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Effects of formulated Nigerian yellow maize, soybean, and crayfish blends on some growth performance and physiological status

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Abstract

In order to develop adequate complementary foods (CFs) to improve infant and young child feeding, the inclusion of animal source foods (ASFs) into plant-based foods (PBFs) is paramount. Unfortunately, the incorporation of ASFs into PBFs to formulate adequate CFs was infrequent in the developing countries, especially Nigeria. Yet, few CFs that were formulated by this strategy lacked adequate studies. This study investigated the compositional quality of CFs formulated from Nigerian yellow maize flour (MF), yellow maize + soybean flour (MSF), yellow maize + soybean + crayfish flour (MSCF), and commercial fortified wheat milk flour (FWMF) and their impact on the growth performance and physiological status of Sprague Dawley rats (SDRs). Compositional quality of CFs and their effects on the health status of SDRs were assessed. MSCF had higher protein and ash contents, its sulphur amino acids content was about 132% higher than that of MSF and astaxanthin was detected only in it. Determined antinutrients much reduced in MSCF compared with other CFs. The body weight gain (23.75 g) in yellow maize + soybean + crayfish diet (MSCD) group was significantly higher than other diet groups, whilst the value of protein efficiency ratio (2.59), feed efficiency ratio (0.30) in MSCD group was nominally higher compared with other groups. Better improvement in some of the biochemical and haematological parameters were observed in MSCD group compared with other groups, but no signs of illness, infection, and organ damage were seen in all the groups. The current study proved that crayfish could be used in a dietary modification to produce an adequate CF that potentiates improved growth performance and positive health outcomes in animals.

Highlights

- Supplementation with crayfish improved sulphur amino acids content of plant-based foods
- Astaxanthin was detected in crayfish-containing food
- Astaxanthin-containing diet improved diet intake and body weight gain in rats
- Serum total protein and albumin could predict normal growth in rats
- A decreased total tannin caused and increased serum calcium and zinc levels

Keywords Complementary foods, Nutrition and health, Total tannin, Phytate, Astaxanthin, Crayfish

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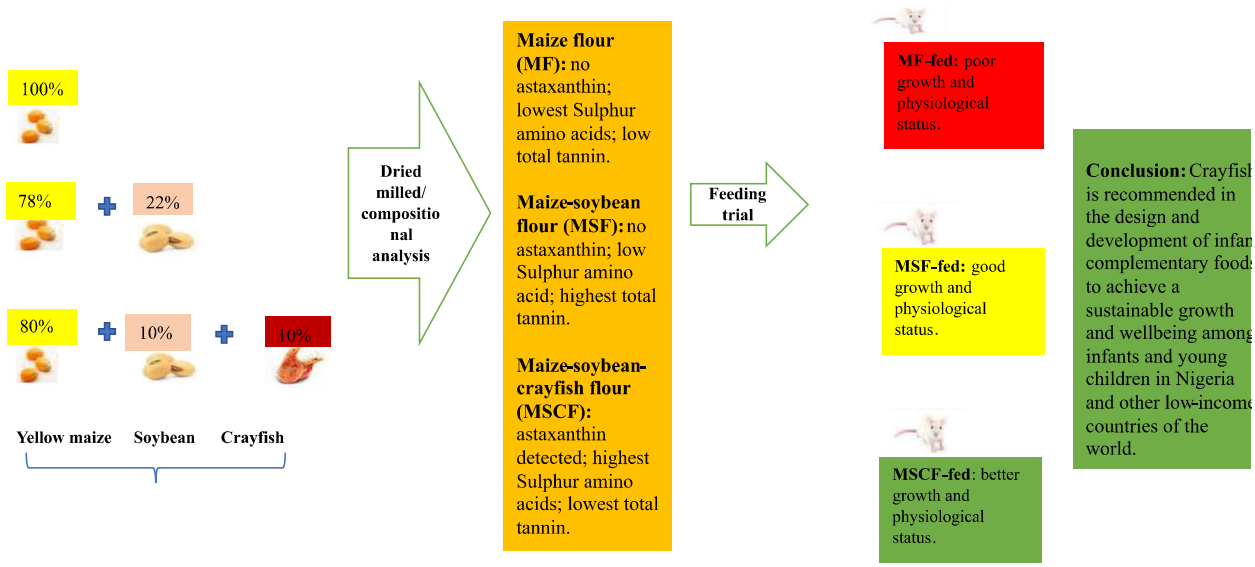
Graphical Abstract



Introduction

Malnutrition is a public health problem in most low-income countries, contributing to 3·1 million deaths annually in children younger than 5 years (Bauserman et al. 2015). Iron, zinc, vitamin A, and protein are the major nutrient deficiencies negatively influencing growth during the complementary feeding period (Agbemafle et al. 2020). Complementary feeding starts with the timely introduction of safe and nutritious foods when breastmilk is no longer sufficient by itself, and this period ranges from 6 to 23 months of age (White et al. 2017). Childhood stunting is the most prevalent form of undernutrition in the world, with a 22% global prevalence in children under 5 years old (Kaimila et al. 2019). In Nigeria, 37% of children under 5 years old were stunted (National Population Commission [Nigeria], (NPC), and (ICF) International Classification of Functioning Disability and Health 2019). Stunting reduces a child's physical, immunological, and cognitive capacities throughout his/her lifetime and was estimated to account for 21% of all disability-adjusted life years (DALYs) in children (Kaimila et al. 2019). Some factors that are associated with stunting include household and family food insecurity, household food allocation, truncated breast feeding, complementary feeding with inadequate traditional foods, reduced dietary diversity and frequent infections such as diarrhea, malaria, respiratory infections, and environmental enteric dysfunction (Kaimila et al. 2019).

Zinc deficiency is associated with stunted growth, anemia, greater susceptibility to infection and other serious health consequences (Agbemafle et al. 2020), iron deficiency has negative effects on physical performance and health status and thus increases healthcare costs (Agbemafle et al. 2020), vitamin A deficiency causes night blindness, while severe protein deficiency causes Kwashiorkor (Agbemafle et al. 2020; Titi-Lartey and Gupta 2022). Dietary proteins provide amino acids for the synthesis of plasma proteins, and play important roles as enzymes, antibodies, and hormones in the body (Oibiokpa et al. 2018).

In traditional sub-Saharan African societies including Nigeria, complementary feeding is dominated by monotonous, low-quality protein and micronutrient-deficient plant-based foods (PBFs) such as maize, millet, cassava, rice, and sorghum (Kaimila et al. 2019; Agbemafle et al. 2020). Animal-source foods (ASFs), contrarily, provide better protein quality and bioavailability of vitamin B12, heme iron, vitamin A, zinc, calcium, and

other minerals (Dror and Allen 2011). Some studies and systematic reviews have demonstrated lower rates of stunting in children that consumed animal-source foods, such as fish, meat, poultry, dairy products and eggs, than children that only consumed PBFs (Bolton 2019; Adesogan et al. 2020). Despite the acclaimed nutritional benefits of ASFs, their consumption among 6–23 months old children were very low in low-income countries especially Nigeria where poverty and lack of nutrition knowledge are rife (Haileselassie et al. 2020). To achieve protein and micronutrient density, complementary feeding guidelines recommends the addition of ASFs to PBFs, because ASFs increase nutrient density of CFs, as well as improve bioavailability of micronutrients (Agbemafle et al. 2020). Fortunately, such unconventional edible ASFs (UNEASFs) as insects, crayfish etc. are accessible in the low-income countries, of which crayfish offer a promising alternative especially in Nigeria. Crayfish are the cheapest source of affordable animal protein and a rich source of lysine, sulphur-containing amino acids, macro- and micronutrients (Ibironke et al. 2014; Iwuchukwu et al. 2017). As shown in Fig. 1, the pinkish-red colour of crayfish is provided by the accumulated astaxanthin in the body, shell, and tissue (Okada et al. 1994; Morrow 2011; Su et al. 2018).

Astaxanthin was reported to have possessed an organoleptic property that aids in diet consumption of animals (Venugopal and Gopakumar 2017). Studies have shown that astaxanthin helped to stabilize lipid in food products, thereby preventing rancidity that could render foods unfit for consumption (Al-Tarifi et al. 2020). Nutrient quality of foods is measured by their nutrients' composition and the bio-availability of those nutrients (World Health Organization (WHO) 1998; Prache et al. 2022). This study was carried out to evaluate the essential amino acids, astaxanthin, phytic acid and tannin compositions of Nigerian yellow maize, soybean, and crayfish blends, and to investigate their impact on the growth performance, and physiological status of Sprague Dawley rats. The study contributed to the improvement of the nutritional quality of traditional plant-based CFs, by harnessing the nutritive value of crayfish as an animal source protein to supplement and improve low and poorly bioavailable nutrients from a combination of maize and soybean flour.

Materials and methods

Sample collection

Raw and dried yellow maize (*Zea mays*) and soybean (*Glycine max*), parboiled and dried crayfish (*Procambarus clarkii*) and a commercial fortified wheat milk powder (Nestle Cerelac) were bought from the main market, Abuja Nigeria, and transported in a separate sealed air-tight plastic bag to the Nutrition Science Laboratory, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia.

Sample preparation

Yellow maize (*Zea mays*), soybean (*Glycine max*) and crayfish (*Procambarus clarkii*) were processed into flours of 710 µm size, as illustrated in Fig. 2, following the treatment procedure of Adegbusi et al. 2022b). Flours of food ingredients were separately packed into sealed polyethylene bags and stored in the refrigerator at 4 °C, pending CF formulation, analytical procedure, and animal trial study.

Formulation of complementary food (CF) products

Protein contents (N × 6.25, for MF and CRF; N × 5.71, for SBF) of MF, SBF, and CRF were determined by Kjeldahl

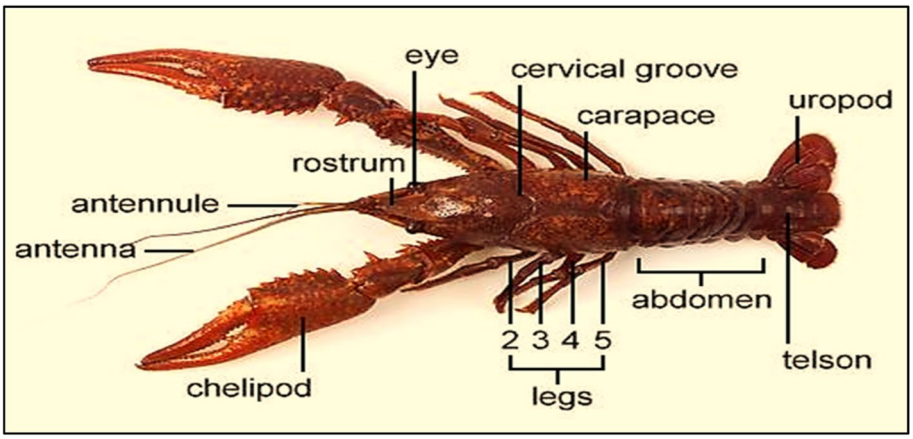


Fig. 1 The external structure of a crayfish Friedman (2017)

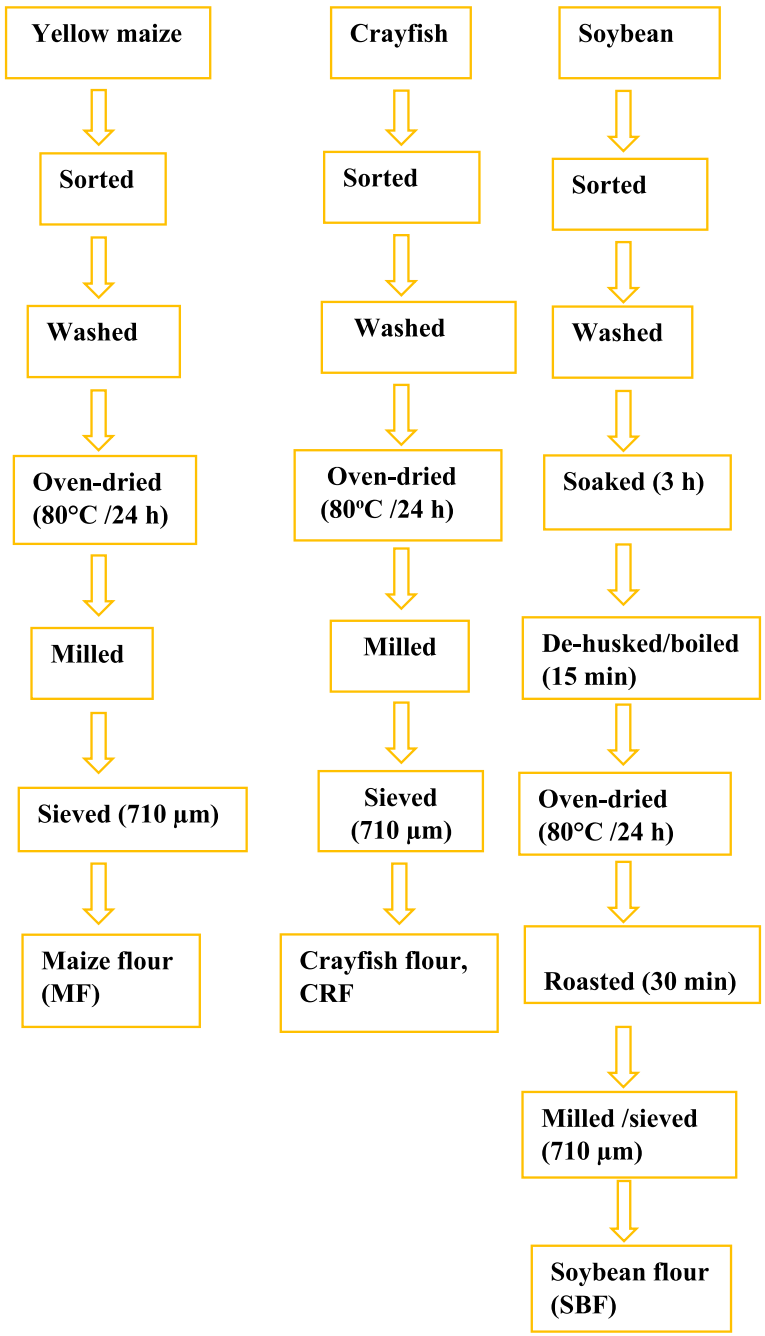


Fig. 2 Processing flow for the preparation of flour. MF = maize flour; CRF = crayfish flour; SBF = soybean flour

method as described in Suksong (2013) and the resultant crude protein contents, based on dry matter (DM), were used to formulate the desired mixed CF products that satisfied protein content of 16% CF requirement for infants (Adegbusi et al. 2022b). Formulated mixed and single CFs, with particle size of 710-µm, are showed by the following expressions.

MSF = maize flour + soybean flour (72:28% w/w),

MSCF = maize flour + soybean flour + crayfish flour (80:10:10% w/w), and.

MF = maize flour (100:0% w/w, used as negative control).

The targeted 16% protein level for the formulated mixed CFs was achieved through material balancing

technique of algebraic food formulation procedure described in Adegbusi et al. 2022b). with expressions.

1. X + (1000 − X) = 1000, i.e., MSF having one unknown amount of food ingredient, X.

2. Y + X + (1000 − X) = 1000, i.e., MSCF having one unknown amount of food ingredient, X, and one fixed amount of food ingredient, Y. In this case, 10% of Y (CRF) supplied best mixture of a CF.

The formulated CFs: MF, MSF, and MSCF were thoroughly mixed into homogenous composites from which analytical samples were drawn for chemical compositional analysis and rat feeding experiment. Analytical samples were also drawn from commercial CF (FWMF) for further analyses.

Chemical compositional analysis of foods

Proximate composition analysis

CFs and their constituent raw materials were analyzed for moisture, ash, crude lipid, and crude fiber contents by the methods described in Food and Agricultural Organisation (FAO) 1994, while the crude protein content was determined by the Kjeldahl method described in Suksong (2013) Total carbohydrate content was evaluated by the method of difference from 100 as described in Suksong (2013) and the energy content was calculated using Atwater's calorie conversion factors of 4 kCal/g for crude protein, 9 kCal/g for crude fat, and 4 kCal/g for carbohydrate described in Food and Agriculture Organization (FAO) (2003). Essential amino acids were determined as described in Adegbusi et al. (2022b).

Total tannin content (TTC) analysis

Total tannin content was determined followed total phenolic and non-tannin phenolic contents determination using the Folin-Ciocalteu method described in Adegbusi et al. (2022a). Before analysis, food samples were extracted with a proper extraction solvent. Into a 25 mL beaker, 500 mg dried ground sample of a food product and 5 mL 50% aqueous acetone were added and subjected to ultrasonic treatment (Power sonic 405, Korea) for 30 min at room temperature, after which the content in the beaker was quantitatively transferred to a 20 mL centrifuge tube for 10 min centrifugation at 3000 rotations per minute (rpm) and 4 °C. The supernatant called original extract was collected into another new 20 mL tube and analysed for tannin.

Determination total phenolic content Into a 20 mL tube, 450 µL of distilled water and 50 µL of a sample's original extract (oE) were added. Subsequently, 250 µL of FC reagent followed by 1250 µL of sodium carbonate solution were added. The mixture was vortexed at each step of addition. After 40 min of incubation in the dark, the absorbance was measured at 725 nm using a UV-Vis spectrophotometer (UV-180, Japan). The concentration of the total phenol was derived through extrapolation from the standard curve shown in Fig. 1. Distilled water in a 1 cm path length quartz cell was used to zero the spectrophotometer, and triplicate extracts of each product were measured. The TPC was calculated as in Formula (Fa) 1.

$$\text{TPC (\% tannic acid equivalent, TAE)} = \left[(A/0.05)/1000\right] \times 1 \quad (1)$$

Where A is the concentration (µg/mL) of the aliquot from the standard curve; 0.05, 1 = (100 mg/mL, 1-fold dilution) and 1000 are conversion factors to percentage.

Determination of non-tannin phenolic content (NTPC) Into a 20 ml tube, 100 mg of Insoluble polyvinyl polypyrrolidone (PVPP; Sigma Aldrich, USA) and 1 mL distilled water were added and vortexed. Subsequently, 1 mL of a sample was added and vortexed. After incubation at 40 °C for 10 min, the tube was centrifuged (2810, Japan) at 3000 rpm and 4 °C for 10 min. After centrifugation, 100 µL of the supernatant was placed in a new 20 mL tube, 400 µL of distilled water was added and vortexed. Afterward, 250 µL Folin-Ciocalteu reagent and 1250 µL of sodium carbonate solution were added and vortexed in succession. The tube was kept in darkness for 40 min; the absorbance was subsequently taken in a spectrophotometer at 725 nm. The concentration of the non-tannin phenolic in the supernatant was interpolated from the standard curve. The interpolated value of unknown (µg/mL) was thus used to determine the non-tannin phenolics content as in Fa 2.

$$\text{NTPC (\%TAE)} = [(B/0.1)/1000] \times 2 \quad (2)$$

Where, B is the concentration (µg/ml) of the aliquot from the standard curve; 0.1 and 1000 are conversion factors to percentage, and 2 is the two-fold dilution.

TTC was determined by the difference between Fa 1 and Fa 2 as in Fa 3.

$$\text{TTC (\%TAE)} = (\text{TPC} - \text{NTPC}) \quad (3)$$

Where TTC = total tannin content; TPC = total phenolic content; NTPC = non-tannin phenolic content.

Absorbance at 725 nm with tannic acid concentrations of 2, 4, 6, 8, and 10 µg/µL was used to obtain a standard linear curve using Microsoft Excel version 2016.

Phytic acid analysis

Phytic acid was determined according to the modified method of Adane et al. (2013). Into a 15 mL tube having 0.075 g of a dried defatted food sample, 10 mL of 2.4% HCl solution was added and allowed to stand for 1 h at 25 °C. The tube was centrifuged (Rotofix 32A, Germany) at 3000 pm and 25 °C for 30 min. After centrifugation, the clear supernatant was collected and used for phytates analysis. One mL of Wade reagent, 0.03% solution of FeC13.6H2O, and 0.3% sulfosalicylic acid in distilled water, were added to 3 ml of the sample clear supernatant in a new 15 mL tube, the mixture was centrifuged at 3000 rpm and 25 °C for 10 min and the absorbance at 500 nm UV–Visible was measured spectrophotometrically (Rotofix 32A, Germany). The absorbance of a control setup having 3 mL of distilled water and 1 mL Wade reagent was equally measured. Distilled water was used to zero the spectrophotometers. Phytic acid content was found in triplicate as in Fa 4.

$$\text{Phytic acid content } (\mu g/g) = \frac{\left[(A_s - A_b) - \text{intercept}\right] \times 10}{\text{Slope} \times W \times 3} \quad (4)$$

Where, A_S = sample absorbance; A_b = blank absorbance; 10 = dilution factor (mL); 3 = aliquot of supernatant used for phytates determination (mL); and W = weight in grams of the food samples; and the intercept and slope were both obtained from the standard curve.

Absorbance at 500 nm with hydrated sodium phytates salt concentrations of 0, 5, 10, 20, 30, and 40 µg/mL was used to obtain a standard linear curve using Microsoft Excel version 2016.

Astaxanthin (ASTX) analysis

As described in Sánchez-Camargo et al. (2011), ASTX determination was followed through a 3-stage procedural quantification of food samples with a modification from the description of Rodriguez-Amaya and Kimura (2004) and Safawo et al. (2010). Into a 250 mL conical flask holding 10 mL of water, an accurately weighed 5 g of a food sample (except for 10 g of MSCF in 15 mL of water) was placed and allowed to rehydrate for 30 min. About 20 mL of cold acetone, in place of dry acetone, was afterward added and allowed to stand for 15 min. Afterward, the mixture was filtered into a new 250 mL conical flask. The residue was put in a mortar and ground with the pestle, in place of the homogenizer, with about 20 mL of cold acetone added, allowed to soak for 5 min, and filtered into the flask holding the earlier extract. Only four extractions–filtrations procedures were carried out when the residue became colourless. The mortar and pestle, funnel, and the residue were finally washed with 20 mL of cold acetone, and the washing was received in the flask with the extract.

The total extract obtained was separated through a 250 mL separating funnel. The joint filtrate of acetone was transferred into a 250 mL separating funnel holding 20 mL, in place of 80 mL petroleum ether (40 °C–60 °C boiling point) of 0.1% Butylated hydroxyl toluene (BHT). Fifty millilitres, in place of 100 mL of 10% NaOH solution were added slowly to the funnel wall for proper separation and to avoid emulsion formation. The upper petroleum ether phase was carefully transferred into a 150 mL evaporating round bottom flask of a known stable dried weight. The acetone phase was extracted two times with 20 mL of petroleum ether of 0.1% BHT. The combined petroleum ether phase in the evaporating flask was evaporated at a temperature of 35 °C and a pressure of 30 mmHg in a rotary evaporator Rodriguez-Amaya and Kimura (2004). The ether-free extract was frozen at -80 °C in a freezer for 24 h and finally freeze-dried for 72 h in a freeze dryer of 50 Hz frequency, instead of nitrogen dried in a vacuum oven.

The freeze-dried extract was dissolved in 6 mL of n-hexane. The absorbance at a wavelength of 472 nm was read in a spectrophotometer zeroed with n-hexane, and the ASTX content as total carotenoid (TC) content for each food was found, using the calibration curve obtained from a series of ASTX standard solutions, as in Fa 5.

$$\text{ASTX}_{\text{TC}}(\mu g/g) = \frac{C(\mu g/mL) \times V(mL)}{E(g)} \quad (5)$$

Where C = carotenoid concentration from the standard curve; V = total extract volume; and E = weight of freeze-dried extract.

Desired concentrations ranging between 1 and 6 µg/mL were prepared from 10 mg AST/100 mL ethyl acetate and diluted with n-hexane for drawing a standard curve using Microsoft Excel version 2016.

Identification of ASTX using thin layer chromatography (TLC)

The n-hexane concentrated carotenoid extract was subjected to TLC using activated 20 × 20 cm silica gel plates (ALUGRAM SIL G/UV254 0.2 mm, Code 818233, Macherey–Nagel, Germany) following the modified procedure described in Lorenz (1998). A TLC tank covered with a lid was saturated for 30 min with 100 mL of a mobile phase of a mixture of acetone and n-hexane in the ratio of 25:75 (% v/v). A TLC Plate that was activated in the air oven for 30 min at 130 °C was loaded, 2 cm from the

Table 1 Composition of formulated diets (CFDs) fed to rats (g/100 g)

Component (g)	CFD				
	MD	MSD	MSCD	FWMD	PFD
MF	100	–	–	–	–
MSF	–	46.3	–	–	–
MSCF	–	–	45.5	–	–
FWMF	–	–	–	55.8	–
Corn starch	–	27	27.8	17.5	73.3
Corn oil	–	10	10	10	10
Cellulose	–	5	5	5	5
Sucrose	–	7	7	7	7
Choline bitartrate	–	0.2	0.2	0.2	0.2
Mineral mix	–	3.5	3.5	3.5	3.5
Vitamin mix	–	1	1	1	1

MD maize diet, *MSD* maize + soybean diet, *MSCD* maize + soybean + crayfish diet, *FWMD* fortified wheat milk diet (Nestle Cerelac diet), *PFD* protein-free diet, *MF* maize flour, *MSF* maize + soybean flour, *MSCF* maize + soybean + crayfish flour, *FWMF* fortified wheat milk flour (Nestle Cerelac powder)

bottom and 1 cm apart, with 50 µL of concentrated carotenoid extract alongside ASTX standard solution. After dryness, the loaded plate was placed inside a lid-covered saturated TLC tank and developed with the tank-saturating mobile phase for 1 h. After 1 h of development, the plate was carefully removed from the tank, the solvent front was quickly and carefully marked, and observable carotenoid spots were circled with a pencil before dried up. The retention factor (R_f) for each observed spot was determined as described in Chemistrylibretexts (2022), as in Fa 6.

$$\mathrm{Rf} = \frac{\text{distance travlled by a spot}}{\text{distance travlled by the solvent front}} \quad (6)$$

Chemicals and reagents

All chemicals and reagents used were of analytical grade obtained from R&M Chemicals, Malaysia, Sigma Aldrich, USA, Pierce Chemicals & Co., USA and Waters, USA.

Formulation of complementary food diets (CFDs)

Complementary food diets (CFDs) for the rat studies were formulated based on the Food and Agricultural Organization protocol as described in Adegbusi et al. (2022b). The compositions of the formulated diets are given in Table 1.

Rat feeding experiment

The rat feeding design followed the procedure described in Adegbusi et al. (2022b). On the 14th day, after 8 h fasting, 2 mL of blood was collected from an individual rat fed the same diet, through cardiac puncturing under the sedative influence of diethyl ether in an anaesthesia induction chamber Nakatsu et al. (2017), into separate ethylenediamine tetra-acetic acid (EDTA) sample bottles (Becton, Dickinson and Company, UK) for haematological analysis, and plain serum bottles (Becton, Dickinson, and Company, UK) for biochemical analysis. The blood from individual rats fed the same diet was analysed for total serum protein (TP), serum albumin (ALB), calcium (Ca), iron (Fe) and zinc (Zn), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and haematological parameters.

Individual blood collected for biochemical parameters was spun using a refrigerated centrifuge (Eppendorf centrifuge 5702, Germany) for 10 min at 503 × g and 4 °C to obtain the serum, which was carefully transferred with Pasteur pipettes into an individual cleaned, dried labelled light-shielding sample bottles. Both whole blood and serum samples were stored at -80 °C freezer pending analysis. The spilled diet of individual rat from each diet group was air-dried for 3 days, weighed, added to the recorded uneaten diet, and deducted from the total diet offered over a four-day balance period for total diet intake determination. Formulated and control diets were also analysed for total nitrogen and moisture contents as described in Suksong (2013).

The Universiti Putra Malaysia code of practice for the care and use of animals for scientific purposes was followed during the feeding study (see Appendix 1 for the animal study approval letter).

Determination of rat growth performance

Data from weight gain, diet and protein intakes of rats fed formulated, control and basal diets were used to determine rat growth performance, in terms of body weight gain (BWG), protein efficiency ratio (PER) and feed efficiency ratio (FER) as described in Oibiokpa et al. (2018) and Adejuwon et al. (2021).

Assays for biochemical parameters

The levels of serum TP, ALB, ALT, AST, ALP, and Ca were found using automated Biolis Premium 24i Chemistry Analyzer Japan, following the procedure of commercial assay kit manufactured by BioREX Mannheim Malaysia Sdn Bhd. The serum iron, zinc, and vitamin A levels were found based on the colorimetric procedure of commercial kits manufactured by Elabscience Biotechnology Inc., USA (E-BC-K139-S, E-BC-K137-M, and E-EL-0135). Similarly, The levels of serum triacylglycerol (TAG), total cholesterol (TCHOL), high-density lipoprotein cholesterol (HDLC), and low-density lipoprotein cholesterol (LDLC) were measured using the automated Siemens Xpand Plus analyzer, USA and following the described procedure of Siemens Dimension Flex Reagent cartridge manufactured by Siemens Healthcare Diagnostics Inc., USA; TAG (Siemens Healthcare, DF69A), TCHOL (Siemens Healthcare, DF27), HDL (Siemens Healthcare, DF48B) and LDL (Siemens Healthcare, DF131).

Assays for haematological parameters

Packed cell volume (PCV) was determined by micro-haematocrit method as described in Solomon (2005). Red blood cell (RBC), mean cell volume (MCV), mean corpuscular haemoglobin concentration (MCHC), haemoglobin (Hb), white blood cells (WBC), the different categories of WBC in respect of neutrophils, lymphocytes, monocytes, eosinophils and basophils, and platelets were measured by automated blood cell analysis, based on the principle of Siemens Advia 2120i haematology auto-analyzer described in Siemens Healthcare Diagnostics Inc., USA.

Assessment of relative organ weight of CFDs-fed rats

On the 14th day of the trial, life-weight of individual rats of a diet group was recorded. Liver, kidneys, heart, and adrenal glands were harvested after blood collection, trimmed free of fat, and weighed. Relative organ weight was calculated for each organ or gland, as in Fa 7, following the description of (He et al. 2019).

$$\text{Relative Organ Weight } (\%) = \frac{(\text{organ weight, g}) \times 100}{\text{body weight g}} \quad (7)$$

At the time of this study blood urea nitrogen and creatinine kinase tests were not conducted.

Statistical analysis

All analyses were conducted in triplicate. Data were statistically analysed by multiple analyses of variance (MANOVA), multiple linear regression and Pearson's correlation analyses. Data significant differences among the formulated and control foods and diets were achieved by Tukey's honestly significant difference test (HSD-test) at the $P \leq 0.05$ level of significance using Statistical Package of Social sciences (SPSS) software, version 25. Thus, significant differences among the means of formulated and control CFs and diets were decided.

Results and discussions

Results

Proximate composition and energy content

The proximate composition and energy content of the individual food ingredients used to formulate CFs are presented in Table 2.

As showed in Table 2, there was a significant difference ($P < 0.5$) among the nutrients and energy contents of the

Table 2 Proximate composition and energy value of individual food ingredients (DW^{-1})

Parameter	Ingredient		
	MF	SBF	CRF
Moisture (g/100 g)	5.20 ± 0.02^{b}	4.87 ± 0.02^{c}	10.38 ± 0.19^{a}
Ash (g/100 g)	0.92 ± 0.11^{c}	3.91 ± 0.03^{b}	14.68 ± 0.11^{a}
Crude protein (g/100 g)	8.22 ± 0.06^{c}	37.01 ± 0.22^{b}	67.04 ± 0.12^{a}
Crude lipid (g/100 g)	6.35 ± 0.16^{b}	23.60 ± 0.23^{a}	6.17 ± 0.07^{b}
Crude fiber (g/100 g)	1.98 ± 0.01^{b}	4.13 ± 0.03^{a}	1.07 ± 0.08^{c}
Carbohydrate (g/100 g)	77.34 ± 0.36^{a}	26.50 ± 0.19^{b}	0.67 ± 0.17^{c}
Energy value (kCal/100 g)	399.38 ± 0.28^{b}	466.41 ± 1.37^{a}	326.38 ± 0.55^{c}

Values are expressed as mean ± standard deviation. Values (a-c) within a row that do not share the same letter are significantly different ($P < 0.5$)

DM dry matter, *MF* maize flour, *SBF* soybean flour, *CRF* crayfish flour

Table 3 Proximate composition and energy value of CFs on a DM basis

Parameter	CFs				
	MF	MSF	MSCF	FWMF	RR[1] from CF
Moisture (%)	5.29 ± 0.04^{c}	4.87 ± 0.18^{c}	6.20 ± 0.46^{b}	7.30 ± 0.10^{a}	≤ 10[2]
Ash (%)	0.94 ± 0.12^{d}	1.99 ± 0.06^{c}	2.72 ± 0.23^{a}	2.31 ± 0.07^{b}	≤ 3[3]
Crude protein (%)	8.38 ± 0.07^{c}	17.28 ± 0.37^{a}	17.59 ± 0.09^{a}	14.31 ± 0.38^{b}	≥ 15[3]
Crude lipid (%)	4.91 ± 0.10^{c}	11.56 ± 0.60^{a}	9.89 ± 0.06^{b}	5.28 ± 0.14^{c}	10–25[3]
Crude fiber (%)	2.03 ± 0.02^{c}	2.77 ± 0.27^{b}	1.41 ± 0.29^{a}	0.67 ± 0.01^{d}	≤ 5[3]
Carbohydrate (%)	80.47 ± 0.24^{a}	64.31 ± 0.67^{c}	63.59 ± 0.47^{c}	70.80 ± 0.30^{b}	NS[3]
Energy value (kCal/100 g)	400.00 ± 0.77^{c}	430.36 ± 3.69^{a}	413.77 ± 1.26^{b}	388.03 ± 0.46^{d}	≥ 400[3]

Values are expressed as mean ± standard deviation. Values (a-d) within a row that do not share the same letter are significantly different ($P < 0.05$)

DM dry matter, *CFs* complementary foods, *MF* maize flour, *MSF* maize + soybean flour, *MSCF* maize + soybean + crayfish flour, *FWMF* fortified wheat milk flour

[1] recommended range

[2] Hayes et al. (1995)

[3] Codex Alimentarius Commission (CAC) (2013)

NS not specified Codex Alimentarius Commission (CAC) (2013)

individual food ingredients. The moisture, ash and protein contents of CRF were significantly higher ($P < 0.5$) than those of MF and SBF. On the other hand, CRF was significantly lower ($P < 0.5$) in respective carbohydrate and crude fiber contents than each of MF and SBF. The respective lipid and energy contents of SBF were significantly higher ($P < 0.5$) than those of MF and CRF, while the carbohydrate content of MF was significantly higher ($P < 0.5$) than those of SBF and CRF.

The results of proximate composition per dry matter (DM) of the formulated {maize flour (MF); maize + soybean flour (MSF); maize + soybean + crayfish flour (MSCF)} and control {fortified wheat milk flour (FWMF)} complementary foods (CFs) are presented in Table 3.

The values of moisture content (per DM) ranged from 4.87–7.30%, of which the MSCF value was significantly higher ($P < 0.05$) than those of MF and MSF, and significantly lower ($P < 0.05$) than that of FWMF. The values of ash content (per DM) varied from 0.94–2.72%, of which the MSCF value was significantly higher ($P < 0.05$) than those of MF, MSF, and FWMF.

The values of crude protein (CP) content (per DM) ranged from 8.38–17.59%, of which MSCF content was significantly higher ($P < 0.05$) than those of MF and FWMF but not significantly different from MSF. The values of crude lipid content (per DM) ranged from 4.91–11.56%, of which MSCF content was significantly lower ($P < 0.05$) than MSF and significantly higher ($P < 0.05$) than those of MF and FWMF. The values of crude fiber

Table 4 The essential amino acid content of CFs compared with RDAs of older infants and young children

EAA (mg/100 g food DM)	MF	MSF	MSCF	FWMF	RDAs (mg/day)	
					7–11 months	12–36 months
Isoleucine	726 ± 26^{c}	1858 ± 109^{a}	1666 ± 30^{a}	1445 ± 136^{b}	387	364
Leucine	2518 ± 83^{b}	3985 ± 162^{a}	3793 ± 95^{a}	2701 ± 229^{b}	837	819
Lysine	591 ± 50^{c}	1576 ± 156.6^{a}	1531 ± 0.06^{a}	1225 ± 159^{b}	801	754
Methionine + Cysteine	124.6 ± 29^{b}	146.4 ± 44^{b}	340 ± 85.3^{ab}	560 ± 153^{a}	387	364
Phenylalanine + Tyrosine	1711 ± 200^{c}	3923 ± 564^{a}	3433 ± 177^{a}	2498 ± 759^{b}	756	702
Threonine	800 ± 53^{c}	1975 ± 155^{a}	1837 ± 62^{a}	1232 ± 373^{b}	441	416
Tryptophan	43.6 ± 2.1^{b}	150 ± 5.3^{a}	156 ± 3.2^{a}	156 ± 4.3^{a}	117	104
Valine	884 ± 8.4^{c}	1830 ± 92.5^{a}	1714 ± 27^{b}	1615 ± 12^{b}	522	481
Histidine	663 ± 77^{b}	$1448^{a} \pm 123^{a}$	1243 ± 54^{a}	841 ± 221^{b}	288	273

Values are expressed as mean ± standard deviation. Values (a-c) within a row that do not share the same letter are significantly different ($P < 0.05$)

DM dry matter, *CFs* complementary foods, *MF* maize flour, *MSF* maize + soybean flour, *MSCF* maize + soybean + crayfish flour, *FWMF* fortified wheat milk flour, *RDAs* recommended dietary allowances derived from RDA (mg/kg per day) × 9 kg (reference bodyweight for 7–11 months old children) and RDA (mg/kg per day) × 13 kg (reference bodyweight for 12–36 months old children) (Institute of Medicine (US) Food and Nutrition Board (IOMFNB), 1998), *EAAs* essential amino acids

Table 5 Phytate, total phenol, total tannin contents, and AST_{TC} contents of CFs, coupled with AST presence at corresponding Rf value on DM basis

Parameters	CFs			
	MF	MSF	MSCF	FWMF
Phytate (mg/100 g)	642 ± 0.04^{a}	640 ± 0.06^{a}	636 ± 0.04^{a}	641 ± 0.03^{a}
Total phenol (% TAE)	0.15 ± 0.00^{b}	0.22 ± 0.01^{a}	0.20 ± 0.01^{a}	0.15 ± 0.01^{b}
Total tannin (% TAE)	0.05 ± 0.00^{a}	0.07 ± 0.01^{a}	0.04 ± 0.02^{a}	0.01 ± 0.00^{b}
AST_{TC} (µg/g DM^{-1})	195.00 ± 3.0^{b}	105.73 ± 5.5^{c}	197.33 ± 4.6^{a}	42.94 ± 4.3^{d}
Presence of AST, and Rf	ND	ND	0.30, 0.59	ND

Values are expressed as mean ± standard deviation. Values (a-d) within a row that do not share the same letter are significantly different ($P < 0.05$)

CFs complementary foods, *MF* maize flour, *MSF* maize + soybean flour, *MSCF* maize + soybean + crayfish flour, *FWMF* fortified wheat milk flour, *TAE* tannin acid equivalent, AST_{TC} astaxanthin as total carotenoid, *AST* astaxanthin, *Rf* retention factor, *ND* not detected, *DM* dry matter

content (per DM) ranged from 0.67–2.77%, of which MSCF content was significantly lower ($P < 0.05$) than the contents of MF, MSF, but higher significantly ($P < 0.05$) than FWMF. The values of total carbohydrate content (per DM) ranged from 63.59–80.47%, of which the MSCF content was not significantly different from MSF content but significantly lower ($P < 0.05$) than the contents of those of MF and FWMF. The overall energy values ranged from 388.03–430.36 kCal/100 g, of which the value of MSCF was significantly lower ($P < 0.05$) than MSF, but significantly higher ($P < 0.05$) than those of MF and FWMF.

Essential amino acid contents of CFs

The results of essential amino acids analysis for formulated and control CFs are as tabulated in Table 4. The values of isoleucine, leucine, lysine, methionine, and cysteine (sulphur amino acids, SAA), phenylalanine and tyrosine (aromatic amino acids, ArAA), threonine, tryptophan, histidine, and valine contents (mg/100 g) of the CFs ranged from 726 to 1858, 2518–3985, 591–1576, 124.6–560, 1711–3923, 800–1975, 43.6–156, 663–1448 and 884–1830 respectively, of which all the essential amino acid contents of MSCF were not significantly different ($P < 0.05$) from MSF, except in the case of valine content. The valine content of MSCF was significantly higher than MF, significantly lower than MSF, and not significantly different from FWMF ($P < 0.05$). In the case of SAA content, MSCF was not significantly different ($P < 0.05$) from MF, FWMF, and the tryptophan content of MSCF was also not significantly different ($P < 0.05$) from FWMF.

Phytate, total phenol, and total tannin contents of CFs

The contents of phytate, total phenol, and total tannin of the formulated (MF, MSF, and MSCF) and control (FWMF) CFs are presented in Table 5. The values of the phytate content (per DM) ranged from 636 to 640 mg/100 g, of which MSCF content was not significantly different ($P < 0.05$) from those of MF, MSF and FWMF.

Astaxanthin as total carotenoid (AST_{TC}) contents of CFs

AST_{TC} contents of formulated and control CFs, coupled with AST presence at the corresponding retention factor (Rf) were represented in Table 5. The values of AST_{TC} content (per DM) which ranged from 42.94–197.33 µg/g had MSCF content significantly higher ($P < 0.05$) than those of MF, MSF and FWMF. Only MSCF was found by thin-layer chromatography to have revealed the presence of AST, in the form of free AST (Rf = 0.30) and AST monoester (Rf = 0.59). The retention factor (Rf) value calculated from the analysed chromatographic plate was used to detect the presence of AST. The Rf values of the various bands (see Appendix 2 for bands on thin-layer plates of extracts from CRF and CFs, and AST standard solution) obtained from the thin layer chromatography (TLC) separation of extracts from formulated and control CFs, and AST standard solution are presented in Table 6.

Growth performance in rats fed CFDs

A feeding trial was carried out to evaluate the effects of feeding on the body weight gain, feed efficiency ratio, and protein efficiency ratio of male Sprague Dawley rats. The results present in Table 7 show that the body weight gained (BWG g) by rats fed formulated, control, and basal diets after 4 days of balance period ranged from −7.00–23.75 g, of which the value of the MSCD-fed group was significantly higher ($P < 0.05$) than those of PFD-, MD-, MSD- and FWMD-fed groups. The amount

Table 6 Retention factors for extracts of CRF, CFs, and AST_{STD}

Band	Rf for CRF, CFs, and AST_{STD}					
	MF	**MSF**	**MSCF**	**FWMF**	**AST_{STD}**	**CRF**
1	0.37	0.37	0.30	NB	0.31	0.15
2	0.98	0.99	0.59	NB	NB	0.20
3	NB	NB	NB	NB	NB	0.30
4	NB	NB	NB	NB	NB	0.51
5	NB	NB	NB	NB	NB	0.73
6	NB	NB	NB	NB	NB	0.88
7	NB	NB	NB	NB	NB	0.97

CFs complementary foods, *MF* maize flour, *MSF* maize + soybean flour, *MSCF* maize + soybean + crayfish flour, *FWMF* fortified wheat milk flour, *CRF* crayfish flour, AST_{STD} astaxanthin standard, *NB* no band

of diet consumed (g) by rats fed formulated, control, and basal diets ranged from 28.00–79.50 g, of which the feed intake by the MSCD-fed group was significantly higher ($P < 0.05$) than that of the PFD-fed group. The feed intake by the MSCD-fed group was not significantly different ($P < 0.05$) from those of MD-, MSD- and FWMD-fed groups. The amount of protein consumed (g) by the experimental rats ranged from 0.11–9.25, and MSCD group was significantly higher ($P < 0.05$) than other diet-fed groups.

Feed efficiency ratio (FER) had values that ranged from 0.07 to 0.30 and there was no significant difference ($P < 0.05$) among the diet-fed groups, nevertheless, the FER of the MSCD group was the highest among other groups. Similarly, the PER values ranged from 0.87 to 2.59 and there was no significant difference ($P < 0.05$) among the diet groups. However, the PER value of the MSCD group was the highest among the diet groups.

Physiological responses in the serum and blood compositions, and relative organ weight of rats fed with CFDs

Serum biochemical, haematological and relative organ weight analyses were used to decide the health status of Sprague Dawley rats fed formulated, control, and basal diets during a 14-day feeding trial. During the feeding trial, none of the experimental rats fed MD or MSD or MSCD, or FWMD or PFD developed any sign of infection, diarrhoea, skin rash, and adrenal, heart, kidney, liver damage. Table 8 presents the effects of CFDs on the serum biochemical parameters of experimental rats.

The values of total serum TP and ALB ranged from 6.40–7.07 g/dl and 2.78–3.10 g/dl respectively, of which the TP and ALB values of the MSCD-fed group were not significantly different ($P < 0.05$) from those of PFD-, MD-, MSD- and FWMD-fed groups. However, both the TP and ALB values of the MSCD-fed group were the highest, while they were lowest in the PFD-fed groups.

There were also no significant changes ($P < 0.05$) in the values of ALT (53.50–130.75 U/L), AST (313.00–737.25 U/L), and ALP (439.16–538.37 U/L) among the diet groups, but the lowest ALT value of 53.50 U/L, AST value of 313.00 U/L and ALP value of 507.52 U/L were recorded for MSCD-fed group compared to other diet groups.

The concentration of Ca ranged from 2.48–2.68 mmol/L, of which the concentrations were not significantly different ($P < 0.05$) among the diet-fed groups. However, the Ca value of the MSCD group was the

Table 7 CFDs and effects on the body weight gain, feed, and protein efficiency ratios of Sprague Dawley rats

Parameter	Rat-fed formulated diets				
	MD ($n = 4$)	**MSD ($n = 4$)**	**MSCD ($n = 4$)**	**FWMD ($n = 4$)**	**PFD ($n = 4$)**
Body Weight gain (g)	4.00 ± 5.48^{b}	8.00 ± 12.30^{b}	23.75 ± 5.62^{a}	4.25 ± 5.38^{b}	-7.00 ± 3.37^{b}
Diet Consumed (g)	53.50 ± 16.34^{ab}	54.25 ± 16.94^{ab}	79.50 ± 16.36^{a}	51.00 ± 10.13^{ab}	28.00 ± 13.43^{b}
Protein Consumed	4.55 ± 1.42^{b}	4.88 ± 1.52^{b}	9.29 ± 1.91^{a}	4.68 ± 0.93^{b}	0.11 ± 0.05^{c}
Feed Efficiency Ratio	0.07 ± 0.10^{a}	0.10 ± 0.20^{a}	0.30 ± 0.06^{a}	0.12 ± 0.14^{a}	0
Protein Efficiency Ratio	0.87 ± 1.18^{a}	1.13 ± 2.19^{a}	2.59 ± 0.51^{a}	1.13 ± 1.57^{a}	0

Values are expressed as mean ± standard deviation. Values (a-c) within a row that do not share the same letter are significantly different ($P < 0.05$)

CFDs Complementary food diets, *MD* maize diet, *MSD* maize + soybean diet, *MSCD* maize + soybean + crayfish diet, *FWMD* fortified wheat milk diet, *PFD* protein-free diet, *n* number of rats

Table 8 Effects of CFDs on the serum biochemical parameters of diet-fed rats during a 14-day feeding trial

Parameter	Rat-fed Diet				
	MD	MSD	MSCD	FWMD	PFD
TP (g/dl)	6.69 ± 0.57^{a}	7.04 ± 0.27^{a}	7.07 ± 0.14^{a}	6.97 ± 0.12^{a}	6.40 ± 0.37^{a}
ALB (g/dl)	2.95 ± 0.18^{a}	3.02 ± 0.12^{a}	3.10 ± 0.06^{a}	3.06 ± 0.13^{a}	2.78 ± 0.20^{a}
ALT (U/L)	78.25 ± 26.37^{a}	130.75 ± 62.06^{a}	53.50 ± 17.06^{a}	100.25 ± 65.02^{a}	68.00 ± 19.44^{a}
AST (U/L)	366.50 ± 121.10^{a}	737 ± 445.41^{a}	313.00 ± 128.55^{a}	490 ± 110.56^{a}	371.50 ± 183.62^{a}
ALP (U/L)	526.07 ± 110.86^{a}	538.37 ± 128.02^{a}	507.52 ± 71.59^{a}	522.85 ± 125.09^{a}	439.16 ± 65.31^{a}
Ca (mmol/L)	2.60 ± 0.08^{a}	2.63 ± 0.10^{a}	2.68 ± 0.12^{a}	2.68 ± 0.36^{a}	2.48 ± 0.05^{a}
Fe (µmol/L)	143.37 ± 48.10^{a}	156.65 ± 56.47^{a}	274.98 ± 348.12^{a}	142.43 ± 49.56^{a}	211.74 ± 91.03^{a}
Zn (µmol/L)	15.79 ± 1.14^{a}	16.34 ± 2.3^{a}	16.44 ± 2.92^{a}	18.21 ± 1.16^{a}	13.83 ± 1.17^{a}
Vit A (ng/mL)	135.67 ± 20.53^{a}	100.00 ± 77.88^{a}	64.40 ± 6.18^{a}	131.68 ± 74.14^{a}	84.50 ± 21.66^{a}
LDLC (mmol/L)	0.43 ± 0.10^{ab}	0.46 ± 0.13^{a}	0.41 ± 0.03^{a}	0.37 ± 0.08^{ab}	0.23 ± 0.05^{b}
HDLC (mmol/L)	1.56 ± 0.3^{b}	2.18 ± 0.5^{ab}	2.40 ± 0.14^{a}	2.12 ± 0.34^{ab}	1.42 ± 0.16^{b}
TCHOL (mmol/L)	1.88 ± 0.40^{ab}	2.58 ± 0.66^{ab}	2.85 ± 0.21^{a}	2.38 ± 0.41^{ab}	1.58 ± 1.19^{b}
TAG (mmol/L)	0.57 ± 0.15^{b}	0.53 ± 0.24^{b}	0.70 ± 0.23^{ab}	1.16 ± 0.25^{a}	0.37 ± 0.12^{b}

Values are expressed as mean ± standard deviation. Values (a-b) within a row that do not share the same letter are significantly different ($P < 0.05$)

CFDs complementary food diets, *MD* maize diet, *MSD* maize + soybean diet, *MSCD* maize + soybean + crayfish diet, *FWMD* fortified wheat milk diet, *PFD* protein-free diet, *TP* total protein, *ALB* albumin, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *ALP* alkaline phosphatases, *Ca* calcium, *Fe* iron, *Zn* zinc, *Vit A* vitamin A, *LDLC* low-density lipoprotein cholesterols, *HDLC* high-lipoprotein cholesterols, *TCHOL* total cholesterol, *TAG* triacylglycerol

highest compared to PFD, MF, MSF groups. The concentrations of Fe and Zn ranged from 142.43–272.98 µM and 13.83–18.21 µM respectively, of which the concentrations of the MSCD-fed group were not significantly different ($P < 0.05$) from other diet-fed groups. However, the concentrations of Fe and Zn in the MSCD-fed group were the highest compared with PFD, MF, MSF groups. The concentrations of Vit A ranged from 64.40–135.67 ng/mL, of which the MSCD-fed group was the lowest and was not significantly different ($P < 0.05$) from other diet-fed groups. However, the Vit A value of the MD-fed group was the highest of the groups.

The values of LDLC (0.23–0.46 mmol/L) and TCHOL (1.58–2.85 mmol/L) did not change significantly among MSCD-, MD-, MSD- and FWMD-fed groups, but the values of LDLC and TCHOL in MSCD group were significantly higher ($P < 0.05$) than that of PFD-fed group. However, the MSCD-fed group had the least value of LDLC compared to MD and MSD groups. The values of HDLC ranged from

Table 9 Effects of CFDs on haematological parameters of diet-fed rats during a 14-day feeding trial

Parameter	Rat–fed CFD				
	MD	MSD	MSCD	FWMD	PFD
RBC ($\times 10^{12}$ / L)	6.77 ± 0.21^{ab}	7.24 ± 1.07^{ab}	8.07 ± 0.29^{a}	7.12 ± 0.18^{ab}	4.91 ± 0.82^{b}
MCV (fl)	62.00 ± 4.76^{a}	58.00 ± 11.05^{a}	64.75 ± 0.96^{a}	63.50 ± 1.00^{a}	61.00 ± 2.94^{a}
MCHC (g / L)	310.00 ± 26.57^{ab}	441.50 ± 149.61^{a}	313.00 ± 7.70^{ab}	308.75 ± 6.70^{ab}	288.75 ± 14.10^{b}
Hb (g / L)	126.25 ± 6.08^{b}	129.50 ± 17.29^{bc}	154.00 ± 6.06^{a}	139.75 ± 5.00^{ac}	113.50 ± 15.89^{bc}
PCV (%)	41 ± 0.05^{bc}	44 ± 0.01^{ac}	49 ± 0.03^{a}	45 ± 0.02^{ac}	37 ± 0.02^{b}
WBC ($\times 10^{9}$ / L)	8.33 ± 4.95^{a}	10.73 ± 0.87^{a}	13.58 ± 2.29^{a}	12.20 ± 4.79^{a}	11.68 ± 3.44^{a}
NEUT (%)	12.00 ± 1.63^{a}	11.50 ± 1.91^{a}	14.50 ± 1.91^{a}	13.50 ± 1.91^{a}	11.50 ± 1.91^{a}
LYMPH (%)	82.00 ± 2.58^{a}	81.25 ± 2.06^{a}	76.25 ± 6.24^{a}	78.75 ± 2.22^{a}	82.00 ± 3.16^{a}
MONO (%)	4.75 ± 0.96^{a}	5.00 ± 0.00^{a}	5.00 ± 0.82^{a}	5.50 ± 0.58^{a}	5.00 ± 0.82^{a}
EOS (%)	0.25 ± 0.50^{a}	1.25 ± 1.26^{a}	3.25 ± 4.57^{a}	1.25 ± 0.50^{a}	0.50 ± 0.58^{a}
BASO (%)	0.00	0.00	0.00	0.00	0.00
PLT ($\times 10^{9}$/ L)	233.25 ± 173.87^{a}	763.25 ± 441.37^{a}	824.50 ± 362.47^{a}	973.25 ± 713.64^{a}	634.25 ± 169.88^{a}

Values are expressed as mean ± standard deviation. Values (a-c) within a row that do not share the same letter are significantly different ($P < 0.05$)

CFDs complementary food diets, *MD* maize diet, *MSD* maize + soybean diet, *MSCD* maize + soybean + crayfish diet, *FWMD* fortified wheat milk diet, *PFD* protein-free diet, *RBC* red blood cells, *MCV* mean cell volume, *MCHC* mean corpuscular hemoglobin concentration, *Hb* hemoglobin, *PCV* pack cell volume, *WBC* white blood cell, *NEUT* neutrophils, *LYMPH* lymphocytes, *MONO* monocytes, *EOS* eosinophils, *BASO* basophils, *PLT* platelets, *fl* femtolitre

1.42–2.40 mmol/L, of which the value of the MSCD-fed group was not significantly different from those of MSD- and FWMD-fed groups, but significantly higher ($P < 0.05$) than those of PFD- and MD-fed groups. However, the MSCD-fed group had the highest value of HDLC. The values of triacylglycerol (TAG) (0.37–1.16 mmol/L) were not significantly different among the diet groups, but the observed TAG value in the FWMD-fed group was the highest among all groups and significantly higher ($P < 0.05$) than those of PFD-, MD- and MSD-fed groups.

Effects of CFDs on the haematological parameters of diet-fed rats are presented in Table 9. The RBC ranged from 4.91–8.07 × 10^{12} /L. Although the MSCD-fed group had the highest value of RBC, it was not significantly different ($P < 0.05$) compared to other diets-fed groups, except for the PFD-fed group. The values of the MCHC ranged from 288.75–441.50 g/L, of which the value of the MSCD-fed group was not significantly different ($P < 0.05$) from other diets-fed groups. However, the MSD-fed group had the highest MCHC value, which was significantly higher ($P < 0.05$) compared to the PFD-fed group. The Hb concentrations ranged from 113.50–154.00 g/L, of which the level of the MSCD-fed group was significantly higher ($P < 0.05$) compared to other diets-fed groups, except for the FWMD-fed group. However, the Hb level of the MSCD-fed group was higher than that of the FWMD group.

The values of PCV ranged from 37 to 49%, of which the value of the MSCD-fed group was not significantly different ($P < 0.05$) from MSD- and FWMD-fed groups, but significantly higher ($P < 0.05$) compared to PFD- and MD-fed groups. The MSCD-fed group had the highest PCV value. The values MCV, WBC and PLT ranged from 58.00–64.75 fl, 8.33–13.58 × 10^9/L, 233.25–973.25 × 10^9/L respectively and there was no significant difference ($P < 0.05$) among diet-fed groups. However, the values of MCV and WBC were the highest for the MSCD-fed group.

The values of NEUT, LYMP), MONO, EOS, and PLT ranged from 11.50–14.50%, 76.25–82.00%, 4.75–5.50%, 0.25–3.25%, 233.25–973.25 × 10^9/L respectively and there was no significant difference among diet-fed groups. However, the values of NEUT and EOS were the highest for the MSCD-fed group, while the value of LYMPH was the highest for PFD-fed group, and the values of MONO and PLT were highest for FWMD-fed group.

Table 10 presents the effects of CFDs on the liver, kidneys, heart, and adrenal glands of experimental rats. In the current study, the values of organs and glands of experimental rats were not significantly different ($P \leq 0.05$) except for the kidney of MSD-fed group that was significantly higher ($P \leq 0.05$) than those of MD-, FWMD- and PFD-fed groups.

Discussions

Considering the proximate composition of MF, SBF, and CRF from Table 2 above, the higher contents in moisture, ash, and protein of CRF than those of maize and soybean were expected, because crayfish is an ASF. This finding is in agreement with the previous report that ASFs are rich sources of protein Agbemafle et al. (2020). The higher moisture content of CRF could be due to its higher water retention capacity, as it was found that crayfish protein concentration had a high-water holding capacity Romero et al. (2014). The lowest content in carbohydrate and fiber observed in CRF is also in conformity with the report that crayfish is generally lower in carbohydrate and fiber than plant-based foods (Venugopal and Gopakumar 2017; Rosemary et al. 2020. The highest lipid content of soybean is in corroboration with its characteristic of being a lipid-rich source Etiosa et al. (2018), whereas the highest carbohydrate content of MF was because starch is its major constituent Michaelsen et al. (2009).

From Table 3 above, the moisture contents of all the CFs were within the ≤10% level recommended for dry CFs Hayes et al. (1995). Low moisture content is desirable in CFs to enhance stability during storage, reduce

Table 10 Relative organ weight of Sprague Dawley rats fed CFDs

Relative organ weight (%)	Rat-fed diet				
	MD	MSD	MSCD	FWMD	PFD
Liver	4.24 ± 0.41^a	4.01 ± 0.42^a	3.70 ± 0.61^a	4.43 ± 0.26^a	3.93 ± 0.54^a
Kidney	0.37 ± 0.03^b	0.63 ± 0.07^a	0.46 ± 0.13^{ab}	0.41 ± 0.04^b	0.43 ± 0.13^b
Heart	0.86 ± 0.15^a	0.77 ± 0.25^a	0.66 ± 0.17^a	0.85 ± 0.11^a	0.93 ± 0.06^a
Adrenal gland	0.04 ± 0.01^a	0.04 ± 0.01^a	0.03 ± 0.00^a	0.05 ± 0.02^a	0.06 ± 0.02^a

Values are expressed as mean ± standard deviation. Values (a-b) within a row that do not share the same letter are significantly different ($P < 0.05$)

CFDs complementary food diets, *MD* maize diet, *MSD* maize + soybean diet, *MSCD* maize + soybean + crayfish diet, *FWMD* fortified wheat milk diet, *PFD* protein-free diet

microbial growth, and increase shelf life. Although the moisture content of MSCF was the highest among the studied CFs, this could be due to its strong water holding capacity affected by the addition of crayfish protein. Ash content for all the CFs was within the ≤3% recommended range of ash content for dry CFs Codex Alimentarius Commission (CAC) (2013). The observed elevated ash content of MSCF could be due to supplementation of maize and soybean blend with crayfish, thereby upholding the previous findings that ASFs such as crayfish are richer in micronutrients than plant-based foods Comerford et al. (2021). Ash content is a measure of the total amount of mineral content of a food Marshall (2010). The higher ash content of MSCF over other CFs in this study indicated the potential of MSCF to provide a much total mineral content for growth and development and thus a higher tendency to prevent micronutrient deficiency diseases (MDDs). Crude protein (CP) contents of MSF and MSCF were not significantly different because the two CFs were maintained at a 16% protein level from the baseline formulation. Crayfish, being an ASF, is endowed with higher-quality protein due to having a complete amino acid profile that is capable of complementing amino acid deficiency from plant-based foods Agbemafle et al. (2020), hence MSCF may produce a higher protein energy percent compared with MSF, thereby constituting a greater nutritional advantage for MSCF to prevent childhood protein energy malnutrition (PEM) on intake with the recommended guideline. The higher lipid content of MSF and MSCF than FWMF was their exclusive inclusion of oil-rich soybean flour Etiosa et al. (2018) in the formulation. The lower lipid content of MSCF compared with MSF may have been due to crayfish supplementation, because crayfish are generally known to have low lipid content Sanusi (2022). However, crayfish contains abundant amount of omega-3 long-chain polyunsaturated fatty acids (PUFAs) particularly eicosapentaenoic acid (EPA) (*cis*-5,8,11,14,17, C20:5) and docosahexaenoic acid (DHA) (*cis*-4,7,10,13,16,19, C22:6) Venugopal and Gopakumar (2017). DHA was reported to be essential for the growth and functional development of the brain and retina in infants, and required to maintain normal brain functioning in adults. Intake of DHA during pregnancy and early life of a child was reported to enhance growth and cognitive performance later in childhood Huffman et al. 2011; Srigley and Mossoba 2017. Both DHA and EPA have beneficial effects on health by lowering serum triacylglycerol levels, increasing membrane fluidity, and reducing the risk of thrombosis, coronary heart disease, hypertension, inflammation, and autoimmune disorders Ayas et al. (2013). These characteristics of crayfish may confer potential growth, immune function, and anti-cardiac disease promoters on MSCF as a CF. The observed lowest fiber content of MSCF among the formulated CFs could be due to crayfish supplementation that was characterized by low fiber content Venugopal and Gopakumar 2017; Rosemary et al. 2020 Thus, crayfish contributed little or no fiber to MSCF content. However, the crude fiber of all the CFs was within the ≤5% recommended range of crude fiber content for dry CFs Codex Alimentarius Commission (CAC) (2013. The low crude fiber content of CFs is paramount to reducing bulkiness, increasing energy density, and nutrient bioavailability Adejuwon et al. (2021). In the overall energy value, only the total energy yield of MSF and MSCF satisfied the recommended range of energy content of ≥400 kCal/100 g for dry CFs Codex Alimentarius Commission (CAC) (2013).

From Table 4, the essential amino acid contents of the formulated and control CFs yielded values that did meet the RDA for children between 7 and 36 months old Institute of Medicine (US) Food and Nutrition Board (IOMFNB) 1998; Institute of Medicine of the National Academies (IOMNA) 2005except for lysine (591 mg/100 g MF), SAA (124.6 mg/100 g MF; 146.4 mg/100 g MSF; 340 mg/100 g MSCF), and tryptophan (43.6 mg/100 g MF) perhaps because they were limiting essential amino acids in maize and soybean Tome (2012). Essential amino acids are paramount for the maintenance of nitrogen balance and other body-building functions, so much that if any one of them is deficient during body protein synthesis, the amino acid in short supply becomes the limiting factor Solomon (2005). In the context of this study, supplementation of MSF with crayfish was to improve the deficient sulphur amino acids in MSF, which are found in abundance in ASFs such as crayfish. By so doing, a better CF as MSCF having a higher quality protein was formulated.

From Table 4, it is observable that sulphur amino acids content was improved by about 132% in MSCF upon supplementation of MSF with 10% crayfish. Considering this improvement, MSCF may have a higher potential for protein synthesis, growth promotion, and hence prevention of PEM when introduce to infants and young children during complementary feeding. Although there was an improvement in sulphur amino acids content of MSCF, further improvement could still be achieved by increasing the quantity of crayfish to about 12.8% for

supplementation, perhaps the yield of sulphur amino acids would satisfy the RDA for children between 6 and 36 months old.

Before the processing of various food ingredients into flours in the current study, such pretreatments as washing, soaking, heating, roasting, boiling, and dehulling were involved. In the report of Samtiya et al. (2020), washing, soaking, roasting, and boiling, could activate such endogenous enzymes as phytase in cereal and legumes to break down phytate into myo-inositol monophosphate, phosphates, minerals, proteins, peptides, and amino acids, thereby helping to reduce the capacity of phytate from binding essential nutrients. Phytate is also soluble in water which can enhance it's being leached into washing or soaking water Mohammadi et al. (2021). Phenolic oxidase produced during soaking could degrade tannin, and dehulling and milling could as well remove the phytate and tannin alongside the bran or seedcoat of maize and soybean Samtiya et al. (2020). The aggregation of these pretreatment processes on food ingredients could have resulted in lowering the contents of phytic acid, polyphenol and tannin observed in MF, MSF, and MSCF, as shown in Table 5. This is corroborated by the findings of Kathuria et al. (2021) that such processes as dehulling, roasting, soaking, and soaking and roasting significantly reduced the contents of phytic acid and total phenolic in soybean. Reduction of the antinutritional factors in MSCF may make it a potential CF to prevent malnutrition in children between 6 and 23 months old when consumed following the recommended guideline.

From Table 5, the significantly higher ($P < 0.05$) content of AST_{TC} in MSCF could be due to the exclusive presence of another form of carotenoid, which was absent in MF, MSF and FWMF (see Table 6). Thin layer chromatography (TLC) analysis of CFs (see Appendix 2 for bands on thin-layer plates of extracts from crayfish and CFs and AST standard solution), revealed that only MSCF had bands 1 and 2 with respective Rf values of 0.30 and 0.59 that showed AST presence. In a comparison of these calculated Rf values with recommended Rf standard as cited in Lorenz, 1998(see Appendix 3 for standard Rf values of carotenoids), the MSCF Rf value of 0.30 was similar to that of the AST standard of 0.31 which corresponded to the recommended Rf value 0.33 of free AST. Similarly, the MSCF Rf value of 0.59 corresponded to the recommended Rf value 0.51 of AST monoester. Consequently, the two AST forms whose presence were detected in MSCF were of free and monoester AST. The exclusive presence of AST in MSCF must have been the supplementation of maize and soybean blend with crayfish that is known to have AST (see Fig. 1). The presence of AST in crayfish and other shellfish food was reported by such authors as Ahmadkelayeh and Hawboldt (2020), Pulcini et al. (2021) and others. Due to the organoleptic property of crayfish, the likeness and acceptance of a child for MSCF may enhance the adequate consumption that can ensure his/her age-specific energy and nutrient needs. Astaxanthin is good for promoting good vision and brain development in infants due to its easy permeation of the blood-brain barrier as well as the blood-retinal barrier to prevent inflammations of these organs (Amengual 2019; Zielinska et al. 2019; Stachowiak & Szulc. 2021. For this, MSCF consumption may help prevent night blindness and low cognitive ability in children, and the eventual antioxidative interactions of astaxanthin in the body due to consistent intake of MSCF might as well help to prevent age-related macular degeneration in adulthood. One of the emerging malnutrition problems in developing countries is childhood overweight and obesity World Health Organization (WHO) (2022), of which the prevalence was about 1.8–2.4% among under-five children in Nigeria UNICEF Global Database (2021). The introduction of astaxanthin-containing MSCF to children may help to prevent the occurrence of childhood obesity, following the report of anti-obesity effect of astaxanthin Yaqoob et al. (2022).

Considering the changes in the BWG of diet-fed groups from Table 7, the significant change showed in the BWG of the MSCD-fed group compared to other diet groups could be due to the astaxanthin part of crayfish contained in MSCD. It was reported that astaxanthin, through its diverse antioxidant activities, possesses an organoleptic quality that aids in the diet consumption of animals Venugopal and Gopakumar (2017). In addition, the MSCD group had a high content of sulphur-containing amino acids, especially methionine which is indispensable for protein synthesis and hence growth Mazor et al. (2018). This is in agreement with the report that the weight gain of animals is partly influenced by the amount of food consumed and the protein quality of that food Umerah et al. 2020; Parikh et al. 2022. Although the FWMD group had a higher sulphur-containing amino acid compared to MSCD, the amount of FWMD consumed by the rats was lesser and thus, showed a lesser weight gain. PFD-fed group was in negative nitrogen balance due to continued reduction in feeding on a protein-fee diet and was therefore at weight deterioration.

Despite that, the feed intake by the test diet-groups was not significantly different ($P < 0.05$), the amount of

MSCD consumed was the highest among the groups. This may be due to the improved palatability and acceptance of MSCD by the rats, which could have been made possible by supplementation with astaxanthin-containing crayfish. This agrees with the finding that feeds intake was a function of palatability, source of nitrogen, and essential amino acid Ene-Obong and Obizoba (1995). During the balance period, the amount of diet consumed by the PFD-fed group was reduced to about half the amount consumed by other diet-fed groups and resulted in a weight loss of 5.86%. A similar trend of weight loss in rats fed a protein-free diet was observed in Ibironke et al. (2018) and Adejuwon et al. (2021).

The increased intake of MSCD by MSCD-fed rats in this study had resulted in higher protein intake (9.29 g) by the MSCD group than in other diet groups. This pattern of consumption may have caused the highest FER value showed in the MSCD group compared to other groups. Hence FER, which is the ability of a diet to support growth, was reflected in significantly higher BWG of the MSCD group compared with other groups. The conversion of MSCD to useful output by MSCD group could also be attributed to the presence of astaxanthin, of which there was a reporting evidence of higher feed efficiency ratio in broiler chickens whose feed were treated with astaxanthin compared with control group placed on astaxanthin deprived feed Awadh and Zangana (2021). The consumption of MSCF by a child may stand a better chance to produce useful nutritional outcomes than other CFs, as the astaxanthin part of MSCF may enhance its palatability, acceptability, consumption, and resultant positive outcomes in the body.

Although the PER value of the MSCD group was not significantly different ($P < 0.05$) from the diet groups but was much higher to have met the minimum recommended value of 2.10 PER for CFs Shiriki et al. (2015). This achievement may be due to the elevated intake of MSCD, elicited by the presence of astaxanthin. The elevated intake of MSCD may have instigated an increased intake of protein that resulted in significant weight gain by rats fed MSCD. Other diet-fed groups were quite below the smallest recommended PER value due to a lower intake diet. This is following the finding of Olu and Adeniran (2015) that when a larger quantity of a diet is consumed, more protein would be made available for the maintenance of body weight and growth, with a resultant higher PER value.

The levels of biochemical parameters from diet-fed rats presented in Table 8 were interpreted by comparing with specified reference standards. Serum TP and ALB results obtained in this study indicated that formulated and control diets did not cause any toxic effects on the rats' livers, as they were, respectively, within the normal ranges of 5.11–7.50 g/dl and 2.69–3.86 g/dl for healthy Sprague Dawley male rats reported in Petterino and Argentino-Storino (2006), Han et al.(2010) and He et al. (2017). Supplementation of MSF with crayfish had greatly improved the sulphur amino acids content of MSCF, especially methionine (see Table 4). This could have influenced the higher TP (7.07 g/dl) and ALB (3.10 g/dl) levels of MSCD compared to MSD groups. The connection of methionine to growth is that cellular methionine status is known to integrate nutrient availability for growth and reproduction Neubauer and Landecker (2021). The involvement of methionine in the protein synthesis in animals is indispensable, as the process of translating genetic code in messenger ribonucleic acid into protein can only be initiated by a transfer ribonucleic acid that carries methionine Neubauer and Landecker (2021). Recently, it was discovered that mTORC1 responded not only to amino acids but also to S-adenosylmethionine which is a key methyl donor derived from methionine Liu and Sabatini (2020). Given the foregoing, the potentiality of MSCF to induce higher protein synthesis in the MSCD-fed group may make it a better alternative to CF for proper growth and prevention of PEM in children when introduced.

Furthermore, based on the multiple linear regression analysis (see Appendix 4, 5 and 6 for model summaries for predicting growth performance in terms BWG, PER and FER in Sprague Dawley rats) as obtained from SPSS version 25, the observed R values of 0.294, 0.482, and 0.486 for BWG, PER, and FER respectively showed some levels of growth were predicted by TP and ALB when the experimental rats were fed on MD, MSD, MSCD, and FWMD. In all the cases, $R = 0.296$ was considered low prediction, whilst $R = 0.482$ or 0,486 was considered moderate prediction. Also, based on the R^2 values from in the 3 cases, TP and ALB had predicted 8.7, 23.3 and 23.7% of the variability in BWG, PER, and FER respectively (see Appendices D, E and F). Thus, in the current study, TP and ALB could insignificantly ($P < 0.05$) predict normal growth in terms of BWG, PER, and FER in Sprague Dawley rats fed on MD, MSD, MSCD, and FWMD.

Serum enzymes such as ALT, AST, and ALP are useful biomarkers of liver function and are thus used for the diagnosis of the health status of the liver Adejuwon et al. (2021). Activities of ALT, AST and ALP in the serum are elevated whenever the integrity of liver cells is disrupted, leading to their release into the bloodstream from such disrupted cells Oibiokpa et al. (2018). The results of

enzyme activities obtained in this study indicated that both formulated and control diets did not cause any alteration in the serum activities of ALT, AST, and ALP of the experimental rats, as their activities were respectively within the normal ranges of 1–223.3, 0.2–838.3 U/L and 160.8–838.3 U/L for healthy Sprague Dawley male rats, as reported in Delwatta et al. (2018). However, exhibition of the lowest values of MSCD-fed group for ALT, AST and ALP could be due to the antioxidative effect of astaxanthin present in MSCD, resulting from crayfish supplementation. Astaxanthin, due to the conjugated double bond in its structure, exerts a potent antioxidant activity by which singlet oxygen is quenched and thus acts as an in vivo scavenger of reactive oxygen species that may pose a threat to the liver cells Venugopal and Gopakumar (2017). The finding in this study may be related to those of Fahmy and Hamdi (2011) and Jasim and Jwad (2021) wherein the administration of astaxanthin extracts from crayfish, following carbon tetrachloride and acetaminophen intoxication respectively, reduced the levels of ALT, AST and ALP in experimental animals as compared to intoxicated groups without astaxanthin intervention.

The observed highest value of Ca in the MSCD group could be due to improved Ca bioavailability resulting from crayfish supplementation. Serum Ca of the diet-fed groups was within the normal ranges of 2.43–2.8 mM for healthy Sprague Dawley male rats, as reported in Petterino and Argentino-Storino (2006). Also, the observed highest values of Fe and Zn of the MSCD group could be due to the highest consumption of protein and improved bioavailability of Fe and Zn due to crayfish supplementation. This supported the finding of Zhang et al. (2016) that Fe and Zn bioavailability from a plant-based diet could improve when supplemented with ASFs, such as crayfish. Generally, the bioavailability of elements is associated with dosage, chemical form, delivery matrix, and pharmacokinetics Zhang et al. (2016). The enhanced bioavailability of calcium, iron, and zinc showed in the MSCD group in the current study could also be due to the joint processing methods for the food ingredients used for CFs formulation, which thus reduced their contents of phytates and tannins, and matrix effects. Moreover, the bioavailability of elements from animal foods is fairly adequate than that from plant foods, this could suggest the rationale for the highest values of serum Fe, Zn, and Ca observed in the MSCD group, as elements from animal origin are usually complemented with absorption promoters that can avoid their precipitation and bioavailability issues Zhang et al. (2021). For instance, the presence of copper-containing protein, hemocyanin, in crayfish was suggested to have been responsible for its copper richness of about 32% (World Healthiest Food (WHF) (2020). Copper is involved in the absorption and metabolism of iron in vertebrates Ems et al. (2021). Copper was found to activate ceruloplasmin in the plasma and hephaestin on the basolateral membrane of the enterocyte. Ceruloplasmin and hephaestin catalyse the oxidation of and subsequent binding of ferrous iron to transferrin in the plasma, prevent the formation of reactive oxygen species, and facilitate the eventual transport of iron into cells Ems et al. (2021). In a related study, after 12 months of feeding, the addition of copper nanopowder into the diet of experimental animals was found to increase serum levels of iron and zinc significantly compared with animals fed with a copper-free diet, but this trial did not affect the calcium level Stepanova et al. (2020).

Furthermore, based on Shapiro-Wilk test, only the data sets of serum calcium, zinc, and total tannin of CFDs were normally distributed ($\alpha > 0.05$), and thus met the condition for correlation test. Subsequently, the Pearson's correlation analysis (see Appendix 7 for Correlation Coefficient between serum calcium or zinc and total tannin in CFDs) conducted between calcium or zinc and total tannin displayed a negative linear relationship between calcium or zinc and total tannin, suggesting a decrease in the level of total tannin in CFDs can elicit an increase in the serum zinc or calcium level of Sprague Dawley rats fed on the CFDs, and vice versa. This association may be liable for the highest levels of serum zinc and calcium showed in MSCD- and FWMD-fed diet groups, as both had the lowest levels of tannin compared with MD and MCD (see Table 5).

The highest value of TCHOL observed in the MSCD group compared with MD and MSD diet groups could have been due to the contribution of crayfish which is known to possess a high level of cholesterol of about 194 mg/100 g World Healthiest Food (WHF) (2020). Although all diet-fed groups showed good nutritional outcomes when compared with normal ranges of serum LDLC (0.54–1.30 mmol/L), HDLC (0.30–1.05 mmol/L), TCHOL (0.37–2.12 mmol/L), and TAG (0.23–1.33 mmol/L) for healthy Sprague Dawley male rats as reported in He et al. (2017) and Delwatta et al. (2018), but the highest and lower occurrences of HDLC and LDLC respectively in MSCD-fed group supported the finding of Adeyemi et al. (2015) that foods with healthy fats, such as monounsaturated fat and omega-3 fatty acids can increase and reduce the respective levels of HDLC and LDLC. More so, supplementation of normal feed with such seafood as crayfish was reported to have influenced positive health outcomes in experimental

rabbits (Ojiako et al., 2018). The presence of astaxanthin in MSCD could have elicited the alternating levels of lipid profile observed in the MSCD group. Astaxanthin, being an antioxidant, was discovered to inhibit low-density lipoprotein oxidation, and increase high-density lipoprotein cholesterol and adiponectin levels Yaqoob et al. (2022). The lower level of LDLC observed in the MSCD group compared with other diet groups could also be attributed to the synergy between other sterols found in crayfish, such as clionasterol and campesterol which function as anti-inflammatory molecules and also are concerned in the association that causes a decrease in the level of serum LDLC World Healthiest Food (WHF) (2020). Although previous studies lack consistent conclusions as to whether astaxanthin is linked to various health benefits as claimed, a meta-analysis of randomized controlled trials found that astaxanthin consumption was associated with an increase in HDL-C Xia et al. (2020).

In addition to the foregoing, the effect of dietary methionine may also play a significant role in the values of lipid profile observed in the MSCD group. In the findings of El-wahab et al. (2016), an increase in the bioavailability of dietary methionine level was associated with increased plasma HDLC level with a corresponding decrease in LDLC, because S-adenosyl methionine derivatized from methionine is a substrate for choline synthesis in the hepatopancreas which can provide enough phospholipids and Acetyl-CoA for cholesterol and lipoprotein synthesis, and that HDLC thus formed is capable of absorbing harmful materials such as LDLC, TCHOL, and TAG for onward transportation to the liver for decomposition and excretion. Consequently, the increase in sulfur amino acids content, especially of methionine in MSCD and the associated increase in the intake and digestibility of MSCD compared with other diets could be responsible for the highest values of HDLC observed MSCD group. A diet that promotes a high level of HDLC and a low level of LDLC in the blood is of nutritional advantage by way of alleviation of the risk factors of childhood obesity Yaqoob et al. (2022). More importantly, the observation by which the serum lipids were within the normal ranges for the diet groups may suggest their zero risk for kidney damage as studies in a variety of animal models have shown that hypercholesterolemia accelerates the rate of progression of kidney disease Trevisan et al. (2006).

As presented in Table 9, haematological parameters from diet-fed rats were also interpreted by comparing with specified reference standards. Most of the haematological parameters recorded for the PFD group were below the specified normal reference range, feasibly due to protein deficiency. The RBC of MD-, MSD-, MSCD- and FWMD-fed groups were within, while that of the PFD-fed group was below the normal range of $6.39–9.65 \times 10^{12}$/L of RBC for healthy Sprague Dawley male rats, as reported in Han et al. (2010) and He et al. (2017). Only the MSD-fed group was above the normal MCHC range of 310–336 g/L for healthy Sprague Dawley male rats. On the other hand, the values of MCHC for MD, MSCD, and FWMD groups were within, and the PFD group was below the normal range of 310–336 g/L for healthy Sprague Dawley male rats, as reported in He et al. He et al. (2017) Only MSCD-fed and FWMD-fed groups were within, and other groups were below the normal Hb range of 135–159 g/L for healthy Sprague Dawley male rats, as reported in He et al. (2017). Only the PFD-fed group had a PCV value below the normal range of 38–52% for healthy Sprague Dawley male rats, while other diet-fed groups were within the normal range, as reported in Han et al. (2010) and He et al. (2017). The values of MCV for all the diet-fed groups were within the normal range of 58–67 fl for healthy Sprague Dawley male rats, as reported in He et al. (2017). The value of WBC for the MD-fed group was below the normal range of $3.00–9.22 \times 10^{9}$ /L for healthy Spraque Dawley male rats, while the WBC values for other diet-fed groups were above the normal range, as reported in He et al. (2017). The values of NEUT, LYMPH, MONO, EOS, and BASO of all the diet-fed groups were respectively within the normal ranges of 4.70–23.30%, 71.10–89.40%, 0.20–8.00%, 0.20–6.80%, and 0.10–5.10% for healthy Spraque Dawley male rats, as reported in Petterino and Argentino-Storino (2006) and Han et al. (2010). The values of PLT for the diet-fed groups, except for the MD-fed group that was below, were within the normal range of $784.00–1500.00 \times 10^{9}$/L for healthy male rats, as reported in Han et al. (2010).

Low PCV, Hb, RBC, and serum TP were found associated with protein deficiency Osundahunsi and Aworh (2003). Whereas the PFD-fed group had the lowest PCV, Hb, RBC, and serum TP values compared with other diet-fed groups, there was no significant difference in PCV, Hb, RBC, and serum TP between MSCD-fed and FWMD-fed groups. The higher concentration of PCV, Hb, RBC, and WBC in MSCD- and FWMD-fed groups showed their nutritional quality, which agrees with the previous report by Adejuwon et al. (2021) that diets containing high-quality protein and iron were found of enhancing the production of haemoglobin and antibody in animals. Contrarily, the low PCV and Hb observed in PFD and MD-fed groups may lead to poor production of haemoglobin and hence, predispose to anaemia Adejuwon et al. (2021). Furthermore,

the improvement in the levels of PCV, Hb, RBC and WBC of the MSCD-fed group compared with other diet-fed groups could be the consequence of crayfish supplementation, as this was evident in the findings of Obimba et al. (2015) wherein supplementation of the respective plant-based CFs with 7 and 10% crayfish at 16 and 20% protein levels caused an improvement in the levels of PVC, WBC and ALB compared with basal and reference diets. The improvement in the methionine content of MSCD due to crayfish supplementation might have enhanced haematopoiesis in MSCD-fed compared with MD-, MSD-, and FWMD-fed groups.

Also, adequate copper supplied by hemocyanin in crayfish may have been liable for the highest values of haematological parameters observed in the MSCD group, as copper functions in haematopoiesis and the formation of haemoglobin Halver (1991). This is in agreement with the findings of Stepanova et al. (2020) in which the addition of copper nano-powder to the diet of experimental animals, for 12 months, affected a significant increase in the levels of RBC, WBC, PVC, Hb and PLT of test group compared with the group fed with copper-free diet. It was reported in related findings that copper deficiency was found to alter the metabolic reprogramming in differentiating hematopoietic stem cells into different cell lineages Ruiz et al. (2021). Furthermore, the chitin found as the main component of crayfish dietary fibre may play a critical role in modulating the haematological parameters of MSCD-fed rats Lopez-Santamarina et al. (2020). Chitin and its derivatives such as chitosan, chitosan oligosaccharide particles have been found to have properties that could elicit immunological effects in mammals (Elieh Ali Komi et al., 2018). The activity of chitin is mainly detected in the lungs or gut where it activates a variety of innate and adaptive immune cells (Elieh Ali Komi et al., 2018). In the report of Elieh Ali Komi et al. (2018), chitin increased the induction of cytokine production, leukocyte recruitment, and alternative macrophage activation in mice fed with dietary chitin compared with mice fed with a chitin-free diet. Considering the above citations, elevated levels of blood cellular components of the MSCD group could have been accounted for by the presence of adequate copper, chitin, and its derivatives, and improved sulphur-containing amino acids in MSCD. Therefore, the feeding of children with MSCF may prevent childhood anaemia, internal bleeding, and offer immune protection.

Organ-body weight ratio is a useful marker of cellular swelling, atrophy, or hypertrophy Akanji et al. (2013). The lack of significant difference ($P < 0.05$) among the organs under study, the liver, kidneys, heart, and adrenal glands, as presented in Table 10, indicated the absence of negative effects caused by rat-fed diets. It can therefore be suggested that the rat feeding trial in the current study did not cause any form of swelling, atrophy, and hypertrophy on the organs of diet-fed rats, hence all the experimental rats may be pronounced free of internal organ diseases or injury He et al. (2019). A similar instance of no significance in the organ-weight ratio among experimental rats was observed in Wadoum et al. (2019) where the protein source of diets was derived from the meat of broiler chickens fed on probiotics and antibiotics. Though the relative weight of kidney from MSD-fed group was significantly higher than those of MD, FWMD, and PFD group, this may be due to higher lipid content of MSF.

Conclusion

The current study proved that 10% crayfish flour could be incorporated into yellow maize (80%) and soybean (10%) flours blend to produce infant complementary food (CF) with adequate nutrient density and quality that could elicit positive impact on the growth, and without negative effect on the health status of animals. The limiting sulphur amino acids in MSF was elevated after crayfish incorporation by about 132% in MSCF. Astaxanthin that could affect organoleptic property in and improve intake and digestibility of foods was exclusively present in MSCF. There were no signs of illness and internal organ diseases among the Sprague Dawley rats fed on CFDs under study. There was a negative linear correlation between tannin content in the CFs and the level of serum calcium or zinc in the experimental rats, reflecting a decreased tannin content as against an increased serum calcium or zinc, and vice versa. More so, TP and ALB could insignificantly ($P < 0.05$) predict normal growth in terms of BWG, PER, and FER in Sprague Dawley rats fed on MD, MSD, MSCD, and FWMD. The incorporation of crayfish flour into yellow maize and soybean flours blend (10:80:10 w/w), showed a strong positive impact on the nutritional quality and health outcomes of the MSCF. Crayfish is therefore highly recommended in the design and development of CFs to achieve a sustainable growth and wellbeing among infants and young children in Nigeria and other low-income countries of the world. It is recommended that the animal study be extended to 28 days with MD-, MSD-, MSCD-, and FWMD-fed groups for possible significant change in some of the growth and physiological parameters. Also recommended for future study are the assessment of renal and muscular health status of the CFD-fed rats by conducting such tests as blood urea nitrogen, creatinine, creatinine kinase and others.

Appendix 1

IACUC Approval Letter

PEJABAT TIMBALAN NAIB CANSELOR (PENYELIDIKAN DAN INOVASI)
OFFICE OF THE DEPUTY VICE CHANCELLOR (RESEARCH AND INNOVATION)

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

Date:	6th February 2020
AUP No.:	UPM/IACUC/AUP-R092/2019
Project Title:	Evaluation of Biological quality of Complementary Food Formulated from a blend of Nigerian Yellow Maize, Soybean and Crayfish powder Investigated in Weanling male Sprague-Dawley rats.
Principal Investigator:	Prof. Dr. Amin bin Ismail
Members:	Assoc. Prof. Dr. Norhaizan Mohd Esa, Dr. Zulfitri Azuan Mat Daud, Halid Sheriff Adegbusi
Attending Veterinarian:	Dr. Mohd Hafidz B. Mohd Izhar
Committee Decision:	The committee has reviewed and approved the proposed animal utilisation protocol, subject to relevant permit and/ or owner's consent.
Project Classification:	Chronic
Category of Invasiveness:	B
Source of Animals:	A Sapphire Enterprise, 22 Jalan Inda 2/8, Taman Universiti Inda 43300 Seri Kembangan.
Number of Animals Approved:	48 Rats.
Housing:	Animal Experimental Unit, Faculty of Medicine and Health Science, Universiti Putra Malaysia
Duration:	6th February 2020 – 6th February 2021

Ethical approval is required in the case of amendments to the approved animal utilisation protocol (AUP). Please apply using Form 105. Kindly submit a final/annual report (Form 106) upon study completion, or before expiry of approval.

PROF. DR. ABDUL RAHMAN OMAR
Chairman
Institutional Animal Care and Use Committee
Universiti Putra Malaysia

Appendix 2

Bands on thin-layer plates from CRF and CFs extracts, and AST_{std} solution

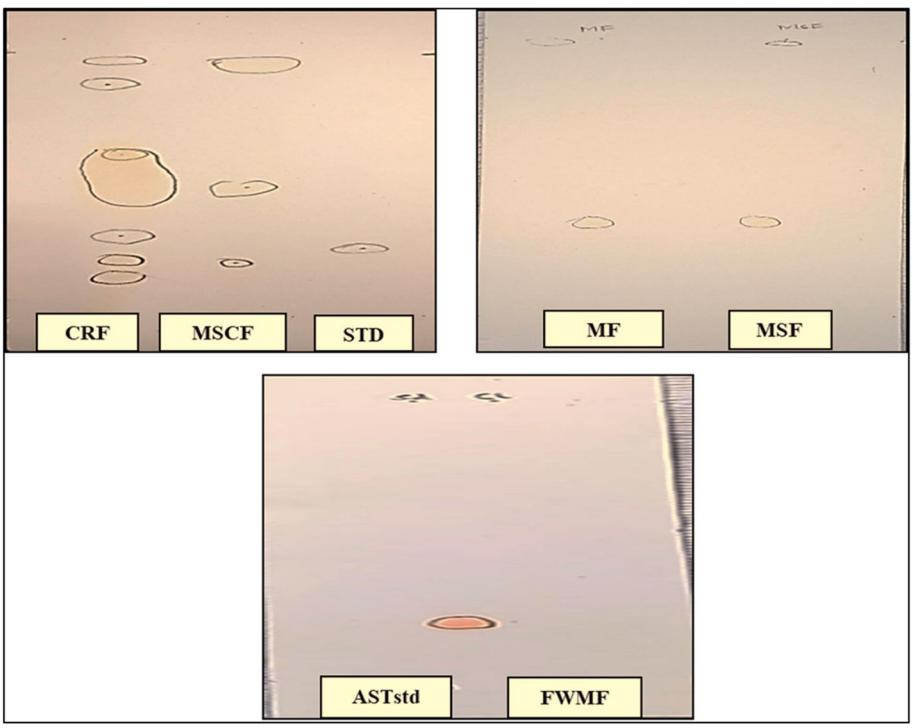


Fig. 3 Thin layer chromatography plates of extracts from CRF, CFs, and AST_{std}. CFs = complementary foods, MF = maize flour, MSF = maize + soybean flour, MSCF = maize + soybean + crayfish flour, FWMF = fortified wheat milk flour, CRF = crayfish flour and ASTstd = astaxanthin standard (STD)

Figure 3

Table 11 Standard Rf values of Carotenoids

Carotenoid	Typical Rf value
B-carotene	0.99
Echinenone	0.87
Astaxanthin Di-esters	0.75
Astaxanthin Monoesters	0.50
Canthaxanthin	0.40
Astaxanthin Free	0.33
Lutein	0.25

Appendix 3
Standard Rf values for astaxanthin
Table 11

Table 12 Model summary for predicting growth performance in terms of body weight (BWG)[b] gain in Sprague Dawley rats

Model	R	R^2	Adjusted R^2	Standard Error of the Estimate
1	0.294[a]	0.087	−0.054	11.16375

[a] Predictors: (Constant), ALB, TP

[b] Dependent variable: BWG

R multiple correlation coefficient, R^2 coefficient of determination, *ALB* albumin, *TP* total protein

The *F*-ratio in the ANOVA table test for BWG was as; $F(2, 13) = 0.617$, $P > 0.05$

Appendix 4
Model summary for predicting growth performance in terms of body weight (BWG)[b] gain in Sprague Dawley rats
Table 12

Table 13 Model summary for predicting growth performance in terms of protein efficiency ratio (PER)[b] in Sprague Dawley rats

Model	R	R^2	Adjusted R^2	Standard Error of the Estimate
1	0.482[a]	0.233	0.114	1.41896

[a] Predictors: (Constant), ALB, TP

[b] Dependent variable: PER

R Multiple correlation coefficient, R^2 = Coefficient of determination, *ALB* Albumin; TP

The *F*-ratio in the ANOVA table test for PER was as; $F(2, 13) = 1.969$, $P > 0.05$

Appendix 5
Model summary for predicting growth performance in terms of protein efficiency ratio (PER)[b] in Sprague Dawley rats
Table 13

Table 14 Model summary for predicting growth performance in terms of food efficiency ratio (FER)[b] in Sprague Dawley rats

Model	R	R^2	Adjusted R^2	Std. Error of the Estimate
1	0.486[a]	0.237	0.119	0.14511

[a] Predictors: (Constant), ALB, TP

[b] Dependent variable: FER

R multiple correlation coefficient, R^2 coefficient of determination, *ALB* albumin, *TP* total protein

The *F*-ratio in the ANOVA table test for FER was as; $F(2, 13) = 2.014$, $P > 0.05$

Appendix 6
Model summary for predicting growth performance in terms of food efficiency ratio (FER)[b] in Sprague Dawley rats

Table 15 Correlation between serum Ca, Zn, and total tannin in CFDs

		Total tannin
Zinc	Pearson Correlation coefficient (r)	−0.080
	Sig. (2-tailed)	0.804
	N	12
Calcium	Pearson Correlation coefficient (r)	−0.273
	Sig. (2-tailed)	0.390
	N	12

N = Sample number; correlation is significant at the 0.05 level (2-tailed); r is between −1 to +1

Table 14

Appendix 7
Correlation between serum Ca, Zn, and total tannin in CFDs
Table 15

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Authors' contributions
All the following contributing authors have read and approved the final manuscript. Halid Sheriff Adegbusi: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing-Original draft preparation, Visualization, and Project administration. Amin Ismail: Lead Supervision, Writing-Reviewing and

Editing. Norhaizan M. Esa: Associate Supervision, Writing-Reviewing and Editing. Zulfitri AM. Daud: Associate Supervision, Writing-Reviewing and Editing.

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Declarations

Ethics approval and consent to participate
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Consent for publication
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Competing interests
The authors declared that there is no conflicting interest.

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