Effects of Composite Maize-Oat-Pigeon Pea Blend on Growth Profile and Biochemical Profile of Albino Rats

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Abstract

The aim of this study was to examine the effects and efficacy of various combinations of pigeon pea, oats, and maize on the blood properties and growth patterns of albino rats. Pigeon peas (Cajanus cajan), oats (Avena sativa), yellow corn (Zea mays), and a control group fed Growers mash chicken feed were among the mainstays used in the study. Standard AOAC techniques were used to assess the blends' chemical and functional characteristics. Carbohydrate was also determined by difference. Additionally, the mixes' impacts on the albino rats' growth profile and biochemical indicators were investigated. Blend X2 had the highest moisture content, measuring 13.56g/100g. At 68.57g/100g, X7 had the highest carbohydrate content of all the blends. When compared to other blends, Blend X5 had the greatest protein level at 13.93g/100g. All of the composite blends had a relatively low fat level. The fibre content of Blend X3 was somewhat higher than that of the other blends. The amount of iron was greater than that of every other mineral examined. Only vitamin A had the greatest content among all the vitamins that were assessed. Following the experimental investigation, the rats fed diet X2 showed a noticeable increase in weight. Notable biochemical parameters were present in the rats. According to the findings, the composite blends may prove to be a useful and nutritious addition to baby food recipes. Additionally, the results demonstrate how these blends might avoid malnutrition and promote appropriate supplemental feeding by utilising locally accessible basic foods.

Keywords

Maize, Oat, Pigeon Pea, Growth Profile, Blood Characteristics, Albino Rats

1. Introduction

It becomes clear that access to sufficient nutrition during the early years of life is a necessary condition for a child to develop all of their potential. It is well known that the period between birth and age two is extremely important for promoting the best possible development of growth, health, and behavioural traits. This age group is especially susceptible to stunted development, deficiencies in essential micronutrients, and common childhood illnesses including diarrhoea, according to longitudinal research [1]. Reversing the consequences of prior stunting becomes extremely difficult once a child reaches the age of two. Impaired cognitive and motor development, as well as high rates of illness and mortality, are the direct consequences of poor nutrition during these critical years. Over time, there is a clear correlation between intellectual deficits and early nutritional inadequacies. Growing infants' dietary demands are only met by breastfeeding until they are four months old [2].

After that, adding supplemental nutrition becomes essential to guaranteeing an infant's good development. The weaning process, which entails switching from breast milk to a wider variety of solid meals and beverages, marks a time of change for the child's general health as well as their diet. The need to supply the necessary food energy, protein, lipids, critical minerals, vitamins, iron, and other micronutrients needed for regular growth, development, and the promotion of optimal health is the scientific basis for weaning [3]. The disease known as protein-energy malnutrition (PEM) typically appears during the crucial time when a kid switches from drinking only breast milk to eating other kinds of food [4].

In many underdeveloped nations, traditional weaning meals consist primarily of locally available staples like grains and tubers. Newborns mostly get their calories and protein from these meals. But because of their high bulk, low protein content, and poor energy density, many staple foods—like the well-liked weaning food ogi—cause PEM symptoms in neonates. While legumes provide a respectable amount of lysine but lack sulfur-containing amino acids like methionine and cysteine, cereals typically lack critical amino acids like tryptophan and lysine. As a result, in terms of nutritional makeup, grains and legumes can enhance one another. Nigeria provides a wide range of nutrient-dense meals from widely grown legumes like soybean and cowpea as well as cereals like maize.

However, the significance of other legumes, like the pigeon pea, is sometimes overlooked in favour of these cereals and legumes. Due to its use as a staple meal and industrial raw material, maize is in great demand and plays a large role as an energy source in complementary feeds. According to reports, 45–55% of supplemental feed is made from maize, hence finding substitute feed sources is essential to lowering production costs. The continuous hunt for alternative feedstuffs is sparked by the rising price of maize [5].

Pigeon peas have attracted a lot of interest lately in the functional food industry. A legume with enormous unrealised potential for both raising the volume and improving the quality of production in Africa is the pigeon pea, or "fiofio" as it is known in southeast Nigeria. Unfortunately, pigeon peas are still largely underutilised in Nigeria, with little attention paid to how they might help poor farming households that rely mostly on starch-based foods for their diet and have little money. Pigeon pea is unique among region-adapted legumes because of its remarkable combination of ideal nutritional profiles, strong resilience to environmental stressors, high biomass productivity, and notable contributions of moisture and nutrients to the soil [6]. Furthermore, the pigeon pea's adaptability permits a variety of methods of use, and the wide genetic diversity seen in both cultivated and wild cousins of the legume promises more research and many applications. Pigeon peas have a substantial amount of protein, especially lysine, an essential sulfur-based amino acid that is frequently lacking in other grains and cereals, according to extensive research [7].

Pigeon pea's incapacity to successfully compete with widely grown legumes like cowpea and soybean is one of its major obstacles. But acknowledging the economic importance of these underutilised crops and the rising demand, especially globally, is the first step towards realising their full potential. According to the National Agricultural Quarantine Service (NAQS), Nigeria has secured an incredible $100 billion deal from India to export legumes, including pigeon peas. This offers a great chance to make a lot of money, which will particularly help women who grow pigeon peas as their primary source of income and ensure nutritional security for their households [8]. Pigeon pea seeds are an important part of the diets of many African, Asian, and South American nations and are a useful addition to staple cereals including corn, maize, wheat, and rice. Pigeon peas provide a well-balanced nutritional profile that can compete with other dense protein sources like whey and soy, in addition to being a cost-effective source of protein, carbs, minerals, and vitamins, especially for vegetarian diets. Pigeon peas are rich in polyphenols and flavonoids, which give them a variety of therapeutic benefits in addition to their nutritional worth. Additionally, a number of studies have demonstrated the ability of pigeon peas to lower the risk of a number of diseases, highlighting their critical role in boosting human health [9]. In Nigeria, pigeon peas are considered an inadequate human nutritional source since they are not as palatable as cowpeas. Its industrial applications are also limited, and it takes longer to cook. As a result, it cannot be regarded as a good alternative to maize.

The seventh most widely cultivated and important cereal in terms of commerce worldwide is oats. Prior to being used as a nutrient-dense food source for both people and animals, it was first grown for its therapeutic qualities. Oats are a naturally gluten-free grain that can be consumed by people with coeliac disease and other gluten-related conditions. More than 60% of the grain weight is made up of starch, which is the main component that determines the physicochemical and structural properties of oats. Beneficial macronutrients found in whole-grain oats include proteins with a well-balanced necessary amino acid composition and lipids with a high degree of unsaturated fatty acids, which account for roughly 40 and 36 percent of total fatty acids, respectively [10]. β-glucan, a soluble form of dietary fibre that has been demonstrated to have a number of physiological benefits, including regulating postprandial blood glucose levels and reducing serum cholesterol levels, is abundant in oats (2–8.5% w/w of oat grain) (Bai et al., 2024). However, bioactive substances such avenanthramides, polyphenols, phenolic acids, carotenoids, vitamin E, and phytosterols are rich in oat grains. [11]

A major obstacle to a child's ideal development and general well-being is inadequate nutrition in the early years of life. For the development of growth, health, and behavioural traits, the period between birth and two years old is especially important. Longitudinal studies, however, continually show how susceptible this age group is to typical childhood illnesses like diarrhoea, nutritional shortages, and stunted development. High rates of morbidity and mortality, along with delayed cognitive and motor development, are the direct results of inadequate nutrition during these critical years. Furthermore, there is a clear correlation between long-term intellectual deficits and early nutritional inadequacies [12].

The limits of traditional weaning diets in many underdeveloped nations, which mostly rely on locally accessible staples like grains and tubers, further exacerbate the issue. Protein-energy malnutrition and its related symptoms are caused by these diets' frequent deficiencies in sufficient protein content, energy density, and vital micronutrients. Because legumes lack certain key amino acids and cereals lack others, it is vital to combine these complimentary food sources in order to meet nutritional needs. Additionally, a major obstacle to food and income security in low-income farming households is the underutilisation of nutrient-dense legumes such pigeon pea [13]. Pigeon peas have significant biomass productivity, tolerance to environmental stressors, an ideal nutritional profile, and soil enriching qualities. But in nations like Nigeria, where farming households mainly rely on starch-based foods and have few other sources of income, its potential is still largely unrealised. [14]

Recent scientific studies provide support for tackling the issue of undernutrition in the early stages of life and investigating the possibilities of underutilised legumes like pigeon pea. The time between birth and two years old is crucial for the best possible growth, health, and behavioural development, according to recent studies. This age group is susceptible to frequent childhood illnesses, nutritional deficits, and delayed development, according to longitudinal studies [15]. Additionally, current studies show how inadequate conventional weaning meals based on locally accessible staples are in meeting infants' nutritional needs. These studies highlight how crucial it is to combine complementary food sources, including legumes and cereals, in order to supply the vital nutrients needed for the best possible growth and development.

Furthermore, new studies highlight how underutilised legumes like pigeon pea might help poor agricultural households increase their food and financial security. Pigeon peas are a promising crop for combating hunger and advancing sustainable agricultural methods because of its nutritional profile, resistance to environmental pressures, biomass productivity, and soil enrichment qualities [16]. We can improve children's health, development, and general well-being by tackling the issue of inadequate nutrition and investigating the possibilities of underutilised legumes, especially in the crucial early years of life.

2.Materials and Methods

2.1 Sample Preparation and Processing

2.1.1 Production of Fermented Maize Flour

The fermented maize flour was produced .The maize grains were sorted, cleaned and soaked in water. It was subjected to 48h fermentation period. The maize grains were allowed to ferment by the microflora inherent in them at temperature of 28±2^{0}C. The fermented grains were dried at 60^{0}C for 12 hours in an air circulating oven, hammer milled and passed through a 0.5 mm mesh size sieve. Afterwhich, it was packaged in an air tight polyethylene bags, store in plastic containers with lids and stored in cool dry place pending usage.

2.1.2 Production of Oats Flour

To prepare the oats for further use, three (3) kilograms of oats were carefully inspected to remove any dirt, stones, and other foreign particles. A cleaning procedure was used on the oats that exhibited no defects. Then, for a total of 12 hours, they underwent drying inside a hot-air oven (Plus11 Sanyo Gallenkamp PLC, Loughborough, Leicestershire, UK) at an average temperature of 55°C. The oats went through drying before being ground in a lab blender (Model KM 901D; Kenwood Electronic, Hertfordshire, UK) and then sieved using a 200 mm mesh (British Standard) screen to yield the flour from them. When the grain flour had been gathered, it was placed in a plastic bag, sealed up and kept at ambient temperatures (27°C) till it was required.

2.1.3 Production of Fermented Pigeon Pea Flour

Three (3) kilograms of pigeon pea seeds were carefully inspected and sorted to eliminate any dirt, stones, insects, defects, and other foreign particles. Subsequently, the seeds were washed thoroughly. After washing, the grains were soaked in water at a volume ratio of 1:3 (seed to water) for duration of 3 hours using a large container. Once the soaking period was complete, the water was drained off. Out of the soaked grains, two kilograms were selected. These grains were then dehulled, crushed, and wrapped in plantain leaves. The wrapped grains were allowed to undergo fermentation for a period of 4 days.

Following fermentation, the grains were dried in an oven at a temperature of 60°C for 7 hours. The fermented and dried cotyledons were then milled into flour using a disc attrition mill (Asiko All, Addis Nigeria) and passed through a standard 1.0mm mesh sieve. The resulting flour was packaged in a polyethylene bag for further studies.

2.2 Formulation of Composite Blend Samples

The blends were formulated using the individual ingredients in accordance with the specified percentages or proportions outlined in Table 1.

Table 1. Composition of blends

Ingredients	**Formulations in g/100g**	**Formulations in g/100g**	**Formulations in g/100g**	
	Fermented Maize Flour	**Oat Flour**	**Fermented Pigeon pea Flour**	
X1	**70**	**25**	**5**	
X2	**60**	**30**	**10**	
X3	**60**	**20**	**20**	
X4	**70**		**30**	
X5	**80**		**20**	
X6	**85**		**15**	**Commercial diet reference (Control)**
X7		**70**	**30**	
X8		**85**	**15**	
X9		**75**	**25**	
X10				

The control sample utilized in the study was Chicken Feed (CF) - Grower mesh.

2.3 Collection of Blood Sample

The Wistar rats underwent a 28-day trial period prior being put through to a nocturnal fast despite still having a supply of water. They were then put to death by heart puncture using chloroform anesthesia, and specimens of their blood were obtained from them. Then, heparinized and non-heparinized tubes were generated based on these blood samples. After allowing the non-heparinized tubes to form a clot, the blood serum was separated by centrifuging them at 3,000 x g for a period of 25 minutes. The blood samples were stored in a deep freezer at -20°C until they could be subjected to hematological and biochemical analyses at the Medical Laboratory Unit of the University Health Centre, Federal University of Technology, Akure. Furthermore, the rats' organs, including the liver, two kidneys, and heart, were excised, cleared of fat, dried with clean tissue paper, and then weighed.

2.4 Biochemical Parameters of Rats Fed on Composite Flour Samples

The biochemical variables were assessed utilizing the procedures described by Jasper. Initially, a sample of their blood was centrifuged at room temperature, specifically at a speed of 1,500 x g for duration of 10 minutes. This centrifugation step facilitated the separation of the serum, which was subsequently employed for evaluating liver function by measuring the levels of enzymes, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP). The tests were carried out using standard method

3.Statistical Analysis

Analysis of variance (ANOVA) was used for statistical evaluation of the results employing SPSS (IBM version 20.0, SPSS Inc., Quarry Bay, Hong Kong). The resultant averages and their standard errors (SEM) are shown. Both the Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT) were used in order to assess disparities among the groups. In accordance with the recommendations made by Yalta and Talha (2008), a significant level of p 0.05 was used.

4. Results

4.1 Effects of these Blends on the Growth Profile of Albino Rats

The rats that were fed with sample X6 had body weight of 56.6g before the experimental study, after feeding for 35 days with an average meal of 47g, the average weight of rat was 46.5g. Nevertheless, the rats fed with diet X2 (46.46g) supported increase in growth after experimental study, followed by rats fed with X1 (45.89g) and X3 (39.31g). The rats weighed nearly 55g after feeding for 35 days at averagely 40g feed. The rats that were given the control diet, X10 did not reduce in weight with about 5g difference after 30 days.

Table 2. Effects of these Blends on the Growth Profile of Albino Rats

Diet Codes	Avg. Initial Weight of Rats (g)	Avg. Feed Consumption (g) for 30 days	Avg. Final Weight of Rats (g)	Avg. Weight gain Percentage (%)
X1	45.89	45	52.57	14.56
X2	46.46	39.5	54.78	17.91
X3	39.31	40	44.98	14.42
X4	49.43	38	40.7	-17.66
X5	51.59	41.5	40	-22.47
X6	56.60	47	46.48	-17.88
X7	53.15	43.5	42.41	-20.20
X8	52.07	39.5	40.52	-22.18
X9	36.22	39.5	40.5	11.82
X10	47.5	45.5	42.5	-10.53

Values are means ± SD. Values on the same column with different superscripts are significantly different.

Keys

X1 - 70% fermented maize flour: 25% oat flour: 5% fermented pigeon pea flour

X2 - 60% fermented maize flour: 30% oat flour: 10% fermented pigeon pea flour

X3 - 60% fermented maize flour: 20% oat flour: 20% fermented pigeon pea flour

X4 - 70% fermented maize flour: 0% oat flour: 30% fermented pigeon pea flour

X5 - 80% fermented maize flour: 0% oat flour: 20% fermented pigeon pea flour

X6 - 85% fermented maize flour: 0% oat flour: 15% fermented pigeon pea flour

X7 - 0% fermented maize flour: 70% oat flour: 30% fermented pigeon pea flour

X8 - 0% fermented maize flour: 85% oat flour: 15% fermented pigeon pea flour

X9 - 0% fermented maize flour: 75% oat flour: 25% fermented pigeon pea flour

X10 - Commercial diet reference

4.2 Effects of These Blends on Some Biochemical Indices Using Rat Bioassay

Table 3 shows the summary for the percentage of hemoglobin; HB (%), packed cell volume; PCV (%), Aspartate Transaminase; AST, Alanine Transaminase; ALT, and Alkaline Phosphatase; ALP. In this study, RX1 showed 8% hemoglobin against initial 6% while these ones; RX6 and RX10 with initial hemoglobin levels of 4% had a slightly increased hemoglobin level of 6% and 7% respectively. The rat with the least recorded final hemoglobin level after the feeding trial was RX9 (4%). Previously, blend X9 has shown tremendous amounts of anti-nutritional factors. The rat RX1 had the highest amount of packed cell volume of 24%. In terms of other liver enzymes; AST, ALT and ALP were highest in rats; RX7 (67.45U/L), RX7 (65.25U/L) and RX4 (478.17U/L) respectively.

Table 3. Effects of these blends on some biochemical indices using rat bioassay

Rat Baseline Data					
Rat Codes	HB (%)	PCV (%)	AST (U/L)	ALT (U/L)	ALP (U/L)
RX1	6	19	19.8	37.74	230.94
RX2	6	19	42.99	41.27	208.57
RX3	6	18	36.08	22.36	372.78
RX4	6	18	29.66	27.82	547.17
RX5	6	19	37.48	35.64	776.18
RX6	4	13	18.72	18.32	576.6
RX7	8	25	68.47	67.63	267.13
RX8	6	19	5.67	10.02	261.67
RX9	8	19	22.95	21.63	467.3
RX10	4	13	56.7	42.23	241.74

Values are means $\pm$ SD. Values on the same column with different superscripts are significantly different.

Keys

X1 - 70% fermented maize flour: 25% oat flour: 5% fermented pigeon pea flour

X2 - 60% fermented maize flour: 30% oat flour: 10% fermented pigeon pea flour

X3 - 60% fermented maize flour: 20% oat flour: 20% fermented pigeon pea flour

X4 - 70% fermented maize flour: 0% oat flour: 30% fermented pigeon pea flour

X5 - 80% fermented maize flour: 0% oat flour: 20% fermented pigeon pea flour

X6 - 85% fermented maize flour: 0% oat flour: 15% fermented pigeon pea flour

X7 - 0% fermented maize flour: 70% oat flour: 30% fermented pigeon pea flour

X8 - 0% fermented maize flour: 85% oat flour: 15% fermented pigeon pea flour

X9 - 0% fermented maize flour: 75% oat flour: 25% fermented pigeon pea flour

X10 - Commercial diet reference

Table 4. Effects of these blends on some biochemical indices using rat bioassay

Rat Experimental Data					
Rat Codes	HB (%)	PCV (%)	AST (U/L)	ALT (U/L)	ALP (U/L)
RX1	8	24	19.42	36.4	240.14
RX2	7	22	40.8	40.2	206
RX3	6.5	19	36.2	22.1	375.26
RX4	7	21	29.42	25.81	478.17
RX5	7	21	36.5	36.5	475.18
RX6	6	19	18.65	19.42	378.13
RX7	6	18	67.45	65.25	250.4
RX8	7.5	22	18.62	16.45	260.14
RX9	4	13	12.67	22.67	370.3
RX10	7	22	14.12	40.12	254.32

Values are means $\pm$ SD. Values on the same column with different superscripts are significantly different.

Keys

X1	-	70% fermented maize flour: 25% oat flour: 5% fermented pigeon pea flour
X2	-	60% fermented maize flour: 30% oat flour: 10% fermented pigeon pea flour
X3	-	60% fermented maize flour: 20% oat flour: 20% fermented pigeon pea flour
X4	-	70% fermented maize flour: 0% oat flour: 30% fermented pigeon pea flour
X5	-	80% fermented maize flour: 0% oat flour: 20% fermented pigeon pea flour
X6	-	85% fermented maize flour: 0% oat flour: 15% fermented pigeon pea flour
X7	-	0% fermented maize flour: 70% oat flour: 30% fermented pigeon pea flour
X8	-	0% fermented maize flour: 85% oat flour: 15% fermented pigeon pea flour
X9	-	0% fermented maize flour: 75% oat flour: 25% fermented pigeon pea flour
X10	-	Commercial diet reference
HB	-	Hemoglobin
PCV	-	Packed cell volume
AST	-	Aspartate aminotransferase
ALT	-	Alanine aminotransferase
ALP	-	Alkaline phosphatase

5. Discussion

The most crucial period for a child's growth and development is the first 1000 days of life. Therefore, to maximise children's potential for growth, development, and well-being, complementary feeding practices should be enhanced [17]. According to the study, Table 4.5 displays the relative initial and final weights of experimental rats fed formulated diets and control samples at the conclusion of the experiment, as well as the final average body weight (g), initial average body weight (g), average feed consumption, and average body weight gain (%).

Rats fed formulated diets increased weight in a range of 40.0 to 54.7 g, with the X2 group gaining the most weight and sample X5 gaining the least. Groups fed maize, oats and pigeon peas acquired the most weight, while the control sample gained the least (42.5 g). The experimental rats who consumed sample X2 showed a 17% weight gain, which may have been caused by balanced nutrients and possibly lower levels of anti-nutrients because there was less pigeon pea added to the mixture. Weight gain may be influenced by the amount of protein consumed. Additionally, compared to bigger portions, the study demonstrated that the rats could tolerate smaller amounts of pigeon pea. Therefore, better feeding and absorption can be encouraged by using enhanced processing techniques. Young animals that lack protein experience anaemia, hypoproteinemia, decreased growth, body protein depletion, wasting, emaciation, and, in extreme cases, death [18]. The rats' food intake varied between 38 and 47g, with the rats fed maize and pigeon peas consuming more. Improved processing methods and palatability may be the cause of this observation. Consequently, the rats' food intake was enhanced by the created blends.

Blood analysis in the lab is crucial for determining an organism's nutritional condition and physiological health since it enables the evaluation of many metabolites and other advantageous compounds in animal bodies [19]. Laboratory measurements of blood protein and micronutrient levels are used in the biochemical examination as a means of determining particular nutritional deficits and evaluating overall nutritional status. [20]

This study examined the impact of different diets on blood using a haematological and biochemical investigation. Haemoglobin test has been proposed as a key metric for assessing circulatory erythrocytes and is important for diagnosing anaemia. In contrast to the baseline period's cumulative average of 6%, the average haemoglobin during the experimental phase was 6.6%. After ingesting the blend (X2), the Rat (RX2) experienced a marked increase in haemoglobin and a large increase in weight. Therefore, nutritional problems can be addressed using enriched supplemental foods [21].

Furthermore, the haemoglobin content of the rats fed control meal increased as well. The liver function tests check for liver dysfunction, and the highest levels in the rat's liver are above the normal reference levels of AST, ALT, and ALP. Important indicators of liver function are these blood enzymes: alkaline phosphatase (ALP), alanine transaminase (ALT), and aspartate transaminase (AST). The degree of tissue damage is closely correlated with blood levels of ALT and AST [22]. Rats (RX7) fed a 70% oat and 30% pigeon pea blend had the highest AST values among the experimental rats, which ranged from 12.67 to 67.45 U/L. Rats (RX9) had the lowest AST values. In healthy animals, the typical range for AST is 10–34 U/L.

The experimental rats' ALT levels ranged from 16.45 to 65.25 U/L, while the usual range is between 10 and 130 U/L. The experimental rats' ALP readings varied from 206 to 478.17 U/L, while the usual range is between 24 and 147 U/L. Even while the observed elevated serum ALP is consistent with hepatocellular damage, there may be other contributing causes that are not necessarily related to the disease or cellular damage. With the exception of ALP values, RX1, which displayed good haemoglobin level and packed cell volume percentages, had normal levels of AST and ALT. This experimental study suggests that the prepared blends are appropriate for supplemental feeding and will therefore promote growth and development**.**

6. Conclusion

This study has shown that maize, oat and pigeon pea blend could serve as a viable complementary feed for infants. The favourable nutritional composition and high protein content of the blend likely contributed of the growth patterns and improved biochemical markers.

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