proximate composition, nutritive and sensory properties of fermented maize, and full fat soy flour blends for "agidi" production. *African Journal of Food Sciience*. 7(12):446-450.

Ingale, S. and Shrivastava, S. K. (2011). Nutritional study of new variety of groundnut (*Arachis hypogaea* L.) JL-24 seeds. *African Journal of Food Science* 5(8): 490 – 498.

Iwe, M.O. (2003). The science and Technology of Soybeans. Rojoint Communication Services, Enugu, Nigeria. 71-98.

Jubb, K.V.F, Kennedy, P.C. and Palmer, N. (1995). Pathology of domestic animals. 3rd edition, Academic Press Inc., New York.

Katina, K., Arendt, E., Liukkonen, K.-H., Autio, K., Flander L. and Poutanen K. (2005). Potential of sourdough for healthier cereal products *Trends in Food Science and Technology*, 16: 104-112.

Laswai , H.S., Pacific, R and Hussein, J. (2017): Suitability of cassava starch in making baked and fried composite flour products. *Tanzania*

Journal of Agricultural Sciences. 16(1): 9-16.

Liu, J.R. and Lin, C.W. (2001). Production of kefir from soymilk with or without added glucose, lactose or sucrose. *Journal of Food Science* 65:716-9.

Mbuya, K., Nkongolo, K.K., and Kalonji-Mbuyi, A. (2011): Nutritional analysis of quality protein maize varieties selected for agronomic characteristics in a breeding program *International Journal of Plant Breeding and Genetics*. 5:317-327.

Ogundeji, T.1, Ayo, J.O., Aluwong, T.1 and Mohammed, A. (2013). Behavioural and haematological studies on effects of lycopene in Wistar rats subjected to psychological stress. *Journal of Neuroscience and Behavioural Health*. 5(2): 30-35.

Omaye, S. T. (2004). Food and nutritional toxicology. CRC Press LLC. 77

Omosebi M.O., Osundahunsi O.F., and Fagbemi T.N. (2018) Effect of extrusion on protein quality, anti-nutritional factors, and digestibility of complementary diet from quality protein maize and soybean protein concentrate. *Journal of Food Biochemistry*. 42(4): e12508. https://doi.org/10.1111/jfbc.12508.

Osundahunsi, O.F. and Aworh, O.C. (2003). Nutritional evaluation, with emphasis on protein quality, of maize based complementary foods enriched with soya bean and cowpea tempe. *International Journal of Food Science and Technology*. 38: 809-813.

Osundahunsi, O.F., Fagbemi, T.N., Kesselman, E. and Shimoni, E. (2003). Comparison of the physicochemical properties and pasting characteristics of flour and starch from red and white sweet potato cultivars. *Journal of Agriculture and Food Chemistry*. 51: 2232-2236.

Osundahunsi, O.F., Seidu, K.T., and Mueller, R. (2011). Dynamics rheological and physicochemical properties of annealed starches from two cultivars of cassava. *Carbohydrate Polymers*, 83(4): 1916-1921.

Osungbaro TO, Esuoso YA, Esuoso KO (2009). Physical and nutritive properties of fermented cereal foods. *African Journal of Food Science*. 3(2):023-027. Available online http://www.academicjournals.org/ajfs.

Robbins, S.L., Cotran, R.S. and Kumar, V., eds. (1984). Environmental pathology. chemical and drug injury. In: Pathological Basis of Disease. 3rd edition, W.B. Saunders and Company, Philadelphia, pp. 452-480.

Savala, N.K., Haritha, P.N. and Narasu, M.L. (2012). Haematological studies of *Emblica officinalis* formulation on Wistar Rats. *International Journal of Medical Health Science*. 1(2): 29-34.

Srivastava, T and Chosdol, K (2007). Clinical Biochemistry: Clinical enzymology and its applications. All India Institute of Medical