ISSN: 2690- 912X

Research Article

Journal of Genetic Engineering and Biotechnology Research

Nutrient Composition of Formulated Infant Complementary Foods and Their Effects on Growth Velocity and Micronutrient Status of Weanling Wistar Rats

Ngozi Mercy Nnam[1], Ifeoma Uzoamaka Onoja[2] and Peace Nwanneka Ani[1]*

[1]*Department of Nutrition and Dietetics, University of Nigeria, Nsukka, Enugu State, Nigeria*

[2]*Department of Nutrition and Dietetics, University of Nigeria Teaching Hospital Ituku Ozalla, Enugu State, Nigeria*

***Corresponding Author**
Peace Nwanneka Ani, Department of Nutrition and Dietetics, University of Nigeria, Nsukka, Enugu State, Nigeria.

Submitted: 2026, May 12; **Accepted:** 2026, Jun 29; **Published:** 2026, Jul 07

Citation: Nnam, N. M., Onoja, I. U., Ani, P. N. (2026). Nutrient Composition of Formulated Infant Complementary Foods and Their Effects on Growth Velocity and Micronutrient Status of Weanling Wistar Rats. *J Gene Engg Bio Res, 8*(2), 01-14.

Abstract

The study evaluated the nutritional composition of infant complementary foods formulated from locally available foods (maize, soybean, carrot, fluted pumpkin leaf, and pawpaw). The study adopted an experimental design. The food samples were purchased from New Market in Enugu, Enugu State, Nigeria. The fruits and vegetables were freeze-dried and processed into flour while maize and soybean were processed into flour using conventional processing methods. Composite flours were developed in varying proportions (nine diets) based on thirteen-gram (13g) protein requirement per day and analyzed for proximate, vitamin, and mineral composition using standard analytical methods. A 28-day feeding trial involving male weanling albino rats were used to assess nutritional quality. The rats were randomly distributed into nine (9) groups of six (6) rats each based on body weight. The nine different groups of rats were fed the flours of the formulated diets ad libitum. At the end of the feeding trial their growth performance, serum micronutrient status, and hematological indices were assessed. Data were analyzed using SPSS, version 27 and presented as means and standard deviations. Paired sample T-test was used to compare the means. Significance was set at $p < 0.05$. The protein (22.56% - 26%), fat (12.93 - 13.96 %), ash (4.42-5.71%) and crude fiber (4.57 - 5.61%) contents of the complementary foods were higher than that of the control (Cerelac – 15%, 10%, 3%, 4.50% respectively). There were significant improvements ($p < 0.05$) in growth performance and micronutrient status following dietary intervention. Complementary foods formulated from locally available cereals, legumes, fruits, and vegetables significantly enhanced biological outcomes.

Keywords: Fortification, Weaning Diets, Growth Performance, Micronutrient Status, Hematological Assessment

1. Introduction

Malnutrition remains one of the most serious global public health concerns, particularly in developing countries where infants and young children are highly vulnerable due to their increased nutritional requirements during periods of rapid growth and development [1,2]. The first 1,000 days of life, spanning from conception to a child's second birthday, represent a critical period for physical growth, brain development, and immune function [3,4]. During this period, adequate nutrition is essential to ensure optimal health outcomes. While exclusive breastfeeding provides sufficient nutrients for the first six months of life, complementary foods become necessary thereafter to meet increasing nutritional demands [5]. However, in many low-and-middle income countries, including Nigeria, complementary feeding practices are often inadequate in terms of timing, frequency, and nutritional quality [6,7].

In Nigeria, traditional complementary foods are predominantly cereal-based, particularly maize, sorghum, and millet. These foods are often prepared as thin porridges with low energy and nutrient density, making it difficult for infants to consume adequate amounts to meet their nutritional requirements [8]. Although these foods provide energy, they are often deficient in high-quality protein, essential vitamins, and minerals such as iron, zinc, calcium, and vitamin A. The presence of anti-nutritional factors such as phytates and tannins in cereal-based diets are also militating factor as they inhibit the bioavailability of micronutrients, particularly iron and zinc, thereby exacerbating the risk of deficiency even when dietary intakes appear sufficient [9]. Children consuming these diets are susceptible to protein-energy malnutrition, micronutrient deficiencies, growth retardation, impaired cognitive development, and increased susceptibility to infections [10].

The persistent prevalence of child malnutrition in Nigeria highlights the urgent need for innovative and sustainable interventions (National Population Commission [11]. According to the 2018 Nigeria Demographic and Health Survey, approximately 32% of children under five years of age are stunted, 7% are wasted, and 22% are underweight. Micronutrient deficiencies are also highly prevalent, with vitamin A deficiency affecting about 30% of children, iron deficiency anaemia affecting approximately 67%, and zinc deficiency being widespread [12]. The situation is compounded by poor dietary diversity, with only 22% of Nigerian children aged 6–23 months receiving a minimum acceptable diet. These alarming statistics underscore the critical need for affordable and nutritionally adequate complementary foods to bridge the nutritional gap during the weaning period.

One promising strategy to achieve this is fortification of traditional complementary foods with locally available nutrient-dense foods such as soybean, carrot, pawpaw, and fluted pumpkin leaves. Soybean (*Glycine max*) is an excellent source of high-quality protein, containing all essential amino acids, as well as significant amounts of healthy fats, calcium, iron, zinc, and folate [13]. Fluted pumpkin leaves (*Telfairia occidentalis*) are exceptionally rich in protein, β-carotene (pro-vitamin A), iron, calcium, magnesium, potassium, and fibre, making them a valuable addition to complementary foods [14,15]. Carrots (*Daucus carota*) are renowned for their high β-carotene content, which serves as a precursor to vitamin A, as well as significant amounts of dietary fibre, potassium, and vitamin K [16,17]. Pawpaw (*Carica papaya*) is widely consumed across Nigeria and is an excellent source of vitamin C, an essential nutrient that enhances iron absorption, supports immune function, and acts as a powerful antioxidant [18,19]. These foods are rich sources of protein, vitamins, minerals, and bioactive compounds that can significantly improve the nutritional quality of cereal-based complementary foods [20]. Utilizing locally available foods offers an affordable, culturally acceptable, and sustainable approach to improving infant nutrition while promoting local agricultural production [21].

Studies have shown that inadequate dietary intake during infancy is strongly associated with growth faltering, impaired cognitive development, weakened immunity, and reduced productivity later in life [22]. Therefore, evaluating the nutritional quality, safety, acceptability, and biological effectiveness of complementary foods formulated from locally available food resources is crucial for developing practical interventions aimed at reducing malnutrition among Nigerian infants and young children. This study was conducted to evaluate the nutrient composition of formulated infant complementary foods and their effects on growth velocity and micronutrient status of weanling Wistar rats.

2. Materials and Methods

2.1. Study Design

This study employed an experimental design.

2.2. Procurement of Samples

The ingredients; maize (*Zea mays*), soybean (*Glycine max*), fluted pumpkin leaves (*Telfairia occidentalis*), carrot (*Daucus carota*), and pawpaw (*Carica papaya*) were purchased from New Market in Enugu North Local Government Area of Enugu State, Nigeria. Upon procurement, they were transported under hygienic conditions to the laboratory at the Department of Nutrition and Dietetics, University of Nigeria, Nsukka, for processing and analysis. Each sample was carefully handled to maintain its integrity and nutritional value.

2.3. Preparation of Samples for Analysis

2.3.1. Maize Flour Preparation

Fifteen kilograms (15 kg) of yellow maize were sorted to remove dirt, stones, and damaged grains. The cleaned grains were washed and soaked in clean tap water in a 1:3 (w/v) ratio at 28 ± 2°C for 48 hours to allow spontaneous fermentation. The soaking water was changed after 24 hours to reduce the sour taste associated with prolonged fermentation. After fermentation, the maize was drained and oven-dried at 55°C for 48 hours. The dried maize was milled into fine flour using a hammer mill (Thomas Wiley Mill Model ED-5) and sieved through a 70 mm mesh sieve. The maize flour was stored in clean, airtight containers at −4°C until required for analysis and formulation. This fermentation procedure was adopted to enhance the nutritional quality and reduce the bulk density of the maize flour, as described by [23].

2.3.2. Soybean Flour Preparation

Fifteen kilograms (15 kg) of soybeans were sorted to remove dirt, stones, and extraneous materials, then washed thoroughly. The cleaned soybeans were boiled in water for 45 minutes to reduce anti-nutritional factors, including trypsin inhibitors and haemagglutinins [24]. After boiling, the soybeans were allowed to cool before being manually dehulled and soaked for 8 hours, with the soaking water changed every 3 hours. The soaked beans were drained and oven-dried at 55°C for 24 hours. The dried seeds were milled into fine flour using a hammer mill and stored in airtight containers at −4°C until required.

2.3.3. Fluted Pumpkin Leaf Powder Preparation

Ten kilograms (10 kg) of fresh fluted pumpkin leaves (*Telfairia occidentalis*) were thoroughly washed with clean water to remove dirt and potential contaminants. The washed leaves were drained and freeze-dried to preserve heat-labile nutrients, including vitamins and antioxidants. The dried leaves were milled into a fine powder using a laboratory hammer mill (*Thomas Wiley Mill*) and sieved through a 0.5 mm mesh to obtain a uniform particle size. The pumpkin leaf powder was stored in airtight plastic containers at −4°C until required.

2.3.4. Carrot Powder Preparation

Fresh carrots were cleaned, peeled, and sliced into 5–6 mm thick pieces. The slices were pre-frozen at −40°C for 16 hours to facilitate faster drying. The pre-frozen slices were arranged in a single layer on freeze-dryer trays, ensuring they did not overlap. Freeze-drying was performed using a laboratory freeze dryer

(Model FD-10, Christ, Germany), operated according to the manufacturer's instructions. The machine first froze the samples to approximately −40°C, after which the pressure was reduced to initiate sublimation, allowing ice to be removed as vapour. Freeze-drying continued until the carrot slices were light, crisp, and fully dry at the centre. The dried carrots were milled into a fine powder using a laboratory grinder (Model HL 3294, Ephilops). The carrot powder was packaged in airtight containers and stored under refrigerated conditions (−4°C) until required for incorporation into the composite flour. Freeze-drying was employed to preserve the carotenoid content and bioactive compounds of the carrots.

2.3.5. Pawpaw Powder Preparation

Fresh pawpaw fruits were cleaned, peeled, and sliced into 5–6 mm thick pieces. The slices were pre-frozen at −40°C for 18 hours. The pre-frozen slices were arranged in a single layer on freeze-dryer trays and freeze-dried using a laboratory freeze dryer (Model FD-10, Christ, Germany). The machine first froze the fruit to approximately −40°C, after which the pressure was reduced to initiate sublimation. Freeze-drying continued until the samples were light, crisp, and fully dry at the center. The dried pawpaw was ground into a fine powder using a laboratory grinder (Model HL 3294, Ephilops). The pawpaw powder was packaged in airtight containers and stored under refrigerated conditions (−4°C) until required for incorporation into the composite flour. Freeze-drying was selected to minimize degradation of heat-labile nutrients such as vitamin C and carotenoids.

2.4. Formulation of Composite Flours

The processed flours from maize, soybean, fluted pumpkin leaves, carrot, and pawpaw were combined in varying proportions to formulate composite flours based on recommended nutritional guidelines for complementary foods (Table 1). The formulation ratios of the complementary food were adjusted to meet the requirements for energy, protein, and micronutrient content, in line with the Codex Alimentarius Commission standards for formulated complementary foods [25]. The final complementary food blends were stored in airtight containers in a cool, dry place to maintain their quality until analysis.

Sample	Ratio	Maize (g)	SoyBean (g)	Carrot (g)	Pawpaw (g)	Fluted Pumpkin Leaf (g)	Total (g)
MS	75:25	139.08 (9.75)	7.73 (3.25)	-	-	-	142.33 (13)
MSC	77:20:3	142.80 (10.01)	6.10 (2.60)	41.94 (0.39)	-	-	190.84 (13)
MSP	77:20:3	142.80 (10.01)	6.10 (2.60)	-	82.98 (0.39)	-	231.88 (13)
MSF	77:20:3	142.80 (10.01)	6.10 (2.60)	-	-	11.14 (0.39)	160.04 (13)
MSCP	74:20:3:3	137.23 (9.62)	6.10 (2.60)	41.94 (0.39)	82.98 (0.39)	-	268.25 (13)
MSCF	74:20:3:3	137.23 (9.62)	6.10 (2.60)	41.94 (0.39)	-	11.14 (0.39)	196.41 (13)
MSPF	74:20:3:3	137.23 (9.62)	6.10 (2.60)	-	82.98 (0.39)	11.14 (0.39)	237.45 (13)
MSCFP	71:20:3:3:3	131.67 (9.23)	6.10 (2.60)	41.94 (0.39)	82.98 (0.39)	11.14 (0.39)	273.83 (13)

Key:
MS = Maize/Soybean
MSC = Maize/Soybean/Carrot
MSP = Maize/Soybean/Pawpaw
MSF =Maize/Soybean/Fluted pumpkin leaf
MSCP = Maize/Soybean/Carrot/Pawpaw
MSCF = Maize/Soybean/Carrot/Fluted pumpkin leaf
MSPF = Maize/Soybean/Pawpaw/Fluted pumpkin leaf
MSCFP = Maize/Soya bean/Carrot/Fluted pumpkin leaf/Pawpaw

Table 1: Formulation and Protein Contribution of Composite Flour Blends Formulated to Provide 13 g of Protein

2.5. Chemical analysis

The formulated complementary food samples were analyzed for proximate composition, vitamins, and mineral content using standard methods.

2.5.1. Proximate Composition

Moisture, protein, fat, crude fibre, ash, and carbohydrate contents were determined according to the methods described by the Association of Official Analytical Chemists [26]. Protein content was determined by the Kjeldahl method (AOAC, 2010), fat content by Soxhlet extraction (AOAC, 2010), crude fibre by acid and alkali digestion (AOAC, 2010), and ash content by incineration in a muffle furnace at 550°C (AOAC, 2010). Carbohydrate content was calculated by difference, and energy values were estimated using Atwater factors.

2.5.2. Micronutrient Composition

Vitamin A (retinol) content was determined using high-performance liquid chromatography (HPLC) according to the method described by AOAC. Riboflavin content was determined using the fluorometric method as described by AOAC. Vitamin C (ascorbic acid) content was determined using the 2,6-dichloroindophenol (DCPIP) titrimetric method as described by AOAC. Iron content was determined using the ortho-phenanthroline colorimetric method as described by AOAC. Calcium content was determined using the dry ashing method as described by AOAC. Zinc content was determined using atomic absorption spectrophotometry (AAS) after dry ashing, according to the method described by AOAC.

2.6. Sourcing and Housing of Animals

Fifty-four (54) healthy weanling rats with no prior drug treatment, weighing between 40–60 g, were used for the experimental study. The rats were purchased from the Department of Veterinary Pathology, University of Nigeria, Nsukka. The rats were randomly distributed into nine (9) groups of six rats each based on body weight. The average body weight of each group did not differ by more than 5 grams. They were allowed to acclimatize for seven days and were fed standard pellets. The rats had access to water and feed ad libitum. Each group was housed in metabolic and standard rat cages equipped to separate feces and urine during the study period. The study was carried out in the animal house (metabolic laboratory) of the Department of Nutrition and Dietetics, University of Nigeria, Nsukka. The rats were maintained under standard environmental conditions (ambient temperature 25 ± 2°C, humidity 45 ± 5%, and12 hours light/12 hours dark) during the study.

2.7. Experimental Design

The study was conducted for twenty-eight (28) days, which consisted of a seven-day acclimatization period and 21 days of feeding trial with the experimental diets, which were: Maize/Soybean, Maize/Soybean/Carrot, Maize/Soybean/Fluted Pumpkin Leaf, Maize/Soybean/Pawpaw, Maize/Soybean/Carrot/Pawpaw, Maize/Soybean/Carrot/Fluted Pumpkin Leaf, Maize/Soybean/Pawpaw/Fluted Pumpkin Leaf, and Maize/Soybean/Carrot/Fluted Pumpkin Leaves/Pawpaw.

MS = 75% Maize /25% Soybean
MSC = 77 %Maize / 20%Soya bean/ 3% carrot
MSF = 77%Maize / 20%Soya bean/ 3 % fluted pumpkin leaves
MSP = 77 %Maize / 20%Soya bean / 3% pawpaw
MSCP = 74%Maize /20%soya bean / 3% carrot / 3% pawpaw
MSCF = 74%Maize/20%soyabean/3%carrot/3%fluted pumpkin leaves
MSPF = 74%Maize /20%soya bean /3% pawpaw /3% fluted pumpkin leaves
MSCFP = 71%Maize /20%Soyabean/3%carrot/3%fluted pumpkin leaves/3%pawpaw

Group one rats (control group) were fed a commercial complementary food (Cerelac) and water ad libitum throughout the experimental period, while the other eight groups were fed the experimental diets for the same period of 28 days. The body weight of the rats was measured every 7 days during the study to determine weight gain using the following equation:

Body weight gain = final weight – initial weight

2.8. Collection of Blood Samples and Biochemical Analysis

At the end of the 28-day experimental period, the rats were anesthetized. Aliquots of blood were collected from the retro-orbital sinus of all animals prior to sacrifice for biochemical and hematological evaluation. The blood samples were collected in dry glass plain and EDTA centrifuge tubes and allowed to coagulate at room temperature. Blood samples were centrifuged at 3500 rpm for 15 minutes at room temperature for serum separation. The clear, non-hemolysed supernatant sera were separated using clear, dry disposable plastic syringes and stored at –20°C for subsequent biochemical analysis. The blood samples were used for the determination of biochemical parameters.

Serum retinol (vitamin A) levels were determined using a validated high-performance liquid chromatography (HPLC) method with reversed-phase C-18 column separation and ultraviolet detection at 313 nm [27]. Serum riboflavin (vitamin B_2) levels were quantified simultaneously using high-performance liquid chromatography (HPLC) with fluorescence detection, following solid-phase extraction sample clean-up [28]. Serum ascorbic acid (vitamin C) levels were measured using reversed-phase HPLC with ultraviolet detection at 245 nm [28].

Serum iron levels were determined using a colorimetric method, in which iron is released from protein binding with hydrochloric acid, proteins are precipitated with trichloroacetic acid, and a coloured complex is formed for spectrophotometric measurement at 440 nm [29]. Serum calcium levels were measured using a colorimetric method with methylthymol blue as the chromogenic reagent [30]. Serum zinc levels were determined using a direct colorimetric method employing a cationic porphyrin reagent, with spectrophotometric detection at 421 nm [31].

2.9. Statistical Analysis

Data obtained were analyzed using IBM-SPSS, version 27. Results were expressed as means with standard deviations and presented in tables. Descriptive statistics were computed to determine the mean and standard deviation of triplicate determinations for each nutrient parameter. For the growth velocity data, descriptive statistics including initial weight, final weight, weight gain, and percentage weight gain were computed for each treatment group. Paired sample t-tests were employed to compare the baseline and endline serum levels of vitamins and minerals within each treatment group. Significance was accepted at $p < 0.05$.

3. Results

Samples	Moisture (%)	Protein (%)	Fat (%)	Fiber (%)	Ash (%)	Carbohydrates (%)	Energy (kcal)
Control	-	15.00g	10.00g	4.50g	3.00g	65.00	410.00
MS	5.84 ± 0.06	22.56 ± 0.04	12.93 ± 0.05	2.76 ± 0.04	4.42 ± 0.04	51.49 ± 0.06	412.58 ± 0.26
MSF	6.28 ± 0.07	25.07 ± 0.08	13.08 ± 0.08	2.98 ± 0.06	5.60 ± 0.04	47.00 ± 0.05	405.35 ± 0.31
MSC	6.00 ± 0.06	22.74 ± 0.07	12.99 ± 0.09	2.87 ± 0.03	4.68 ± 0.06	50.79 ± 0.12	410.62 ± 0.18
MSP	6.52 ± 0.05	22.64 ± 0.04	12.95 ± 0.02	2.80 ± 0.04	4.44 ± 0.05	50.66 ± 0.04	409.71 ± 0.37
MSCP	6.72 ± 0.06	22.90 ± 0.11	12.99 ± 0.08	2.89 ± 0.04	4.71 ± 0.04	49.79 ± 0.07	407.81 ± 0.14
MSCF	6.40 ± 0.04	25.31 ± 0.05	13.25 ± 0.06	3.26 ± 0.08	5.68 ± 0.05	46.00 ± 0.08	404.89 ± 0.31
MSPF	6.79 ± 0.06	13.16 ± 0.13	13.16 ± 0.06	3.14 ± 0.05	5.42 ± 0.03	46.52 ± 0.05	404.41 ± 0.29
MSCFP	7.08 ± 0.09	26.05 ± 0.07	13.96 ± 0.04	3.61 ± 0.05	5.71 ± 0.03	43.62 ± 0.05	404.26 ± 0.39

Values are expressed as Mean ± SD (n = 3).

Key

Control = Cerelac
MS = Maize/Soybean (75:25)
MSF =Maize/Soybean/Fluted pumpkin leaf (77:20:3)
MSC = Maize/Soybean/Carrot (77:20:3)
MSP = Maize/Soybean/Pawpaw (77:20:3)
MSCP = Maize/Soybean/Carrot/Pawpaw (74:20:3:3)
MSCF = Maize/Soybean/Carrot/Fluted pumpkin leaf (74:20:3:3)
MSPF = Maize/Soybean/Pawpaw/Fluted pumpkin leaf (74:20:3:3)
MSCFP = Maize/Soya bean/Carrot/Fluted pumpkin leaf/Pawpaw (71:20:3:3:3)

Table 2: Proximate composition of the formulated infant complementary foods

Table 2 shows the proximate composition of the formulated infant complementary foods. The blend that contains all the ingredients (MSCFP) recorded the highest protein (26.05%), fat (13.96%), fibre (3.61%), and ash (5.71%) contents, while containing the lowest carbohydrate content (43.62%). In contrast, the MS sample had the lowest moisture (5.84%), fibre (2.76) and ash (4.42%) content. All formulated blends had comparable energy density to the control, ranging from 404.26 to 412.58 Kcal.

Sample	Beta Carotene (µg/100g)	Riboflavin (Vitamin B_2) (mg/100g)	Vitamin C (mg/100g)	Iron (mg/100g)	Calcium (mg/100g)	Zn (mg/100g)
Control	-	0.45	65.00	10.00	600.00	7.00
MS	394.37 ± 0.03	0.17 ± 0.06	7.04 ± 0.06	9.05 ± 0.09	10.96 ± 0.05	2.89 ± 0.09
MSF	415.95 ± 0.05	0.36 ± 0.04	15.80 ± 0.06	10.17 ± 0.15	14.85 ± 0.05	3.18 ± 0.07
MSC	430.81 ± 0.03	0.28 ± 0.03	10.22 ± 0.03	10.57 ± 0.19	11.98 ± 0.61	3.49 ± 0.07
MSP	426.00 ± 0.10	0.33 ± 0.05	12.38 ± 0.05	9.85 ± 0.13	13.78 ± 0.27	3.16 ± 0.06
MSCP	460.58 ± 0.04	0.39 ± 0.04	16.99 ± 0.07	11.42 ± 0.39	17.71 ± 0.44	3.26 ± 0.15
MSCF	448.79 ± 0.06	0.40 ± 0.03	18.43 ± 0.07	12.15 ± 0.15	15.81 ± 0.04	3.96 ± 0.14
MSPF	440.56 ± 0.06	0.44 ± 0.02	19.90 ± 0.06	11.77 ± 0.15	15.13 ± 0.10	3.80 ± 0.06
MSCFP	494.92 ± 0.04	0.48 ± 0.04	22.96 ± 0.09	12.43 ± 0.13	19.07 ± 0.08	4.53 ± 0.42

Values are expressed as Mean ± SD (n = 3).

Key

Control=Cerelac
MS = Maize/Soybean (75:25)
MSF =Maize/Soybean/Fluted pumpkin leaf (77:20:3)
MSC = Maize/Soybean/Carrot (77:20:3)
MSP =Maize/Soybean/Pawpaw(77:20:3)
MSCP = Maize/Soybean/Carrot/Pawpaw (74:20:3:3)
MSCF = Maize/Soybean/Carrot/Fluted pumpkin leaf (74:20:3:3)
MSPF = Maize/Soybean/Pawpaw/Fluted pumpkin leaf (74:20:3:3)
MSCFP = Maize/Soya bean/Carrot/Fluted pumpkin leaf/Pawpaw (71:20:3: 3:3)

Table 3: Vitamin and mineral content of the formulated infant complementary foods

Table 3 shows the vitamin and mineral composition of the formulated complementary foods. The MSCFP blend had the highest beta-carotene (494.92 µg/100g), vitamin C (22.96 mg/100g), iron (12.43 mg/100g), and zinc (4.53 mg/100g) content among all test samples. The iron content of MSCFP was higher than that of the commercial control (12.43 mg vs. 10.00 mg). However, the control remained superior in calcium and vitamin C due to commercial fortification.

Groups	**Initial weight (g)**	**Final weight (g)**	**Weight gain (g)**	**Percentage Weight gain (%)**
A	58.00	123.50	65.50	112.93
B	56.00	70.50	14.50	25.89
C	53.50	98.00	44.50	83.18
D	52.50	90.00	37.50	71.43
E	57.00	88.00	31.00	54.39
F	55.50	90.00	34.50	62.16
G	55.00	108.00	53.00	96.36
H	50.50	109.00	58.50	115.82
I	50.50	114.00	63.50	125.74

Values are expressed as Mean ± SD (n = 3).
Where; Group A: Rats fed with Cerelac
Group B: Rats fed with MS = Maize, Soyabean (75:25)
Group C: Rats fed with MSF = Maize, Soyabean, fluted pumpkin leaf (77:20:3)
Group D: Rats fed with MSC = Maize, Soyabean, Carrot (77:20:3)
Group E: Rats fed with MSP = Maize, Soyabean, Paw-paw (77:20:3)
Group F: Rats fed with MSCP = Maize, Soyabean, Carrot, Paw-paw (74:20:3:3)
Group G: Rats fed with MSCF = Maize, Soyabean, Carrot, fluted pumpkin leaf (74:20:3:3)
Group H: Rats fed with MSPF = Maize, Soyabean, Paw-paw, fluted pumpkin leaf (74:20:3:3)
Group I: Rats fed with MSCFP = Maize, Soyabean, Carrot, fluted pumpkin leaf, Paw-paw (71:20:3:3:3).

Table 4: Effects of Formulated Infant Complementary Foods on the Growth Velocity of Weanling Rats

Table 4 shows the effects of the formulated infant complementary foods on the growth velocity of weanling rats. The initial weights of the rats across all groups ranged from 50.50 g to 58.00 g. At the end of the study, Group A (control) recorded the highest final weight of 123.50 g, followed closely by Group I (MSCFP) with 114.00 g, and Group H (MSPF) with 109.00 g. The lowest final weight was recorded in Group B (MS) at 70.50 g. The absolute weight gain was highest in Group A (65.50 g), followed by Group I (63.50 g), while Group B recorded the least weight gain (14.50 g). In terms of percentage weight gain, Group I had the highest value (125.74%), followed by Group H (115.82%), with Group A recording 112.93%. The lowest percentage weight gain was observed in Group B (25.89%).

Groups	**Baseline (µg/dl)**	**Endline (µg/dl)**	**Mean Difference (µg/dl)**	**% Difference**	**t-value**
A	18.88	25.61	6.73	35.64	30.59**
B	19.90	30.48	10.58	53.17	34.16**
C	30.45	36.78	6.33	20.79	48.69**
D	24.69	34.59	9.90	40.07	8.53**
E	29.00	36.58	7.58	26.14	11.48**
F	40.80	47.15	6.35	15.56	12.21**

G	26.44	34.41	7.97	30.14	42.00**
H	33.66	33.73	0.07	0.21	1.40
I	53.17	53.02	-0.15	-0.28	-3.75*

Values are expressed as Mean ± SD (n = 3).
Where; Group A: Rats fed with Cerelac
Group B: Rats fed with MS = Maize, Soyabean (75:25)
Group C: Rats fed with MSF = Maize, Soyabean, fluted pumpkin leaf (77:20:3)
Group D: Rats fed with MSC = Maize, Soyabean, Carrot (77:20:3)
Group E: Rats fed with MSP = Maize, Soyabean, Paw-paw (77:20:3)
Group F: Rats fed with MSCP = Maize, Soyabean, Carrot, Paw-paw (74:20:3:3)
Group G: Rats fed with MSCF = Maize, Soyabean, Carrot, fluted pumpkin leaf (74:20:3:3)
Group H: Rats fed with MSPF = Maize, Soyabean, Paw-paw, fluted pumpkin leaf (74:20:3:3)
Group I: Rats fed with MSCFP = Maize, Soyabean, Carrot, fluted pumpkin leaf, Paw-paw (71:20:3:3:3).

The effects of the formulated diets on the serum vitamin A levels of the weanling rats are presented in Table 5. At endline, all groups showed an increase in serum vitamin A except Group I, which showed a slight decrease from 53.17 µg/dL to 53.02 µg/dL. The highest mean difference was observed in Group B (10.58 µg/dL), representing a 53.17% increase ($p < 0.01$). Group H showed the least positive change (0.07 µg/dL; 0.21%), which was not statistically significant ($p > 0.05$). The t-test analysis revealed that all groups except Group H showed significant increase ($p < 0.05$).

Groups	Baseline (µg/dl)	Endline (µg/dl)	Mean Difference (µg/dl)	% Difference	t-value
A	0.29	0.38	0.09	31.03	9.00**
B	0.19	0.23	0.04	21.05	4.00**
C	0.30	0.36	0.06	20.00	6.00**
D	0.30	0.44	0.14	46.67	14.00**
E	0.29	0.43	0.14	48.28	14.00**
F	0.34	0.48	0.14	41.18	7.00**
G	0.47	0.49	0.02	4.26	2.00*
H	0.53	0.53	0.00	0.00	0.00
I	0.58	0.59	0.01	1.72	1.00

Values are expressed as Mean ± SD (n = 3).
Where; Group A: Rats fed with Cerelac
Group B: Rats fed with MS = Maize, Soyabean (75:25)
Group C: Rats fed with MSF = Maize, Soyabean, fluted pumpkin leaf (77:20:3)
Group D: Rats fed with MSC = Maize, Soyabean, Carrot (77:20:3)
Group E: Rats fed with MSP = Maize, Soyabean, Paw-paw (77:20:3)
Group F: Rats fed with MSCP = Maize, Soyabean, Carrot, Paw-paw (74:20:3:3)
Group G: Rats fed with MSCF = Maize, Soyabean, Carrot, fluted pumpkin leaf (74:20:3:3)
Group H: Rats fed with MSPF = Maize, Soyabean, Paw-paw, fluted pumpkin leaf (74:20:3:3)
Group I: Rats fed with MSCFP = Maize, Soyabean, Carrot, fluted pumpkin leaf, Paw-paw (71:20:3:3:3).

Table 6: Effects of Formulated Infant Complementary Foods on the Serum Vitamin B_2 Levels of Weanling Rats

The effects of the formulated complementary foods on serum thiamin (vitamin B_2) levels are shown in Table 6. Groups D and E had the most percentage increases (~46–48%). Group H showed no change between baseline and endline. Group I had only a slight change of (0.01) and the change observed was not statistically significant.

Groups	Baseline (µg/dl)	Endline (µg/dl)	Mean Difference (µg/dl)	% Difference	t-value
A	1.61	1.76	0.15	9.32	7.50**
B	0.81	0.95	0.14	17.28	4.67**
C	1.74	1.90	0.16	9.20	16.00**

D	2.39	2.38	-0.01	-0.42	-0.14
E	1.65	1.78	0.13	7.88	4.33**
F	2.11	2.20	0.09	4.27	2.25*
G	2.24	2.22	-0.02	-0.89	-0.17
H	2.10	2.12	0.02	0.95	2.00*
I	3.16	3.18	0.02	0.63	2.00*

Values are expressed as Mean ± SD (n = 3).
Where; Group A: Rats fed with Cerelac
Group B: Rats fed with MS = Maize, Soyabean (75:25)
Group C: Rats fed with MSF = Maize, Soyabean, fluted pumpkin leaf (77:20:3)
Group D: Rats fed with MSC = Maize, Soyabean, Carrot (77:20:3)
Group E: Rats fed with MSP = Maize, Soyabean, Paw-paw (77:20:3)
Group F: Rats fed with MSCP = Maize, Soyabean, Carrot, Paw-paw (74:20:3:3)
Group G: Rats fed with MSCF = Maize, Soyabean, Carrot, fluted pumpkin leaf (74:20:3:3)
Group H: Rats fed with MSPF = Maize, Soyabean, Paw-paw, fluted pumpkin leaf (74:20:3:3)
Group I: Rats fed with MSCFP = Maize, Soyabean, Carrot, fluted pumpkin leaf, Paw-paw (71:20:3:3:3).

Table 7: Effects of Formulated Infant Complementary Foods on the Serum Vitamin C Levels of Weanling Rats

Table 7 shows the effects of the formulated infant complementary foods on serum vitamin C levels. The highest endline value was observed in group I (3.18 µg/dL), while Group C showed the most significant increase ($p < 0.01$). Groups D and G exhibited minor non-significant decreases, indicating adequate maintenance of vitamin C status.

Groups	Baseline (mg/dL)	Endline (mg/dL)	Mean Difference (mg/dL)	% Difference	t-value
A	13.15	13.08	-0.07	-0.53	-0.23
B	10.87	10.96	0.09	0.83	0.69
C	15.00	14.88	-0.12	-0.80	-0.32
D	13.43	11.75	-1.68	-12.51	-2.51*
E	12.62	13.45	0.83	6.58	1.36
F	15.44	17.21	1.77	11.47	6.32**
G	14.24	15.80	1.56	10.95	2.89*
H	15.43	15.12	-0.31	-2.01	-1.55
I	18.04	18.04	0.00	0.00	0.00

Values are expressed as Mean ± SD (n = 3).
Where; Group A: Rats fed with Cerelac
Group B: Rats fed with MS = Maize, Soyabean (75:25)
Group C: Rats fed with MSF = Maize, Soyabean, fluted pumpkin leaf (77:20:3)
Group D: Rats fed with MSC = Maize, Soyabean, Carrot (77:20:3)
Group E: Rats fed with MSP = Maize, Soyabean, Paw-paw (77:20:3)
Group F: Rats fed with MSCP = Maize, Soyabean, Carrot, Paw-paw (74:20:3:3)
Group G: Rats fed with MSCF = Maize, Soyabean, Carrot, fluted pumpkin leaf (74:20:3:3)
Group H: Rats fed with MSPF = Maize, Soyabean, Paw-paw, fluted pumpkin leaf (74:20:3:3)
Group I: Rats fed with MSCFP = Maize, Soyabean, Carrot, fluted pumpkin leaf, Paw-paw (71:20:3:3:3).

Table 8: Effects of Formulated Infant Complementary Foods on the Serum Calcium Levels of Weanling Rats

The effects of formulated infant complementary foods on serum calcium levels are shown in Table 8. The baseline serum calcium levels ranged from 10.87 mg/dL in Group B to 18.04 mg/dL in Group I. At endline, the values ranged from 10.96 mg/dL in Group B to 18.04 mg/dL in Group I. Group I maintained the highest values throughout the study, showing no change (0.00 mg/dL; 0.00%). The highest mean difference was recorded in Group F (1.77 mg/dL), which corresponds to an 11.47% increase ($p < 0.01$). Group G also recorded a significant increase of 1.56 mg/dL ($p < 0.05$). on the other hand, Group D showed a significant decrease from 13.43 mg/dL to 11.75 mg/dL, which was statistically significant ($t = -2.51$, $p < 0.05$).

Groups	Baseline (mg/dL)	Endline (mg/dL)	Mean Difference (mg/dL)	% Difference	t-value
A	5.08	7.07	1.99	39.17	4.23**
B	8.20	9.05	0.85	10.37	3.70**
C	9.30	10.23	0.93	10.00	2.02*
D	9.39	10.51	1.12	11.93	2.55*
E	8.53	9.79	1.26	14.77	3.32**
F	10.97	11.37	0.40	3.65	1.38
G	11.08	12.14	1.06	9.56	3.79**
H	9.51	11.76	2.25	23.66	5.00**
I	10.96	12.43	1.47	13.41	21.00**

Values are expressed as Mean ± SD (n = 3).
Where; Group A: Rats fed with Cerelac
Group B: Rats fed with MS = Maize, Soyabean (75:25)
Group C: Rats fed with MSF = Maize, Soyabean, fluted pumpkin leaf (77:20:3)
Group D: Rats fed with MSC = Maize, Soyabean, Carrot (77:20:3)
Group E: Rats fed with MSP = Maize, Soyabean, Paw-paw (77:20:3)
Group F: Rats fed with MSCP = Maize, Soyabean, Carrot, Paw-paw (74:20:3:3)
Group G: Rats fed with MSCF = Maize, Soyabean, Carrot, fluted pumpkin leaf (74:20:3:3)
Group H: Rats fed with MSPF = Maize, Soyabean, Paw-paw, fluted pumpkin leaf (74:20:3:3)
Group I: Rats fed with MSCFP = Maize, Soyabean, Carrot, fluted pumpkin leaf, Paw-paw (71:20:3:3:3).

Table 9: Effects of Formulated Infant Complementary Foods on the Serum Iron Levels of Weanling Rats

Table 9 shows the effects of the formulated infant complementary foods on the serum iron levels of the weanling rats. The highest endline value was observed in Group I (12.43 mg/dL), followed by Group G (12.14 mg/dL). The highest mean difference was recorded in Group H (2.25 mg/dL), representing a 23.66% increase, which was highly significant ($t = 5.00$, $p < 0.01$). Group A showed a mean difference of 1.99 mg/dL ($p < 0.01$). The t-test analysis revealed that all groups recorded significant increases except for Group F which showed no statistically significant change ($p > 0.05$).

Groups	Baseline (mg/dL)	Endline (mg/dL)	MD (mg/dL)	%D	Std error	Df	t-value
A	2.03	2.60	0.57	28.08	0.08	4	7.13**
B	2.62	2.89	0.27	10.31	0.09	4	3.00**
C	2.62	3.23	0.61	23.28	0.09	4	6.78**
D	2.73	3.47	0.74	27.11	0.13	4	5.69**
E	2.73	3.16	0.43	15.75	0.22	4	1.95*
F	2.69	3.24	0.55	20.45	0.46	4	1.20
G	2.50	3.88	1.38	55.20	0.35	4	3.94**
H	2.86	3.80	0.94	32.87	0.13	4	7.23**
I	3.45	4.60	1.15	33.33	0.41	4	2.80*

Values are expressed as Mean ± SD (n = 3).
Where; Group A: Rats fed with Cerelac
Group B: Rats fed with MS = Maize, Soyabean (75:25)
Group C: Rats fed with MSF = Maize, Soyabean, fluted pumpkin leaf (77:20:3)
Group D: Rats fed with MSC = Maize, Soyabean, Carrot (77:20:3)
Group E: Rats fed with MSP = Maize, Soyabean, Paw-paw (77:20:3)
Group F: Rats fed with MSCP = Maize, Soyabean, Carrot, Paw-paw (74:20:3:3)
Group G: Rats fed with MSCF = Maize, Soyabean, Carrot, fluted pumpkin leaf (74:20:3:3)
Group H: Rats fed with MSPF = Maize, Soyabean, Paw-paw, fluted pumpkin leaf (74:20:3:3)
Group I: Rats fed with MSCFP = Maize, Soyabean, Carrot, fluted pumpkin leaf, Paw-paw (71:20:3:3:3).

Table 10: Effects of formulated infant complementary foods on the serum zinc levels of weanling rats

In Table 10 all groups recorded an increase in serum zinc levels. The highest endline value was recorded in Group I (4.60 mg/dL), followed by Group G (3.88 mg/dL) and Group H (3.80 mg/dL). The highest mean difference was observed in Group G (1.38 mg/dL), corresponding to a 55.20% increase, which was statistically significant ($p < 0.01$). Group I also recorded a substantial increase of 1.15 mg/dL (33.33%) ($p < 0.05$). Group H showed a mean difference of 0.94 mg/dL (32.87%) ($p < 0.01$). The analysis further revealed that all Groups had significant increase except for Group F which showed no significant difference ($p > 0.05$).

4. Discussion

4.1.Proximate Composition of the Formulated Infant Complementary Foods

The proximate composition of the formulated infant complementary foods revealed significant improvements in nutrient density compared to the control sample, particularly in protein, fat, ash, and crude fibre contents. These findings align with current recommendations that complementary foods should be nutrient-dense to meet the high nutritional demands of infants during the complementary feeding period. The higher protein content of the formulated samples (22.56% to 26.05%) relative to the control (15g) could be attributed to the inclusion of soybean, a high-quality plant protein source rich in essential amino acids. Similar findings have been reported by, who observed significant increases in protein content in maize-based complementary foods fortified with legumes [34]. Also reported that incorporation of legumes into cereal-based formulations significantly enhanced protein quality and quantity, thereby improving their suitability for infant feeding. Adequate protein intake is essential for growth, tissue repair, and overall development, particularly in low-resource settings where protein-energy malnutrition is prevalent [35]. The higher fat content of the formulated complementary foods (12.93-13.96%) relative to the control (10.0g) reflects the contribution of soybean and other plant ingredients. Dietary fat is crucial for infants as it provides concentrated energy and supports neurological development. Studies have shown that legume-enriched complementary foods contain higher fat levels compared to cereal-only formulations, thereby improving energy density [36,37]. This is particularly important given the limited gastric capacity of infants, which necessitates nutrient- and energy-dense foods. The higher ash content (4.42-5.71%) an indicator of total mineral composition in the composites in relation to the control (3.0g) could be linked to the inclusion of micronutrient-rich ingredients such as fluted pumpkin leaf, carrot, and pawpaw. Similar increases in ash content have been reported in studies involving vegetable-fortified complementary foods, indicating enhanced mineral availability. Minerals such as calcium, iron, and zinc are critical for bone development, immune function, and cognitive growth in infants. The higher crude fibre content (4.76-5.61%) of the formulated samples when compared to that of the control (4.5g) may likely be due to the addition of plant-based ingredients. This observation is consistent with findings by, who reported increased fibre content in complementary foods enriched with vegetable powders [38]. While moderate fibre intake supports gastrointestinal health, excessive fibre may interfere with nutrient absorption in infants. However, the fibre levels observed in this study remained within acceptable limits, suggesting suitability for infant consumption. The formulated samples had lower (43.62-51.49%) carbohydrate when compared to the control (65g). This inverse relationship between carbohydrate and protein/fat content is consistent with previous studies, where increasing protein- and fat-rich ingredients resulted in a proportional reduction in carbohydrate levels. This shift reflects an improved macronutrient balance, enhancing the overall nutritional quality of the complementary foods.

Moisture content in the formulated samples was relatively low (5.84-7.08%), which is advantageous for shelf stability and microbial safety. Low moisture levels reduce the risk of spoilage and microbial growth, thereby extending shelf life. This finding is in agreement with studies reporting that properly processed complementary foods typically exhibit low moisture content suitable for storage [39]. Despite variations in macronutrient composition, the energy values of the formulated complementary foods were comparable to the control. This observation aligns with findings that nutrient enhancement does not necessarily compromise caloric adequacy. Maintaining adequate energy density is essential to ensure that infants meet their daily energy requirements during complementary feeding. The proximate composition results demonstrate that the incorporation of cereals, legumes, and vegetables significantly improved most of the nutrient contents of complementary foods. These findings revealed that evidence that food-to-food fortification using locally available ingredients is an effective and sustainable strategy for addressing infant malnutrition in low-resource settings.

4.2. Effects of Formulated Infant Complementary Foods on the Growth Velocity of Weanling Rats

Growth velocity in weanling rats is a widely accepted indicator of the nutritional adequacy, digestibility, and biological utilization of formulated diets. The results presented in Table 4 demonstrate marked variations in weight gain and percentage growth across the experimental groups, reflecting differences in nutrient composition and dietary diversity of the complementary food formulations. The control group (Group A), fed with Cerelac, exhibited a high weight gain of 65.50 g (112.93%), which is consistent with the known nutritional quality of commercial complementary foods that are typically fortified with essential nutrients. However, rats fed maize soybean carrot pawpaw fluted pumpkin leaves recorded the highest weight gain (63.50 g; 125.74%), surpassing even the control. Similarly, rats fed maize soybean pawpaw fluted pumpkin leaves) also demonstrated a high growth response (115.82%). These findings suggest that the multi-ingredient formulations were highly effective in supporting growth, likely due to improved nutrient density and synergistic interactions among ingredients.

The superior growth observed in rats fed maize soybean carrot pawpaw fluted pumpkin leaves can be attributed to the combined inclusion of maize, soybean, carrot, pawpaw, and fluted pumpkin leaf. This combination provides an adequate supply of macronutrients (protein and energy) and micronutrients (vitamins and minerals) essential for growth. According to World Health Organization

(2023), dietary diversity is a key determinant of nutrient adequacy in complementary feeding and plays a critical role in preventing growth faltering in infants. The enhanced growth performance observed in this study supports this recommendation. However, rats fed maize soybean only, recorded the lowest weight gain (14.50 g; 25.89%), indicating poor growth performance relative to other groups. Although soybean is a good source of plant protein, the absence of micronutrient-rich components such as fruits and vegetables likely limited overall growth. This finding aligns with the report by Hambidge, which emphasizes that adequate intake of micronutrients such as iron, zinc, and vitamins is essential for optimal growth and metabolic function, beyond protein intake alone [40]. Rats fed (maize soybean fluted pumpkin leaves), (maize soybean carrot), (maize soybean pawpaw), and (maize soybean carrot pawpaw) showed moderate weight gains ranging from 54.39% to 83.18%, reflecting incremental improvements with the addition of individual or combined plant ingredients. For instance, carrot contributes provitamin A carotenoids, pawpaw provides vitamin C and natural sugars, while fluted pumpkin leaf is rich in iron and folate. These nutrients are essential for cellular growth, immune function, and hematopoiesis, which collectively support weight gain. Similar findings have been reported by Beal, who identified micronutrient deficiencies as a major limiting factor in complementary feeding, particularly in low- and middle-income countries [41].

The high growth performance observed in rats (maize- soybean-carrot -fluted pumpkin leaves), (maize- soybean -pawpaw -fluted pumpkin leaves), and (maize- soybean -carrot -pawpaw fluted pumpkin leaves), with percentage weight gains ranging from 96.36% to 125.74%, suggests a strong synergistic effect of combining multiple nutrient-dense ingredients. This synergy likely improved both nutrient intake and utilization. Studies indicate that diets combining cereals and legumes with fruits and vegetables enhance amino acid balance, vitamin availability, and overall nutrient utilization, leading to improved growth outcomes.

The results indicate that the formulated complementary foods, particularly the multi-component blends (maize -soybean- carrot -fluted pumpkin leaves, maize- soybean -pawpaw fluted pumpkin leaves, and maize- soybean -carrot- pawpaw fluted pumpkin leaves), significantly enhanced growth in weanling rats. The highest growth observed in rats (maize -soybean -carrot pawpaw-fluted pumpkin leaves) underscores the importance of dietary diversity, nutrient synergy, and bioavailability in promoting optimal growth. Conversely, the poor performance of the maize-soybean formulation highlights the limitations of less diversified diets. The study demonstrates that nutrient-dense, diversified complementary foods can effectively support growth and may serve as viable alternatives to commercial products, especially in resource-limited setting.

4.3. Effects of Formulated Infant Complementary Foods on the Serum Vitamin Levels of Weanling Rats

The study demonstrated that feeding weanling rats with formulated complementary foods significantly influenced serum vitamin profiles, indicating the nutritional efficacy and bioavailability of micronutrients from the composite formulations. Most experimental diets showed significant improvements in serum levels of vitamins A, B_2, and C although variations were observed across groups depending on ingredient composition and possible nutrient interactions. With respect to serum vitamin A, the significant increases observed in most rats fed most of the diets suggest that the formulated diets effectively supplied provitamin A carotenoids and supported their metabolic conversion to retinol. The inclusion of carotenoid-rich ingredients such as carrot and pawpaw likely contributed to these improvements. This finding is consistent with reports that plant-based complementary foods enriched with β-carotene sources can significantly improve vitamin A status due to enzymatic conversion in the intestinal mucosa [42]. However, the non-significant change in rats fed maize-soybean -pawpaw- fluted pumpkin leaves and slight decrease in rats fed maize -soybean carrot- pawpaw- fluted pumpkin leaves may be attributed to nutrient–nutrient interactions or possible saturation effects, where excessive intake of carotenoids does not proportionally increase serum retinol due to regulatory mechanisms controlling vitamin A homeostasis. Additionally, bioavailability of carotenoids is influenced by dietary fat and food matrix, which may vary across formulations.

Serum vitamin B_2 (riboflavin) levels showed significant increases in rats fed most diets, with higher increments observed in diets containing carrot and pawpaw. Riboflavin plays a crucial role in oxidative metabolism and cellular growth. However, the minimal changes in maize soybean carrot pawpaw fluted pumpkin leaves and maize soybean carrot pawpaw fluted pumpkin leaves may again reflect nutrient saturation or limited absorption efficiency at higher intake levels. Serum vitamin C levels showed mild increases, with some groups exhibiting non-significant changes or slight decreases. This observation is consistent with the known instability of vitamin C during processing and storage. Although freeze-drying was employed which is generally effective in preserving heat-sensitive nutrients, some losses may still occur due to oxidation during handling and storage [43]. The relatively low contribution of vitamin C aligns with earlier findings in complementary food formulations where ascorbic acid degradation limits its bioavailability.

4.4. Effects of Formulated Infant Complementary Foods on the Mineral Levels of Weanling Rats

The evaluation of serum mineral profiles in weanling rats provides important insight into the bioavailability and physiological utilization of nutrients in the formulated complementary foods. Minerals such as iron, calcium and zinc are critical for growth, bone development, enzymatic function, and overall metabolic regulation in early life. The findings revealed largely non-significant changes in serum calcium levels across rats fed most of the diets, except for significant increases observed in maize soybean carrot pawpaw and maize soybean carrot fluted pumpkin leaves, and a significant decrease in maize soybean carrot. The minimal changes in most groups suggest tight homeostatic regulation of calcium in the body, which is consistent with established physiological mechanisms that maintain serum calcium within a narrow range through hormonal control involving parathyroid hormone and vitamin D [44]. The significant increases observed in rats fed maize soybean carrot pawpaw and maize soybean carrot may be attributed to improved calcium bioavailability resulting from dietary diversification and nutrient synergy, particularly from combinations of legumes and vegetables. The significant decrease observed in rats fed maize soybean carrot may be linked to inhibitory interactions or insufficient calcium density in the carrot-based formulation, as carotenoid-rich foods alone do not substantially contribute to calcium intake.

There were significant increases in serum iron levels of rats fed all the diets, particularly in rats fed the control diet (Cerelac), maize soybean pawpaw, maize soybean carrot fluted pumpkin leaves, maize soybean pawpaw fluted pumpkin leaves, and maize soybean carrot pawpaw fluted pumpkin leaves. Iron is a critical micronutrient for hemoglobin synthesis, cognitive development, and immune function in early life [45]. The improved iron status observed in the rats fed formulated diets may be attributed to the inclusion of soybean and green leafy vegetables, which are known sources of non-heme iron. Furthermore, the presence of vitamin C from pawpaw and carrot likely enhanced iron absorption by reducing ferric to ferrous iron, thereby improving bioavailability [46]. The relatively lower or non-significant improvement in rats fed maize soybean carrot pawpaw may be due to nutrient interactions or competition for absorption, highlighting the complexity of micronutrient bioavailability in composite diets.

Serum zinc levels increased significantly across most groups, with particularly high increases observed in rats fed maize soybean carrot fluted pumpkin leaves, maize soybean pawpaw fluted pumpkin leaves, and maize soybean carrot pawpaw fluted pumpkin leaves. Zinc is essential for growth, immune function, and enzyme activity, and its deficiency is a major concern in complementary feeding in developing countries [47]. The significant improvements in zinc status may be attributed to the inclusion of soybean and leafy vegetables, which contribute to dietary zinc intake. Again, food processing methods such as soaking, drying, and milling can reduce phytate levels and improve zinc bioavailability (Gibson et al., 2010). The higher increases observed in rats fed multi-component diets further emphasize the benefits of dietary diversity in improving micronutrient status. These findings highlight the importance of ingredient diversification and appropriate processing techniques, such as freeze-drying, in enhancing nutrient retention and bioavailability.

5. Conclusion

This study established the potential of the formulated complementary foods as nutritionally adequate, locally adaptable alternatives to commercially available products. The formulated complementary foods, particularly the MSCFP blend, demonstrated superior nutritional quality and growth-promoting potential compared to the maize-soybean-only blend. The inclusion of locally available, nutrient-dense vegetables significantly enhanced both the macronutrient and micronutrient profiles of the formulations. This study, therefore, provides a scientific basis for the utilization of these indigenous plant materials in the development of affordable, culturally acceptable, and nutritionally adequate complementary foods for infants and young children in resource-limited settings.

Declarations

Funding
This research received no specific grant from any funding agency.

Conflict of Interest

The authors declare no conflict of interest.

Ethical Approval

Ethical Approval [MOUAU/CVM/REC/202601] was obtained from the Research Ethics Committee of the College of Veterinary Medicine, Michael Okpara University of Agriculture Umudike, Abia State, Nigeria.

Author Contributions

NMN, IUO and PNA conceived and designed the study, including the formulation of the research objectives and experimental framework. PNA and IUO procured and prepared the samples, and performed laboratory analysis. PNA and IUO performed the statistical analysis and data curation under the supervision of NMN. PNA and IUO wrote the first draft of the manuscript. IUO, NMN and PNA interpreted the findings. All the authors critically reviewed, edited and approved the final manuscript for submission.

Data Availability Statement: Data available upon reasonable request.

AI Usage Statement: AI tools not used at all.

References

1. World Health Organization. (2021). *Guideline: Updates on the Management of Severe Acute Malnutrition in Infants and Children.* WHO.
2. United Nations Children's Fund, World Health Organization, & World Bank. (2023). *Levels and Trends in Child Malnutrition: Unicef/Who/World Bank Group Joint Child Malnutrition Estimates*. WHO/UNICEF/World Bank.
3. Victora, C. G., Christian, P., Vidaletti, L. P., Gatica-Domínguez, G., Menon, P., & Black, R. E. (2021). Revisiting Maternal and Child Undernutrition in Low-Income and Middle-Income Countries: Variable Progress Towards an Unfinished Agenda. *The Lancet, 397*(10282), 1388–1399.
4. Black, R. E., Victora, C. G., Walker, S. P., Bhutta, Z. A., Christian, P., de Onis, M., Ezzati, M., Grantham-McGregor, S., Katz, J., Martorell, R., & Uauy, R. (2013). Maternal and Child Undernutrition and Overweight in Low-Income and Middle-Income Countries. *The Lancet, 382*(9890), 427–451.
5. World Health Organization. (2023). WHO Guideline for Complementary Feeding of Infants and Young Children 6–23 Months of Age.
6. United Nations Children's Fund. (2021). *The State of the World's Children 2021: On My Mind.* UNICEF.

7. Ogbo, F. A., Page, A., Idoko, J., Claudio, F., & Agho, K. E. (2015). Trends in Complementary Feeding Indicators in Nigeria, 2003–2013. *BMJ Open, 5*(10), e008467.
8. Abeshu, M. A., Lelisa, A., & Geleta, B. (2016). Complementary Feeding: Review of Recommendations, Feeding Practices, And Adequacy of Homemade Complementary Food Preparations in Developing Countries – Lessons from Ethiopia. *Frontiers in Nutrition, 3,* 41.
9. Gibson, R. S., Bailey, K. B., Gibbs, M., & Ferguson, E. L. (2010). A Review of Phytate, Iron, Zinc, And Calcium Concentrations in Plant-Based Complementary Foods Used in Low-Income Countries and Implications for Bioavailability. *Food and Nutrition Bulletin, 31*(2), 134–146.
10. Dewey, K. G., & Adu-Afarwuah, S. (2008). Systematic Review of The Efficacy and Effectiveness of Complementary Feeding Interventions in Developing Countries. *Maternal & Child Nutrition, 4*(s1), 24–85.
11. National Population Commission & ICF. (2019). *Nigeria Demographic and Health Survey 2018.* NPC and ICF.
12. Akombi, B. J., Agho, K. E., Hall, J. J., Merom, D., Astell-Burt, T., & Renzaho, A. M. N. (2017). Stunting, Wasting and Underweight in Sub-Saharan Africa: A Systematic Review. *International Journal of Environmental Research and Public Health, 14*(8), 863.
13. Messina, M. (2016). Soy and Health Update: Evaluation of The Clinical and Epidemiologic Literature. *Nutrients, 8*(12), 754.
14. Nnam, N. M., & Obiakor, P. N. (2003). Effect of Fermentation on The Nutrient and Antinutrient Composition of African Breadfruit (*Treculia Africana*) and Pumpkin (*Telfairia Occidentalis*) Leaves. *Journal of Food Science and Technology, 40*(3), 296–299.
15. Mohammed, A. M., Idris, M., & Abdulrasheed, A. (2016). The Mineral Composition and Proximate Analysis of T. Occidentalis (Fluted Pumpkin) Leaves Consumed in Kano Metropolis, Northern Nigeria. American Chemical Science Journal, 10(1), 1–4.
16. Sharma, K. D., Karki, S., Thakur, N. S., & Attri, S. (2012). Chemical Composition, Functional Properties and Processing of Carrot-A Review. *Journal of Food Science and Technology, 49*(1), 22–32.
17. Boadi, N. O., Badu, M., Kortei, N. K., Saah, S. A., Annor, B., Mensah, M. B., Okyere, H., Fiebor, A. (2021). Nutritional Composition and Antioxidant Properties of Three Varieties of Carrot (Daucus Carota), Scientific African, 12, e00801.
18. Akinyele, I. O., & Keshinro, O. O. (1980). Tropical Fruits as Sources of Vitamin C. *Food Chemistry, 5*(2), 163–167.
19. Shofian, N. M., Hamid, A. A., Osman, A., Saari, N., Anwar, F., Dek, M. S. P., & Hairuddin, M. R. (2011). Effect of Freeze-Drying on The Antioxidant Compounds and Antioxidant Activity of Selected Tropical Fruits. *International Journal of Molecular Sciences, 12*(7), 4678–4692.
20. Nnam, N. M., Ani, P. N., & Chukwuemeka, N. E. (2025). Effect of Locally Formulated Complementary Foods on Anthropometric Parameters and Micronutrient Status in Children (6-23 Months) in Enugu State, Nigeria: A Randomized Controlled Trial. *Preventive nutrition and food science, 30*(2), 110–119.
21. Food and Agriculture Organization. (2017). *The Future of Food and Agriculture: Trends and Challenges.* FAO.
22. Popkin, B. M., Corvalan, C., & Grummer-Strawn, L. M. (2020). Dynamics of the Double Burden of Malnutrition and The Changing Nutrition Reality. *The Lancet, 395*(10217), 65–74.
23. Ejigui, J., Savoie, L., Marin, J., & Desrosiers, T. (2005). Improvement of the Nutritional Quality of a Traditional Complementary Porridge Made of Fermented Yellow Maize (Zea Mays): Effect of Maize-Legume Combinations and Traditional Processing Methods. *Food and Nutrition Bulletin, 28*(1), 50–64.
24. Egounlety, M., & Aworh, O. C. (2003). Effect of Soaking, Dehulling, Cooking and Fermentation with Rhizopus Oligosporus On the Oligosaccharides, Trypsin Inhibitor, Phytic Acid and Tannins of Soybean (Glycine Max Merr.), Cowpea (Vigna Unguiculata L. Walp) and Groundbean (Macrotyloma Geocarpa Harms). *Journal of Food Engineering, 56*(2–3), 249–252.
25. Codex Alimentarius Commission. (2013). *Guidelines for Formulated Complementary Foods for Older Infants and Young Children* (CAC/GL 8-1991). World Health Organization/Food and Agriculture Organization.
26. Association of Official Analytical Chemists. (2010). *Official Methods of Analysis of AOAC International* (18th ed.). AOAC International.
27. Nierenberg, D. W. (1984). Determination of Serum and Plasma Concentrations of Retinol Using High-Performance Liquid Chromatography. *Journal of Chromatography, 311*(2), 239–248.
28. Bötticher, B., Bötticher, D., & Bässler, K. H. (1987). A New HPLC-Method for the Simultaneous Determination of B1-, B2- and B6-Vitamers in Serum and Whole Blood. *International Journal for Vitamin and Nutrition Research, 57*(3), 273–278.
29. Cerhata, D., Bauerová, A., & Ginter, E. (1994). Determination of Ascorbic Acid in Blood Serum Using High-Performance Liquid Chromatography and Its Correlation with Spectrophotometric (Colorimetric) Determination. *Česká a Slovenská Farmacie, 43*(4), 166–168.
30. Guirgis, F. K., & Habib, Y. A. (1970). A Simple Colorimetric Method for The Determination of Iron in Serum. *The Analyst, 95*(1131), 614–618.
31. Ripoll, J. P. (1976). Colorimetric Determination of Calcium in Serum Using Methylthymol Blue. *Clinica Chimica Acta, 72*(1), 133–139.
32. Makino, T. (1999). A Simple and Sensitive Colorimetric Assay of Zinc in Serum Using Cationic Porphyrin. *Clinica Chimica Acta, 282*(1-2), 65–76.
33. World Health Organization. (2023). *Infant and Young Child Feeding: Model Chapter for Textbooks for Medical Students and Allied Health Professionals*. WHO.
34. Omah, E., Nwaudah, E., Asogwa, I., & Eze, C. (2021). Quality Evaluation of Powdered Ogi Produced from Maize-Sorghum

and Soybean Flour Blends in Nigeria. African Journal of Food, Agriculture, Nutrition and Development, 21(10), 18839+.

35. Aynalem, E. G., & Duraisamy, R. (2022). Formulation and Optimization of Complementary Food Based on Its Nutritional and Antinutritional Analysis. *International journal of food science, 2022,* 1126031.
36. Ijarotimi, O. S. (2022). Macronutrient Composition, Amino Acid Profiles and Acceptability of Maize-Based Complementary Foods Enriched with Defatted White Melon Seed and Moringa Oleifera Leaf Powder. Croatian Journal of Food Science and Technology, 14(1), 7–17.
37. Adepeju, A. B., Adewa, T. T., Oni, K. O., Oyinlove, A. M., & Olugbuyi, A. O. (2024). Nutrient Rich Complementary Food Formulation Using Locally Sourced Compositions. *FUOYE Journal of Pure and Applied Sciences, 9*(1), 1–12.
38. Duraisamy, R., Bekele, B., Haile, B., Mulugeta, E., & Mada, T. (2025). Carrot Powder and Jaggery Supplemented Germinated Oat, Teff Based Complementary Infant Food: Formulation, Optimization, and Characterizations. Food Science & Nutrition, 13: e70264.
39. Mohammed, Z. K., Petrol, B. B., & Ahmad, U. (2021). Formulation and Nutritional Evaluation of a Complementary Food Blend Made from Fermented Yellow Maize (Improved Variety), Soybean and African Cat Fish Meal. *Nigerian Journal of Biotechnology, 38*(1), 1–9.
40. Hambidge, K. M., & Krebs, N. F. (2018). Strategies for Optimizing Maternal Nutrition to Promote Infant Development. *Reproductive Health, 15*(Suppl 1), 87.
41. Beal, T., White, J. M., Arsenault, J. E., Okronipa, H., Hinnouho, G. M., Murira, Z., Torlesse, H., & Garg, A. (2021). Micronutrient Gaps During the Complementary Feeding Period in South Asia: A Comprehensive Nutrient Gap Assessment. *Nutrition Reviews, 79*(Suppl 1), 26–34.
42. Buzigi, E., Pillay, K., & Siwela, M. (2022). Potential of Pumpkin to Combat Vitamin A Deficiency During Complementary Feeding in Low- And Middle-Income Countries: Variety, Provitamin A Carotenoid Content and Retention, and Dietary Reference Intakes. Critical Reviews in Food Science and Nutrition, 62(22), 6103–6112.
43. Znamirowska, A., Szajnar, K., & Pawlos, M. (2021). Effect of Vitamin C Source on Its Stability during Storage and the Properties of Milk Fermented by *Lactobacillus Rhamnosus. Molecules (Basel, Switzerland), 26*(20), 6187.
44. Weaver, C. M., & Peacock, M. (2019). Calcium. Advances in Nutrition, 10(3), 546–548.
45. Zimmermann, M. B., & Hurrell, R. F. (2007). Nutritional Iron Deficiency. *The Lancet, 370*(9586), 511–520.
46. Hurrell, R., & Egli, I. (2010). Iron Bioavailability and Dietary Reference Values. *The American journal of clinical nutrition, 91*(5), 1461S–1467S.
47. Osendarp, S. J. M., Broersen, B., van Liere, M. J., De-Regil, L. M., Bahirathan, L., Klassen, E., & Neufeld, L. M. (2016). Complementary Feeding Diets Made of Local Foods Can Be Optimized, But Additional Interventions Will Be Needed to Meet Iron and Zinc Requirements in 6- to 23-Month-Old Children in Low- and Middle-Income Countries. *Food and Nutrition Bulletin, 37*(4), 544–570.